EMERGING CHALLENGES OF CARDIOVASCULAR AND METABOLIC DYSFUNCTIONS IN CARDIO-ONCOLOGY: FROM BENCH TO BEDSIDE

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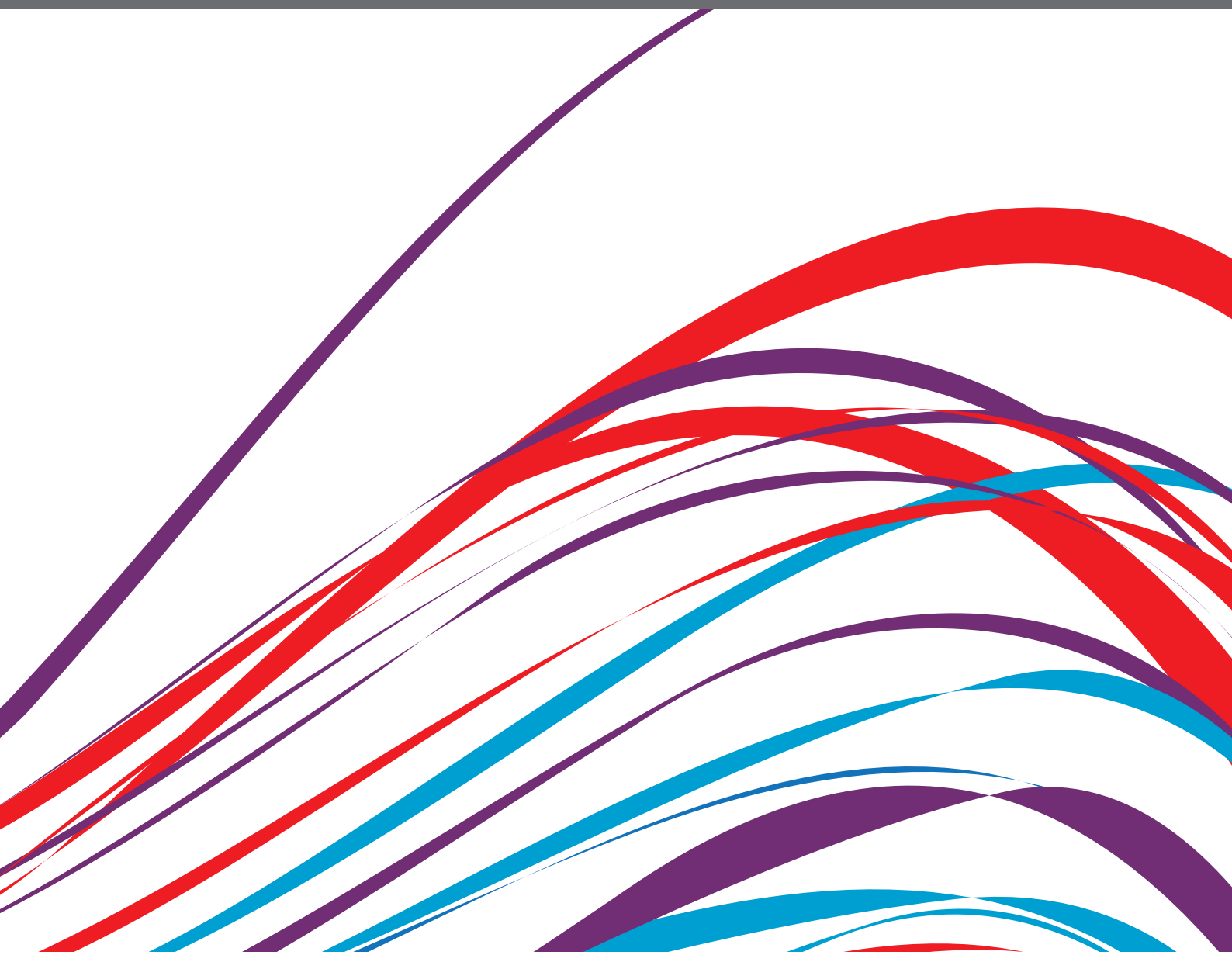

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EMERGING CHALLENGES OF CARDIOVASCULAR AND METABOLIC DYSFUNCTIONS IN CARDIO-ONCOLOGY: FROM BENCH TO BEDSIDE

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EDITORIAL
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Editorial: Emerging Challenges of Cardiovascular and Metabolic Dysfunctions in Cardio-Oncology: From Bench to Bedside

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Keywords: cardiooncology, cardiotoxicity, antracyclines, biomarkers, GPCR (G protein coupled receptors), epigenetics (methylation/demethylation), HPSC-cardiomyocytes, HDLP

Editorial on the Research Topic

Emerging Challenges of Cardiovascular and Metabolic Dysfunctions in Cardio-Oncology: From Bench to Bedside

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INTRODUCTION

The number of cancer survivors is increasing and up to 30 new cancer therapies are approved each year with only incompletely characterized side effects (1). Many anti-cancer drugs including traditional, new targeted kinase inhibitors and immunotherapies are associated with cardiovascular and metabolic adverse effects and may have dramatic impact on morbidity and mortality (2). The exact mechanisms how chemotherapeutics induce metabolic disturbances are mostly unclear. Chemotherapeutics-induced oxidative stress and mitochondrial dysfunction may promote metabolic disturbance (3).

Cardio-oncology is a relatively new discipline, aiming at finding an optimal balance between the efficacy of anticancer treatments and the management of their adverse cardiovascular and metabolic effects. It includes the prevention, diagnosis, and treatment of these complications in cancer patients. Identifying markers/predictors of disease risk, ensuring safety of novel cancer therapeutics, developing cardioprotective drugs are the emerging challenges in cardio-oncology (4).

Cardiovascular disease and cancer not only share common genetic, cellular, and signaling mechanisms such as chronic inflammation but also exhibit common risk factors such as obesity and diabetes (5). Dyslipidemia, hypertriglyceridemia, altered levels of low-density lipoprotein cholesterol (LDL-C), and low high-density lipoprotein cholesterol (HDL-C) have been observed in cancer survivals (6). In this regard, about half of the cancer survivors have obesity issue (7). Hypertension, Insulin resistance, hyperinsulinemia, and impaired glucose control that directly affect insulin sensitivity have been observed in cancer survivals (8). Chemotherapies that adversely affect metabolism may amplify cardiac and vascular toxicity, and patient management represents a major economical and clinical burden.

In this edition, we covered the expert reviews on biomarkers and signaling pathways of cardiovascular toxicity and metabolic alteration, and characterization of possible target molecules to prevent or treat cardiovascular damages induced by the cancer therapy.

Kumari et al., focused on epigenetic modifications by doxorubicin (DOX) that can either be used as molecular markers for cancer prognosis or represent molecular targets to attenuate DOX-induced cardiotoxicity in cancer patients.

Kluck et al., outlined emerging preclinical evidence that high density lipoprotein and its precursor protein apolipoprotein A1 may also protect against doxorubicin-induced cardiotoxicity.

Schwach et al., described that human pluripotent stem cell derived cardiomyocytes can be used as a screening platform to test cardioprotective agents against anti-cancer mediated oxidative stress generation and mitochondrial dysfunction, disruption of calcium homeostasis, and changes in transcriptome and proteome, triggering apoptotic cell death.

Mrotzek et al., underlined new studies on the mechanisms and severity of radiation-induced cardiovascular side effects and clinical management and treatment options.

Cardinale et al., discussed in particular troponins as a biomarker of subclinical cardiotoxicity and angiotensin-converting enzyme inhibitors (mainly enalapril) to prevent LVEF reduction in case of early detection of cardiotoxicity and prompt heart failure treatment.

Parichatikanond et al., discussed the molecular mechanisms of TGF-β in the pathogenesis of cardiac fibrosis and cancer and provide *in vitro* and *in vivo* evidences regarding antifibrotic and anticancer actions of TGF-β inhibitors.

Schlaak et al. outlined how inherited genetic variants promote differences in mitochondrial gene expression that may contribute to susceptibility of cancer patients to mediated cardiotoxicity.

Lee's et al. group recommended combined managements with control of comorbidities (such as hypertension, hypercholesterolemia, and diabetes, smoking cessation), and close monitoring and discussed use of statins and angiotensin-converting enzyme inhibitors (ACEIs) in the treatment of cardiovascular disorders induced by anti-cancer drugs.

Livingston et al., presented the evidence that understanding of mitochondria-dependent mechanisms of radiation-induced heart dysfunction can help to develop potential therapeutic targets to assist in prevention and treatment of radiation-induced heart damage.

Audebrand et al. emphasized newly identified cardioprotective agents targeting G protein coupled receptors (GPCRs) of adrenalin, adenosine, melatonin, ghrelin, galanin, apelin, prokineticin, and cannabidiol, provoking further drug development studies for the treatment of human heart failure induced by anticancer drugs.

CONCLUSION AND FUTURE CHALLENGES

The adverse effects of anticancer treatments including cardiovascular toxicity and metabolic syndrome and their relations with the genetic and environmental factors are still needs to be discovered. Research in cardio-oncology should aim at elucidating the mechanisms involved in cardiovascular toxicity as well as metabolic disturbances. A better understanding of the mechanisms of these adverse effects of anti-cancer therapies may lead to the identification of novel targets for drug development. Currently, evaluation of anticancer therapy-induced cardiovascular toxicity and metabolic disturbance have limitations. Therefore, identification of new early biomarkers of subclinical cardiovascular dysfunctions and metabolic disorders is a key challenge. Research in cardio-oncology should also aim at elucidating the efficacity and toxicity of the new cancer treatments. Thus, the coordinated efforts of oncologists, endocrinologists, and cardiologist are required to overcome these life-threatening problems especially in cancer survivals.

We believe that the topic of "Emerging challenges of cardiovascular and metabolic dysfunctions in cardio-oncology" provides new challenges and potential future directions to the readers from basic scientists, cardiologist, endocrinologists, and oncologist in developing field of cardio-oncology.

AUTHOR CONTRIBUTIONS

CN wrote the editorial information. MC and TR have been corrected and edited. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Rassaf T, Totzeck M, Backs J, Bokemeyer C, Hallek M, Hilfiker-Kleiner D, et al. Onco-cardiology: consensus paper of the german cardiac society, the german society for pediatric cardiology and congenital heart defects and the german society for hematology and medical oncology. *Clin Res Cardiol.* (2020). doi: 10.1007/s00392-020-01636-7
2. Nebigil CG, Désaubry L. Updates in anthracycline-mediated cardiotoxicity. *Front Pharmacol.* (2018) 9:1262. doi: 10.3389/fphar.2018.01262
3. Tocchetti CG, Cadeddu C, Di Lisi D, Femminò S, Madonna R, Mele D, et al. From molecular mechanisms to clinical management of antineoplastic drug-induced cardiovascular toxicity: a translational overview. *Antioxid Redox Signal.* (2019) 30:2110–53. doi: 10.1089/ars.2016.6930

4. Graffagnino J, Kondapalli L, Arora G, Hawi R, Lenneman CG. Strategies to prevent cardiotoxicity. *Curr Treat Options Oncol.* (2020) 21:32. doi: 10.1007/s11864-020-0722-6
5. Masoudkabir F, Sarrafzadegan N, Gotay C, Ignaszewski A, Krahn AD, Davis MK, et al. Cardiovascular disease and cancer: evidence for shared disease pathways and pharmacologic prevention. *Atherosclerosis.* (2017) 263:343–51. doi: 10.1016/j.atherosclerosis.2017.06.001
6. Rosen GP, Nguyen HT, Shaibi GQ. Metabolic syndrome in pediatric cancer survivors: a mechanistic review. *Pediatr Blood Cancer.* (2013) 60:1922–8. doi: 10.1002/pbc.24703
7. Chueh HW, Yoo JH. Metabolic syndrome induced by anticancer treatment in childhood cancer survivors. *Ann Pediatr Endocrinol Metab.* (2017) 22:82–9. doi: 10.6065/apem.2017.22.2.82
8. Barbosa-Cortés L, López-Alarcón M, Mejía-Aranguré JM, Klünder-Klünder M, Del Carmen Rodríguez-Zepeda M, Rivera-Márquez H, et al. Adipokines, insulin resistance, and adiposity as a predictors of metabolic syndrome in child survivors of lymphoma and acute lymphoblastic leukemia of a developing country. *BMC Cancer.* (2017) 17:125. doi: 10.1186/s12885-017-3097-8

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REVIEW
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Targeting GPCRs Against Cardiotoxicity Induced by Anticancer Treatments

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Novel anticancer medicines, including targeted therapies and immune checkpoint inhibitors, have greatly improved the management of cancers. However, both conventional and new anticancer treatments induce cardiac adverse effects, which remain a critical issue in clinic. Cardiotoxicity induced by anti-cancer treatments compromise vasospastic and thromboembolic ischemia, dysrhythmia, hypertension, myocarditis, and cardiac dysfunction that can result in heart failure. Importantly, none of the strategies to prevent cardiotoxicity from anticancer therapies is completely safe and satisfactory. Certain clinically used cardioprotective drugs can even contribute to cancer induction. Since G protein coupled receptors (GPCRs) are target of forty percent of clinically used drugs, here we discuss the newly identified cardioprotective agents that bind GPCRs of adrenalin, adenosine, melatonin, ghrelin, galanin, apelin, prokineticin and cannabidiol. We hope to provoke further drug development studies considering these GPCRs as potential targets to be translated to treatment of human heart failure induced by anticancer drugs.

Keywords: GPCRs, cardiotoxicity, melatonin, ghrelin, galanin, apelin, prokineticin, cannabidiol

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INTRODUCTION

New anticancer treatments have improved overall mortality (1). However, most of the anticancer drugs display a wide array of cardiovascular toxicities, leading to interruption of cancer therapies and maladaptive remodeling in hearts, affecting the short- and long-term quality of life (2–4). Oxidative stress and inflammation are inter-reliant processes involved in cardiovascular diseases and cancers (5, 6), along with apoptosis (7, 8) and necrosis (9). Tissue resident and circulating inflammatory cells (such as macrophages, mast cells, neutrophils, and monocytes) can also release both reactive oxygen species (ROS) and reactive nitrogen species (RNS) to induce an oxidative stress (6). Due to negligible detoxification capacity, the heart is particularly susceptible to ROS and RNS injury (10). Thus, high levels of ROS and RNS can debilitate cardiac cellular signaling pathways and can augment the gene expression of proinflammatory (11) and antioxidant defenses as the major cause for necrosis and apoptosis.

Classic chemotherapeutics particularly anthracyclines are the prototype of drugs causing cardiotoxicity (12). They can induce acute cardiotoxicity, including reversible hypotension, pericarditis and transient electrocardiographic abnormalities (changes in the ST-T waves, QT prolongation), and vasodilatation (13). However, after completion of cumulative dose regimens, anthracyclines promote irreversible cardiomyopathy (classified as type (1) cardiotoxicity), leading to heart failure (HF) (13, 14). Doxorubicin (DOX), the most frequently used anthracyclines can

cause irreversible type 1 cardiotoxicity via accumulation of ROS and RNS (15, 16). They also target Topoisomerase IIβ (Top IIβ) in cardiomyocytes to induce DNA damage and apoptosis. Recently, the anthracycline mediated cardiotoxicity has been reviewed by Nebigil (17).

Targeted therapies also provoke some degree of cardiotoxicity. Targeting key tyrosine kinases (TKs) with TK antibodies and inhibitors has a remarkable achievement in cancer management. However, they also induce cardiotoxicity, because they block pathways that also regulate myocardial function (18). This cardiotoxicity is often reversible, and thus classified as type 2 cardiotoxicity (19, 20). It results in ultrastructural changes in cardiomyocytes, with reversible cardiac dysfunctions such as elevated blood pressure, thromboembolism, pericardial thickening, and arrhythmia (21). Type 1 and 2 forms of cardiotoxicity can overlap, when the classic and targeted therapeutics used together or subsequently. For example, in patient treated with anthracyclines earlier, trastuzumab, a monoclonal antibody anti-HER-2 can cause irreversible cardiac damage and left ventricular (LV) dysfunction (18, 22, 23). On the other hand, 27%, of patients who received both anthracycline and trastuzumab encountered cardiac dysfunction, while this rate was of 2-16% for patients treated with anthracyclines alone (24).

Recent studies have demonstrated that patients treated with immune checkpoint inhibitors (25) also develop myocarditis due to immune-related adverse events (6, 26). The therapeutic mechanisms of inhibitors mostly rely on blocking either the cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) or programmed cell death protein-1 (PD-1) pathways, while activating the host's immune system against cancer (27). CTLA-4 and PD-1 act as immune response inhibitors (6, 28). They suppress the T-cell response in order to prevent autoimmunity and maintain T-cell tolerance. Cardiac immune-related adverse events appear more frequently in patients treated with CTLA-4 antagonists compared with PD-1 inhibitors (29) and the myocarditis risk increases with combination therapy, leading to discontinuation in approximately 50% of patients (30, 31) probably due to targeting PD-1 and CTLA-4 in cardiomyocytes as well.

Abbreviations: GPCR, G protein-coupled receptor; DOX, Doxorubicin; HF, Heart failure; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; Top Iiβ, Topoisomerase I*i*β; LVEF, LV ejection fraction; HER2, Hergulin2; CTLA-4, T-lymphocyte associated antigen-4; PD-1, Programmed cell death protein-1; LDL, low-density lipoprotein; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; PI3K, Phosphoinositide 3-kinases; MAPK, Mitogen-activated protein kinases; β-ARs, β-adrenergic receptors; ? -ARs???-adrenergic receptors; CaMKII, Calmodulin-dependent protein kinase II; Ang-II, Angiotensin II; AT-1R and AT-2R, Angiotensin receptors; RAS, Renin-angiotensin system; ARB, Angiotensin-II receptor blockers; IP3, Inositol trisphosphate; DAG, Diacylglycerol; PKD1, Anchored protein kinase D1; ATP, Adenosine-triphosphate; A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R, Adenosine receptors; MT1 and MT2, Melatonin receptors; mPTP, Mitochondrial permeability transition pore; GHS-R, Ghrelin receptor, growth hormone secretagogue receptor; VEGF, vascular endothelial growth factor; GalR1, GalR2 and GalR3, Galanin receptors; APJ, Apelin receptor; ACE2, Angiotensin-converting enzyme 2; PARP, Poly(ADP-ribose) polymerase; PROK1 and PROK2, Prokineticins 1 and 2; PKR1 and PKR2, Prokineticin receptors; hiPSC-CMs, Inducible pluripotent stem cell derived cardiomyocytes.

CLINICALLY USED CARDIOPROTECTIVE AGENTS AGAINST CARDIOTOXICITY

There are several cardioprotective therapeutics that have been used against anticancer-mediated cardiotoxicity. Their properties are summarized in **Table 1**.

Antioxidants

Beneficial effects of antioxidants on LV remodeling and amelioration of contractility have been demonstrated in many experimental models of HF. For example, vitamin C effectively mitigates DOX-induced oxidative stress and apoptosis in rats (35). Resveratrol, a polyphenolic compound has also both prophylactic and therapeutic benefits in reversing DOX induced apoptosis and fibrosis in rat myocardium (36). Baicalein, a bioflavonoid can alleviate cardiotoxicity in mice (37). However, elimination of ROS and RNS by antioxidant drugs may be detrimental and even impair physiological cellular functions (58). There is also a risk of loss of oncological efficacy, because of the overlapping mechanisms with cardioprotective effects. Nevertheless, in clinic these approaches did not significantly improve survival rate and they may even increase mortality if they do not have other pharmacological properties (32, 59).

Dexrazoxane

Dexrazoxane is an iron chelator and detoxifying agent that can prevent anthracycline-associated cardiotoxicity. It also acts on Topoisomerase IIβ to promote cardioprotective effects. Dexrazoxane is the only Food and Drug Administration (FDA) and the European Medicines Agency (EMA) approved cardioprotective drug to against chemotherapeutics-mediated HF (38, 60). However, its use in children and adolescent were forbidden by EMA in 2011, because it increases risk of infection, myelosuppression and second primary malignancies. These restrictions by EMA have been partially altered based on the new findings in 2018 (39). Only use of dexrazoxane was allowed in patients who have received a cumulative DOX at the dose of 300 mg/m (2) and are continuing with this medicine. Although dexrazoxane is a valuable option to prevent cardiotoxicity, it induces a severe leukopenia in 78% of cancer patients (40). Use of dexrazoxane is not recommended with non-anthracycline chemotherapy regimens.

Statin

Statins are used to lower low-density lipoprotein (LDL) and cholesterol amount in the blood on patients suffering to arterosclerosis (61). The mechanism involved in this action is due to inhibition of HMG-CoA reductase, which is involved the biosynthesis of cholesterol. Statins also display significant vasodilatation, platelet inhibition, anti-inflammatory, and antioxidant effects due to their pleiotropic effects (62, 63). Statin (atorvastatin) could be effective in maintenance of LV ejection fraction (LVEF) in patients treated with anthracycline (42). Moreover, it could limit oxidative stress and vascular inflammation (64) and activate autophagy (43) to promote cardioprotective effects against dasatinib. Statins also inhibits Top IIβ mediated DNA damage via Rac1 inhibition. Recent

TABLE 1 | Prophylactic cardioprotective agents.

Clinically used cardioprotective agents	Mechanism of cardioprotection	Name of molecules	Anti-tumor effect	Study limitations
Antioxidants	⇓ROS and RNS (32–34)	Vitamin C (35) Resveratrol (36) Bicalein (37)	A risk of loss of oncological efficacy	No improvement in survival rate (32)
Dexrazoxane	Iron chelator and detoxifying agent, ⇓ Topoisomerase Iiβ (25, 38–41)	Topotect Zinecard Cardioxane	It increases risk of infection and myelosuppression second primary malignancies, leukopenia (78%) (40)	No improvement in survival rate (39)
Statin	⇑Vasodilatation, anticoagulation, ⇓platelet, antioxidant and anti-inflammatory functions; ⇓Topoisomerase II via Rac1 inhibition (42–45)	Lipitor Simvastatin Lovastatin Zocor Lescol Crestor Livalo	The meta-analyses suggested that statin can reduce cancer (expecially breast cancer)-mediated mortality (46)	40% patients use ACEIs and β-blockers together with statin, thus it is difficult to estimate the cardioprotective effectiveness of statin. Decreasing synthesis of mevalonic acid It can lead to muscle injury and diabetes (47)
Beta-AR blokers β1-AR acts through Gs and Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) β2-AR acts through the Gi and Akt pathway	⇓ROS generation ⇓Apoptosis in cardiomyocyte ⇓Mitochondrial complex-I (carvedilol)(48, 49) and vasodilatory effects (nebivolol) (50)	Carvedilol Nebivolol Metoprolol	The role of β-blockers on cancer-specific survival rate resulted in conflicting results (51, 52)	The benefit of the use of prophylactic beta-blockers for prevention of chemo-induced cardiotoxicity remains unclear (53). The non-selective β1 and β2 blockers could be more beneficial due to antioxidant effects (28)
ACEIs and angiotensin receptor blokers AT1R uses $G_{q/11}$, G_i, G_{12} and G_{13} coupled to PLCβ and Rho/ROCK. ⇑ROS generation, transactivation of growth factor receptors (IGF-1R).	⇓Vasoconstriction, ⇓Inflammation, ⇓Fibrosis, ⇓Hypertrophy ⇓Catecholamine and aldosterone release (54, 55)	Valsartan Candesartan Cilexetil	Antitumor effect is conflicting (56, 57)	Human trials are not conclusive yet. Combination of enalapril with metoprolol or candesartan has no clear beneficial effects (48)

meta-analyses suggest that statins are at least equally potent as dexrazoxane in the prevention of anthracycline-induced cardiotoxicity (65). Calvillo-Argüelles and colleagues have found that in $HER2^+$ breast cancer patients treated with trastuzumab with or without anthracycline, the concomitant statin use was associated with a lower risk of cardiotoxicity (44). Although, several studies on the influence of statin therapy on development of cancer risk resulted in conflicting results, the recent meta-analyses suggested that statin can reduce cancer-mediated mortality (46). However, there are some studies show that statin induces myopathies that may be due to decreased synthesis of mevalonic acid, leading to decreased energy generation and muscle injury. Another side effect associated with statin usage is new-onset diabetes (47). Many of the beneficial effects of a statin is due to inhibition of heterotrimeric G proteins, including Ras and Rho or Rac1 signaling (45). Thus, the specific Rho and Rac inhibitors may be more preferable targets for future chemo-preventive strategies.

GPCRs

As seven transmembrane (7TM) domain proteins, G protein-coupled receptors (GPCRs) represent the largest family of cell surface proteins (66). GPCRs regulate many physiological processes in every tissue, making the GPCR superfamily a major target for therapeutic intervention (67). The binding of agonists to GPCRs not only initiates the "classical," signaling cascades through heterotrimeric G proteins (composed of the three subunits, Gα, Gβ, and Gγ). It can also activate G-protein-independent pathways involving β-arrestin (68, 69). Indeed, β-arrestins are identified as scaffolding proteins for MAP kinases and serine/threonine kinases cascades (70). The discovery that some GPCRs prefer to activate G-protein- or arrestin-mediated pathways has given rise to efforts to produce signal biased drugs (71). The drug discovery efforts aim to produce "biased" and/or allosteric ligands with less adverse effects without compromising their efficacy (72). In cardiovascular system, GPCRs can lead to hypertrophy, apoptosis, contraction, and cardiomyocytes survival. Some of the GPCR targeted therapeutics are used in clinic for treatment of heart failure and cardiotoxicity (**Table 1**).

Preventive and Prophylactic Strategies Targeting GPCRs Against Anticancer-Induced Cardiotoxicity

β-Blockers

β-adrenergic receptors (β-ARs) play a crucial role in cardiovascular regulation. It exists 3 types of β-ARs: β_1, β_2 and β_3. Cardiac adrenergic receptor corresponding to β_1-ARs

whereas β_2-ARs are localized on blood vessels. β_1-ARs, are coupled to the $G\alpha_s$ and activate adenylyl cyclase to exert a positive inotropic, chronotropic and dromotropic effects in the heart. Indeed, β_1-ARs increase heart rate, cardiac contractility and myocardial oxygen demand, thus promoting myocardial ischemia in patients with coronary heart disease. More importantly, persistent β_1-ARs induce myocyte apoptosis and hypertrophy by activating CaMKII. On the opposite, persistent β_2-ARs activation protects myocardium through a Gαi-mediated pathway, and activating PI3K, and Akt kinase probably via small G proteins (73). Administration of β2-AR agonist and β1-AR antagonist seems to be better than β2-AR antagonist in HF prevention. Interestingly, β3-AR is activated by catecholamines at higher concentration than those required to activate β1-AR and β2-AR (73). Thus, β3-AR plays an important protective role in the cardiovascular system during sympathetic over-stimulation.

It exists three mains β-AR blockers. The first generation of β-blockers, such as propranolol, inhibits both β_1 and β_2-ARs. The second generation of β-blockers (metoprolol) are cardioselective (β_1-ARs).

The third generation of β-blockers (carvedilol and nebivolol) are vasodilators that not only inhibit β1 and α_1-adrenoreceptors, but they also activate β_3-adrenergic receptors (74). Carvedilol also reduces ROS generation and apoptosis in cardiomyocyte (49). Nebivolol has a vasodilatory effect mediated by nitric oxide release and avoid vasoconstriction to decrease blood pressure in hypertensive patients (50). Two clinical studies showed that carvedilol prevent cardiotoxicity in female patients diagnosed with breast cancer (75, 76). This cardioprotective effects has been attributed to its antioxidant and anti-apoptotic properties rather than its β-AR blocking activity, because carvedilol inhibits mitochondrial complex-I that promotes cardiotoxicity (77). This cardioprotective effect of carvedilol is superior than metoprolol and atenolol for preventing cardiomyocytes against DOX-induced apoptosis (78). In contrast, Avila and his colleague showed that carvedilol has no impact on the LVEF reduction induced by anthracycline in breast cancer patients (53). The recent meta-analyses on cancer patients have demonstrated that the use of β-blockers is not associated with cancer prognosis (51). Indeed, several studies on the influence of β-blockers on cancer-specific survival rate resulted in conflicting results (51, 52). The beneficial effects of non-selective β1 and β2 blockers could be due to their antioxidant effects (28).

Angiotensin Converting Enzyme Inhibitors (ACEI) and Angiotensin (AngII) Receptor Blockers (ARB)

Renin-angiotensin-aldosterone (RAAS) system regulates the cardiac and renal functions. Ang-II interacts with two GPCRs: AT-1R and AT-2R that are associated with opposite functions (79). However, most of the effects of renin-angiotensin system (RAS) are mediated by AT-1R, which promotes vasoconstriction, inflammation, fibrosis, hypertrophy, and releasing of catecholamine and aldosterone. AT-2 is implicated to vasodilatations, inhibition on cell growth, apoptosis, and bradykinin releasing. Increasing of Ang-II also stimulates sympathetic system and the production of aldosterone, leading to LV hypertrophy (80). Reduction of excessive Ang-II and aldosterone decrease cardiovascular morbidity and mortality. Indeed, AT-1R blockers ACE inhibitors are of paramount importance in treatment of cardiovascular diseases, including hypertension (54).

Several clinical trials indicate that Angiotensin-II receptor blockers (ARB) alleviate anthracycline cardiotoxicity (55), however, prospective trials are still needed for further validation. The expression of AngII and AT-1R have been found in many cell types of the tumor microenvironment (56). Thus, the RAS may alter remodeling of the tumor microenvironment and the immuno-suppressive milieu, thereby affecting tumor growth. In contrast, meta-analysis derived from the results of a group of trials demonstrated that ARB may promote the occurrences of new tumors (especially lung cancer) (57). These findings warrant further investigation.

The cardioprotective effects of combined ACEIs/ARBs and β-blockers have been evaluated during anthracycline, trastuzumab, or sequential chemotherapy. The combination of carvedilol and enalapril has been shown to preserve the LV function in adult patients treated with anthracyclines (81). However, other trials with combination of enalapril with metoprolol (82) or candesartan with metoprolol (83), ended up with disappointing results. Indeed, Guglin and his colleague recently demonstrated that both lisinopril and carvedilol do not prevent the cardiotoxicity of trastuzumab monotherapy in breast cancer patients (48). However, both drugs significantly alleviated the cardiotoxicity of anthracycline and trastuzumab sequential therapy. Although, ARBs, ACEIs, and β-blockers are necessary for treatment of HF, long-term studies are essential to validate whether ARBs have cardioprotective effects against the chronic or late-onset types of cardiotoxicities induced by cancer treatments.

Newly Discovered GPCR Agonist Against Anticancer-Mediated Cardiotoxicity

We discus here newly identified GPCR agonists that exhibit cardioprotective effects against anti-cancer drugs in *in vitro* and *in vivo* preclinical models (**Figure 1** and **Table 2**).

Alpha Adrenergic Receptor (Dabuzalgron)

Both the adrenergic receptors alpha 1 (α-AR1) and alpha 2 (α-AR2) bind catecholamines (epinephrine and norepinephrine). The α-AR1 couples to Gαq type, resulting in activation of phospholipase C, increasing Inositol trisphosphate (IP3) and diacylglycerol (DAG), and ultimately increasing the intracellular Ca^{2+} levels, leading to smooth muscle contraction and glycogenolysis (104). Cardiac α_1-ARs activate phospholipase C and MAPK to promote ischemic preconditioning (105), cardiac hypertrophy (106)and cardiac cell survival (107). The knockout of α_{1A}/α_{1B}-adrenoceptor in mice develops small hearts (108) and aggravates the pressure overload–induced HF. In support of this study a large-scale clinical trial showed that doxazosin, an inhibitor of α-AR1 signaling, increases HF in hypertension patients (109). The α2-AR acts via Gαi/o to an inhibit adenylyl cyclase, decreasing the available cAMP (110). It also decreases neurotransmitter release and central vasodilation.

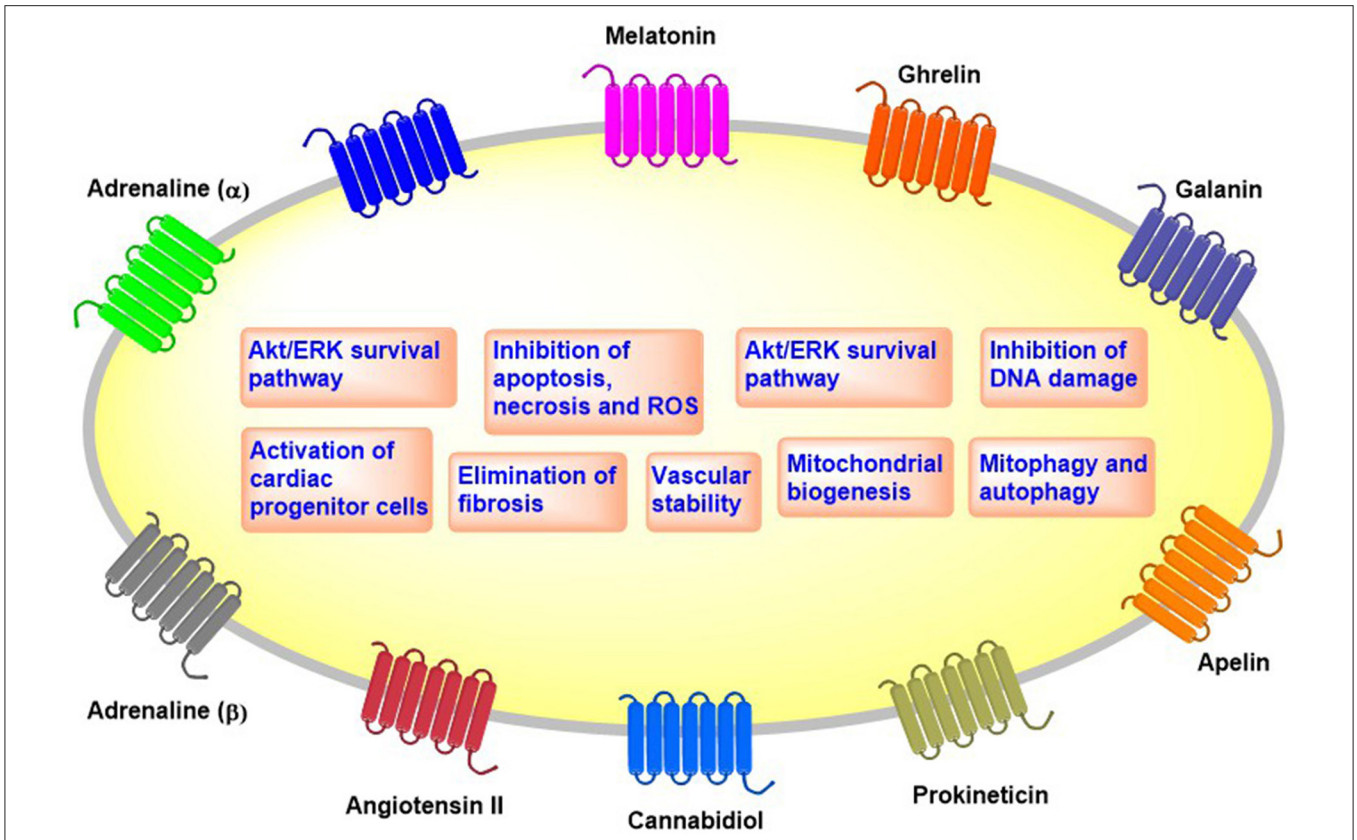

FIGURE 1 | Overview of the cellular effects of cardioprotective GPCRs.

Dabuzalgron is a selective α1AR agonist that has been clinically examined against urinary incontinence (111). Recent study in mice showed that dabuzalgron displayed a strong cardioprotection against DOX-induced cardiotoxicity (84). It reduces ROS production and fibrosis, enhances contractile function, and preserves myocardial ATP content via regulating mitochondrial function, in DOX-treated mice. Cardioprotective signaling pathways of α1-AR is not limited to activation of MAPK1/2 pathways (84), it also activates pro-survival pathways such as A kinase anchoring protein-Lbc (AKAP-Lbc) and its anchored protein kinase D1 (PKD1) in cardiotoxicity mice models (112). Future studies should determine whether dabuzalgron can be used to treat chemotherapeutics-mediated HF in cancer patients.

Adenosine Receptor Agonists

Adenosine is a naturally occurring nucleoside formed by the degradation of ATP. Extracellular adenosine concentrations rise in response to hypoxia and other stress (113). However, chronic adenosine elevation can increase inflammation, cytokine release, and induces brain dopamine depletion, fibrosis and kidney damage (114). The adenosine receptors A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R can sense an imbalance of demand and supply of oxygen and nutrients (115). Adenosine exerts a significant cardioprotective effect during cardiac ischemia by activation of the A_1R and A_3R (86, 116). However, full A_1R agonists have promote several cardiovascular adverse effects due to its off-target activation as well as desensitization of A_1R, leading to tachyphylaxis (117). In contrast, a selective partial agonist for A_1AR improves cardiac function without promoting atrioventricular blocks, bradycardia, or unfavorable effect on blood pressure (118, 119).

A selective A_3R agonist (Cl-IB-ME) mitigates bradycardia, elevated serum creatine kinase levels and cardiac histopathological changes in DOX-treated mice. Cardioprotective effect of Cl-IB-ME involves the inhibition of ROS production and inflammation induced by DOX *in vivo* (85). A_3AR activation also prevents perioperative myocardial ischemic injury (120), protects ischemic cardiomyocytes by preconditioning (121), and induces ischemic tolerance that is dependent on K_{ATP} channels (122). This cardioprotective effects A_3R agonists were absence in A_3AR deficient mouse cardiomyocytes, showing an A_3AR-mediated effect. On the opposite to A_1AR, A_3AR is expressed at very low levels in adult ventricular cardiomyocytes. The efficacy of two A_3AR agonists is currently examined in multiple clinical trials (123).

Melatonin Receptor Agonists

Melatonin is a pineal gland hormone synthesized from the amino acid tryptophan and is secreted into both the bloodstream and cerebrospinal fluid. It regulates circadian, seasonal, and transgenerational time cycles. Melatonin acts through 2 GPCRs, MT1, and MT2 that are linked to $G\alpha_i/G\alpha_o$ or $G\alpha_q/G\alpha_{11}$ to induce

TABLE 2 | Newly discovered cardioprotective agents targeting GPCRs.

Newly discovered cardioprotective agents targeting GPCRs	Mechanism of cardioprotection against anticancer-mediated cardiotoxicity	Name of molecules	Tumor effect	Study limitations
Alpha adrenergic receptor (α 1AR) Via $G\alpha_q/G_{11}$ $\Uparrow$PLC/Ca^{+2}	$\Downarrow$ROS,$\Uparrow$ mitochondrial function, $\Uparrow$ATP content, $\Uparrow$ERK 1/2 phosphorylation (84)	Dabuzalgron α 1AR agonist	No effect on anticancer efficacity in animal models (84)	While dabuzalgron a well-tolerated oral α1A-AR agonist, there has been no clinical trial on its cardioprotective role yet
Adenosine (A_1R and A_3R) Via $G\alpha_{i/o}$ $\Downarrow$ cAMP /PKA /CREB. Via Gαq $\Uparrow$PKC $\Downarrow$cardiac K^+ channels and voltage sensitive Ca^{2+} channels	$\Downarrow$oxidant/$\Uparrow$antioxidant $\Downarrow$inflammation, $\Downarrow K_{ATP}$ channels,$\Uparrow$neovascularization (85, 86)	Neladenoson (BAY 1067197) A_1AR agonist Cl-IB-MECA CP-608,039 34 CP-608,039 35 A3AR agonist	Highly selective receptor subtype agents are necessary Their effects on anticancer efficacity is not known	Multiple clinical trials with two A_3AR agonists are ongoing
Melatonin (MT1 and MT2) MT1 via Gαi $\Downarrow$AC/AMPK/PGC1α, $\Uparrow$PLC/PKC via Gαq. MT2 couples Gαs They dimerize with 5-$HT_{2c,}$ GPR61, GPR62, GPR50, GPR135	$\Downarrow$ROS $\Downarrow$mitochondrial permeability transition pore (mPTP) $\Downarrow$ lipid peroxidation (87–93)	Circadin ™ Country Life® Melatonin	Melatonin increases anticancer efficacity of anthracycline in animal models (93)	Receptor oligomerization may contribute to the functional diversity of Melatonin It needs to be further exploded in human trials
Ghrelin (GHS-R) $\Uparrow$PI3K, Akt, and NOS and p38-MAPK and $\Downarrow$AMPK activity. It dimerizes with SSTR5, DR2, MC3R, 5-HT2C	$\Uparrow$Autophagy $\Downarrow$ROS and mTOR induction (94, 95)	Hexarelin and GHRP-6 agonist	The role of ghrelin administration on antitumor efficacity of anticancer drugs is not known	Receptor oligomerization may contribute to the functional diversity of ghrelin Clinical trials are needed
Galanin (GalR1, 2, 3) GalR1-3 couple to Gαi/Gαo, $\Uparrow$Rho	$\Uparrow$ Functional and metabolic tolerance of the heart (96, 97)	GalR1-3 agonist Spexin (GalR3 agonist)	The role of galanin administration on antitumor efficacity of anticancer drugs is not known	It needs to be further exploded in human trials
Apelin (APJ) $\Uparrow$AMPK and PI3K, and MAPK/ERK kinase 1/2	$\Downarrow$ROS and SOD $\Downarrow$DNA damage $\Downarrow$PARP cleavage and caspases activation (98, 99)	Apelin-13 (APJ agonist)	The role of apelin administration on antitumor efficacity of anticancer drugs is not known	It needs to be further exploded in human trials
Prokineticin (PKR1 and PKR2) PKR1 couple to Gαq/11 activates Akt, MAPK, detoxification pathways. PKR2 couple to $G\alpha_{12/13}$ and Gs.	$\Downarrow$ROS, $\Uparrow$detoxification sytem, $\Downarrow$DNA damage, $\Downarrow$Cleavage of caspases Protects endothelial cells, cardiomyocytes and cardiac progenitor cells via Akt and MAPK activation (100)	IS20, PKR1 agonist	It does not alter anti-tumor efficacity of chemotherapeutics in animal models (100)	It needs to be further exploded in human trials
Cannabidiol (CB_1 and CB_2) CB1 couples to Gαi/o, CB2 couples to $G\alpha_s$ and activates MAPK, inhibit Na^+/Ca^{2+} exchange It activates GPR55, TRPV1, α_1-AR, μ opioid and 5HT $_{1A}$	$\Downarrow$ ROS and RNS, $\Uparrow$mitochondrial function $\Downarrow$ inflammation (101, 102)	Rimonabant, AM281 (CB1 receptor antagonist), AM1241 and JWH-133 (CB2R agonist)	Cannabidiol has antitumor effects in a large variety of cancer cell lines (103)	Cannabidiol can be used glioblastoma multiforme and childhood epilepsy in humans Receptor oligomerization should be clarified

anti-adrenergic effects (124). These melatonin receptors are ubiquitously present in central and peripheral organs, including the cardiovascular system. Melatonin regulates blood pressure and heart rate either normalizing the circadian rhythm of blood pressure and ameliorating nocturnal hypertension, or directly acting on heart and blood vessels (125). They also regulate the renin-angiotensin system (126) and mitochondrial function (127).

Melatonin inhibits necrosis and apoptosis, and improves DOX-mediated cardiac dysfunction without compromising the antitumor effect of DOX in mice (87) and rats (88). The mechanism involved in cardioprotective

effect against DOX-cardiotoxicity has been attributed to its antioxidant effect (89) and suppression of lipid peroxidation (90). Recent studies showed that melatonin activates AMPK, PGC1α (91), and sirtuins (92) to attenuate acute DOX-cardiotoxicity via alleviating mitochondrial oxidative damage and apoptosis. Indeed, high doses of melatonin are essential to reach adequate subcellular concentrations to exert these cardioprotective effects (128).

Ramelteon, is a dual MT1 and MT2 melatonin receptor agonist used for insomnia that displays a strong cardioprotective effect in the models of ischemic HF induced by the coronary artery ligation (129), chronic intermittent hypoxia-induced HF (130), and isoproterenol-induced myocardial infarction (131, 132). Unfortunately, the effect of ramelteon in anticancer-mediated cardiotoxicity has not been studied yet. Melatonin can also enhance antitumor effects of anthracycline in animal model (93). Thus, the combined treatment of anthracyclines and melatonin needs to be further explored in cancer patients.

Ghrelin Receptor Agonists

Ghrelin is a growth hormone-releasing and orexigenic peptide that acts through growth hormone secretagogue receptor (GHS-R) in the brain. However, expression of GHS-R in cardiovascular system is controversial. Ghrelin regulates energy balance, body weight maintenance, and metabolism (133). Roles of ghrelin in protecting heart function and reducing mortality after myocardial infarction are partly due to its role on the cardiac vagal afferent nerve terminals (inhibition of cardiac sympathetic and activation of cardiac parasympathetic nerve activity) (134). Ghrelin significantly decreased blood pressure and heart rate in healthy human (135) and prevents the arrhythmia in the mice model of myocardial infarction (136).

Ghrelin significantly improves LV functions and attenuates fibrosis (137) and development of cachexia (138) in rat HF model. Ghrelin inhibits the DOX -induced cardiotoxicity in mice hearts and cardiomyocytes by blocking AMPK activity and activating the p38-MAPK pathway, which suppresses excessive autophagy (94). A ghrelin-containing salmon extract given per os was found to alleviate the cardiotoxicity of DOX in mice, mimicking cardioprotective effect of synthetic ghrelin (95). Cardioprotective effect of ghrelin can also be due to its angiogenic properties in ischemic tissue (139–141). Ghrelin via GHS-R ameliorates impaired angiogenesis by increasing VEGF levels in the ischemic hearts of diabetic rats (140) and in a rat myocardial infarction model (142). Despite the potent synthetic agonist of GHS-R, RM-131 plays an anticatabolic effect in chronic HF models of rat (143), its role in anti-cancer drug mediated cardiotoxicity has not been studied yet.

Galanin Receptor Agonists

Galanin is a neuropeptide present in the nervous system and some organs (144) that uses 3 kinds of GPCRs called GalR1, GalR2 and GalR3 that are all expressed in the cardiovascular system (145). The elevated sympathetic activity during cardiac failure stimulates the release of galanin. This neuropeptide is a one of the sympathetic co-transmitters together with ATP and neuropeptide Y (NPY), in addition to norepinephrine. Galanin released by sympathetic nerves may diminish vagal neurotransmission (146). Indeed, galanin via GalR1 inhibits vagal bradycardia (147). In accord with this study, GalR1 inhibitor, M40 improves cardiac function and attenuate remodeling after myocardial infarction in rats (148). In contrast, an peptide agonist of galanin receptors and the full-length galanin reduce infarct size and the cardiac damage markers in ischemia and reperfusion rat model (96). Indeed, the natural N fragments of Galanin that have more affinity to GalR2 than GalR1 and GalR3 (145) limit acute myocardial infarction in rats *in vivo* (149). Moreover, natural galanin and GalR2 agonist have shown to increase cell viability by suppressing caspase-3 and 9 activity against hypoxic insults in other cells (97).

The GalR1-3 agonist [RAla14, His15]-galanin (2-15) exhibits cardioprotective properties against DOX-mediated cardiac injury in rats. Coadministration of this agonist with DOX has prevented the increase in plasma CK-MB activity and improved the parameters of cardiac function and caused weight gain. The obtained results demonstrate the ability of a novel agonist of galanin receptors GalR1-3 to attenuate DOX-induced cardiotoxicity (150). To conclude, galanin peptides via GalR1-3 alleviate the cardiac dysfunctions induced by DOX. The role of GalR1-3 agonist on anti-tumor effect of DOX in cancer mice model needs to be studied.

Apelin Receptor Agonists

Apelin is an endogenous peptide that acts trough the APJ receptor that is 54% identical with AngII receptor. However, angiotensin II does not bind to APJ (151). Mature apelin, apelin-36, and its shorter forms (apelin-17, -12, and -13) result from the cleavage of pre-pro-apelin. Apelin itself can also be cleaved *in vitro* by the angiotensin-converting enzyme 2 (ACE2) (152). Apelin has a positive inotropic effect *in vitro* (153) and is involved in lowering arterial blood pressure (154), inducing arterial vasodilation (155), and improving cardiac output (156). It protects the heart against ischemia/reperfusion-mediated injury and promotes angiogenesis (157).

Moreover, in APJ knockout mice exhibited more severe heart injury, including impaired contractility functions and survival rate after DOX treatments as compare to wild type mice receiving DOX (98). On the other hand, apelin protects H9c2 cardiomyocytes overexpressing APJ against DOX-mediated cell death. These findings all together have suggested that the suppression of APJ expression can worsen DOX-induced cardiotoxicity. Impairment of the endogenous apelin-APJ system may partially depress the protective signaling in DOX-treated hearts (98). Apelin-13 pretreatment attenuates cisplatin-induced cardiotoxicity by inhibiting apoptosis in cardiomyocytes via activation of MAPKs and PI3K/Akt signaling *in vitro* and *in vivo* in mice heart (99). The mechanism of cardioprotection *in vivo* involves an attenuation of the ROS and superoxide anion accumulation, inhibition of DNA damage, and suppression of PARP and caspases as well as an improvement in angiogenesis.

Importantly, high levels of apelin and APJ have been found in several cancer types that may be connected with obesity. For example, increase levels of Apelin-12 in colon cancer patients with obesity (158), or elevated levels of apelin-36 in endometrial and breast cancer patients with obesity (159–161) have been

found. The role of AJP agonist on anti-tumor effect of anti-cancer agents in cancer mice model needs to be studied. Thus, promoting APJ signaling in heart may represent an interesting strategy to alleviate the cardiotoxicity of anticancer treatments.

Prokineticin Receptor Agonists

Prokineticins are peptides found in milk and macrophages (162). These peptides are called prokineticin because of their first identified biological activity was a prokinetic effect on smooth muscle cells of the gastrointestinal tract (163). Prokineticins exist as two isoforms, PROK1 and PROK2 that are expressed in all mammalian tissues (164). They are angiogenic factors (165) and induce mitogenic and survival pathway in lymphocytes and hematopoietic stem cells (166), neuronal cells (167, 168), cardiomyocytes (169), and endothelial cells (170). PROK1 and PROK2 exert their biological activity on prokineticin receptors 1 and 2 (PKR1 and PKR2) (171).

We have showed that PROK2/PKR1 can induce angiogenesis, while PROK2/PKR2 signaling promotes endothelial cell fenestration and disorganization (170). In cardiomyocytes PKR1 signaling activates $G\alpha_{11}$/Akt pathway to reduce cardiomyocyte death (169), while PKR2 signaling induces hypertrophic cardiomyopathy (172). Indeed, PKR1 gene therapy promotes resistance to ischemia, protects heart against myocardial infarction, and ameliorates heart structure and function (169). Overexpression of PKR1 in transgenic mice hearts promotes neovascularization, suggesting a novel myocardial-epicardial interaction that is involved in differentiation of epicardial progenitor cells (EPDCs) in to vasculogenic cells type by a paracrine PROK2/PKR1 signaling (173).

PKR1 signaling controls epithelial mesenchymal transformation (EMT) during heart (174) and kidney development (175). PKR1 controls fate of $tcf21^{+}$ fibroblast (176) and $Wt1^{+}$ epicardial cells (174). PKR1 epigenetically controls stemness and differentiation of these cells, unraveling a new neovasculogenic pathway vs. adipogenesis (177). PKR1 inhibits adipogenesis and reduce adipocyte accumulation under high fat diet regime of mice (178, 179). PKR1 controls

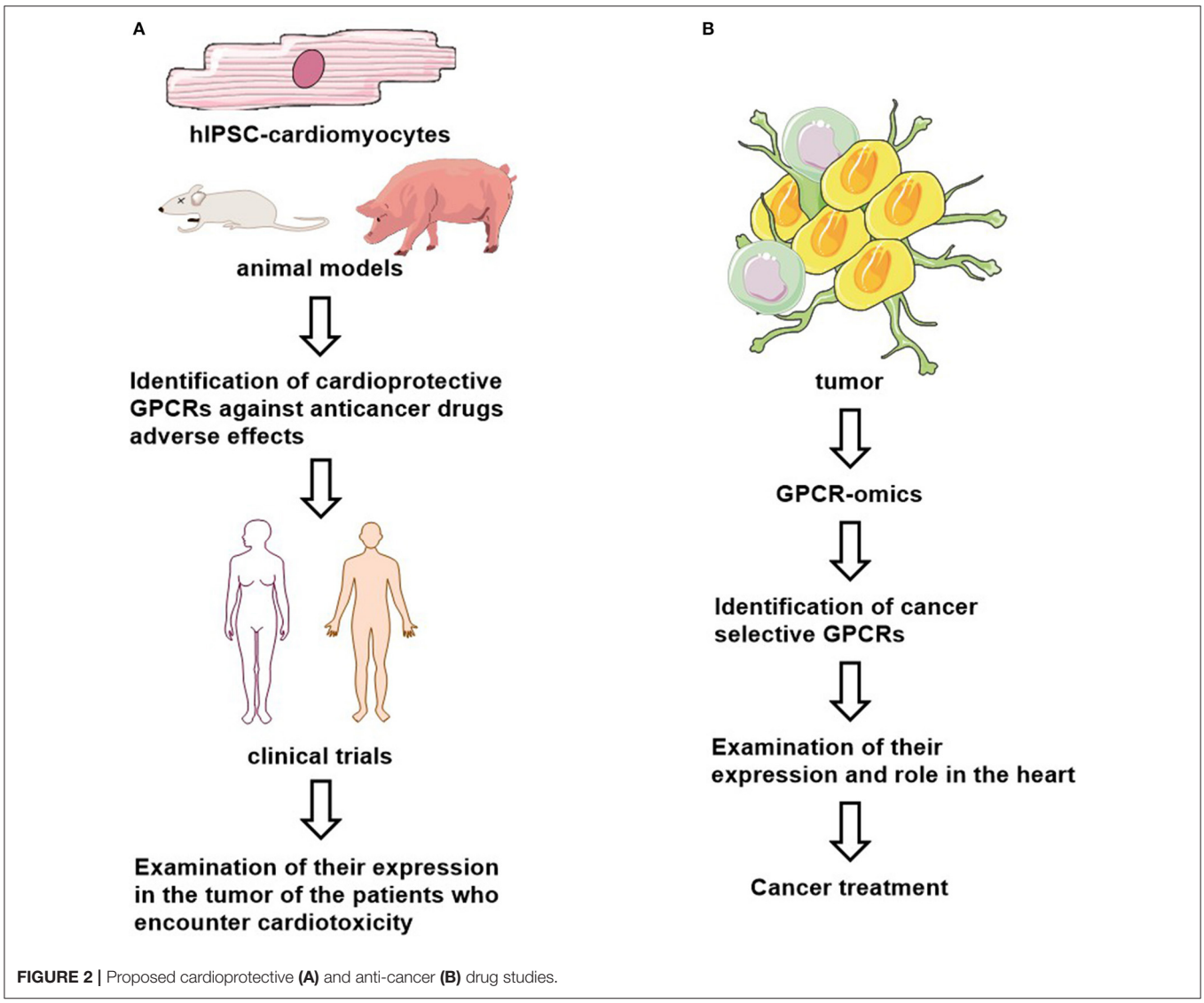

FIGURE 2 | Proposed cardioprotective **(A)** and anti-cancer **(B)** drug studies.

trans-endothelial insulin uptake, preadipocyte proliferation and adipogenesis (180). Lack of PKR1 in mice induces developmental defect in heart and kidney and in adult stage insulin resistance and obesity (181, 182).

In 2015, Gasser et al. discovered the first PKR1 agonists called IS20 (183). This agonist prevents the formation of cardiac lesions and ameliorates the cardiac function and survival after myocardial infarction in mice. IS20 inhibits DOX-mediated cardiotoxicity in cultured cardiac cells including cardiomyocytes, endothelial and progenitor cell as well as in mice models of acute and chronic cardiotoxicity. Importantly, these small molecules did not alter cytotoxic effect of DOX in cancer cells and *in vivo* cancer cell line- derived xenograft mice model (100). This study also described how classic chemotherapeutics, anthracyclines affect cardiac cells in dose-and time-dependent manner and how they impair NFR2 defense mechanism. These results indicate that PKR1 is a target for development of cardioprotective drugs.

Cannabidiol

Cannabidiol is the most abundant non-psychoactive, derived cannabinoid (184). In the low nanomolar range, cannabidiol act as an antagonist of cannabinoid 1 receptor (CB_1R) and cannabinoid 2 receptor (CB_2R), while it has agonist/inverse agonist actions at micromolar concentrations (185, 186). Cannabidiol activate TRPV1 channel and several GPCRs, including the orphan receptor GPR55, the putative Abn-CBD receptor, α_1-adrenoreceptors, $5HT_{1A}$ receptors and μ opioid receptors (187). Several studies showed cardioprotective effects of cannabidiol in animal models of myocardial ischemic reperfusion injury (188), and myocardial infarction (189). It also ameliorates cardiac functions in diabetic cardiomyopathy (186).

Cannabidiol protects hearts against DOX-induced cardiac injury, in rats (101) and in mice (102). It improves cardiac dysfunction by (i) attenuating ROS /RNS accumulation, (ii) preserving mitochondrial function and biogenesis, (iii) promoting cell survival, and (v) decreasing myocardial inflammation. The involvement of CB_1 and CB_2 signaling were not clarified in these studies. Recent data has shown that CB_1R and CB_2R receptors have opposite effects. Indeed CB_1R antagonists and CB2R agonists both protect the heart against clozapine-toxicity (190). Thus, CB_1R antagonist reduces DOX-induced cardiotoxicity and decreased cortical cerebral infarction (191). By contrast, two CB_2R agonists JWH-133, AM 1241 alleviate quetiapine cardiotoxicity (192). Moreover, cannabidiol by itself display cytotoxicity in many cancer cell lines, and anti-tumor effects in cancer mice models (103), suggesting that cannabidiol may have a synergistic effect with antineoplastic drugs in the use of cardioprotective agents. In fact, the cannabinoid HU-331 has been shown to be more potent and less cardiotoxic than DOX (193). Indeed, Insys Therapeutics has obtained FDA orphan drug designation for Cannabidiol for the treatment of multiform glioblastoma and childhood epilepsy.

CONCLUSION

Cardiotoxicity induced by anti-cancer therapy may occur when the anticancer agent targets a common signaling pathway that are essential to maintain the functions of both cardiac and cancer cells. It can also involve off-target effects due to non-selective actions of anti-cancer agents. The choice of the cardioprotective therapeutic approach relies on the delicate balance between the efficiency of anti-neoplastic drugs and the management of cardiovascular complication.

Cardioprotective utility of GPCR ligands will require validation of preferentially expression of these GPCRs in both cancer and cardiac cells, and identification of their signaling (e.g., G-protein- or arrestin-mediated pathways) and functional roles (**Figure 2A**). Whether these cardioprotective ligands interfere with the anti-tumor effect of the chemotherapeutics should be studied as well. The human inducible pluripotent stem cell derived cardiomyocytes (hiPSC-CMs), iPSC-CM-derived 3D cultures and organoids provide human-based model systems to explore the molecular mechanisms of cardiotoxicity and cardioprotection (194). They may also serve as a platform for personalized medicine. Thus, GPCR ligand efficacy can be optimized and their side-effects can be examined in hiPSC-CMs and organoids.

In addition, most of the data regarding the efficacy of cardioprotective GPCR-ligands against cancer therapy mediated-cardiotoxicity have been obtained from small animal models of cardiotoxicity and cancer cell-derived xenograft mice models. Therefore, further studies in bigger animals are necessary to examine their efficacy and adverse effects before these findings can be translated to a human study.

Interestingly, certain cancer cell types may retain a GPCR expression pattern via serving novel biomarkers and/or as valuable therapeutic targets. For example, GPR161 is functionally expressed in breast cancer (195) and GPRC5A in pancreatic cancer (196) and GPR68 in the tumor microenvironment (197). However, both CD97 and GPR56 are highly express in multiple cancer types and in normal tissues (198). Moreover, many mutated GPCRs such as GPR110, GPR112, GPR125, GPR126, GPR98, and GPR110 have been found in certain cancers (199). These findings suggest that different types of cancers may be characterized by a specific onco-GPCR-ome (67). It could be interesting to examine if there is a "GPCR signature" in heart as well. In precision medicine, selectively targeting GPCRs in specific cancers can lead to a novel class of anti-cancer drugs with less adverse cardiac effects, after defining their expression and their role in heart (**Figure 2B**).

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AA, LD, and CN participated in writing and drawings of the manuscript.

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REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* (2011) 61:69–90. doi: 10.3322/caac.20107
2. Moslehi JJ, Deininger M. Tyrosine kinase inhibitor-associated cardiovascular toxicity in chronic myeloid leukemia. *J Clin Oncol.* (2015) 33:4210–8. doi: 10.1200/JCO.2015.62.4718
3. Mercurio V, Pirozzi F, Lazzarini E, Marone G, Rizzo P, Agnetti G, et al. Models of Heart Failure Based on the Cardiotoxicity of Anticancer Drugs. *J Card Fail.* (2016) 22:449–58. doi: 10.1016/j.cardfail.2016.04.008
4. Armenian SH, Lacchetti C, Lenihan D. Prevention and monitoring of cardiac dysfunction in survivors of adult cancers: american society of clinical oncology clinical practice guideline summary. *J Oncol Pract.* (2017) 13:270–5. doi: 10.1200/JOP.2016.018770
5. Galdiero MR, Varricchi G, Marone G. The immune network in thyroid cancer. *Oncoimmunology.* (2016) 5:e1168556. doi: 10.1080/2162402X.2016.1168556
6. Varricchi G, Galdiero MR, Tocchetti CG. Cardiac toxicity of immune checkpoint inhibitors: cardio-oncology meets immunology. *Circulation.* (2017) 136:1989–92. doi: 10.1161/CIRCULATIONAHA.117.029626
7. Priya LB, Baskaran R, Huang CY, Padma VV. Neferine ameliorates cardiomyoblast apoptosis induced by doxorubicin: possible role in modulating NADPH oxidase/ROS-mediated NFkappaB redox signaling cascade. *Sci Rep.* (2017) 7:12283. doi: 10.1038/s41598-017-12060-9
8. Zhao L, Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. *Sci Rep.* (2017) 7:44735. doi: 10.1038/srep44735
9. Dhingra R, Margulets V, Chowdhury SR, Thliveris J, Jassal D, Fernyhough P, et al. Bnip3 mediates doxorubicin-induced cardiac myocyte necrosis and mortality through changes in mitochondrial signaling. *Proc Natl Acad Sci USA.* (2014) 111:E5537–44. doi: 10.1073/pnas.1414665111
10. Minotti G, Salvatorelli E, Menna P. Pharmacological foundations of cardio-oncology. *J Pharmacol Exp Ther.* (2010) 334:2–8. doi: 10.1124/jpet.110.165860
11. Biswas SK. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxid Med Cell Longev.* (2016) 2016:5698931. doi: 10.1155/2016/5698931
12. Ghigo A, Li M, Hirsch E. New signal transduction paradigms in anthracycline-induced cardiotoxicity. *Biochim Biophys Acta.* (2016) 1863:1916–25. doi: 10.1016/j.bbamcr.2016.01.021
13. Licata S, Saponiero A, Mordente A, Minotti G. Doxorubicin metabolism and toxicity in human myocardium: role of cytoplasmic deglycosidation and carbonyl reduction. *Chem Res Toxicol.* (2000) 13:414–20. doi: 10.1021/tx000013q
14. Takemura G, Fujiwara H. Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management. *Prog Cardiovasc Dis.* (2007) 49:330–52. doi: 10.1016/j.pcad.2006.10.002
15. Ewer MS, Ewer SM. Cardiotoxicity of anticancer treatments: what the cardiologist needs to know. *Nat Rev Cardiol.* (2010) 7:564–75. doi: 10.1038/nrcardio.2010.121
16. Scott JM, Khakoo A, Mackey JR, Haykowsky MJ, Douglas PS, Jones LW. Modulation of anthracycline-induced cardiotoxicity by aerobic exercise in breast cancer current evidence and underlying mechanisms. *Circulation.* (2011) 124:642–50. doi: 10.1161/CIRCULATIONAHA.111.021774
17. Nebigil CG, Désaubry L. Updates in anthracycline-mediated cardiotoxicity. *Front Pharmacol.* (2018) 9:1262. doi: 10.3389/fphar.2018.01262
18. Suter TM, Ewer MS. Cancer drugs and the heart: importance and management. *Eur Heart J.* (2013) 34:1102–11. doi: 10.1093/eurheartj/ehs181
19. Ewer MS, Lippman SM. Type II chemotherapy-related cardiac dysfunction: time to recognize a new entity. *J Clin Oncol.* (2005) 23:2900–2. doi: 10.1200/JCO.2005.05.827
20. Ewer MS, Vooletich MT, Durand JB, Woods ML, Davis JR, Valero V, et al. Reversibility of trastuzumab-related cardiotoxicity: new insights based on clinical course and response to medical treatment. *J Clin Oncol.* (2005) 23:7820–6. doi: 10.1200/JCO.2005.13.300
21. Chen ZI, Ai DI. Cardiotoxicity associated with targeted cancer therapies. *Mol Clin Oncol.* (2016) 4:675–81. doi: 10.3892/mco.2016.800
22. Timolati F, Ott D, Pentassuglia L, Giraud MN, Perriard JC, Suter TM, et al. Neuregulin-1 beta attenuates doxorubicin-induced alterations of excitation-contraction coupling and reduces oxidative stress in adult rat cardiomyocytes. *J Mol Cell Cardiol.* (2006) 41:845–54. doi: 10.1016/j.yjmcc.2006.08.002
23. Zamorano JL, Lancellotti P, Rodriguez Munoz D, Aboyans V, Asteggiano R, Galderisi M, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). *Eur J Heart Fail.* (2017) 19:9–42. doi: 10.1002/ejhf.654
24. Bengala C, Zamagni C, Pedrazzoli P, Matteucci P, Ballestrero A, Da Prada G, et al. Cardiac toxicity of trastuzumab in metastatic breast cancer patients previously treated with high-dose chemotherapy: a retrospective study. *Br J Cancer.* (2006) 94:1016–20. doi: 10.1038/sj.bjc.6603060
25. Swain SM, Vici P. The current and future role of dexrazoxane as a cardioprotectant in anthracycline treatment: expert panel review. *J Cancer Res Clin Oncol.* (2004) 130:1–7. doi: 10.1007/s00432-003-0498-7
26. Altan M, Toki MI, Gettinger SN, Carvajal-Hausdorf DE, Zugazagoitia J, Sinard JH, et al. Immune checkpoint inhibitor-associated pericarditis. *J Thorac Oncol.* (2019) 14:1102–8. doi: 10.1016/j.jtho.2019.02.026
27. Tajiri K, Ieda M. Cardiac complications in immune checkpoint inhibition therapy. *Front Cardiovasc Med.* (2019) 6:3. doi: 10.3389/fcvm.2019.00003
28. Varricchi G, Galdiero MR, Marone G, Criscuolo G, Triassi M, Bonaduce D, et al. Cardiotoxicity of immune checkpoint inhibitors. *ESMO Open.* (2017) 2:e000247. doi: 10.1136/esmoopen-2017-000247
29. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. *Curr Opin Immunol.* (2012) 24:207–12. doi: 10.1016/j.coi.2011.12.009
30. Johnson DB, Balko JM, Compton ML, Chalkias S, Gorham J, Xu Y, et al. Fulminant myocarditis with combination immune checkpoint blockade. *N Engl J Med.* (2016) 375:1749–55. doi: 10.1056/NEJMoa1609214
31. Tawbi HA, Bolejack V, Burgess M, Schuetze S. PD-1 inhibition in sarcoma still needs investigation - Authors' reply. *Lancet Oncol.* (2018) 19:e7. doi: 10.1016/S1470-2045(17)30922-1
32. Arcaro A, Pirozzi F, Angelini A, Chimenti C, Crotti L, Giordano C, et al. Novel perspectives in redox biology and pathophysiology of failing myocytes: modulation of the intramyocardial redox milieu for therapeutic interventions-a review article from the working group of cardiac cell biology, italian society of cardiology. *Oxid Med Cell Longev.* (2016) 2016:6353469. doi: 10.1155/2016/6353469
33. Aon MA, Cortassa S, O'Rourke B. Redox-optimized ROS balance: a unifying hypothesis. *Biochim Biophys Acta.* (2010) 1797:865–77. doi: 10.1016/j.bbabio.2010.02.016
34. Nickel A, Kohlhaas M, Maack C. Mitochondrial reactive oxygen species production and elimination. *J Mol Cell Cardiol.* (2014) 73:26–33. doi: 10.1016/j.yjmcc.2014.03.011
35. Akolkar G, da Silva Dias D, Ayyappan P, Bagchi AK, Jassal DS, Salemi VMC, et al. Vitamin C mitigates oxidative/nitrosative stress and inflammation in doxorubicin-induced cardiomyopathy. *Am J Physiol Heart Circ Physiol.* (2017) 313:H795–809. doi: 10.1152/ajpheart.00253.2017
36. Shoukry HS, Ammar HI, Rashed LA, Zikri MB, Shamaa AA, Abou Elfadl SG, et al. Prophylactic supplementation of resveratrol is more effective than its therapeutic use against doxorubicin induced cardiotoxicity. *PLoS ONE.* (2017) 12:e0181535. doi: 10.1371/journal.pone.0181535
37. Sahu BD, Kumar JM, Kuncha M, Borkar RM, Srinivas R, Sistla R. Baicalein alleviates doxorubicin-induced cardiotoxicity via suppression of myocardial oxidative stress and apoptosis in mice. *Life Sci.* (2016) 144:8–18. doi: 10.1016/j.lfs.2015.11.018
38. Deng S, Yan T, Jendrny C, Nemecek A, Vincetic M, Godtel-Armbrust U, et al. Dexrazoxane may prevent doxorubicin-induced DNA damage via depleting both topoisomerase II isoforms. *BMC Cancer.* (2014) 14:842. doi: 10.1186/1471-2407-14-842
39. Reichardt P, Tabone MD, Mora J, Morland B, Jones RL. Risk-benefit of dexrazoxane for preventing anthracycline-related cardiotoxicity: re-evaluating the European labeling. *Future Oncol.* (2018) 14:2663–76. doi: 10.2217/fon-2018-0210

40. Cvetkovic RS, Scott LJ. Dexrazoxane : a review of its use for cardioprotection during anthracycline chemotherapy. *Drugs.* (2005) 65:1005–24. doi: 10.2165/00003495-200565070-00008
41. Shaikh F, Dupuis LL, Alexander S, Gupta A, Mertens L, Nathan PC. Cardioprotection and second malignant neoplasms associated with dexrazoxane in children receiving anthracycline chemotherapy: a systematic review and meta-analysis. *J Natl Cancer Inst.* (2016) 108:djv357. doi: 10.1093/jnci/djv357
42. Acar Z, Kale A, Turgut M, Demircan S, Durna K, Demir S, et al. Efficiency of atorvastatin in the protection of anthracycline-induced cardiomyopathy. *J Am Coll Cardiol.* (2011) 58:988–9. doi: 10.1016/j.jacc.2011.05.025
43. Andres AM, Hernandez G, Lee P, Huang C, Ratliff EP, Sin J, et al. Mitophagy is required for acute cardioprotection by simvastatin. *Antioxid Redox Signal.* (2014) 21:1960–73. doi: 10.1089/ars.2013.5416
44. Calvillo-Arguelles O, Abdel-Qadir H, Michalowska M, Billia F, Suntheralingam S, Amir E, et al. Cardioprotective effect of statins in patients with her2-positive breast cancer receiving trastuzumab therapy. *Can J Cardiol.* (2019) 35:153–9. doi: 10.1016/j.cjca.2018.11.028
45. Henninger C, Fritz G. Statins in anthracycline-induced cardiotoxicity: Rac and Rho, and the heartbreakers. *Cell Death Dis.* (2017) 8:e2564. doi: 10.1038/cddis.2016.418
46. Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Statin use and mortality in cancer patients: Systematic review and meta-analysis of observational studies. *Cancer Treat Rev.* (2015) 41:554–67. doi: 10.1016/j.ctrv.2015.04.005
47. Sattar N, Preiss D, Murray HM, Welsh P, Buckley BM, de Craen AJ, et al. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *Lancet.* (2010) 375:735–42. doi: 10.1016/S0140-6736(09)61965-6.
48. Guglin M, Krischer J, Tamura R, Fink A, Bello-Matricaria L, McCaskill-Stevens W, et al. Randomized trial of lisinopril versus carvedilol to prevent trastuzumab cardiotoxicity in patients with breast cancer. *J Am Coll Cardiol.* (2019) 73:2859–68. doi: 10.1016/j.jacc.2019.03.495
49. Spallarossa P, Garibaldi S, Altieri P, Fabbi P, Manca V, Nasti S, et al. Carvedilol prevents doxorubicin-induced free radical release and apoptosis in cardiomyocytes *in vitro*. *J Mol Cell Cardiol.* (2004) 37:837–46. doi: 10.1016/j.yjmcc.2004.05.024
50. Mason RP, Kalinowski L, Jacob RF, Jacoby AM, Malinski T. Nebivolol reduces nitroxidative stress and restores nitric oxide bioavailability in endothelium of black Americans. *Circulation.* (2005) 112:3795–801. doi: 10.1161/CIRCULATIONAHA.105.556233
51. Na Z, Qiao X, Hao X, Fan L, Xiao Y, Shao Y, et al. The effects of beta-blocker use on cancer prognosis: a meta-analysis based on 319,006 patients. *Onco Targets Ther.* (2018) 11:4913–44. doi: 10.2147/OTT.S167422
52. Weberpals J, Jansen L, Carr PR, Hoffmeister M, Brenner H. Beta blockers and cancer prognosis - The role of immortal time bias: a systematic review and meta-analysis. *Cancer Treat Rev.* (2016) 47:1–11. doi: 10.1016/j.ctrv.2016.04.004
53. Avila MS, Ayub-Ferreira SM, de Barros Wanderley MR Jr, das Dores Cruz F, Goncalves Brandao SM, Rigaud VOC, et al. Carvedilol for prevention of chemotherapy-related cardiotoxicity: the CECCY trial. *J Am Coll Cardiol.* (2018) 71:2281–90. doi: 10.1016/j.jacc.2018.02.049
54. Ferrario CM, Mullick AE. Renin angiotensin aldosterone inhibition in the treatment of cardiovascular disease. *Pharmacol Res.* (2017) 125:57–71. doi: 10.1016/j.phrs.2017.05.020
55. Pinter M, Jain RK. Targeting the renin-angiotensin system to improve cancer treatment: Implications for immunotherapy. *Sci Transl Med.* (2017) 9:eaan5616. doi: 10.1126/scitranslmed.aan5616
56. Cortez-Retamozo V, Etzrodt M, Newton A, Ryan R, Pucci F, Sio SW, et al. Angiotensin II drives the production of tumor-promoting macrophages. *Immunity.* (2013) 38:296–308. doi: 10.1016/j.immuni.2012.10.015
57. Sipahi I, Debanne SM, Rowland DY, Simon DI, Fang JC. Angiotensin-receptor blockade and risk of cancer: meta-analysis of randomised controlled trials. *Lancet Oncol.* (2010) 11:627–36. doi: 10.1016/S1470-2045(10)70106-6
58. Cortassa S, O'Rourke B, Aon MA. Redox-optimized ROS balance and the relationship between mitochondrial respiration and ROS. *Biochim Biophys Acta.* (2014) 1837:287–95. doi: 10.1016/j.bbabio.2013.11.007
59. Kirk JA, Paolocci N. New redox-related arrows in the arsenal of cardiac disease treatment. *Antioxid Redox Signal.* (2014) 21:1945–8. doi: 10.1089/ars.2014.6124
60. van Dalen EC, Caron HN, Dickinson HO, Kremer LC. Cardioprotective interventions for cancer patients receiving anthracyclines. *Cochrane Database Syst Rev.* (2005) 2005:Cd003917. doi: 10.1002/14651858.CD003917.pub2
61. Reddy R, Chahoud G, Mehta JL. Modulation of cardiovascular remodeling with statins: fact or fiction? *Curr Vasc Pharmacol.* (2005) 3:69–79. doi: 10.2174/1570161052773915
62. Liao JK, Laufs U. Pleiotropic effects of statins. *Annu Rev Pharmacol Toxicol.* (2005) 45:89–118. doi: 10.1146/annurev.pharmtox.45.120403.095748
63. Oesterle A, Laufs U, Liao JK. Pleiotropic effects of statins on the cardiovascular system. *Circ Res.* (2017) 120:229–43. doi: 10.1161/CIRCRESAHA.116.308537
64. Jones PJ. Statin rebound effect? *J Lab Clin Med.* (2003) 141:235–6. doi: 10.1067/mlc.2003.34
65. Kalam K, Marwick TH. Role of cardioprotective therapy for prevention of cardiotoxicity with chemotherapy: a systematic review and meta-analysis. *Eur J Cancer.* (2013) 49:2900–9. doi: 10.1016/j.ejca.2013.04.030
66. Lefkowitz RJ. Seven transmembrane receptors: something old, something new. *Acta Physiol.* (2007) 190:9–19. doi: 10.1111/j.1365-201X.2007.01693.x
67. Insel PA, Sriram K, Wiley SZ, Wilderman A, Katakia T, McCann T, et al. GPCRomics: GPCR expression in cancer cells and tumors identifies new, potential biomarkers and therapeutic targets. *Front Pharmacol.* (2018) 9:431. doi: 10.3389/fphar.2018.00431
68. Azzi M, Charest PG, Angers S, Rousseau G, Kohout T, Bouvier M, et al. Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc Natl Acad Sci USA.* (2003) 100:11406–11. doi: 10.1073/pnas.1936664100
69. Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by beta-arrestins. *Science.* (2005) 308:512–7. doi: 10.1126/science.1109237
70. Zhou XE, Melcher K, Xu HE. Understanding the GPCR biased signaling through G protein and arrestin complex structures. *Curr Opin Struct Biol.* (2017) 45:150–9. doi: 10.1016/j.sbi.2017.05.004
71. Shukla AK, Xiao K, Lefkowitz RJ. Emerging paradigms of beta-arrestin-dependent seven transmembrane receptor signaling. *Trends Biochem Sci.* (2011) 36:457–69. doi: 10.1016/j.tibs.2011.06.003
72. Oakley RH, Laporte SA, Holt JA, Caron MG, Barak LS. Differential affinities of visual arrestin, beta arrestin1, and beta arrestin2 for G protein-coupled receptors delineate two major classes of receptors. *J Biol Chem.* (2000) 275:17201–10. doi: 10.1074/jbc.M910348199
73. Zheng M, Zhu W, Han Q, Xiao RP. Emerging concepts and therapeutic implications of beta-adrenergic receptor subtype signaling. *Pharmacol Ther.* (2005) 108:257–68. doi: 10.1016/j.pharmthera.2005.04.006
74. do Vale GT, Ceron CS, Gonzaga NA, Simplicio JA, Padovan JC. Three generations of beta-blockers: history, class differences and clinical applicability. *Curr Hypertens Rev.* (2019) 15:22–31. doi: 10.2174/1573402114666180918102735
75. Nabati M, Janbabai G, Baghyari S, Esmaili K, Yazdani J. Cardioprotective effects of carvedilol in inhibiting doxorubicin-induced cardiotoxicity. *J Cardiovasc Pharmacol.* (2017) 69:279–85. doi: 10.1097/FJC.0000000000000470
76. Tashakori Beheshti A, Mostafavi Toroghi H, Hosseini G, Zarifian A, Homaei Shandiz F, Fazlinezhad A. Carvedilol administration can prevent doxorubicin-induced cardiotoxicity: a double-blind randomized trial. *Cardiology.* (2016) 134:47–53. doi: 10.1159/000442722
77. Oliveira PJ, Goncalves L, Monteiro P, Providencia LA, Moreno AJ. Are the antioxidant properties of carvedilol important for the protection of cardiac mitochondria? *Curr Vasc Pharmacol.* (2005) 3:147–158. doi: 10.2174/1570161053586903
78. Kalay N, Basar E, Ozdogru I, Er O, Cetinkaya Y, Dogan A, et al. Protective effects of carvedilol against anthracycline-induced cardiomyopathy. *J Am Coll Cardiol.* (2006) 48:2258–62. doi: 10.1016/j.jacc.2006.07.052
79. Porrello ER, Delbridge LM. Cardiomyocyte autophagy is regulated by angiotensin II type 1 and type 2 receptors. *Autophagy.* (2009) 5:1215–6. doi: 10.4161/auto.5.8.10153

80. Turu G, Balla A, Hunyady L. The role of beta-arrestin proteins in organization of signaling and regulation of the AT1 angiotensin receptor. *Front Endocrinol.* (2019) 10:519. doi: 10.3389/fendo.2019.00519
81. Bosch X, Rovira M, Sitges M, Domenech A, Ortiz-Perez JT, de Caralt TM, et al. Enalapril and carvedilol for preventing chemotherapy-induced left ventricular systolic dysfunction in patients with malignant hemopathies: the OVERCOME trial (preventiOn of left Ventricular dysfunction with Enalapril and caRvedilol in patients submitted to intensive ChemOtherapy for the treatment of Malignant hEmopathies). *J Am Coll Cardiol.* (2013) 61:2355–62. doi: 10.1016/j.jacc.2013.02.072
82. Georgakopoulos P, Roussou P, Matsakas E, Karavidas A, Anagnostopoulos N, Marinakis T, et al. Cardioprotective effect of metoprolol and enalapril in doxorubicin-treated lymphoma patients: a prospective, parallel-group, randomized, controlled study with 36-month follow-up. *Am J Hematol.* (2010) 85:894–6. doi: 10.1002/ajh.21840
83. Gulati G, Heck SL, Ree AH, Hoffmann P, Schulz-Menger J, Fagerland MW, et al. Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 x 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol. *Eur Heart J.* (2016) 37:1671–80. doi: 10.1093/eurheartj/ehw022
84. Beak J, Huang W, Parker JS, Hicks ST, Patterson C, Simpson PC, et al. An oral selective Alpha-1A adrenergic receptor agonist prevents doxorubicin cardiotoxicity. *JACC Basic Transl Sci.* (2017) 2:39–53. doi: 10.1016/j.jacbts.2016.10.006
85. Galal A, El-Bakly WM, Al Haleem EN, El-Demerdash E. Selective A3 adenosine receptor agonist protects against doxorubicin-induced cardiotoxicity. *Cancer Chemother Pharmacol.* (2016) 77:309–22. doi: 10.1007/s00280-015-2937-y
86. Shneyvays V, Mamedova L, Zinman T, Jacobson K, Shainberg A. Activation of A(3)adenosine receptor protects against doxorubicin-induced cardiotoxicity. *J Mol Cell Cardiol.* (2001) 33:1249–61. doi: 10.1006/jmcc.2001.1387
87. Liu X, Chen Z, Chua CC, Ma YS, Youngberg GA, Hamdy R, et al. Melatonin as an effective protector against doxorubicin-induced cardiotoxicity. *Am J Physiol Heart Circ Physiol.* (2002) 283:H254–63. doi: 10.1152/ajpheart.01023.2001
88. Aygun H, Gul SS. Cardioprotective effect of melatonin and agomelatine on doxorubicin-induced cardiotoxicity in a rat model: an electrocardiographic, scintigraphic and biochemical study. *Bratisl Lek Listy.* (2019) 120:249–55. doi: 10.4149/BLL_2019_045
89. Xu MF, Ho S, Qian ZM, Tang PL. Melatonin protects against cardiac toxicity of doxorubicin in rat. *J Pineal Res.* (2001) 31:301–7. doi: 10.1034/j.1600-079X.2001.310403.x
90. Balli E, Mete UO, Tuli A, Tap O, Kaya M. Effect of melatonin on the cardiotoxicity of doxorubicin. *Histol Histopathol.* (2004) 19:1101–8. doi: 10.14670/HH-19.1101.
91. Liu D, Ma Z, Di S, Yang Y, Yang J, Xu L, et al. AMPK/PGC1alpha activation by melatonin attenuates acute doxorubicin cardiotoxicity via alleviating mitochondrial oxidative damage and apoptosis. *Free Radic Biol Med.* (2018) 129:59–72. doi: 10.1016/j.freeradbiomed.2018.08.032
92. Govender J, Loos B, Marais E, Engelbrecht AM. Melatonin improves cardiac and mitochondrial function during doxorubicin-induced cardiotoxicity: A possible role for peroxisome proliferator-activated receptor gamma coactivator 1-alpha and sirtuin activity? *Toxicol Appl Pharmacol.* (2018) 358:86–101. doi: 10.1016/j.taap.2018.06.031
93. Kim C, Kim N, Joo H, Youm JB, Park WS, Cuong DV, et al. Modulation by melatonin of the cardiotoxic and antitumor activities of adriamycin. *J Cardiovasc Pharmacol.* (2005) 46:200–10. doi: 10.1097/01.fjc.0000171750.97822.a2
94. Wang X, Wang XL, Chen HL, Wu D, Chen JX, Wang XX, et al. Ghrelin inhibits doxorubicin cardiotoxicity by inhibiting excessive autophagy through AMPK and p38-MAPK. *Biochem Pharmacol.* (2014) 88:334–50. doi: 10.1016/j.bcp.2014.01.040
95. Kihara M, Kaiya H, Win ZP, Kitajima Y, Nishikawa M. Protective effect of dietary ghrelin-containing salmon stomach extract on mortality and cardiotoxicity in doxorubicin-induced mouse model of heart failure. *J Food Sci.* (2016) 81:H2858–65. doi: 10.1111/1750-3841.13526
96. Studneva I, Serebryakova L, Veselova O, Pal'keeva M, Molokoedov A, Ovchinnikov M, et al. Galanin receptors activation modulates myocardial metabolic and antioxidant responses to ischaemia/reperfusion stress. *Clin Exp Pharmacol Physiol.* (2019) 46:1174–82. doi: 10.1111/1440-1681.13164
97. Sun J, Xu S, Li H, Li L, Xu ZD. Galanin protects rat cortical astrocyte from oxidative stress: involvement of GalR2 and pERK1/2 signal pathway. *Med Inflamm.* (2019) 2019:2716028. doi: 10.1155/2019/2716028
98. Hamada J, Baasanjav A, Ono N, Murata K, Kako K, Ishida J, et al. Possible involvement of downregulation of the apelin-APJ system in doxorubicin-induced cardiotoxicity. *Am J Physiol Heart Circ Physiol.* (2015) 308:H931–41. doi: 10.1152/ajpheart.00703.2013
99. Zhang P, Yi LH, Meng GY, Zhang HY, Sun HH, Cui LQ. Apelin-13 attenuates cisplatin-induced cardiotoxicity through inhibition of ROS-mediated DNA damage and regulation of MAPKs and AKT pathways. *Free Radic Res.* (2017) 51:449–59. doi: 10.1080/10715762.2017.1313414
100. Gasser AYWC, Audebrand YW, Daglayan A, Charavin A, Escoubet M, Karpov B, et al. Prokineticin receptor-1 signaling inhibits dose- and time-dependent anthracycline-induced cardiovascular toxicity via myocardial and vascular protection. *JACC Cardio Oncol.* (2019) 1:84–102. doi: 10.1016/j.jaccao.2019.06.003
101. Fouad AA, Albuali WH, Al-Mulhim AS, Jresat I. Cardioprotective effect of cannabidiol in rats exposed to doxorubicin toxicity. *Environ Toxicol Pharmacol.* (2013) 36:347–57. doi: 10.1016/j.etap.2013.04.018
102. Hao E, Mukhopadhyay P, Cao Z, Erdelyi K, Holovac E, Liaudet L, et al. Cannabidiol protects against doxorubicin-induced cardiomyopathy by modulating mitochondrial function and biogenesis. *Mol Med.* (2015) 21:38–45. doi: 10.2119/molmed.2014.00261
103. Massi P, Solinas M, Cinquina V, Parolaro D. Cannabidiol as potential anticancer drug. *Br J Clin Pharmacol.* (2013) 75:303–12. doi: 10.1111/j.1365-2125.2012.04298.x
104. Biazi GR, Frasson IG, Miksza DR, de Morais H, de Fatima Silva F, Bertolini GL, et al. Decreased hepatic response to glucagon, adrenergic agonists, and cAMP in glycogenolysis, gluconeogenesis, and glycolysis in tumor-bearing rats. *J Cell Biochem.* (2018) 119:7300–9. doi: 10.1002/jcb.27027
105. Kudej RK, Shen YT, Peppas AP, Huang CH, Chen W, Yan L, et al. Obligatory role of cardiac nerves and alpha1-adrenergic receptors for the second window of ischemic preconditioning in conscious pigs. *Circ Res.* (2006) 99:1270–6. doi: 10.1161/01.RES.0000251282.79411.44
106. Sabri A, Pak E, Alcott SA, Wilson BA, Steinberg SF. Coupling function of endogenous alpha(1)- and beta-adrenergic receptors in mouse cardiomyocytes. *Circ Res.* (2000) 86:1047–53. doi: 10.1161/01.RES.86.10.1047
107. Wright CD, Chen Q, Baye NL, Huang Y, Healy CL, Kasinathan S, et al. Nuclear alpha1-adrenergic receptors signal activated ERK localization to caveolae in adult cardiac myocytes. *Circ Res.* (2008) 103:992–1000. doi: 10.1161/CIRCRESAHA.108.176024
108. O'Connell TD, Ishizaka S, Nakamura A, Swigart PM, Rodrigo MC, Simpson GL, et al. The alpha(1A/C)- and alpha(1B)-adrenergic receptors are required for physiological cardiac hypertrophy in the double-knockout mouse. *J Clin Invest.* (2003) 111:1783–91. doi: 10.1172/JCI200316100
109. O'Connell TD, Block RC, Huang SP, Shearer GC. omega3-Polyunsaturated fatty acids for heart failure: Effects of dose on efficacy and novel signaling through free fatty acid receptor 4. *J Mol Cell Cardiol.* (2017) 103:74–92. doi: 10.1016/j.yjmcc.2016.12.003
110. Durkee CA, Covelo A, Lines J, Kofuji P, Aguilar J, Araque A. Gi/o protein-coupled receptors inhibit neurons but activate astrocytes and stimulate gliotransmission. *Glia.* (2019) 67:1076–93. doi: 10.1002/glia.23589
111. Musselman DM, Ford AP, Gennevois DJ, Harbison ML, Laurent AL, Mokatrin AS, et al. A randomized crossover study to evaluate Ro 115-1240, a selective alpha1A/1L-adrenoceptor partial agonist in women with stress urinary incontinence. *BJU Int.* (2004) 93:78–83. doi: 10.1111/j.1464-410X.2004.04560.x
112. Caso S, Maric D, Arambasic M, Cotecchia S, Diviani D. AKAP-Lbc mediates protection against doxorubicin-induced cardiomyocyte toxicity. *Biochim Biophys Acta Mol Cell Res.* (2017) 1864:2336–46. doi: 10.1016/j.bbamcr.2017.09.007

113. Chen JF, Eltzschig HK, Fredholm BB. Adenosine receptors as drug targets–what are the challenges? *Nat Rev Drug Discov.* (2013) 12:265–86. doi: 10.1038/nrd3955
114. Borea PA, Gessi S, Merighi S, Vincenzi F, Varani K. Pathological overproduction: the bad side of adenosine. *Br J Pharmacol.* (2017) 174:1945–60. doi: 10.1111/bph.13763
115. Sobrevia L, Fredholm BB. Adenosine – from molecular mechanisms to pathophysiology. *Mol Aspects Med.* (2017) 55:1–3. doi: 10.1016/j.mam.2017.06.003
116. McIntosh VJ, Lasley RD. Adenosine receptor-mediated cardioprotection: are all 4 subtypes required or redundant? *J Cardiovasc Pharmacol Ther.* (2012) 17:21–33. doi: 10.1177/1074248410396877
117. Deb PK. Therapeutic potentials of adenosine receptors: the state of the art. *Curr Pharm Des.* (2019) 25:2789–91. doi: 10.2174/1381612825261910071439 42
118. Meibom D, Albrecht-Kupper B, Diedrichs N, Hubsch W, Kast R, Kramer T, et al. Neladenoson bialanate hydrochloride: a prodrug of a partial adenosine A1 receptor agonist for the chronic treatment of heart diseases. *ChemMedChem.* (2017) 12:728–37. doi: 10.1002/cmdc.201700151
119. Voors AA, Dungen HD, Senni M, Nodari S, Agostoni P, Ponikowski P, et al. Safety and tolerability of neladenoson bialanate, a novel oral partial adenosine A1 receptor agonist, in patients with chronic heart failure. *J Clin Pharmacol.* (2017) 57:440–51. doi: 10.1002/jcph.828
120. DeNinno MP, Masamune H, Chenard LK, DiRico KJ, Eller C, Etienne JB, et al. 3'-Aminoadenosine-5'-uronamides: discovery of the first highly selective agonist at the human adenosine A3 receptor. *J Med Chem.* (2003) 46:353–5. doi: 10.1021/jm0255724
121. Lasley RD. Adenosine receptor-mediated cardioprotection-current limitations and future directions. *Front Pharmacol.* (2018) 9:310. doi: 10.3389/fphar.2018.00310
122. Wan TC, Tampo A, Kwok WM, Auchampach JA. Ability of CP-532,903 to protect mouse hearts from ischemia/reperfusion injury is dependent on expression of A3 adenosine receptors in cardiomyoyctes. *Biochem Pharmacol.* (2019) 163:21–31. doi: 10.1016/j.bcp.2019.01.022
123. Jacobson KA, Tosh DK, Jain S, Gao ZG. Historical and current adenosine receptor agonists in preclinical and clinical development. *Front Cell Neurosci.* (2019) 13:124. doi: 10.3389/fncel.2019.00124
124. Baltatu OC, Senar S, Campos LA, Cipolla-Neto J. Cardioprotective melatonin: translating from proof-of-concept studies to therapeutic use. *Int J Mol Sci.* (2019) 20:4342. doi: 10.3390/ijms20184342
125. Stroethoff M, Christoph I, Behmenburg F, Raupach A, Bunte S, Senpolat S, et al. Melatonin receptor agonist ramelteon reduces ischemia-reperfusion injury through activation of mitochondrial potassium channels. *J Cardiovasc Pharmacol.* (2018) 72:106–11. doi: 10.1097/FJC.0000000000000600
126. Campos LA, Cipolla-Neto J, Amaral FG, Michelini LC, Bader M, Baltatu OC. The Angiotensin-melatonin axis. *Int J Hypertens.* (2013) 2013:521783. doi: 10.1155/2013/521783
127. Baltatu OC, Amaral FG, Campos LA, Cipolla-Neto J. Melatonin, mitochondria and hypertension. *Cell Mol Life Sci.* (2017) 74:3955–64. doi: 10.1007/s00018-017-2613-y
128. Govender J, Loos B, Marais E, Engelbrecht AM. Mitochondrial catastrophe during doxorubicin-induced cardiotoxicity: a review of the protective role of melatonin. *J Pineal Res.* (2014) 57:367–80. doi: 10.1111/jpi.12176
129. Pei H, Du J, Song X, He L, Zhang Y, Li X, et al. Melatonin prevents adverse myocardial infarction remodeling via Notch1/Mfn2 pathway. *Free Radic Biol Med.* (2016) 97:408–17. doi: 10.1016/j.freeradbiomed.2016.06.015
130. Yeung HM, Hung MW, Lau CF, Fung ML. Cardioprotective effects of melatonin against myocardial injuries induced by chronic intermittent hypoxia in rats. *J Pineal Res.* (2015) 58:12–25. doi: 10.1111/jpi.12190
131. Mukherjee D, Roy SG, Bandyopadhyay A, Chattopadhyay A, Basu A, Mitra E, et al. Melatonin protects against isoproterenol-induced myocardial injury in the rat: antioxidative mechanisms. *J Pineal Res.* (2010) 48:251–62. doi: 10.1111/j.1600-079X.2010.00749.x
132. Patel V, Upaganlawar A, Zalawadia R, Balaraman R. Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: a biochemical, electrocardiographic and histoarchitectural evaluation. *Eur J Pharmacol.* (2010) 644:160–8. doi: 10.1016/j.ejphar.2010.06.065
133. Tokudome T, Otani K, Miyazato M, Kangawa K. Ghrelin and the heart. *Peptides.* (2019) 111:42–6. doi: 10.1016/j.peptides.2018.05.006
134. Soeki T, Kishimoto I, Schwenke DO, Tokudome T, Horio T, Yoshida M, et al. Ghrelin suppresses cardiac sympathetic activity and prevents early left ventricular remodeling in rats with myocardial infarction. *Am J Physiol Heart Circ Physiol.* (2008) 294:H426–32. doi: 10.1152/ajpheart.00643.2007
135. Zhang CJ, Bidlingmaier M, Altaye M, Page LC, D'Alessio D, Tschop MH, et al. Acute administration of acyl, but not desacyl ghrelin, decreases blood pressure in healthy humans. *Eur J Endocrinol.* (2017) 176:123–32. doi: 10.1530/EJE-16-0789
136. Soeki T, Niki T, Uematsu E, Bando S, Matsuura T, Kusunose K, et al. Ghrelin protects the heart against ischemia-induced arrhythmias by preserving connexin-43 protein. *Heart Vessels.* (2013) 28:795–801. doi: 10.1007/s00380-013-0333-2
137. Yang C, Liu J, Liu K, Du B, Shi K, Ding M, et al. Ghrelin suppresses cardiac fibrosis of post-myocardial infarction heart failure rats by adjusting the activin A-follistatin imbalance. *Peptides.* (2018) 99:27–35. doi: 10.1016/j.peptides.2017.10.018
138. Nagaya N, Uematsu M, Kojima M, Ikeda Y, Yoshihara F, Shimizu W, et al. Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. *Circulation.* (2001) 104:1430–5. doi: 10.1161/hc3601.095575
139. Katare R, Rawal S, Munasinghe PE, Tsuchimochi H, Inagaki T, Fujii Y, et al. Ghrelin promotes functional angiogenesis in a mouse model of critical limb ischemia through activation of proangiogenic MicroRNAs. *Endocrinology.* (2016) 157:432–45. doi: 10.1210/en.2015-1799
140. Wang L, Chen Q, Li G, Ke D. Ghrelin ameliorates impaired angiogenesis of ischemic myocardium through GHSR1a-mediated AMPK/eNOS signal pathway in diabetic rats. *Peptides.* (2015) 73:77–87. doi: 10.1016/j.peptides.2015.09.004
141. Yuan MJ, Wang T, Kong B, Wang X, Huang CX, Wang D. GHSR-1a is a novel pro-angiogenic and anti-remodeling target in rats after myocardial infarction. *Eur J Pharmacol.* (2016) 788:218–25. doi: 10.1016/j.ejphar.2016.06.032
142. Yuan MJ, He H, Hu HY, Li Q, Hong J, Huang CX. Myocardial angiogenesis after chronic ghrelin treatment in a rat myocardial infarction model. *Regul Pept.* (2012) 179:39–42. doi: 10.1016/j.regpep.2012.08.013
143. Lenk K, Palus S, Schur R, Datta R, Dong J, Culler MD, et al. Effect of ghrelin and its analogues, BIM-28131 and BIM-28125, on the expression of myostatin in a rat heart failure model. *J Cachexia Sarcopenia Muscle.* (2013) 4:63–9. doi: 10.1007/s13539-012-0085-3
144. Tatemoto K, Rokaeus A, Jornvall H, McDonald TJ, Mutt V. Galanin - a novel biologically active peptide from porcine intestine. *FEBS Lett.* (1983) 164:124–8. doi: 10.1016/0014-5793(83)80033-7
145. Webling KE, Runesson J, Bartfai T, Langel U. Galanin receptors and ligands. *Front Endocrinol.* (2012) 3:146. doi: 10.3389/fendo.2012.00146
146. Potter EK, Smith-White MA. Galanin modulates cholinergic neurotransmission in the heart. *Neuropeptides.* (2005) 39:345–8. doi: 10.1016/j.npep.2004.12.006
147. Herring N, Cranley J, Lokale MN, Li D, Shanks J, Alston EN, et al. The cardiac sympathetic co-transmitter galanin reduces acetylcholine release and vagal bradycardia: implications for neural control of cardiac excitability. *J Mol Cell Cardiol.* (2012) 52:667–76. doi: 10.1016/j.yjmcc.2011.11.016
148. Chen A, Li M, Song L, Zhang Y, Luo Z, Zhang W, et al. Effects of the galanin receptor antagonist M40 on cardiac function and remodeling in rats with heart failure. *Cardiovasc Ther.* (2015) 33:288–93. doi: 10.1111/1755-5922.12144
149. Serebryakova LI, Studneva IM, Ovchinnikov MV, Veselova OM, Molokoedov AS, Arzamastsev EV, et al. [Cardiometabolic efficacy and toxicological evaluation of a pharmacological galanin receptor agonist]. *Biomed Khim.* (2019) 65:231–8. doi: 10.18097/PBMC20196503231
150. Studneva IM, Palkeeva ME, Veselova OM, Molokoedov AS, Lubimov RO, Ovchinnikov MV, et al. [Protective action of a modified fragment of galanine in rats with doxorubicin-induced heart failure]. *Biomed Khim.* (2019) 65:51–6. doi: 10.18097/PBMC20196501051

151. Lee DK, Cheng R, Nguyen T, Fan T, Kariyawasam AP, Liu Y, et al. Characterization of apelin, the ligand for the APJ receptor. *J Neurochem.* (2000) 74:34–41. doi: 10.1046/j.1471-4159.2000.0740034.x
152. Sato T, Suzuki T, Watanabe H, Kadowaki A, Fukamizu A, Liu PP, et al. Apelin is a positive regulator of ACE2 in failing hearts. *J Clin Invest.* (2013) 123:5203–11. doi: 10.1172/JCI69608
153. Szokodi I, Tavi P, Foldes G, Voutilainen-Myllyla S, Ilves M, Tokola H, et al. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circ Res.* (2002) 91:434–40. doi: 10.1161/01.RES.0000033522.37861.69
154. Tatemoto K, Takayama K, Zou MX, Kumaki I, Zhang W, Kumano K, et al. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regul Pept.* (2001) 99:87–92. doi: 10.1016/S0167-0115(01)00236-1
155. Japp AG, Cruden NL, Amer DA, Li VK, Goudie EB, Johnston NR, et al. Vascular effects of apelin *in vivo* in man. *J Am Coll Cardiol.* (2008) 52:908–13. doi: 10.1016/j.jacc.2008.06.013
156. Japp AG, Cruden NL, Barnes G, van Gemeren N, Mathews J, Adamson J, et al. Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure. *Circulation.* (2010) 121:1818–27. doi: 10.1161/CIRCULATIONAHA.109.911339
157. Mughal A, O'Rourke ST. Vascular effects of apelin: mechanisms and therapeutic potential. *Pharmacol Ther.* (2018) 190:139–47. doi: 10.1016/j.pharmthera.2018.05.013
158. Zuurbier L, Rahman A, Cordes M, Scheick J, Wong TJ, Rustenburg F, et al. Apelin: a putative novel predictive biomarker for bevacizumab response in colorectal cancer. *Oncotarget.* (2017) 8:42949–61. doi: 10.18632/oncotarget.17306
159. Altinkaya SO, Nergiz S, Kucuk M, Yuksel H. Apelin levels are higher in obese patients with endometrial cancer. *J Obstet Gynaecol Res.* (2015) 41:294–300. doi: 10.1111/jog.12503
160. Cabia B, Andrade S, Carreira MC, Casanueva FF, Crujeiras AB. A role for novel adipose tissue-secreted factors in obesity-related carcinogenesis. *Obes Rev.* (2016) 17:361–76. doi: 10.1111/obr.12377
161. Uribesalgo I, Hoffmann D, Zhang Y, Kavirayani A, Lazovic J, Berta J, et al. Apelin inhibition prevents resistance and metastasis associated with anti-angiogenic therapy. *EMBO Mol Med.* (2019) 11:e9266. doi: 10.15252/emmm.201809266
162. Nebigil CG. Prokineticin receptors in cardiovascular function: foe or friend? *Trends Cardiovasc Med.* (2009) 19:55–60. doi: 10.1016/j.tcm.2009.04.007
163. Li M, Bullock CM, Knauer DJ, Ehlert FJ, Zhou QY. Identification of two prokineticin cDNAs: recombinant proteins potently contract gastrointestinal smooth muscle. *Mol Pharmacol.* (2001) 59:692–8. doi: 10.1124/mol.59.4.692
164. Ngan ES, Tam PK. Prokineticin-signaling pathway. *Int J Biochem Cell Biol.* (2008) 40:1679–84. doi: 10.1016/j.biocel.2008.03.010
165. LeCouter J, Lin R, Tejada M, Frantz G, Peale F, Hillan KJ, et al. The endocrine-gland-derived VEGF homologue Bv8 promotes angiogenesis in the testis: localization of Bv8 receptors to endothelial cells. *Proc Natl Acad Sci USA.* (2003) 100:2685–90. doi: 10.1073/pnas.0337667100
166. LeCouter J, Zlot C, Tejada M, Peale F, Ferrara N. Bv8 and endocrine gland-derived vascular endothelial growth factor stimulate hematopoiesis and hematopoietic cell mobilization. *Proc Natl Acad Sci USA.* (2004) 101:16813–18. doi: 10.1073/pnas.0407697101
167. Landucci E, Lattanzi R, Gerace E, Scartabelli T, Balboni G, Negri L, et al. Prokineticins are neuroprotective in models of cerebral ischemia and ischemic tolerance *in vitro*. *Neuropharmacology.* (2016) 108:39–48. doi: 10.1016/j.neuropharm.2016.04.043
168. Gordon R, Neal ML, Luo J, Langley MR, Harischandra DS, Panicker N, et al. Prokineticin-2 upregulation during neuronal injury mediates a compensatory protective response against dopaminergic neuronal degeneration. *Nat Commun.* (2016) 7:12932. doi: 10.1038/ncomms12932
169. Urayama K, Guilini C, Messaddeq N, Hu K, Steenman M, Kurose H, et al. The prokineticin receptor-1 (GPR73) promotes cardiomyocyte survival and angiogenesis. *Faseb j.* (2007) 21:2980–93. doi: 10.1096/fj.07-8116com
170. Guilini C, Urayama K, Turkeri G, Dedeoglu DB, Kurose H, Messaddeq N, et al. Divergent roles of prokineticin receptors in the endothelial cells: angiogenesis and fenestration. *Am J Physiol Heart Circ Physiol.* (2010) 298:H844–52. doi: 10.1152/ajpheart.00898.2009
171. Kaser A, Winklmayr M, Lepperdinger G, Kreil G. The AVIT protein family. Secreted cysteine-rich vertebrate proteins with diverse functions. *EMBO Rep.* (2003) 4:469–73. doi: 10.1038/sj.embor.embor830
172. Urayama K, Dedeoglu DB, Guilini C, Frantz S, Ertl G, Messaddeq N, et al. Transgenic myocardial overexpression of prokineticin receptor-2 (GPR73b) induces hypertrophy and capillary vessel leakage. *Cardiovasc Res.* (2009) 81:28–37. doi: 10.1093/cvr/cvn251
173. Urayama K, Guilini C, Turkeri G, Takir S, Kurose H, Messaddeq N, et al. Prokineticin receptor-1 induces neovascularization and epicardial-derived progenitor cell differentiation. *Arterioscl Thromb Vasc Biol.* (2008) 28:841–9. doi: 10.1161/ATVBAHA.108.162404
174. Arora H, Boulberdaa M, Qureshi R, Bitirim V, Gasser A, Messaddeq N, et al. Prokineticin receptor-1 signaling promotes Epicardial to Mesenchymal Transition during heart development. *Sci Rep.* (2016) 6:25541. doi: 10.1038/srep25541
175. Arora H, Boulberdaa M, Qureshi R, Bitirim V, Messadeq N, Dolle P, et al. Prokineticin receptor 1 is required for mesenchymal-epithelial transition in kidney development. *FASEB J.* (2016) 30:2733–40. doi: 10.1096/fj.201600181R
176. Qureshi R, Kindo M, Arora H, Boulberdaa M, Steenman M, Nebigil CG. Prokineticin receptor-1-dependent paracrine and autocrine pathways control cardiac tcf21(+) fibroblast progenitor cell transformation into adipocytes and vascular cells. *Sci Rep.* (2017) 7:12804. doi: 10.1038/s41598-017-13198-2
177. Qureshi R, Kindo M, Boulberdaa M, von Hunolstein JJ, Steenman M, Nebigil CG. A prokineticin-driven epigenetic switch regulates human epicardial cell stemness and fate. *Stem Cells.* (2018) 36:1589–602. doi: 10.1002/stem.2866
178. Szatkowski C, Vallet J, Dormishian M, Messaddeq N, Valet P, Boulberdaa M, et al. Prokineticin receptor 1 as a novel suppressor of preadipocyte proliferation and differentiation to control obesity. *PLoS ONE.* (2013) 8:e81175. doi: 10.1371/journal.pone.0081175
179. Von Hunolstein JJ, Nebigil CG. Can prokineticin prevent obesity and insulin resistance? *Curr Opin Endocrinol Diabet Obesity.* (2015) 22:367–73. doi: 10.1097/MED.0000000000000185
180. Dormishian M, Turkeri G, Urayama K, Nguyen TL, Boulberdaa M, Messaddeq N, et al. Prokineticin receptor-1 is a new regulator of endothelial insulin uptake and capillary formation to control insulin sensitivity and cardiovascular and kidney functions. *J Am Heart Assoc.* (2013) 2:e000411. doi: 10.1161/JAHA.113.000411
181. Boulberdaa M, Turkeri G, Urayama K, Dormishian M, Szatkowski C, Zimmer L, et al. Genetic inactivation of prokineticin receptor-1 leads to heart and kidney disorders. *Arterioscl Thromb Vasc Biol.* (2011) 31:842–50. doi: 10.1161/ATVBAHA.110.222323
182. Boulberdaa M, Urayama K, Nebigil CG. Prokineticin receptor 1 (PKR1) signalling in cardiovascular and kidney functions. *Cardiovasc Res.* (2011) 92:191–8. doi: 10.1093/cvr/cvr228
183. Gasser A, Brogi S, Urayama K, Nishi T, Kurose H, Tafi A, et al. Discovery and cardioprotective effects of the first non-Peptide agonists of the G protein-coupled prokineticin receptor-1. *PLoS ONE.* (2015) 10:e0121027. doi: 10.1371/journal.pone.0121027
184. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev.* (2006) 58:389–462. doi: 10.1124/pr.58.3.2
185. Stanley CP, Hind WH, O'Sullivan SE. Is the cardiovascular system a therapeutic target for cannabidiol? *Br J Clin Pharmacol.* (2013) 75:313–22. doi: 10.1111/j.1365-2125.2012.04351.x
186. Rajesh M, Mukhopadhyay P, Hasko G, Liaudet L, Mackie K, Pacher P. Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. *Br J Pharmacol.* (2010) 160:688–700. doi: 10.1111/j.1476-5381.2010.00712.x
187. Pertwee RG. Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. *Addict Biol.* (2008) 13:147–59. doi: 10.1111/j.1369-1600.2008.00108.x
188. Durst R, Danenberg H, Gallily R, Mechoulam R, Meir K, Grad E, et al. Cannabidiol, a nonpsychoactive Cannabis constituent, protects against myocardial ischemic reperfusion injury. *Am J Physiol Heart Circ Physiol.* (2007) 293:H3602–7. doi: 10.1152/ajpheart.00098.2007

189. Walsh SK, Hepburn CY, Kane KA, Wainwright CL. Acute administration of cannabidiol *in vivo* suppresses ischaemia-induced cardiac arrhythmias and reduces infarct size when given at reperfusion. *Br J Pharmacol.* (2010) 160:1234–42. doi: 10.1111/j.1476-5381.2010.00755.x
190. Williams DW, Askew LC, Jones E, Clements JE. CCR2 signaling selectively regulates IFN-alpha: role of beta-arrestin 2 in IFNAR1 internalization. *J Immunol.* (2019) 202:105–18. doi: 10.4049/jimmunol.1800598
191. Lim SY, Davidson SM, Yellon DM, Smith CC. The cannabinoid CB1 receptor antagonist, rimonabant, protects against acute myocardial infarction. *Basic Res Cardiol.* (2009) 104:781–92. doi: 10.1007/s00395-009-0034-2
192. Li X, Peng Z, Zhou Y, Wang J, Lin X, Dong X, et al. Quetiapine induces myocardial necroptotic cell death through bidirectional regulation of cannabinoid receptors. *Toxicol Lett.* (2019) 313:77–90. doi: 10.1016/j.toxlet.2019.06.005
193. Kogan NM, Schlesinger M, Peters M, Marincheva G, Beeri R, Mechoulam R. A cannabinoid anticancer quinone, HU-331, is more potent and less cardiotoxic than doxorubicin: a comparative *in vivo* study. *J Pharmacol Exp Ther.* (2007) 322:646–53. doi: 10.1124/jpet.107.120865
194. Nair P, Prado M, Perea-Gil I, Karakikes I. Concise review: precision matchmaking: induced pluripotent stem cells meet cardio-oncology. *Stem Cells Transl Med.* (2019) 8:758–67. doi: 10.1002/sctm.18-0279
195. Feigin ME, Xue B, Hammell MC, Muthuswamy SK. G-protein-coupled receptor GPR161 is overexpressed in breast cancer and is a promoter of cell proliferation and invasion. *Proc Natl Acad Sci USA.* (2014) 111:4191–6. doi: 10.1073/pnas.1320239111
196. Zhou H, Telonis AG, Jing Y, Xia NL, Biederman L, Jimbo M, et al. GPRC5A is a potential oncogene in pancreatic ductal adenocarcinoma cells that is upregulated by gemcitabine with help from HuR. *Cell Death Dis.* (2016) 7:e2294. doi: 10.1038/cddis.2016.169
197. Wiley SZ, Sriram K, Liang W, Chang SE, French R, McCann T, et al. GPR68, a proton-sensing GPCR, mediates interaction of cancer-associated fibroblasts and cancer cells. *FASEB J.* (2018) 32:1170–83. doi: 10.1096/fj.201700834R
198. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. *Science.* (2015) 347:1260419. doi: 10.1126/science.1260419
199. Wu V, Yeerna H, Nohata N, Chiou J, Harismendy O, Raimondi F, et al. Illuminating the Onco-GPCRome: novel G protein-coupled receptor-driven oncocrine networks and targets for cancer immunotherapy. *J Biol Chem.* (2019) 294:11062–86. doi: 10.1074/jbc.REV119.005601

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The Role of Mitochondrial Dysfunction in Radiation-Induced Heart Disease: From Bench to Bedside

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Radiation is a key modality in the treatment of many cancers; however, it can also affect normal tissues adjacent to the tumor, leading to toxic effects. Radiation to the thoracic region, such as that received as part of treatment for breast and lung cancer, can result in incidental dose to the heart, leading to cardiac dysfunction, such as pericarditis, coronary artery disease, ischemic heart disease, conduction defects, and valvular dysfunction. The underlying mechanisms for these morbidities are currently being studied but are not entirely understood. There has been increasing focus on the role of radiation-induced mitochondrial dysfunction and the ensuing impact on various cardiac functions in both preclinical models and in humans. Cardiomyocyte mitochondria are critical to cardiac function, and mitochondria make up a substantial part of a cardiomyocyte's volume. Mitochondrial dysfunction can also alter other cell types in the heart. This review summarizes several factors related to radiation-induced mitochondrial dysfunction in cardiomyocytes and endothelial cells. These factors include mitochondrial DNA mutations, oxidative stress, alterations in various mitochondrial function-related transcription factors, and apoptosis. Through improved understanding of mitochondria-dependent mechanisms of radiation-induced heart dysfunction, potential therapeutic targets can be developed to assist in prevention and treatment of radiation-induced heart damage.

Keywords: mitochondria, radiation-adverse effects, radiation-induced cardiovascular toxicity, oxidative stress, radiation, cardiomyocyte, endothelial cell, apoptosis

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RADIATION-INDUCED CARDIAC DISEASE

It has long been recognized that high-dose radiation exposure to the heart can cause cardiac dysfunction, manifesting months to decades following treatment. In 1924, radiation-induced histologic changes to the heart were first reported following radiation treatment of a patient for Hodgkin's lymphoma (1). Since that time, it has been established that therapeutic radiation to the thoracic region, for treatment of lymphomas, breast and lung cancers and pediatric malignancies can cause cardiac injuries (2). Even low doses of radiation can lead to radiation-induced heart dysfunction (RIHD), as demonstrated in epidemiologic cohorts of atomic bombing survivors and occupational exposures (1, 3, 4). Radiation can cause various structural changes to cardiac

tissue, including the cardiac vasculature, leading to complications, such as pericarditis, coronary artery disease, ischemic heart disease, congestive heart failure, conduction defects and valvular dysfunction (5, 6).

Darby et al. completed a population-based case-control study of women who underwent radiotherapy for breast cancer. In this study, for every gray (Gy) of mean dose to the heart (the average mean dose was 4.9 Gy), the rate of major coronary events (myocardial infarction, coronary revascularization, or death from ischemic cardiac disease) increased by 7.4% with no upper limit. Cardiac events occurred within the first 5 years and continued several decades post-radiotherapy (7). Other studies have also examined the association between mean heart dose and cardiac events/disease, finding an ∼4–16% increased risk per Gy of mean heart dose (7–9). Studies have also suggested RIHD can occur in non-small cell lung cancer patients within 2 years post-radiation exposure (8–11). In a number of lung cancer studies, mortality rates were cardiac dose-dependent, either based on mean heart dose (12) or with the percent of heart receiving 5 Gy (13), 30 Gy (13), or 50 Gy (10). In pediatric and young adult cancer patients who received cardiac radiation, there is over a 6-fold relative risk of RIHD, defined as congestive heart failure, myocardial infarction, pericardial disease, and/or valvular abnormalities (14). Cardiac events were also found to be dose-dependent, with the highest risk of events found when the mean heart dose was >30 Gy (15).

As demonstrated from these studies, cardiac exposure should be minimized when possible for radiation therapy to the thoracic region. There have been many advances in reducing cardiac exposure by improving both imaging and radiotherapy techniques (16–21). However, heart radiation exposure often remains unavoidable. There are currently no widely used methods to reverse RIHD, thus the primary way to reduce cardiotoxicity is through improved treatment planning. There is a need for preclinical studies to understand radiation-induced changes on a cellular and molecular level, with the hope of discovering new targetable pathways. Currently, there are several hypotheses on the predominant causes of RIHD, with most identified using animal models. One cause is the formation of fibrosis, distinguished by collagen deposition in and surrounding cardiomyocytes (1, 6, 22). An additional cause is macrovascular and microvascular injury, developed in a multifactorial manner by endothelial cell damage and adhesion, and activation of inflammatory and atherosclerotic responses (1, 6, 23–28). Signaling pathways, including apoptosis and mitochondrial dysfunction, have also been linked to RIHD (29, 30). This review will focus on the role of radiation-induced mitochondrial dysfunction in RIHD. The biologic pathways described in this review are illustrated in **Figure 1**, and the discussed clinical and preclinical studies are summarized in **Table 1**.

MITOCHONDRIA AND OXIDATIVE STRESS

The role of cardiomyocyte mitochondria is critical to cardiac function, with each cardiomyocyte having abundant mitochondria that make up ∼30% of cell volume (43, 44). Mitochondria have a role in stress responses, cell death, and metabolic processes. They are essential for energy production, which is created by products of glycolysis and fatty acid metabolism via oxidative phosphorylation in the respiratory chain, yielding reactive oxygen species (ROS) biproducts, such as superoxide, peroxide and hydroxyl radicals. Wang et al. reviewed the normal mitochondrial mechanisms, as well as manners in which equilibrium can be interrupted in the heart after radiation. In homeostasis, ROS facilitate cellular functions, including immune responses, signal transduction and apoptosis. ROS can be neutralized by antioxidants when their concentrations are in excess (29). If this highly regulated process is disrupted, increased production or decreased removal of ROS can lead to cellular and DNA damage (29).

Stress-induced mitochondrial damage can cause a loss of mitochondrial membrane potential, leading to the mitochondria undergoing either fission or fusion. Fission helps mitigate stress by fusing parts of damaged mitochondria with normal mitochondria and is regulated by proteins including Opa1 and Mitofusin-1 and−2 (Mfn-1 and Mfn-2). Fission is needed to help create new mitochondria, but also serves as quality control through facilitating apoptosis during high levels of cellular stress. Fission is mediated by proteins including Drp1 and Mft (45, 46). If damaged mitochondria need to be eliminated, kinase PINK1 yields as a sensor of mitochondrial damage and signals to induce mitophagy (46). These complex dynamic mitochondrial processes are imperative in cardiomyocytes, as these cells obtain more than 90% of their energy from mitochondrial respiration (35, 40). This makes mitochondria within these energy-demanding cells an ideal study model to characterize mitochondrial changes that occur after radiation exposure.

It was discovered in the late 1960s that radiation can drastically alter the structural appearance of mitochondria, both short- and long-term. Seven years after high dose radiation (52 Gy) to the mediastinal region in a human patient, electron microscopy revealed cardiomyocyte mitochondria that were variably swollen with decreased number and disorganization of cristae, often with fused outer double membranes (31). Changes in mitochondrial structural integrity occurred as early as 48 h following exposure in rabbit myocardial cells which had received a single dose of either 10 or 13 Gy (32). These findings have led to additional studies on the role of mitochondrial function in RIHD.

On a molecular level, ionizing radiation directly modifies DNA, including single- and double-stranded breaks, base damage, and cross-links, all of which can lead to cell death if not repaired properly. Indirectly, radiation can lead to ROS formation, which can cause cellular stress and death (1). Mitochondrial DNA (mtDNA) is a major radiation target because it lacks the protective effects of histones (47). In addition, it is generally repaired less efficiently than nuclear DNA (48) and has a mutation rate 10–1,000 times higher than nuclear DNA, making it an ideal model to study the mutational effects of radiation (33, 48, 49). This has been most notably reflected with the mutation called common deletion—a 4,977 base pair deletion within mtDNA that has become a marker for oxidative damage. Increased levels of the common

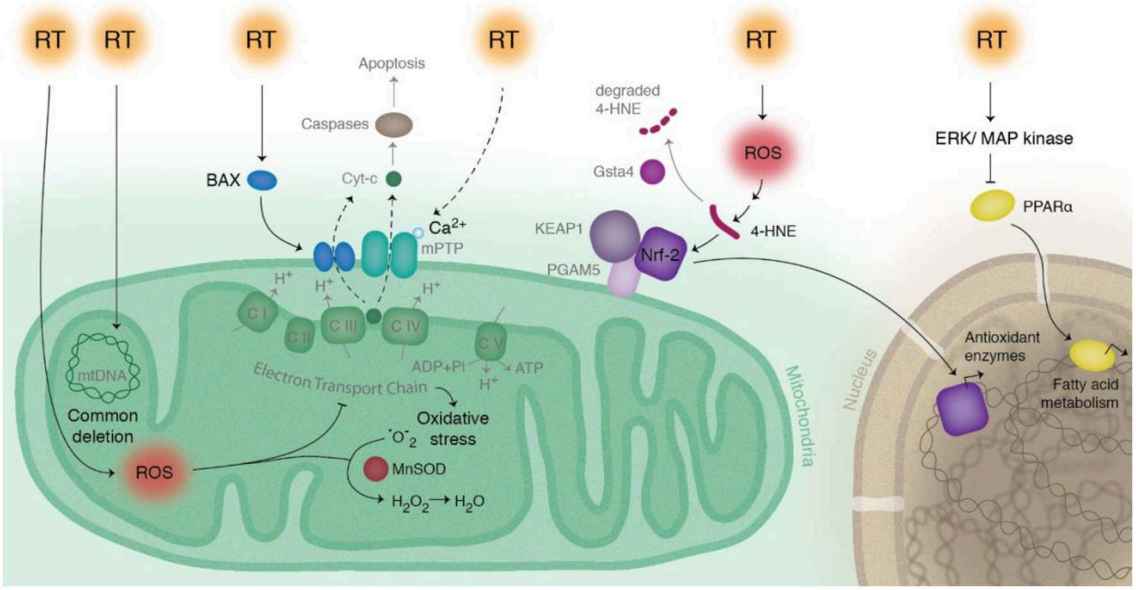

FIGURE 1 | Schematic of radiation-induced effects on pathways related to mitochondria in cardiac cells. Radiation therapy (RT) directly modifies mitochondrial DNA, as seen most notably with the common deletion mutation. RT also indirectly modifies mitochondrial dysfunction by production of reactive oxygen species (ROS), leading to a disruption in the electron transport chain and increased levels of 4-HNE and increased production of antioxidant enzymes via Nrf2. Manganese superoxide dismutase (MnSOD) decreases ROS concentrations by converting superoxide (O_2^-) to hydrogen peroxide (H_2O_2). RT decreases fatty acid energy production via activation of ERK/MAP kinase pathway, which inhibits PPAR-α. RT causes activation of Bax and release of cytochrome c, initiating the intrinsic pathway of apoptosis.

deletion have been noted in human cardiac cells undergoing oxidative stress secondary to atrial fibrillation (50). Other studies have noted radiation-induced development of common deletion within human cell lines, both in low (0.1 Gy) and therapeutic doses (>1 Gy) (33, 48, 51), although no studies to date have specifically analyzed radiation-induced common deletion in cardiomyocytes. All mtDNA genes are essential for the biogenesis and function of mitochondria, so mutations leading to altered overall gene expression would be expected to cause a deficiency in energy metabolism and enhanced production of ROS, leading to oxidative stress (40).

Alterations in proteins that affect ROS generation and oxidative stress may also enhance RIHD. Manganese superoxide dismutase (MnSOD), a mitochondrial matrix enzyme that protects against oxidative stress by converting superoxide to H_2O_2, can decrease cardiac injury severity. Mice deficient in MnSOD died within the first 10 days of life and exhibited dilated cardiomyopathy, among other abnormalities. A study by Nojiri et al. generated cardiac-specific MnSOD-deficient mice, and these mice developed congestive heart failure with severe cardiac muscle degeneration and significantly reduced ATP production, demonstrating that alterations in enzymes important for maintenance of ROS levels can lead to oxidative stress-dependent heart disease (52). Other studies have shown that MnSOD can play an important role in ischemia-reperfusion cardiac injury as well (52).

G protein-coupled receptor kinase (GRK) is another protein reported to regulate ROS in response to stress. Studies have shown data that GRK may act both a protector against and a promotor for death following ischemic injury (45, 53). Removal of cardiac-specific GRK2 has been linked to embryonic cardiovascular development, adult cardiac dilatation, early atherosclerosis and inhibited angiogenesis in mice (42, 54–57). Franco et al. evaluated cell cultures with knockdown or overexpressed GRK2 3–8 h after exposure of a single dose of 4 Gy. Knockdown of GRK caused morphologic mitochondrial changes, reduced membrane potential and reduced mitochondrial function (42). The overexpression of GRK2 protected mitochondria from radiation damage (42). Furthermore, it was demonstrated that in the presence of heat shock proteins, GRK2 interacts with mitofusins (MFN-1 and MFN-2), key regulators of mitochondrial fission and fusion (42, 58). Additional studies are required to understand the chronic response of GRK to radiation, but GRK-related pathways may be important targets to drive mitochondrial protection from radiation-induced damage.

Several other studies have noted radiation-induced mitochondrial changes in terms of oxidative stress and respiratory capacity in mice (34). These changes have been noted h to months following radiation exposure. Five and 24 h after 3 Gy total body irradiation, C57BL/6 mice had immediate changes in cardiac structure and function. On murine cardiac tissue proteomic analysis, mitochondrial proteins represented the protein class most sensitive to radiation, with increased levels of proteins involved in oxidative phosphorylation, including ATP synthase, NADH dehydrogenase and cytochrome *c* oxidase (34).

Chronic low dose exposure of ionizing radiation can cause heart disease, as noted in atomic bomb survivors and nuclear power industry workers (3, 4, 59). As previously mentioned,

TABLE 1 | Summary of studies investigating the role of mitochondria in RIHD.

References	Tissue type/Study subject	Methods/intervention	Radiation	Result
Burch et al. (31)	*in vivo*; human cardiomyocyte	Electron microscopy of irradiated tissue of mediastinum	52 Gy, unknown fractionation	Mito swollen; reduced and disorganized cristae; fused double membrane
Khan (32)	*in vivo*; rabbit myocardial cells	Electron microscopy of irradiated heart tissue	10 or 13 Gy, single dose	Altered mito structure 48 h post-RT exposure
Prithivirajsingh et al. (33)	*in vitro*; human cell lines (dermal fibroblasts, AT, KSS, DNA glioblastoma, and colon carcinoma cell lines)	Evaluation of common deletion	Cesium-137, 4.17 Gy/min, total of 5, 10 or 20 Gy	Increased levels of common deletion 72 h post-RT; dose-independent
Azimzadeh et al. (34)	*in vivo*; C57BL/6 mice; cardiac tissue protein lysates	Proteomic analysis of irradiated mito proteins	TBI, 3 Gy, single dose	5 and 24 h post-RT—increased levels of proteins involved in oxidative phosphorylation (ATP synthase, NADH dehydrogenase, cytochrome c oxidase)
Barjaktarovic et al. (35)	*in vivo*; C57BL/6N mice; isolated cardiac mito	Mito proteomic and functional analysis of low dose RT localized to heart (4 weeks)	0.2 or 2 Gy, single dose	4 weeks post-RT, 2 Gy (functional and proteomic changes); 0.2 Gy functional changes only)
Barjaktaroic et al. (36)	*in vivo*; C57BL/6N mice; isolated cardiac mito	Mito proteomic and functional analysis of late effects (40 weeks) of low dose RT localized to heart	0.2 or 2 Gy, single dose	40 weeks post-RT: 2 Gy (functional and proteomic changes); 0.2 Gy (no significant effect)
Boerma et al. (37)	*in vivo*; *Gsta4*-null vs. WT mice; cardiac tissue	Analysis of cardiac function and proteomics following local heart RT	18 Gy, single dose	Reduced CO, SV and EF in WT. Increased levels of PGAM5 and Nrf2 in *Gsta4*-null-mice
Azimzadeh et al. (38)	*in vivo*; C57BL/6 mice; cardiac tissue protein lysates	Analysis of PPAR-α activity following local radiation to the heart	8 or 16 Gy, single dose	PPAR-α inactivated post-RT with increased FFA, decreased mito complexes I, III, V
Azimzadeh et al. (4)	*in vivo*; human cardiomyocytes	Epidemiologic proteomic analysis following chronic occupational exposures	100 mcGy−5 Gy, chronic exposure	Dose-dependent increase phosphorylation of PPAR-α and decrease in mito complex I and III and Nrf2
Salata et al. (39)	Wistar rats; left ventricular cardiac tissue	Analysis of apoptotic factors 5 months post-cardiac RT	20 Gy, single dose	Increased expression Bax/Bcl2, increased apoptotic nuclei
Sridharan et al. (40)	Male Sprague-Dawley rats; isolated left ventricular cardiac mito	Analysis of time course of RT mito apoptotic changes (at 2 h−9 months post-RT)	3–21 Gy, single dose	Bax/Bcl2 ratio elevated (6 h−6 months). Apoptotic nuclei (6 and 24 h and 2 weeks) Increased calcium-induced swelling/ MPT susceptibility (6 h−9 months)
Ferreira-Machado et al. (41)	Female Wistar rat cardiomyocytes	Analysis of caspase activity 13 months post heart RT	15 Gy, single dose	Cleaved/activated caspase at 13 months post-radiation
Franco et al. (42)	HEK-293 cells	Analysis of GRK activity post-RT	4 Gy, single dose	Overexpression of GRK preserved mito morphology, maintained membrane potential and enhanced respiration (3–8 h post-RT)

RT, Radiation therapy; mito, mitochondria; AT, ataxia telangiectasia; KSS, Kearns Sayre Syndrome; MnSOD, manganese superoxide dismutase; TBI, total body irradiation; WT, wildtype; CO, cardiac output; SV, stroke volume; EF, ejection fraction; FFA, free fatty acids, MPT, membrane permeability transition; RT, radiation therapy.

doses as low as 0.1 Gy caused accumulation of the common deletion in human cell lines (51). However, other studies have shown minimal effect with these low doses. Barjaktarovic et al. studied C57BL/6N mice that received either 0.2 Gy, 2 Gy to the heart or sham radiation. Four weeks post-exposure, cardiac mitochondria were examined for proteomic and functional alterations. After 2 Gy, both functional and proteomic alterations were observed. Proteomic analysis revealed a total of 25 downregulated proteins, in three biological areas: oxidative phosphorylation, pyruvate metabolism and cytoskeletal structures. Functional impairment was reflected as partial deactivation of mitochondrial Complex I and III, decreased succinate-driven respiratory capacity, increased ROS levels and enhanced oxidation of mitochondrial proteins. At the lower dose (0.2 Gy), only proteomic changes were identified, suggesting a dose-dependence of mitochondrial dysfunction after cardiac radiation (35). This group then investigated the late cardiac effects at 40 weeks post-exposure, at which time respiratory capacity of the mitochondria was still reduced after 2 Gy. This suggests that radiation can cause non-transient alterations of oxidative stress in mitochondria (36).

GLUTATHIONE S-TRANSFERASE ALPHA 4 (GSTA4-4)/Nrf2 PATHWAY

Another method of measuring oxidative stress is by quantifying downstream transcription factors. During the process of lipid peroxidation, 4-hydroxynonenal (4-HNE) concentrations

increase, which directly activates nuclear factor erythroid 2 [NF-E2]-related factor 2 (Nrf2, gene name *NFE2L2*), a transcription factor that targets a number of antioxidant proteins. Nrf2 is a redox-sensitive factor that controls oxidative responses within cells and has a role in endothelial function and cardiac protection (22, 60). The repressor protein Keap1 binds and sequesters Nrf2, promoting Nrf2 ubiquitin-mediated degradation. The protein phosphoglycerate mutase family member-5 (PGAM5) is attached to the mitochondrial membrane and can form a complex with Keap1 and Nrf2 (37, 61, 62). Under oxidative conditions, Nrf2 is released from the complex, allowing nuclear accumulation of Nrf2 (60, 62). The role of Nrf2 was indirectly verified by the impact of glutathione S-transferase alpha 4 (GSTA4-4), which is an enzyme that removes 4-HNE. A study with *Gsta4*-null mice noted enhanced resistance to the cardiotoxic effects from doxorubicin, suggesting a compensatory mechanism may have caused cardiac protection (37). Boerma et al. conducted a similar study with the *Gsta4*-null mice with local heart irradiation to a total dose of 18 Gy. Six months post-radiation exposure, the wild-type mice had reduced cardiac output, stroke volume and ejection fraction, with associated increased levels of cardiac troponin-I levels when compared to the *Gsta4*-null mice. Additionally, the *Gsta4*-null mice had increased mRNA levels of PGAM5 and Nrf2. Nrf2 was also significantly elevated in the sham-irradiated *Gsta4*-null mice when compared to wild-type mice. When comparing the levels of 14 different Nrf2 target genes, none were significantly elevated in wild-type irradiated mice; seven genes were significantly elevated in irradiated *Gsta4*-null mice compared to non-irradiated *Gsta4*-null mice, suggesting a stronger activation of the Nrf2 pathway in the irradiated *Gsta4*-null mice (37).

Another study found that genes on rat chromosome 3 can alter RIHD by using consomic rat strains to identify genetic variants that cause differences in cardiac radiosensitivity. One week after 24 Gy of localized cardiac radiation, changes in expression of numerous gene pathways, including mitochondrial function, were seen between the sensitive and resistant rat hearts. Nrf2 was found to be an upstream regulator of many of the enriched pathways (61). Other preclinical investigations have studied the protective role of the Nrf2 pathway on radiation injury to cardiomyocytes and other cell lines, including embryonic fibroblasts and breast and lung epithelial cells (37). These results taken together suggest Nrf2 and GSTA-4 pathways may be promising targets for reducing mitochondrial dysfunction from cardiac radiation exposure.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-α (PPAR-α)

Cardiac muscle preferentially relies on fatty acid energy production via oxidative phosphorylation over glucose metabolism. Peroxisome proliferator-activated receptor-α (PPAR-α) is a highly expressed transcription factor in tissues with elevated lipid metabolic turnover, including cardiac tissue. When PPAR-α is downregulated, lipid metabolism is impaired, as noted in PPAR-α-null mice (38), suggesting PPAR-α regulates energy equilibrium. When C57BL/6 mice were exposed to 8 or 16 Gy of cardiac radiation, PPAR-α was phosphorylated by ERK-MAPK causing decreased transcriptional activity, leading to increased free fatty acid levels and reduced levels of mitochondrial complexes I, III and V (38). The finding that radiation-induced PPAR-α alterations cause decreased expression of energy metabolism and mitochondrial respiration-related genes has been corroborated in human subjects with chronic radiation exposure. In the 1940s, the Mayak Production Association built a nuclear facility in Russia, where workers were chronically exposed to incidental radiation during their occupational duties. Epidemiologic cohorts were analyzed to identify workers who died from heart disease and individual dosimetric monitors were used to determine radiation exposure. Studies in this cohort of individuals found significant increases in heart disease associated with total external gamma-ray doses, even after adjusting for confounding factors, such as smoking exposure (63–65). In a separate study, the protein expression from post-mortem heart samples were examined from a subset of workers exposed only to external gamma rays who had a diagnosis of ischemic heart disease and a primary cause of death of ischemic heart disease. Total doses of external exposure in this cohort ranged from 100 mcGy to more than 5 Gy. Proteomic analysis from 29 individuals identified a dose-dependent increase in phosphorylation of PPAR-α with a corresponding dose-dependent decrease in mitochondrial proteins, such as complexes I, III, and Nrf2 (4). PPAR-α has already shown an effect in other cardiovascular risk factors in preclinical and clinical studies (for dyslipidemia and diabetes mellitus) and is now an encouraging targetable agent for potential mitigation of RIHD (6, 66).

MITOCHONDRIA AND APOPTOSIS

Mitochondria are critical for some methods of programmed cell death, or apoptosis. For the intrinsic pathway, the inner and outer mitochondrial membranes must be permeabilized to release apoptotic factors, such as cytochrome *c*. The Bcl-2/Bax family of proteins help regulate and stabilize the membranes and govern the predilection for mitochondrial membrane permeabilization (29, 67). Once Bax is activated, it translocates from the cytoplasm to the mitochondrial membrane, where it can induce membrane permeability transition (MPT). MPT is characterized by mitochondrial swelling, depolarization of the membrane and uncoupled oxidative phosphorylation. It can also be induced by calcium influx and ROS. Radiation may also cause mitochondrial-mediated apoptosis due to the close association between the endoplasmic reticulum and mitochondria. When cardiomyocytes are irradiated, the endoplasmic reticulum releases a flux of excess calcium ions (Ca^{2+}) that facilitate permeabilization of mitochondria (29). Animal studies have shown increased levels of the Bax/Bcl2 ratio expression following irradiation. In one analysis, increased expression levels of Bax/Bcl2 and increased apoptotic nuclei were seen in Wistar rats 5 months after cardiac radiation (20 Gy), with an associated increase of fibrotic tissue and cardiomyocyte hypertrophy (39). Sridharan et al. investigated the time course of radiation-induced changes to mitochondria in rats sacrificed 2 h to 9 months

following a single dose of radiation, ranging from 3 to 21 Gy. Levels of Bax and Bcl2 were significantly increased by 6 h post-exposure. The Bax/Bcl2 ratio was elevated from 6 h to 6 months after irradiation, but not significantly elevated at 9 months. These findings were associated with apoptotic nuclei at 6 and 24 h and 2 weeks following radiation (40). One study identified cleaved caspase 3, an apoptosis activator, as late as 13 months post local heart radiation (15 Gy) to Wistar rats (40, 41). Additionally, the study completed by Sridharan et al. noted increased radiation-induced susceptibility to MPT, measured by increased calcium-stimulated mitochondrial swelling. At time points ranging from 6 h to 9 months post-radiation, in a dose-dependent manner, irradiated cardiac mitochondria were more susceptible to calcium-induced swelling. Previously, studies have noted only a transient depolarization of the mitochondrial membrane potential and MPT after radiation. This suggests that radiation may cause an enhancement in the susceptibility of MPT and pore opening in mitochondria to subsequent stressors (40).

ENDOTHELIAL CELL MITOCHONDRIA

Radiation exposure can induce endothelial cell activation, shifting endothelial cells into a pro-inflammatory state. When exposure is repeated or prolonged, the endothelium can alter its protective physiology, which can lead to exhaustion and a decrease in vascular function. This endothelial dysfunction leads to decreased vascular tone, inflammation and atherosclerosis, all of which may contribute to cardiovascular disease (28). Concentrations of mitochondria are relatively low in endothelial cells compared to cardiomyocytes and mitochondria produce a lower portion of total endothelial cell energy. However, endothelial cell mitochondria have been found to play important roles in cellular signaling (28). Radiation-induced endothelial cell mitochondrial dysfunction may contribute to RIHD, though data on this topic is currently limited. Endothelial cell functions that can be altered by radiation include Ca^{2+} regulation, apoptosis and oxidative stress signaling. Radiation-induced release of Ca^{2+} from the endoplasmic reticulum leads to increased mitochondrial Ca^{2+} uptake, yielding membrane swelling and release of apoptotic factors (29). However, Ca^{2+} plays numerous roles in signaling pathways and intracellular functions that theoretically may be affected by radiation (e.g., inner membrane calcium uniporter, mitochondrial Ca^{2+} activation of dehydrogenase enzymes and ATP synthase and TNF-α-induced inflammation). These concepts have just begun to be addressed preclinically in the setting of radiation exposure (28).

Baselet et al. illustrated that dysregulation of the Bcl2 pathway (intrinsic apoptosis pathway) yields endothelial inflammation, apoptosis and senescence, all of which are coupled with atherosclerotic development (28). Along similar lines, cells can undergo senescence, the irreversible arrest of endothelial cell renewal, after extensive cell division or exposure to stressors, including radiation. Previous *in vitro* and *in vivo* studies have noted evidence of endothelial cell senescence following local radiation exposure. In human umbilical vein endothelial cells (HUVECs), mitochondrial membrane potential was altered 2 days after irradiation with 1.5, 4, and 10 Gy. The membrane potential returned to baseline levels at days 5 and 6 with 1.5 and 4 Gy, respectively; however, mitochondrial activity remained reduced in cells irradiated with 10 Gy (28). The underlying mechanisms of radiation-induced senescence are not fully established, though mechanisms may involving the p53-p21 and the IGF1-PI3K-Akt/mTOR pathways that may be attributable to the downregulation of Silent Information Regulator-1 (SIRT1) (68). SIRT1 is a NAD-dependent deacetylase that regulates many proteins involved in mitigating oxidative stress, and although its relationship to RIHD has not been explored, SIRT1-deficiency increased the sensitivity of thymocytes to apoptosis (69). Similar to cardiomyocytes, studies of *in vitro* endothelial cells noted increased production of ROS 24–72 h post-radiation exposure (5–20 Gy) (28). In addition, Nrf2 upregulation has also been implicated in oxidative stress-induced endothelial dysfunction (1, 28). Furthermore, proteomic data on C57BL/6 mice receiving 8 or 16 Gy of local heart irradiation revealed expression of proteins associated with mitochondrial dysfunction within endothelial cells (70).

CONCLUSION

Radiation exposure to the thoracic region can cause a variety of cardiac injuries. Numerous preclinical animal and cell models have studied the mechanisms behind RIHD, though these are not yet fully elucidated. Here we have reviewed several factors related to radiation-induced cardiomyocyte and endothelial cell mitochondrial dysfunction, including mtDNA mutations, oxidative stress, alterations in various transcription factors and apoptosis. These factors ultimately play a role in the complex mitochondrial dynamics that can change the fate of cardiac cells. Through further understanding of mitochondria-dependent mechanisms of RIHD, potential therapeutic targets can be developed to prevent and/or treat radiation-induced heart damage.

AUTHOR CONTRIBUTIONS

All authors contributed to conception and design of the review, wrote sections of the manuscript, contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. Baselet B, Rombouts C, Benotmane AM, Baatout S, Aerts A. Cardiovascular diseases related to ionizing radiation: the risk of low-dose exposure (Review). *Int J Mol Med.* (2016) 38:1623–41. doi: 10.3892/ijmm.2016.2777
2. Stewart FA. Mechanisms and dose-response relationships for radiation-induced cardiovascular disease. *Ann ICRP.* (2012) 41:72–9. doi: 10.1016/j.icrp.2012.06.031
3. Howe GR, Zablotska LB, Fix JJ, Egel J, Buchanan J. Analysis of the mortality experience amongst U.S. nuclear power industry workers after chronic low-dose exposure to ionizing radiation. *Radiat Res.* (2004) 162:517–26. doi: 10.1667/RR3258
4. Azimzadeh O, Azizova T, Merl-Pham J, Subramanian V, Bakshi MV, Moseeva M, et al. A dose-dependent perturbation in cardiac energy metabolism is linked to radiation-induced ischemic heart disease in Mayak nuclear workers. *Oncotarget.* (2017) 8:9067–78. doi: 10.18632/oncotarget.10424
5. Stewart JR, Fajardo LF. Radiation-induced heart disease: an update. *Prog Cardiovasc Dis.* (1984) 27:173–94. doi: 10.1016/0033-0620(84)90003-3
6. Tapio S. Pathology and biology of radiation-induced cardiac disease. *J Radiat Res.* (2016) 57:439–48. doi: 10.1093/jrr/rrw064
7. Darby SC, Ewertz M, McGale P, Bennet AM, Blom-Goldman U, Bronnum D, et al. Risk of ischemic heart disease in women after radiotherapy for breast cancer. *N Engl J Med.* (2013) 368:987–98. doi: 10.1056/NEJMoa1209825
8. Haque W, Verma V, Fakhreddine M, Butler EB, Teh BS, Simone CB II. Trends in cardiac mortality in patients with locally advanced non-small cell lung cancer. *Int J Radiat Oncol Biol Phys.* (2018) 100:470–7. doi: 10.1016/j.ijrobp.2017.10.031
9. Wang K, Pearlstein KA, Patchett ND, Deal AM, Mavroidis P, Jensen BC, et al. Heart dosimetric analysis of three types of cardiac toxicity in patients treated on dose-escalation trials for Stage III non-small-cell lung cancer. *Radiother Oncol.* (2017) 125:293–300. doi: 10.1016/j.radonc.2017.10.001
10. Speirs CK, DeWees TA, Rehman S, Molotievschi A, Velez MA, Mullen D, et al. Heart dose is an independent dosimetric predictor of overall survival in locally advanced non-small cell lung cancer. *J Thorac Oncol.* (2017) 12:293–301. doi: 10.1016/j.jtho.2016.09.134
11. Yegya-Raman N, Wang K, Kim S, Reyhan M, Deek MP, Sayan M, et al. Dosimetric predictors of symptomatic cardiac events after conventional-dose chemoradiation therapy for inoperable NSCLC. *J Thorac Oncol.* (2018) 13:1508–18. doi: 10.1016/j.jtho.2018.05.028
12. Dess RT, Sun Y, Matuszak MM, Sun G, Soni PD, Bazzi L, et al. Cardiac events after radiation therapy: combined analysis of prospective multicenter trials for locally advanced non-small-cell lung cancer. *J Clin Oncol.* (2017) 35:1395–402. doi: 10.1200/JCO.2016.71.6142
13. Bradley JD, Paulus R, Komaki R, Masters G, Blumenschein G, Schild S, et al. Standard-dose versus high-dose conformal radiotherapy with concurrent and consolidation carboplatin plus paclitaxel with or without cetuximab for patients with stage IIIA or IIIB non-small-cell lung cancer (RTOG 0617): a randomised, two-by-two factorial phase 3 study. *Lancet Oncol.* (2015) 16:187–99. doi: 10.1016/S1470-2045(14)71207-0
14. Mulrooney DA, Yeazel MW, Kawashima T, Mertens AC, Mitby P, Stovall M, et al. Cardiac outcomes in a cohort of adult survivors of childhood and adolescent cancer: retrospective analysis of the Childhood Cancer Survivor Study cohort. *BMJ.* (2009) 339:b4606. doi: 10.1136/bmj.b4606
15. Haddy N, Diallo S, El-Fayech C, Schwartz B, Pein F, Hawkins M, et al. Cardiac diseases following childhood cancer treatment: cohort study. *Circulation.* (2016) 133:31–8. doi: 10.1161/CIRCULATIONAHA.115.016686
16. Bergom C, Currey A, Desai N, Tai A, Strauss JB. Deep inspiration breath hold: techniques and advantages for cardiac sparing during breast cancer irradiation. *Front Oncol.* (2018) 8:87. doi: 10.3389/fonc.2018.00087
17. Bergom C, Kelly T, Morrow N, Wilson JF, Walker A, Xiang Q, et al. Prone whole-breast irradiation using three-dimensional conformal radiotherapy in women undergoing breast conservation for early disease yields high rates of excellent to good cosmetic outcomes in patients with large and/or pendulous breasts. *Int J Radiat Oncol Biol Phys.* (2012) 83:821–8. doi: 10.1016/j.ijrobp.2011.08.020
18. Bradley JA, Mendenhall NP. Novel radiotherapy techniques for breast cancer. *Annu Rev Med.* (2018) 69:277–88. doi: 10.1146/annurev-med-042716-103422
19. Modiri A, Sabouri P, Gu X, Timmerman R, Sawant A. Inversed-planned respiratory phase gating in lung conformal radiation therapy. *Int J Radiat Oncol Biol Phys.* (2017) 99:317–24. doi: 10.1016/j.ijrobp.2017.05.039
20. Bernard ME, Glaser SM, Gill BS, Beriwal S, Heron DE, Luketich JD, et al. Results of a single institution experience with dose-escalated chemoradiation for locally advanced unresectable non-small cell lung cancer. *Front Oncol.* (2017) 7:1. doi: 10.3389/fonc.2017.00001
21. Ehrbar S, Perrin R, Peroni M, Bernatowicz K, Parkel T, Pytko I, et al. Respiratory motion-management in stereotactic body radiation therapy for lung cancer - a dosimetric comparison in an anthropomorphic lung phantom (LuCa). *Radiother Oncol.* (2016) 121:328–34. doi: 10.1016/j.radonc.2016.10.011
22. Boerma M, Sridharan V, Mao XW, Nelson GA, Cheema AK, Koturbash I, et al. Effects of ionizing radiation on the heart. *Mutat Res.* (2016) 770:319–27. doi: 10.1016/j.mrrev.2016.07.003
23. Vos J, Aarnoudse MW, Dijk F, Lamberts HB. On the cellular origin and development of atheromatous plaques. A light and electron microscopic study of combined X-ray and hypercholesterolemia-induced atheromatosis in the carotid artery of the rabbit. *Virchows Arch B Cell Pathol Incl Mol Pathol.* (1983) 43:1–16. doi: 10.1007/BF02932938
24. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y, Ali F. Atherosclerotic cardiovascular disease: a review of initiators and protective factors. *Inflammopharmacology.* (2016) 24:1–10. doi: 10.1007/s10787-015-0255-y
25. Hoving S, Heeneman S, Gijbels MJ, te Poele JA, Russell NS, Daemen MJ, et al. Single-dose and fractionated irradiation promote initiation and progression of atherosclerosis and induce an inflammatory plaque phenotype in ApoE(-/-) mice. *Int J Radiat Oncol Biol Phys.* (2008) 71:848–57. doi: 10.1016/j.ijrobp.2008.02.031
26. Stewart FA, Heeneman S, Te Poele J, Kruse J, Russell NS, Gijbels M, et al. Ionizing radiation accelerates the development of atherosclerotic lesions in ApoE-/- mice and predisposes to an inflammatory plaque phenotype prone to hemorrhage. *Am J Pathol.* (2006) 168:649–58. doi: 10.2353/ajpath.2006.050409
27. Boerma M, Hauer-Jensen M. Preclinical research into basic mechanisms of radiation-induced heart disease. *Cardiol Res Pract.* (2010) 2011:858262. doi: 10.4061/2011/858262
28. Baselet B, Sonveaux P, Baatout S, Aerts A. Pathological effects of ionizing radiation: endothelial activation and dysfunction. *Cell Mol Life Sci.* (2019) 76:699–728. doi: 10.1007/s00018-018-2956-z
29. Wang H, Wei J, Zheng Q, Meng L, Xin Y, Yin X, et al. Radiation-induced heart disease: a review of classification, mechanism and prevention. *Int J Biol Sci.* (2019) 15:2128–38. doi: 10.7150/ijbs.35460
30. Koleini N, Kardami E. Autophagy and mitophagy in the context of doxorubicin-induced cardiotoxicity. *Oncotarget.* (2017) 8:46663–80. doi: 10.18632/oncotarget.16944
31. Burch GE, Sohal RS, Sun SC, Miller GC, Colcolough HL. Effects of radiation on the human heart. An electron microscopic study. *Arch Intern Med.* (1968) 121:230–4. doi: 10.1001/archinte.1968.03640030022003
32. Khan MY. Radiation-induced cardiomyopathy: an electron microscopic study of cardiac muscle cells. *Am J Pathol.* (1973) 73:131–46.
33. Prithivirajsingh S, Story MD, Bergh SA, Geara FB, Ang KK, Ismail SM, et al. Accumulation of the common mitochondrial DNA deletion induced by ionizing radiation. *FEBS Lett.* (2004) 571:227–32. doi: 10.1016/j.febslet.2004.06.078
34. Azimzadeh O, Scherthan H, Sarioglu H, Barjaktarovic Z, Conrad M, Vogt A, et al. Rapid proteomic remodeling of cardiac tissue caused by total body ionizing radiation. *Proteomics.* (2011) 11:3299–311. doi: 10.1002/pmic.201100178
35. Barjaktarovic Z, Schmaltz D, Shyla A, Azimzadeh O, Schulz S, Haagen J, et al. Radiation-induced signaling results in mitochondrial impairment in mouse heart at 4 weeks after exposure to X-rays. *PLoS ONE.* (2011) 6:e27811. doi: 10.1371/journal.pone.0027811
36. Barjaktarovic Z, Shyla A, Azimzadeh O, Schulz S, Haagen J, Dorr W, et al. Ionising radiation induces persistent alterations in the cardiac mitochondrial function of C57BL/6 mice 40 weeks after local heart exposure. *Radiother Oncol.* (2013) 106:404–10. doi: 10.1016/j.radonc.2013.01.017

37. Boerma M, Singh P, Sridharan V, Tripathi P, Sharma S, Singh SP. Effects of local heart irradiation in a glutathione S-transferase alpha 4-null mouse model. *Radiat Res.* (2015) 183:610–9. doi: 10.1667/RR13979.1
38. Azimzadeh O, Sievert W, Sarioglu H, Yentrapalli R, Barjaktarovic Z, Sriharshan A, et al. PPAR alpha: a novel radiation target in locally exposed Mus musculus heart revealed by quantitative proteomics. *J Proteome Res.* (2013) 12:2700–14. doi: 10.1021/pr400071g
39. Salata C, Ferreira-Machado SC, De Andrade CB, Mencalha AL, Mandarim-De-Lacerda CA, de Almeida CE. Apoptosis induction of cardiomyocytes and subsequent fibrosis after irradiation and neoadjuvant chemotherapy. *Int J Radiat Biol.* (2014) 90:284–90. doi: 10.3109/09553002.2014.887869
40. Sridharan V, Aykin-Burns N, Tripathi P, Krager KJ, Sharma SK, Moros EG, et al. Radiation-induced alterations in mitochondria of the rat heart. *Radiat Res.* (2014) 181:324–34. doi: 10.1667/RR13452.1
41. Ferreira-Machado SC, Salata C, Rocha NN, Correa AF, Corte-Real S, Peregrino AA, et al. Caspase-3 activation and increased procollagen type I in irradiated hearts. *An Acad Bras Cienc.* (2013) 85:215–22. doi: 10.1590/S0001-37652013005000009
42. Franco A, Sorriento D, Gambardella J, Pacelli R, Prevete N, Procaccini C, et al. GRK2 moderates the acute mitochondrial damage to ionizing radiation exposure by promoting mitochondrial fission/fusion. *Cell Death Discov.* (2018) 4:25. doi: 10.1038/s41420-018-0028-7
43. Piquereau J, Caffin F, Novotova M, Lemaire C, Veksler V, Garnier A, et al. Mitochondrial dynamics in the adult cardiomyocytes: which roles for a highly specialized cell? *Front Physiol.* (2013) 4:102. doi: 10.3389/fphys.2013.00102
44. Barth E, Stammler G, Speiser B, Schaper J. Ultrastructural quantitation of mitochondria and myofilaments in cardiac muscle from 10 different animal species including man. *J Mol Cell Cardiol.* (1992) 24:669–81. doi: 10.1016/0022-2828(92)93381-S
45. Franco A, Ciccarelli M, Sorriento D, Napolitano L, Fiordelisi A, Trimarco B, et al. Rays sting: the acute cellular effects of ionizing radiation exposure. *Transl Med UniSa.* (2016) 14:42–53.
46. Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. *Science.* (2012) 337:1062–5. doi: 10.1126/science.1219855
47. Ljungman M, Hanawalt PC. Efficient protection against oxidative DNA damage in chromatin. *Mol Carcinog.* (1992) 5:264–9. doi: 10.1002/mc.2940050406
48. Wang L, Kuwahara Y, Li L, Baba T, Shin RW, Ohkubo Y, et al. Analysis of Common Deletion (CD) and a novel deletion of mitochondrial DNA induced by ionizing radiation. *Int J Radiat Biol.* (2007) 83:433–42. doi: 10.1080/09553000701370878
49. Richard SM, Bailliet G, Paez GL, Bianchi MS, Peltomaki P, Bianchi NO. Nuclear and mitochondrial genome instability in human breast cancer. *Cancer Res.* (2000) 60:4231–7.
50. Lin PH, Lee SH, Su CP, Wei YH. Oxidative damage to mitochondrial DNA in atrial muscle of patients with atrial fibrillation. *Free Radic Biol Med.* (2003) 35:1310–8. doi: 10.1016/j.freeradbiomed.2003.07.002
51. Schilling-Toth B, Sandor N, Kis E, Kadhim M, Safrany G, Hegyesi H. Analysis of the common deletions in the mitochondrial DNA is a sensitive biomarker detecting direct and non-targeted cellular effects of low dose ionizing radiation. *Mutat Res.* (2011) 716:33–9. doi: 10.1016/j.mrfmmm.2011.07.018
52. Nojiri H, Shimizu T, Funakoshi M, Yamaguchi O, Zhou H, Kawakami S, et al. Oxidative stress causes heart failure with impaired mitochondrial respiration. *J Biol Chem.* (2006) 281:33789–801. doi: 10.1074/jbc.M602118200
53. Sorriento D, Ciccarelli M, Santulli G, Illario M, Trimarco B, Iaccarino G. Trafficking GRK2: cellular and metabolic consequences of GRK2 subcellular localization. *Transl Med UniSa.* (2014) 10:3–7.
54. Matkovich SJ, Diwan A, Klanke JL, Hammer DJ, Marreez Y, Odley AM, et al. Cardiac-specific ablation of G-protein receptor kinase 2 redefines its roles in heart development and beta-adrenergic signaling. *Circ Res.* (2006) 99:996–1003. doi: 10.1161/01.RES.0000247932.71270.2c
55. Jaber M, Koch WJ, Rockman H, Smith B, Bond RA, Sulik KK, et al. Essential role of beta-adrenergic receptor kinase 1 in cardiac development and function. *Proc Natl Acad Sci USA.* (1996) 93:12974–9. doi: 10.1073/pnas.93.23.12974
56. Ciccarelli M, Sorriento D, Franco A, Fusco A, Del Giudice C, Annunziata R, et al. Endothelial G protein-coupled receptor kinase 2 regulates vascular homeostasis through the control of free radical oxygen species. *Arterioscler Thromb Vasc Biol.* (2013) 33:2415–24. doi: 10.1161/ATVBAHA.113.302262
57. Rivas V, Carmona R, Munoz-Chapuli R, Mendiola M, Nogues L, Reglero C, et al. Developmental and tumoral vascularization is regulated by G protein-coupled receptor kinase 2. *J Clin Invest.* (2013) 123:4714–30. doi: 10.1172/JCI67333
58. Sorriento D, Gambardella J, Fiordelisi A, Iaccarino G, Illario M. GRKs and beta-arrestins: "Gatekeepers" of mitochondrial function in the failing heart. *Front Pharmacol.* (2019) 10:64. doi: 10.3389/fphar.2019.00064
59. Little MP. Cancer and non-cancer effects in Japanese atomic bomb survivors. *J Radiol Prot.* (2009) 29:A43–59. doi: 10.1088/0952-4746/29/2A/S04
60. Holmstrom KM, Kostov RV, Dinkova-Kostova AT. The multifaceted role of Nrf2 in mitochondrial function. *Curr Opin Toxicol.* (2016) 1:80–91. doi: 10.1016/j.cotox.2016.10.002
61. Schlaak RA, Frei A, Schottstaedt AM, Tsaih SW, Fish BL, Harmann L, et al. Mapping genetic modifiers of radiation-induced cardiotoxicity to rat chromosome 3. *Am J Physiol Heart Circ Physiol.* (2019) 316:H1267–80. doi: 10.1152/ajpheart.00482.2018
62. Kansanen E, Kuosmanen SM, Leinonen H, Levonen AL. The Keap1-Nrf2 pathway: mechanisms of activation and dysregulation in cancer. *Redox Biol.* (2013) 1:45–9. doi: 10.1016/j.redox.2012.10.001
63. Azizova TV, Grigoryeva ES, Haylock RG, Pikulina MV, Moseeva MB. Ischaemic heart disease incidence and mortality in an extended cohort of Mayak workers first employed in 1948–1982. *Br J Radiol.* (2015) 88:20150169. doi: 10.1259/bjr.20150169
64. Azizova TV, Muirhead CR, Druzhinina MB, Grigoryeva ES, Vlasenko EV, Sumina MV, et al. Cardiovascular diseases in the cohort of workers first employed at Mayak PA in 1948-1958. *Radiat Res.* (2010) 174:155–68. doi: 10.1667/RR1789.1
65. Simonetto C, Azizova TV, Grigoryeva ES, Kaiser JC, Schollnberger H, Eidemuller M. Ischemic heart disease in workers at Mayak PA: latency of incidence risk after radiation exposure. *PLoS ONE.* (2014) 9:e96309. doi: 10.1371/journal.pone.0096309
66. Grygiel-Gorniak B. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications–a review. *Nutr J.* (2014) 13:17. doi: 10.1186/1475-2891-13-17
67. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev.* (2007) 87:99–163. doi: 10.1152/physrev.00013.2006
68. Wang Y, Boerma M, Zhou D. Ionizing radiation-induced endothelial cell senescence and cardiovascular diseases. *Radiat Res.* (2016) 186:153–61. doi: 10.1667/RR14445.1
69. Tanno M, Kuno A, Horio Y, Miura T. Emerging beneficial roles of sirtuins in heart failure. *Basic Res Cardiol.* (2012) 107:273. doi: 10.1007/s00395-012-0273-5
70. Azimzadeh O, Sievert W, Sarioglu H, Merl-Pham J, Yentrapalli R, Bakshi MV, et al. Integrative proteomics and targeted transcriptomics analyses in cardiac endothelial cells unravel mechanisms of long-term radiation-induced vascular dysfunction. *J Proteome Res.* (2015) 14:1203–19. doi: 10.1021/pr501141b

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Emerging Challenges of Radiation-Associated Cardiovascular Dysfunction (RACVD) in Modern Radiation Oncology: Clinical Practice, Bench Investigation, and Multidisciplinary Care

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Radiotherapy (RT) is a crucial treatment modality in managing cancer patients. However, irradiation dose sprinkling to tumor-adjacent normal tissues is unavoidable, generating treatment toxicities, such as radiation-associated cardiovascular dysfunction (RACVD), particularly for those patients with combined therapies or pre-existing adverse features/comorbidities. Radiation oncologists implement several efforts to decrease heart dose for reducing the risk of RACVD. Even applying the deep-inspiration breath-hold (DIBH) technique, the risk of RACVD is though reduced but still substantial. Besides, available clinical methods are limited for early detecting and managing RACVD. The present study reviewed emerging challenges of RACVD in modern radiation oncology, in terms of clinical practice, bench investigation, and multidisciplinary care. Several molecules are potential for serving as biomarkers and therapeutic targets. Of these, miRNAs, endogenous small non-coding RNAs that function in regulating gene expression, are of particular interest because low-dose irradiation, i.e., 200 mGy (one-tenth of conventional RT daily dose) induces early changes of pro-RACVD miRNA expression. Moreover, several miRNAs, e.g., miR-15b and miR21, involve in the development of RACVD, further demonstrating the potential bio-application in RACVD. Remarkably, many RACVDs are late RT sequelae, characterizing highly irreversible and progressively worse. Thus, multidisciplinary care from oncologists and cardiologists is crucial. Combined managements with commodities control (such as hypertension, hypercholesterolemia, and diabetes), smoking cessation, and close monitoring are recommended. Some agents show abilities for preventing and managing RACVD, such as statins and angiotensin-converting enzyme inhibitors (ACEIs); however, their real roles should be confirmed by further prospective trials.

Keywords: radiation, cardiovascular dysfunction, miRNA, late sequelae, toxicity

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INTRODUCTION

Radiotherapy (RT) is an essential treatment modality in managing cancer patients (1, 2). Biologically, RT delivers ionizing radiation (IR) to eradicate cancer cells mainly through reacting with H_2O to generate reactive oxygen species (ROS) to target multiple intra-cellular organelles, such as nucleus (mainly DNA), mitochondria, and cell membrane (3–5). Many IR-associated normal tissue damages are acute toxicities, characterizing potentially reversible and self-limited; however, some types of damages develop late sequelae, which are highly irreversible and progressively worse (4–6). For example, though the incidence is rare, irradiated cancer patients who had IR dose sprinkling to the cardiovascular system may encounter radiation-associated cardiovascular dysfunctions (RACVDs) (7–9), including blood pressure reduction (10), carotid stenosis (11), pericardial disease (12), myocardial infarction (13), pericardial/myocardial fibrosis (14, 15), valvular heart disease (16), arrhythmia (17), and subsequent heart failure (18–20). On clinical presentation, many RACVDs are late RT sequelae, developing a few years later after RT (21). Notably, as time elapsed, the risk of RACVD is larger in the third decade than that of the first two decades after IR exposure (22).

RACVD is a well-known treatment-related toxicity in the field of cardio-oncology (23–25). Other anti-cancer therapies, such as chemotherapy (26–29), targeted therapy (30–33), and immunotherapy (34–36), may also induce cardiovascular dysfunctions (37–39). As a result, when these therapies are prescribed concurrently or sequentially with RT, the risk of RACVD is increased substantially, especially in vulnerable pediatric (40, 41) or elderly cancer patients (42, 43). Besides, other RT-associated adverse events may occur with RACVD, such as ischemic stroke (44, 45) and lung fibrosis (46, 47), which may further impair patients' survival and life quality.

Several cardiovascular pathophysiological dysfunctions are associated with RT, such as late fibrosis/stenosis in the irradiated cardiovascular structures, mainly the endothelium (including endothelial cells and its stroma) and smooth muscle cells (2, 4, 5, 48). Epigenetic dysregulation, e.g., DNA methylation regulating gene expression without changes of sequence, demonstrates profound effects on the development of RACVD. For instance, differentially methylated enhancer of diacylglycerol kinase alpha (DGKA) reduces pro-fibrotic fibroblast activation, involving in radiation-associated tissue fibrosis and vascular stenosis (49). Similarly, microRNAs (miRNAs) also have been found to regulate the innate endothelium response to IR (50).

Clinically, moderate- to high-dose IR to the cardiovascular system increases the risk of RACVD (2, 4, 5). More notably, low-dose IR with a single 200 mGy (i.e., one-tenth of conventional RT daily dose of 200 cGy) has been observed to induce early damage of RACVD, demonstrating expression changes of miRNAs, e.g., miR-21 and miR-146b, and their regulated proteins in primary human coronary artery endothelial cells (51). This finding suggests that miRNAs as potential biomarkers for early detecting RACVD. Furthermore, some miRNAs have been reported as potential targets in managing RACVD, e.g., miR-15b (52), miR-21 (51–54), and miR-126-5p (55).

Hence, the present study aimed to review clinical challenges, potential biomarkers, and therapeutic targets of RACVD, with a focus on the role of miRNA. Emerging challenges of multidisciplinary care and example agents for prevention are also reviewed.

CLINICAL CHALLENGES AND EMERGING ISSUES FOR DETECTING, MANAGING, AND PREVENTING RACVD

Clinical Challenges in Improving Detection, Management, and Prevention of RACVD

Several factors affect the risk of RACVD (**Table 1**). As a result, current treatment guidelines recommend several methods to reduce the risk of RACVD (1, 2, 56). For example, in patients at high risk, radiation oncologists always consider alternative treatment choice of deferred RT, adopt rigorous dose constraints on the cardiovascular system, or implement advanced irradiation techniques. However, even implementing advanced techniques, the occurrence of RACVD cannot be avoided totally. Several issues are still challenging in clinical practice.

Clinical Challenges of Decreasing the Risk of RACVD in Modern Radiation Oncology

Clinically, the overall incidence of RACVDs is rare but substantially encountered in irradiated patients with mediastinum lymphoma (8, 44, 66), head and neck (10, 45), esophagus (63), lung (13, 61, 62), and breast (12, 21, 56, 64) cancers. High-risk features of RACVD development are as follows: left-side breast irradiation (21, 65), combination with anthracycline-based chemotherapy (65), and vulnerable patient populations [e.g., pre-existing cardiac risk factors/heart disease (21, 57) and BRCA1/2 mutation carriers (60)]. For example, for a typical 50-year-old woman with pre-existing cardiac risk factors, an estimated 20-year risk of death from ischemic heart disease after breast RT is up to 1.6%, which is higher than that of those patients with no RT (i.e., 0.9%) (21, 56, 75). Moreover, in irradiated left breast cancer patients, each additional Gray (Gy) of the mean heart dose (MHD) increases the relative risk of major cardiac events by 7.4% (21).

Radiation oncologists implement several methods to decrease IR dose to the heart for minimizing the risk of RACVD (76), such as prone positioning (77), heart block with electronic compensation (57), heart-sparing three-dimensional printing technique (78), continuous positive airway pressure (CPAP) (79), real-time position management (RPM) inspiration gating (80, 81), proton-beam irradiation (82–85), and deep-inspiration breath-hold technique (DIBH) (86–90). However, even with the highly recommended visual-guided DIBH technique, residual variations of the heart position are still noticeable (91). As a result, the actual heart dose may be underestimated, burdening a higher risk of cardiac toxicities than that of estimation from the RT treatment planning.

For reducing the risk of cardiac toxicities, modern irradiation techniques aim to decrease irradiation dose to the heart. Diminishing the mean heart dose (MHD) is the main goal

TABLE 1 | Factors affect the risk of RACVD.

Factors	Description	References
PATIENT FACTOR		
Pre-existing cardiovascular risk factors	Patients with pre-existing cardiovascular risk factors, such as prior cardiovascular disease, diabetes, COPD, smoking history, and high BMI (obesity), increase the risk of RACVD.	(21, 56–59)
BRCA1/2 mutation carriers	Patients with BRCA1/2 mutation demonstrate a higher risk of CVD than that of control patients.	(60)
Vulnerable populations	Pediatric and elderly cancer patients are vulnerable to RTCVD.	(40–43)
CANCER FACTOR		
Lung cancer	RT to lung cancers increases the risk of RTCVD.	(13, 61, 62)
Esophagus cancer	RT to esophagus cancers, especially the middle/lower third tumors, has a high risk of RTCVD.	(63)
Breast cancer	RT to breast cancers, especially the left side breast, burdens a substantial risk of RTCVD that may develop in decades.	(12, 21, 64, 65)
Head and neck cancers	RT to head and neck cancers increases the risk of RTCVD, mainly carotid stenosis and subsequent ischemic stroke.	(10, 45)
Lymphoma	RT to lymphomas that involved the thorax or head and neck regions demonstrates a high risk of RTCVD.	(8, 44, 66)
RT HEART DOSE CONSTRAINS		
*Lung SABR	**1. 50 Gy in 4 fractions:** V40 $\leq$ 1c.c.; V20 $\leq$ 5c.c.; Dmax $\leq$ 45 Gy. **2. 70 Gy in 10 fractions:** V45 $\leq$ 1c.c.; Dmax $\leq$ 60 Gy.	(67–69)
*Lung RT	V30 $\leq$ 45%; MHD $<$ 26 Gy.	(67, 70)
*Breast RT	V5 $<$ 10%; V25 $<$ 5%; MHD $<$ 4 Gy.	(67)
*Esophagus RT	Dmax (0.03 cc) $\leq$ 52 Gy; V40 $<$ 50%; MHD $<$ 26 Gy.	(67)
*Lymphoma RT	MHD $<$ 5 Gy ideal, no higher than 15 Gy.	(67)
COMBINED THERAPY		
**Chemotherapy	Some regimens demonstrate cardiotoxicities, e.g., anthracycline agents.	(26–29, 65)
**Targeted therapy	Some targeted therapy has cardiotoxicities, e.g., anti-Her2 and anti-VEGF agents.	(30–33)
**Immunotherapy	Some immunotherapeutic drugs have cardiotoxicities, e.g., anti-PD1/PDL1 agents.	(34–36)
OTHER FACTORS		
***Statins	Statins use may decrease the risk of RACVD in irradiated cancer patients.	(71)
****ACEI and angiotensin II receptor antagonist	These agents may decrease the risk of RACVD in irradiated cancer patients.	(72, 73)

The dose to OARs is different according to the irradiated sites and cancer disease extension. Radiation oncologists always judge the pros and cons of RT to achieve better tumor control and fewer toxicities, i.e., judging for maximum tolerated dose (MTD) or as low as reasonably achievable (ALARA) (67, 74).

**Multimodality treatment is the cornerstone in managing cancer patients. However, combined treatments irreversibly enhance the risk of RTCVD.*

***Statin used in irradiated cancer patients with hypercholesterolemia may demonstrate double benefits of decreasing the blood level of cholesterol and the risk of RACVD.*

****ACEIs and angiotensin II receptor antagonists used in irradiated patients with hypertension may have double benefits of controlling blood pressure and decreasing the risk of RACVD.*

"V5" represents the percent volume of organ at risk (i.e., the heart) that is irradiated with an IR dose of ≥5 Gy. V25, V30, V40, and V45 are similar representations.

ACEI, angiotensin-converting enzyme inhibitor; ALARA, as low as reasonably achievable; BMI, body mass index; COPD, chronic obstructive pulmonary disease; Dmax, maximal dose; Gy, gray; MHD, mean heart dose; MTD, maximum tolerated dose; OAR, organ at risk; RACVD, radiation-associated cardiovascular dysfunction; RT, radiotherapy; SABR, stereotactic ablative body radiotherapy; VEGF, vascular endothelial growth factor.

based on data estimated from conventional tangential technique (21, 92–94). Nevertheless, attenuating IR doses to coronary artery (95–97) and other cardiac substructures, such as left anterior descending artery (LAD) and left ventricle (LV), are more reasonable and suitable in modern precise RT departments (2, 66, 95, 98). However, long-term results investigated dose effects on these cardiac substructures are pending.

Another emerging challenge in clinical radiation oncology is the concept-shifting on treatment consideration. Previously, radiation oncologists always apply IR dose to organs at risk (OARs) according to the principle of "as low as reasonably achievable (ALARA) (99)." However, in some patient populations that required very aggressive managements, the treatment concept frequently shifts to maximum tolerated dose (MTD) for gaining the ultimate tumor control (67, 74). Undoubtedly, adopting MTD increases the heart dose and then burdens a higher risk of RTCVD than that of ALARA.

Challenges of Clinical Detection for RACVD

Early detection of RACVD is challenging. Some clinical predictors have been reported for stratifying patients at risk, such as dosimetric parameters of RT (61), cardiac risk index (100), and coronary calcium score (101). Moreover, biomarkers are clinically helpful for detecting RACVD (102), such as cardiac troponins (e.g., troponin I or T) and natriuretic peptides (e.g., B-type natriuretic peptide (BNP) or pro-BNP) (103). On imaging, echocardiography is the pivotal method to detect cardiac anatomic and functional changes of RACVD (104–106). Profound RACVD may show a reduction of LV ejection fraction, and subclinical disease may reveal early signs of decreased global longitudinal strain (107–109). Recently, other advanced imaging modalities are attractive for detecting RACVD (110), such as cardiac computed tomography (111–113) and cardiovascular magnetic resonance (CMR) (114–116).

In recent precision cardio-oncology, it is a promise direction that applies combined omic-data and metabolic-function nuclear images (117), such as single-photon emission computed tomography (SPECT) (118) and positron emission tomography (PET) (119–122). Of these, PET that demonstrated metabolic changes of the heart is the most expecting image marker for detecting RACVD. However, identifying suitable isotopes of PET for early detecting RACVD is still challenging.

Challenge of Clinical Managements for RACVD

Unfortunately, there is still no effective method to restore RT-associated late sequelae, including RACVD, because their disease courses are generally irreversible (2, 4, 6, 56). However, several pre-clinic studies have suggested potential targets for therapeutic interventions, such as HMGB1 (123) and miR-212 (124). Moreover, selective irradiation to the heart induces early overexpression of pro-hypertrophic miR-212, leading the miR-212 intervention as a reasonable approach for RACVD (124).

Clinical Prevention for RACVD and Future Challenges

Some clinical agents may be used to prevent the occurrence of RACVD. For instance, statins, HMG-CoA reductase inhibitors prescribed for managing hypercholesterolemia, significantly reduces the risk of stroke [hazard ratio (HR) = 0.68; 95% confidence interval (CI), 0.48–0.98; $P = 0.0368$] and demonstrates a trend to decrease the risk of RACVD (HR = 0.85; 95% CI, 0.69–1.04; $P = 0.0811$) in irradiated cancer patients (71).

The detailed mechanism of statin in protecting the cardiovascular system is unclear. Some potential mechanisms are proposed. Firstly, statin inhibits RhoA GTPase (125), which is essential to mediate the irradiation inhibition of endothelial cell migration (126–128). Secondly, statin decreases cardiac endothelial cell permeability via activating ERK5 (129). Thirdly, statin enhances the release of Nitric Oxide (NO), which is crucial for improving endothelial function via regulating miR-221/222 (130). Fourthly, statin diminishes IR-induced responses of cardiac Connexin-43 and miR-21 (53) that involves in the process of cardiac fibrosis (52).

Clinical strategies, such as close monitoring, smoking cessation (58, 131), prescribing angiotensin-converting enzyme inhibitors (ACEIs), and β-blockers, are useful to prevent anthracycline-associated cardiac toxicities (132, 133). In the literature, ACEIs also showed a potential for preventing RACVD. For example, Captopril, one of ACEIs prescribed for hypertension or heart failure, has been found to decrease pulmonary endothelial dysfunction in irradiated rats (72). Similarly, Candesartan, an Angiotensin II Receptor Antagonist, has been reported to reduce the risk of RACVD in left breast irradiated patients (73). Thus, a potential mechanism of ACEI for cardioprotection may be demarcated reasonably by inhibiting angiotensin II to decrease the expression of TGF-β, a well-known pro-fibrogenic factor of post-IR late fibrosis (134, 135). However, these methods required further data support to demarcate their real roles in preventing the development of RACVD.

Future Challenge: Mixed-Agent-Associated Cardiotoxicity in Combined Treatments

The major clinical problem is that many cancer patients were managed with multimodality treatments. As a result, the incidence of multi-treatment-associated CVDs, such as combined anthracycline-based chemotherapy and RT (136), is much higher than that of isolated RACVD. This phenomenon increases the difficulty of prevention and management, mostly requiring combined care from multidisciplinary team members, including radiation oncologists, medical oncologists, and cardiologists.

Emerging Challenge of Bench Studies to Improve Early Detection, Management, and Prevention of RACVD, Focusing on the Role of miRNA in Acting as a Biomarker and Therapeutic Target

As mentioned above, in addition to currently clinical use biomarkers, such as cardiac troponins (e.g., troponin I) and natriuretic peptides (e.g., BNP) (103), several pre-clinical studies have been investigated to explore underlying mechanisms of RACVD, such as TGF-β and PPAR-α signaling pathways (137, 138), damage-associated molecular patterns (DAMPs) (139), and miRNA modulations (138). Of these, endogenous small non-coding miRNAs that function in regulating gene expression (140) grasp more interest in terms of biomarkers (141–143) and therapeutic targets (144–146) (**Table 2**).

Emerging Challenges for Investigating Biological Mechanisms of RACVD

Detail mechanisms of RACVD are not well-recognized. Some potential mechanisms and pathways have been proposed. For example, IR may impair corin function and inhibit natriuretic peptides to accelerate senescence of cardiac and endothelial cells, contributing to the development of RACVD (151). Besides, several pathways have been identified with involvement into the process of RACVD, such as the 5-lipoxygenase (5-LO)/leukotriene pathway (152), the miRNA-34a/sirtuin-1 signaling pathway (149), the Reactive Oxygen Species (ROS)-mediated p16 pathway (153), and the TGF-β-associated signaling (154).

TABLE 2 | Examples of miRNAs involved in the process of RACVD that are potential for severing as biomarkers or therapeutic targets.

miRNA	Description	References
miR-1	1. miR-1 involved in cardiac hypertrophy. 2. IR decreased miR-1 in the rat myocardium. 3. HRW attenuated post-IR miR-1 decrease.	(52)
miR-15b	1. miR-15b showed anti-fibrotic, anti-hypertrophic, and anti-oxidative profiles. 2. IR decreased miR-15b value. 3. HRW restored miR-15b value.	(52)
miR-21	1. IR increases miR-21 expression in the irradiated rat hearts. 2. miR-21 involves in the process of cardiac fibrosis. 3. HRW diminishes post-IR myocardial miR-21 levels.	(52)
	4. Statins decrease IR-induced cardiac miR-21 response.	(53)
	5. A single low-dose 200 mGy induces expression changes of miR-21 and its modulated proteins in primary human coronary artery endothelial cells.	(51)
	6. On the contrast, miR-21 may play a cardioprotective role through Per2-dependent mechanisms.	(54)
miR-29b	miR-29b is one of pro-RACVD miRNAs.	(147)
miR-30	miR-30, miR-155, and miR-210 involve in the process of vascular calcification, which is one of the end events of RACVD that induces coronary artery stenosis and ischemic heart disease, via exosome delivery to vascular smooth muscle cells.	(148)
miR-34a	MIF inhibits miR-34a to protect from radiation-induced cardiomyocyte senescence via targeting SIRT1.	(149)
miR-126-5p	Applying miR-126-5p therapy represents a potential to improve endothelial recovery and prevent post-IR vascular re-stenosis.	(55)
miR-146a	At 24 h after 2-Gy IR, miR-146a is significantly overexpressed.	(150)
miR-146b	Low-dose IR with a single 200 mGy induces expression changes of miR-146b and its modulated proteins in primary human coronary artery endothelial cells.	(51)
miR-155	miR-30, miR-155, and miR-210 involve in the process of vascular calcification, which is one of the end events of RACVD that induces coronary artery stenosis and ischemic heart disease, via exosome delivery to vascular smooth muscle cells.	(148)
	At 2 h after 2-Gy IR, the level of miR-155 is decreased. At 24 h after 2-Gy IR, miR-155 is significantly overexpressed.	(150)
miR-210	miR-30, miR-155, and miR-210 involve in the process of vascular calcification, which is one of the end events of RACVD that induces coronary artery stenosis and ischemic heart disease, via exosome delivery to vascular smooth muscle cells.	(148)
miR-212	1. Selective irradiation to the heart induced overexpression of pro-hypertrophic miR-212. As a result, miR-212 is a potential therapeutic target.	(124)
miR-221	1. Statins conduct cardiovascular protection through enhancing the release of NO that is associated mainly with an improvement of endothelial function via regulating miR-221/222.	(130)
	2. At 2 h after 2-Gy IR, the expression of miR-221 is significantly increased.	(150)
miR-222	1. Statins conduct cardiovascular protection through enhancing the release of NO that is associated mainly with an improvement of endothelial function via regulating miR-221/222.	(130)
	2. At 2 h after 2-Gy IR, the expression of miR-222 is significantly increased. At 24 h after 2-Gy IR, miR-222 is significantly down-regulated.	(150)

HRW, hydrogen-risk water (H_2 water); IR, ionizing radiation; mGy, micro-Gray; MIF, macrophage migration inhibitory factor; NO, Nitric Oxide; RACVD, radiation-associated cardiovascular dysfunction.

Moreover, some molecules may play roles in the process of RACVD, such as peroxisome proliferator-activated receptor gamma (PPAR-γ) (155), Growth differentiation factor 15 (GDF15) (153), and RhoA GTPase (125) that is essential to mediate the irradiation inhibition of endothelial cell migration. More recently, by using RNA-seq, differential gene-expression profiles have been identified in mice models, such as Nrf2, PDK1, and sirtuins (156). However, despite these lines of evidence, the whole picture of RACVD development is still not well-demarcated.

Another emerging challenge of investigating bio-mechanisms of RACVD comes from the difference of biological effects among different irradiation sources, e.g., proton vs. photon beams. Although proton and photon beams activate similar canonical radiation response pathways, distinct vascular genomic responses have been observed in the murine aorta (157). That is, models established according to photon radiation may not accurately predict the risk of RACVD associated with proton radiation.

Emerging Challenge of Bench Studies for Early Detecting and Managing RACVD, Focusing on the Example Role of miRNA

In the literature, many clinical studies assessed circulating miRNA levels in peripheral blood for diagnosing, predicting, and monitoring human diseases (158–163), including cardiac and vascular disease (CVD) (164–169). For example, the combination

of miR-34a-5p and fibrinogen levels have been reported as a useful tool in differentiating pre-thrombotic status in patients with stable coronary artery disease (165). Moreover, the plasma expression level of miR-423-5p has been reported to serve as a promising biomarker for stratifying patients with coronary artery disease (168).

Similarly, several miRNAs have been found to involve in the process of RACVD (147, 170, 171). For example, via exosomes-based delivery to vascular smooth muscle cells, miR-30, miR-210, and miR-155 play roles in developing vascular calcification, which is one of the end events of RACVD that induces coronary artery stenosis and ischemic heart disease (148). Remarkably, IR-induced miRNAs expression behaves in a dose- and time-depended manner (150, 172). For instance, at 2 h after 2-Gy IR, the expression of miRNA-221 and miRNA-222 are significantly increased, but the level of miRNA-155 is decreased. On the other hand, at 24 h after 2-Gy IR, miRNA-146a and miRNA-155 are significantly overexpressed, but miRNA-222 is down-regulated (150). These patterns of miRNA expression changes require attention in further prospective studies that intend to demarcate the role of miRNAs in association with RACVD.

Although it requires further efforts to bridge miRNAs from bench to bedside, some miRNAs are attractive in early detecting and managing RACVD (124). For instance, applying miR-126-5p therapy potentially improves endothelial recovery and prevents post-irradiation vascular re-stenosis (55). Besides, inhibiting miR34a by macrophage migration inhibitory factor (MIF) has been reported to reduce radiation-induced cardiomyocyte senescence via targeting SIRT1, implicating a novel strategy for managing RACVD (149). Moreover, molecular hydrogen, i.e., hydrogen-rich water (HRW; H_2 water), shows protective effects on IR-induced heart damage via regulating miRNA-1, -15b, and -21 (52).

In conjunction with miRNAs, circular RNAs (circRNAs) have been identified to involve in the regulatory network of the cardiovascular system. In biological function, circular RNAs may interact with RNA-binding proteins and act as miRNA sponges that inhibit the function of correspondingly matched miRNAs (173), demonstrating an ability for serving as novel biomarkers to early detect cardiovascular disease (174).

Applying circulating miRNA levels of peripheral blood is an immediately translatable mean for screening/monitoring RACVD. When researchers selected their miRNA targets by a literature review (such as targets that listed in the present study), miRNA database search, or miRNA-specific sequencing, they can subsequently conduct prospective clinical studies to validate their targets of interest under the pre-defined purpose of detecting, screening, or monitoring RACVD by using blood samples. However, testing details of circulating miRNAs (such as measuring methods, timing, and cut-off point values) are still required to be validated by prospectively clinical trials.

In the ClinicalTrials.gov (175), two actively recruiting trials integrate circulating miRNA as predicting biomarkers to detect RACVD in irradiated breast cancer patients, entitled: (1), Pre- or Postoperative Accelerated Radiotherapy (POP-ART; Identifier: NCT03783364) and (2), Breast Cancer and Cardiotoxicity Induced by Radiotherapy: the BACCARAT Study (Identifier: NCT02605512). Of these, the BACCARAT study investigates the role of several types of circulating biomarkers in detecting RACVD, including B-type natriuretic peptide, TGF-β1, and several miRNAs (e.g., miR-1, miR-34, miR-126, and miR-155). The results of the two trials are highly anticipated.

One potential limitation of applying miRNA in clinical practice is that the expression level of specific miRNAs would be varied in different tissues and testing time points. Therefore, the studies proceeding on the ClinicalTrials.gov may be very informative. Before the information of these clinical trials is available, in the authors' consensus opinion, integrating miRNAs as a component of circulating biomarkers for detecting RACVD may be critically considered in future clinical trials and practice that apply RT. Several measuring time points that similar to the protocol of the BACCARAT study are suggested as follows: before RT, the middle term of the RT course, and five time points after RT (i.e., 1 day, 6 months, 2, 5, and 10 years).

Why the time points of 2, 5, and 10 years should be considered testing and measuring? The main reason is that RACVD is a well-known RT toxicity; it characterizes not only acute cardiovascular damage but also late sequelae of cardiovascular dysfunction that may be encountered a few years or decades after RT (21, 56, 75). Thus, long-term series measuring (i.e., 2-, 5-, and 10-years after RT) of target miRNA levels is useful for early detecting and monitoring the occurrence and severity altering of RACVD.

Emerging Challenge: Novel Agents and Managements for Treating RACVD

As mentioned above, TGF-β-associated signaling gains a substantial interest in investigating the process of RACVD. For example, reducing irradiation-induced TGF-β1 production through blocking the NF-kB signaling pathway has been reported to provide a new insight in inhibiting irradiation-induced myocardial fibrosis (154). Besides, Protein Kinase C (PKC) has been reported to play a role in the process of RACVD (48). Remarkably, inhibiting PKC, such as applying RNA-interference techniques (176), could be a reasonable approach for managing IR-induced vascular dysfunction (48).

Some radioprotection agents, such as L-arginine, show protection effects on blood vessels of urinary bladder wall in patients treated with pelvic RT (177). Furthermore, IR-damaged vascular dysfunction has been observed to be restored by quercetin-filled phosphatidylcholine liposomes and mesenchymal stem cell injection (48). However, the real clinical roles of these agents and interventions on the cardiovascular system require further evidence to define.

Emerging Challenge: Further Multidisciplinary Cooperation Among Radiation Oncologists, Cardiologists, and Molecular Biologists

Multidisciplinary care is required for preventing, detecting, and managing RACVD in irradiated cancer patients (178). In conjunction with the improvement of detection methods, increasing awareness and integrating works between oncologists and cardiologists are essential (179). Managing comorbidities

adequately [e.g., hypertension, hypercholesterolemia, and diabetes control (180)], exercise therapy (181), and smoking cessation (58, 131) are all useful to decrease the risk of anti-cancer-treatment-related CVD (182), including RACVD. For multidisciplinary management, standard recommendation and structure/infra-structure requirements for patient care are ongoing established (183–188). For example, establishing consensus guidance to train RT staffs to delineate cardiac substructures decreases inter-observer variation and increases the accuracy of dose estimation, helping in implementing further randomized clinical trials and then daily clinical practice (189, 190).

Remarkably, several radiation-associated toxicities, including RACVDs, are diagnosed by a ruling-out—not ruling-in—way (2, 6). That is, diagnosing RACVD requires excluding other heart diseases, such as infectious disease or prior-existing subclinical cardiovascular dysfunctions. This work requires tight cooperation and interaction among multidisciplinary team members, such as radiation oncologists, medical oncologists, and cardiologists. Further consensus and recommendations are encouraged to establish in a multidisciplinary manner.

CONCLUSION

Overall, the incidence of RACVD is rare in irradiated cancer patients. When it happened, however, RACVD may significantly impair patients' survival and life quality, particularly in vulnerable patient populations. Radiation oncologists implement many clinical efforts to reduce the risk; the incidence of RACVD is decreased but still substantial.

Further efforts from bench studies are emergently required to improve early detection, management, and prevention. For example, miRNAs play active roles in serving as biomarkers and therapeutic targets. Remarkably, integrating cooperation among multidisciplinary team members, such as oncologists and cardiologists, is encouraged and ongoing.

In the ClinicalTrials.gov (175), more than 20 clinical trials are actively or not yet recruiting for investigating challenging issues of RACVD, mainly focusing on early detection (e.g., circulating and imaging biomarkers) and aggressively avoidance/prevention (e.g., DIBH and proton therapy). Results from these ongoing trials are hopeful for resolving clinical obstacles of RACVD in the future.

AUTHOR CONTRIBUTIONS

All authors contributed to the brainstorming of the ideas generation. M-SL and D-WL also contributed to first draft writing. S-KH, C-CY, C-LC, W-YC, L-CC, R-IL, L-WH, C-HC, and F-CH also contributed to literature review and interpretation. H-YL and MC also co-corresponded to overall manuscript communication and final approval.

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REFERENCES

1. NCCN.org. *Clinical Practice Guidelines in Oncology*. Available online at: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp (accessed November 09, 2019).
2. Halperin EC, Wazer DE, Perez CA, Brady LW. *Perez & Brady's Principles and Practice of Radiation Oncology*. 7th ed. Philadelphia, PA: Lippincott Williams & Wilkins (LWW) (2018).
3. Islam MT. Radiation interactions with biological systems. *Int J Radiat Biol.* (2017) 93:487–93. doi: 10.1080/09553002.2017.1286050
4. Hall EJ, Giaccia AJ. *Radiobiology for the Radiologist*. Philadelphia, PA: Wolters Kluwer (2018).
5. Joiner MC, van der Kogel AJ. *Basic Clinical Radiobiology*. Boca Raton, FL: CRC Press (2018).
6. Koontz BF. *Radiation Therapy Treatment Effects: An Evidence-Based Guide to Managing Toxicity*. New York, NY: Springer Publishing Company (2017).
7. Armanious MA, Mohammadi H, Khodor S, Oliver DE, Johnstone PA, Fradley MG. Cardiovascular effects of radiation therapy. *Curr Probl Cancer.* (2018) 42:433–42. doi: 10.1016/j.currproblcancer.2018.05.008
8. Nolan MT, Russell DJ, Marwick TH. Long-term risk of heart failure and myocardial dysfunction after thoracic radiotherapy: a systematic review. *Can J Cardiol.* (2016) 32:908–20. doi: 10.1016/j.cjca.2015.12.020
9. Marmagkiolis K, Finch W, Tsitlakidou D, Josephs T, Iliescu C, Best JF, et al. Radiation toxicity to the cardiovascular system. *Curr Oncol Rep.* (2016) 18:15. doi: 10.1007/s11912-016-0502-4
10. Leibowitz A, Grossman E, Berkovitch A, Levartovski M, Appel S, Sharabi Y, Gluck I. The effect of head and neck radiotherapy on blood pressure and orthostatic hypotension in patients with head and neck tumors. *Am J Hypertens.* (2018) 31:235–39. doi: 10.1093/ajh/hpx158
11. Mahmood SS, Nohria A. Cardiovascular complications of cranial and neck radiation. *Curr Treat Options Cardiovasc Med.* (2016) 18:45. doi: 10.1007/s11936-016-0468-4
12. Marinko, T. Pericardial disease after breast cancer radiotherapy. *Radiol Oncol.* (2018) 53:1–5. doi: 10.2478/raon-2018-0035
13. Lee CC, Zheng H, Soon YY, Foo LL, Koh WY, Leong CN, et al. Association between radiation heart dosimetric parameters, myocardial infarct and overall survival in stage 3 non-small cell lung cancer treated with definitive thoracic radiotherapy. *Lung Cancer.* (2018) 120:54–9. doi: 10.1016/j.lungcan.2018.03.024
14. Darby SC, Cutter DJ, Boerma M, Constine LS, Fajardo LF, Kodama K, et al. Radiation-related heart disease: current knowledge and future prospects. *Int J Radiat Oncol Biol Phys.* (2010) 76:656–65. doi: 10.1016/j.ijrobp.2009.09.064
15. Wu SP, Tam M, Vega,RM, Perez CA, Gerber NK. Effect of breast irradiation on cardiac disease in women enrolled in BCIRG-001 at 10-year follow-up. *Int J Radiat Oncol Biol Phys.* (2017) 99:541–8. doi: 10.1016/j.ijrobp.2017.06.018
16. Stewart MH, Jahangir E, Polin NM. Valvular heart disease in cancer patients: etiology, diagnosis, and management. *Curr Treat Options Cardiovasc Med.* (2017) 19:53. doi: 10.1007/s11936-017-0550-6
17. Viganego F, Singh R, Fradley MG. Arrhythmias and other electrophysiology issues in cancer patients receiving chemotherapy or radiation. *Curr Cardiol Rep.* (2016) 18:52. doi: 10.1007/s11886-016-0730-0
18. Marangou J, Redfern A, Haddad T, Rankin JM, Dwivedi G. Heart failure following oncological treatment. *Curr Opin Cardiol.* (2018) 33:237–44. doi: 10.1097/HCO.0000000000000488
19. Saiki H, Petersen IA, Scott CG, Bailey KR, Dunlay SM, Finley RR, et al. Risk of heart failure with preserved ejection fraction in older women after

contemporary radiotherapy for breast cancer. *Circulation.* (2017) 135:1388–96. doi: 10.1161/CIRCULATIONAHA.116.025434
20. Nolan MT, Russell DJ, Negishi K, Marwick TH. Meta-analysis of association between mediastinal radiotherapy and long-term heart failure. *Am J Cardiol.* (2016) 118:1685–91. doi: 10.1016/j.amjcard.2016.08.050
21. Darby SC, Ewertz M, McGale P, Bennet AM, Blom-Goldman U, Bronnum D, et al. Risk of ischemic heart disease in women after radiotherapy for breast cancer. *N Engl J Med.* (2013) 368:987–98. doi: 10.1056/NEJMoa1209825
22. Henson KE, McGale P, Taylor C, Darby SC. Radiation-related mortality from heart disease and lung cancer more than 20 years after radiotherapy for breast cancer. *Br J Cancer.* (2013) 108:179–82. doi: 10.1038/bjc.2012.575
23. Lee Chuy K, Nahhas O, Dominic P, Lopez C, Tonorezos E, Sidlow R, et al. Cardiovascular complications associated with mediastinal radiation. *Curr Treat Options Cardiovasc Med.* (2019) 21:31. doi: 10.1007/s11936-019-0737-0
24. Steingart, R.M, Yadav, N, Manrique, C, Carver, J.R, Liu, J. Cancer survivorship: cardiotoxic therapy in the adult cancer patient; cardiac outcomes with recommendations for patient management. *Semin Oncol.* (2013) 40:690–708. doi: 10.1053/j.seminoncol.2013.09.010
25. Spetz J, Moslehi J, Sarosiek K. Radiation-induced cardiovascular toxicity: mechanisms, prevention, and treatment. *Curr Treat Opt Cardiovasc Med.* (2018) 20:31. doi: 10.1007/s11936-018-0627-x
26. Saunders S, Anwar M. Capecitabine-induced myopericarditis - A case report and review of literature. *J Oncol Pharm Pract.* (2019) 25:1006–10. doi: 10.1177/1078155218774871
27. Nicol M, Baudet M, Cohen-Solal A. Subclinical left ventricular dysfunction during chemotherapy. *Card Fail Rev.* (2019) 5:31–6. doi: 10.15420/cfr.2018.25.1
28. Kanduri J, More LA, Godishala A, Asnani A. Fluoropyrimidine-associated cardiotoxicity. *Cardiol Clin.* (2019) 37:399–405. doi: 10.1016/j.ccl.2019.07.004
29. Zhang KW, Finkelman BS, Gulati G, Narayan HK, Upshaw J, Narayan V, et al. Abnormalities in 3-dimensional left ventricular mechanics with anthracycline chemotherapy are associated with systolic and diastolic dysfunction. *JACC Cardiovasc Imaging.* (2018) 11:1059–68. doi: 10.1016/j.jcmg.2018.01.015
30. Tsugu T, Nagatomo Y, Nakajima Y, Kageyama T, Akise Y, Endo J, et al. Cancer therapeutics-related cardiac dysfunction in a patient treated with abiraterone for castration-resistant prostate cancer. *J Med Ultrason.* (2019) 46:239–43. doi: 10.1007/s10396-018-0897-7
31. Shah CP, Moreb JS. Cardiotoxicity due to targeted anticancer agents: a growing challenge. *Ther Adv Cardiovasc Dis.* (2019) 13:1753944719843435. doi: 10.1177/1753944719843435
32. Hussain Y, Drill E, Dang CT, Liu JE, Steingart RM, Yu AF. Cardiac outcomes of trastuzumab therapy in patients with HER2-positive breast cancer and reduced left ventricular ejection fraction. *Breast Cancer Res Treat.* (2019) 175:239–46. doi: 10.1007/s10549-019-05139-6
33. Guha A, Armanious M, Fradley MG. Update on cardio-oncology: novel cancer therapeutics and associated cardiotoxicities. *Trends Cardiovasc Med.* (2019) 29:29–39. doi: 10.1016/j.tcm.2018.06.001
34. Ball S, Ghosh RK, Wongsaengsak S, Bandyopadhyay D, Ghosh GC, Aronow WS, et al. Cardiovascular toxicities of immune checkpoint inhibitors: JACC review topic of the week. *J Am Coll Cardiol.* (2019) 74:1714–27. doi: 10.1016/j.jacc.2019.07.079
35. Salem JE, Manouchehri A, Moey M, Lebrun-Vignes B, Bastarache L, Pariente A, et al. Cardiovascular toxicities associated with immune checkpoint inhibitors: an observational, retrospective, pharmacovigilance study. *Lancet Oncol.* (2018) 19:1579–89. doi: 10.1016/S1470-2045(18)30608-9
36. Lyon AR, Yousaf N, Battisti NML, Moslehi J, Larkin J. Immune checkpoint inhibitors and cardiovascular toxicity. *Lancet Oncol.* (2018) 19:e447–58. doi: 10.1016/S1470-2045(18)30457-1
37. Totzeck M, Schuler M, Stuschke M, Heusch G, Rassaf T. Cardio-oncology - strategies for management of cancer-therapy related cardiovascular disease. *Int J Cardiol.* (2019) 280:163–75. doi: 10.1016/j.ijcard.2019.01.038
38. Perez IE, Taveras Alam S, Hernandez GA, Sancassani R. Cancer therapy-related cardiac dysfunction: an overview for the clinician. *Clin Med Insights Cardiol.* (2019) 13:1179546819866445. doi: 10.1177/1179546819866445
39. Zheng PP, Li J, Kros JM. Breakthroughs in modern cancer therapy and elusive cardiotoxicity: critical research-practice gaps, challenges, and insights. *Med Res Rev.* (2018) 38:325–76. doi: 10.1002/med.21463
40. Ryan TD, Nagarajan R, Godown J. Pediatric cardio-oncology: development of cancer treatment-related cardiotoxicity and the therapeutic approach to affected patients. *Curr Treat Options Oncol.* (2019) 20:56. doi: 10.1007/s11864-019-0658-x
41. Mulcahy Levy JM, Tello T, Giller R, Wilkening G, Quinones R, Keating AK, et al. Late effects of total body irradiation and hematopoietic stem cell transplant in children under 3 years of age. *Pediatr Blood Cancer.* (2013) 60:700–4. doi: 10.1002/pbc.24252
42. Findlay SG, Gill JH, Plummer R, DeSantis C, Plummer C. Chronic cardiovascular toxicity in the older oncology patient population. *J Geriatr Oncol.* (2019) 10:685–9. doi: 10.1016/j.jgo.2019.01.018
43. Accordino MK, Neugut AI, Hershman DL. Cardiac effects of anticancer therapy in the elderly. *J Clin Oncol.* (2014) 32:2654–61. doi: 10.1200/JCO.2013.55.0459
44. Huang R, Zhou Y, Hu S, Ren G, Cui F, Zhou PK. Radiotherapy exposure in cancer patients and subsequent risk of stroke: a systematic review and meta-analysis. *Front Neurol.* (2019) 10:233. doi: 10.3389/fneur.2019.00233
45. Chu CN, Chen PC, Bai LY, Muo CH, Sung FC, Chen SW. Young nasopharyngeal cancer patients with radiotherapy and chemotherapy are most prone to ischaemic risk of stroke: a national database, controlled cohort study. *Clin Otolaryngol.* (2013) 38:39–47. doi: 10.1111/coa.12064
46. Omer H, Sulieman A, Alzimami K. Risks of lung fibrosis and pneumonitis after postmastectomy electron radiotherapy. *Radiat Prot Dosimetry.* (2015) 165:499–502. doi: 10.1093/rpd/ncv111
47. Bentzen SM, Skoczylas JZ, Overgaard M, Overgaard J. Radiotherapy-related lung fibrosis enhanced by tamoxifen. *J Natl Cancer Inst.* (1996) 88:918–22. doi: 10.1093/jnci/88.13.918
48. Soloviev AI, Kizub IV. Mechanisms of vascular dysfunction evoked by ionizing radiation and possible targets for its pharmacological correction. *Biochem Pharmacol.* (2019) 159:121–39. doi: 10.1016/j.bcp.2018.11.019
49. Weigel C, Veldwijk MR, Oakes CC, Seibold P, Slynko A, Liesenfeld DB, et al. Epigenetic regulation of diacylglycerol kinase alpha promotes radiation-induced fibrosis. *Nat Commun.* (2016) 7:10893. doi: 10.1038/ncomms10893
50. Wagner-Ecker M, Schwager C, Wirkner U, Abdollahi A, Huber PE. MicroRNA expression after ionizing radiation in human endothelial cells. *Radiat Oncol.* (2010) 5:25. doi: 10.1186/1748-717X-5-25
51. Barjaktarovic Z, Anastasov N, Azimzadeh O, Sriharshan A, Sarioglu H, Ueffing M, et al. Integrative proteomic and microRNA analysis of primary human coronary artery endothelial cells exposed to low-dose gamma radiation. *Radiat Environ Biophys.* (2013) 52:87–98. doi: 10.1007/s00411-012-0439-4
52. Kura B, Kalocayova B, LeBaron TW, Frimmel K, Buday J, Surovy J, et al. Regulation of microRNAs by molecular hydrogen contributes to the prevention of radiation-induced damage in the rat myocardium. *Mol Cell Biochem.* (2019) 457:61–72. doi: 10.1007/s11010-019-03512-z
53. Viczenczova C, Kura B, Egan Benova T, Yin C, Kukreja RC, Slezak J, et al. Irradiation-induced cardiac Connexin-43 and miR-21 responses are hampered by treatment with atorvastatin and aspirin. *Int J Mol Sci.* (2018) 19:E1128. doi: 10.3390/ijms19041128
54. Bartman CM, Oyama Y, Brodsky K, Khailova L, Walker L, Koeppen M, et al. Intense light-elicited upregulation of miR-21 facilitates glycolysis and cardioprotection through Per2-dependent mechanisms. *PLoS ONE.* (2017) 12:e0176243. doi: 10.1371/journal.pone.0176243
55. Zhou Z, Schober A, Nazari-Jahantigh M. Dicer promotes endothelial recovery and limits lesion formation after vascular injury through miR-126–5p. *Int J Cardiol.* (2018) 273:199–202. doi: 10.1016/j.ijcard.2018.09.006
56. Wright JL. *Toxicities of Radiation Treatment for Breast Cancer: Risks and Management Strategies.* Cham: Springer Nature Switzerland (2019). doi: 10.1007/978-3-030-11620-0
57. Kang HJ, Kim SW, Son SH. The feasibility of a heart block with an electron compensation as an alternative whole breast radiotherapy technique in patients with underlying cardiac or pulmonary disease. *PLoS ONE.* (2017) 12:e0184137. doi: 10.1371/journal.pone.0184137

58. The American Cancer Society. *The American Cancer Society's Principles of Oncology: Prevention to Survivorship*. Oxford, UK; Hoboken, NJ; Chichester: Wiley (2018).
59. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. *JAMA*. (1999) 282:1523–9. doi: 10.1001/jama.282.16.1523
60. van Westerop LL, Arts-de Jong M, Hoogerbrugge N, de Hullu JA, Maas AH. Cardiovascular risk of BRCA1/2 mutation carriers: a review. *Maturitas*. (2016) 91:135–9. doi: 10.1016/j.maturitas.2016.06.012
61. Borkenhagen JF, Bergom C, Rapp CT, Klawikowski SJ, Rein LE, Gore EM. Dosimetric predictors of cardiotoxicity in thoracic radiotherapy for lung cancer. *Clin Lung Cancer*. (2019) 20:435–41. doi: 10.1016/j.cllc.2019.05.014
62. Ming X, Feng Y, Yang C, Wang W, Wang P, Deng J. Radiation-induced heart disease in lung cancer radiotherapy: a dosimetric update. *Medicine*. (2016) 95:e5051. doi: 10.1097/MD.0000000000005051
63. Frandsen J, Boothe D, Gaffney DK, Wilson BD, Lloyd S. Increased risk of death due to heart disease after radiotherapy for esophageal cancer. *J Gastrointest Oncol*. (2015) 6:516–23.
64. Piroth MD, Baumann R, Budach W, Dunst J, Feyer P, Fietkau R, et al. Heart toxicity from breast cancer radiotherapy: current findings, assessment, and prevention. *Strahlenther Onkol*. (2019) 195:1–12. doi: 10.1007/s00066-018-1378-z
65. Rehammar JC, Jensen MB, McGale P, Lorenzen EL, Taylor C, Darby SC, et al. Risk of heart disease in relation to radiotherapy and chemotherapy with anthracyclines among 19,464 breast cancer patients in Denmark, 1977–2005. *Radiother Oncol*. (2017) 123:299–305. doi: 10.1016/j.radonc.2017.03.012
66. Hoppe BS, Bates JE, Mendenhall NP, Morris CG, Louis D, Ho MW, et al. The meaningless meaning of mean heart dose in mediastinal lymphoma in the modern radiation therapy era. *Pract Radiat Oncol*. (2019) doi: 10.1016/j.prro.2019.09.015. [Epub ahead of print].
67. Ozyigit G, Selek U. *Radiation Oncology: A Case-Based Review*. Cham: Springer Nature Switzerland (2019). doi: 10.1007/978-3-319-97145-2
68. Chang JY, Bezjak A, Mornex F, IASLC Advanced Radiation Technology Committee. Stereotactic ablative radiotherapy for centrally located early stage non-small-cell lung cancer: what we have learned. *J Thorac Oncol*. (2015) 10:577–85. doi: 10.1097/JTO.0000000000000453
69. Zhao L, Zhou S, Balter P, Shen C, Gomez DR, Welsh JD, et al. Planning target volume D95 and mean dose should be considered for optimal local control for stereotactic ablative radiation therapy. *Int J Radiat Oncol Biol Phys*. (2016) 95:1226–35. doi: 10.1016/j.ijrobp.2016.01.065
70. Marks LB, Yorke ED, Jackson A, Ten Haken RK, Constine LS, Eisbruch A, et al. Use of normal tissue complication probability models in the clinic. *Int J Radiat Oncol Biol Phys*. (2010) 76:S10–9. doi: 10.1016/j.ijrobp.2009.07.1754
71. Boulet J, Pena J, Hulten EA, Neilan TG, Dragomir A, Freeman C, et al. Statin use and risk of vascular events among cancer patients after radiotherapy to the thorax, head, and neck. *J Am Heart Assoc*. (2019) 8:e005996. doi: 10.1161/JAHA.117.005996
72. Ward WF, Kim YT, Molteni A, Solliday NH. Radiation-induced pulmonary endothelial dysfunction in rats: modification by an inhibitor of angiotensin converting enzyme. *Int J Radiat Oncol Biol Phys*. (1988) 15:135–40. doi: 10.1016/0360-3016(88)90357-4
73. Gulati G, Heck SL, Ree AH, Hoffmann P, Schulz-Menger J, Fagerland MW, et al. Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 x 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol. *Eur Heart J*. (2016) 37:1671–80. doi: 10.1093/eurheartj/ehw022
74. Ohri N. Radiotherapy dosing for locally advanced non-small cell lung carcinoma: "MTD" or "ALARA"? *Front Oncol*. (2017) 7:205. doi: 10.3389/fonc.2017.00205
75. Taylor CW, Kirby AM. Cardiac side-effects from breast cancer radiotherapy. *Clin Oncol*. (2015) 27:621–9. doi: 10.1016/j.clon.2015.06.007
76. Padegimas A, Clasen S, Ky B. Cardioprotective strategies to prevent breast cancer therapy-induced cardiotoxicity. *Trends Cardiovasc Med*. (2019) 30:22–8. doi: 10.1016/j.tcm.2019.01.006
77. Freedman GM, Lin L. Cardiac-sparing radiation therapy for breast cancer. *Appl Radiat Oncol*. (2016) 5:6–11.
78. Yang K, Park W, Ju SG, Chung Y, Choi DH, Cha H, et al. Heart-sparing radiotherapy with three-dimensional printing technology after mastectomy for patients with left breast cancer. *Breast J*. (2019) 25:682–6. doi: 10.1111/tbj.13304
79. Kil WJ, Pham T, Kim K. Heart sparing breast cancer radiotherapy using continuous positive airway pressure (CPAP) and conventional supine tangential fields: an alternative method for patients with limited accessibility to advanced radiotherapy techniques. *Acta Oncol*. (2019) 58:105–9. doi: 10.1080/0284186X.2018.1503711
80. Kaplinsky A, Pyatigorskaya V, Granot H, Gelernter I, Ben-Ayun M, Alezra D, et al. RPM inspiration gating: improving radiotherapy for left breast cancer patients with anterior heart position. *Isr Med Assoc J*. (2018) 20:548–52.
81. Becker-Schiebe M, Stockhammer M, Hoffmann W, Wetzel F, Franz H. Does mean heart dose sufficiently reflect coronary artery exposure in left-sided breast cancer radiotherapy?: influence of respiratory gating. *Strahlenther Onkol*. (2016) 192:624–31. doi: 10.1007/s00066-016-1011-y
82. Speleers BA, Belosi FM, De Gersem WR, Deseyne PR, Paelinck LM, Bolsi A, et al. Comparison of supine or prone crawl photon or proton breast and regional lymph node radiation therapy including the internal mammary chain. *Sci Rep*. (2019) 9:4755. doi: 10.1038/s41598-019-41283-1
83. Braunstein LZ, Cahlon O. Potential morbidity reduction with proton radiation therapy for breast cancer. *Semin Radiat Oncol*. (2018) 28:138–49. doi: 10.1016/j.semradonc.2017.11.009
84. Kammerer E, Guevelou JL, Chaikh A, Danhier S, Geffrelot J, Levy C, et al. Proton therapy for locally advanced breast cancer: a systematic review of the literature. *Cancer Treat Rev*. (2018) 63:19–27. doi: 10.1016/j.ctrv.2017.11.006
85. Tommasino F, Durante M, D'Avino V, Liuzzi R, Conson M, Farace P, et al. Model-based approach for quantitative estimates of skin, heart, and lung toxicity risk for left-side photon and proton irradiation after breast-conserving surgery. *Acta Oncol*. (2017) 56:730–6. doi: 10.1080/0284186X.2017.1299218
86. Simonetto C, Eidemuller M, Gaasch A, Pazos M, Schonecker S, Reitz D, et al. Does deep inspiration breath-hold prolong life? Individual risk estimates of ischaemic heart disease after breast cancer radiotherapy. *Radiother Oncol*. (2019) 131:202–7. doi: 10.1016/j.radonc.2018.07.024
87. Duma MN, Baumann R, Budach W, Dunst J, Feyer P, Fietkau R, et al. Heart-sparing radiotherapy techniques in breast cancer patients: a recommendation of the breast cancer expert panel of the German society of radiation oncology (DEGRO). *Strahlenther Onkol*. (2019) 195:861–71. doi: 10.1007/s00066-019-01495-w
88. Wikstrom K, Isacsson U, Nilsson K, Ahnesjo A. Reproducibility of heart and thoracic wall position in repeated deep inspiration breath holds for radiotherapy of left-sided breast cancer patients. *Acta Oncol*. (2018) 57:1318–24. doi: 10.1080/0284186X.2018.1490027
89. Corradini S, Ballhausen H, Weingandt H, Freislederer P, Schonecker S, Niyazi M, et al. Left-sided breast cancer and risks of secondary lung cancer and ischemic heart disease: effects of modern radiotherapy techniques. *Strahlenther Onkol*. (2018) 194:196–205. doi: 10.1007/s00066-017-1213-y
90. Al-Hammadi N, Caparrotti P, Naim C, Hayes J, Rebecca Benson K, Vasic A, et al. Voluntary deep inspiration breath-hold reduces the heart dose without compromising the target volume coverage during radiotherapy for left-sided breast cancer. *Radiol Oncol*. (2018) 52:112–20. doi: 10.1515/raon-2018-0008
91. Koivumaki T, Tujunen J, Viren T, Heikkila J, Seppala J. Geometrical uncertainty of heart position in deep-inspiration breath-hold radiotherapy of left-sided breast cancer patients. *Acta Oncol*. (2017) 56:879–83. doi: 10.1080/0284186X.2017.1298836
92. Niska JR, Thorpe CS, Allen SM, Daniels TB, Rule WG, Schild SE, et al. Radiation and the heart: systematic review of dosimetry and cardiac endpoints. *Expert Rev Cardiovasc Ther*. (2018) 16:931–50. doi: 10.1080/14779072.2018.1538785
93. Drost L, Yee C, Lam H, Zhang L, Wronski M, McCann C, et al. A systematic review of heart dose in breast radiotherapy. *Clin Breast Cancer*. (2018) 18:e819–24. doi: 10.1016/j.clbc.2018.05.010
94. Taylor CW, Wang Z, Macaulay E, Jagsi R, Duane F, Darby SC. Exposure of the heart in breast cancer radiation therapy: a systematic review of heart doses published during 2003 to 2013. *Int J Radiat Oncol Biol Phys*. (2015) 93:845–53. doi: 10.1016/j.ijrobp.2015.07.2292
95. Milgrom SA, Varghese B, Gladish GW, Choi AD, Dong W, Patel ZS, et al. Coronary artery dose-volume parameters predict risk of

calcification after radiation therapy. *J Cardiovasc Imaging.* (2019) 27:268–79. doi: 10.4250/jcvi.2019.27.e38
96. Tan W, Liu D, Xue C, Xu J, Li B, Chen Z, et al. Anterior myocardial territory may replace the heart as organ at risk in intensity-modulated radiotherapy for left-sided breast cancer. *Int J Radiat Oncol Biol Phys.* (2012) 82:1689–97. doi: 10.1016/j.ijrobp.2011.03.009
97. Kole TP, Aghayere O, Kwah J, Yorke ED, Goodman KA. Comparison of heart and coronary artery doses associated with intensity-modulated radiotherapy versus three-dimensional conformal radiotherapy for distal esophageal cancer. *Int J Radiat Oncol Biol Phys.* (2012) 83:1580–6. doi: 10.1016/j.ijrobp.2011.10.053
98. Jacob S, Camilleri J, Derreumaux S, Walker V, Lairez O, Lapeyre M, et al. Is mean heart dose a relevant surrogate parameter of left ventricle and coronary arteries exposure during breast cancer radiotherapy: a dosimetric evaluation based on individually-determined radiation dose (BACCARAT study). *Radiat Oncol.* (2019) 14:29. doi: 10.1186/s13014-019-1234-z
99. Hiniker SM, Donaldson SS. ALARA: in radiation oncology and diagnostic imaging alike. *Oncology.* (2014) 28:247–8.
100. Sung K, Choi YE, Lee KC. Cardiac risk index as a simple geometric indicator to select patients for the heart-sparing radiotherapy of left-sided breast cancer. *J Med Imaging Radiat Oncol.* (2017) 61:410–7. doi: 10.1111/1754-9485.12567
101. Tjessem KH, Bosse G, Fossa K, Reinertsen KV, Fossa SD, Johansen S, et al. Coronary calcium score in 12-year breast cancer survivors after adjuvant radiotherapy with low to moderate heart exposure - relationship to cardiac radiation dose and cardiovascular risk factors. *Radiother Oncol.* (2015) 114:328–34. doi: 10.1016/j.radonc.2015.01.006
102. Riddell E, Lenihan D. The role of cardiac biomarkers in cardio-oncology. *Curr Probl Cancer.* (2018) 42:375–85. doi: 10.1016/j.currproblcancer.2018.06.012
103. Shah KS, Yang EH, Maisel AS, Fonarow GC. The role of biomarkers in detection of cardio-toxicity. *Curr Oncol Rep.* (2017) 19:42. doi: 10.1007/s11912-017-0602-9
104. Negishi T, Miyazaki S, Negishi K. Echocardiography and Cardio-Oncology. *Heart Lung Circ.* (2019) 28:1331–8. doi: 10.1016/j.hlc.2019.04.023
105. Liu J, Banchs J, Mousavi N, Plana JC, Scherrer-Crosbie M, Thavendiranathan P, et al. Contemporary role of echocardiography for clinical decision making in patients during and after cancer therapy. *JACC Cardiovasc Imaging.* (2018) 11:1122–31. doi: 10.1016/j.jcmg.2018.03.025
106. Bordun KA, Premecz S, daSilva M, Mandal S, Goyal V, Glavinovic T, et al. The utility of cardiac biomarkers and echocardiography for the early detection of bevacizumab- and sunitinib-mediated cardiotoxicity. *Am J Physiol Heart Circ Physiol.* (2015) 309:H692–701. doi: 10.1152/ajpheart.00172.2015
107. Laufer-Perl M, Arnold JH, Mor L, Amrami N, Derakhshesh M, Moshkovits Y, et al. The association of reduced global longitudinal strain with cancer therapy-related cardiac dysfunction among patients receiving cancer therapy. *Clin Res Cardiol.* (2020) 109:255–62. doi: 10.1007/s00392-019-01508-9
108. Laufer-Perl M, Derakhshesh M, Milwidsky A, Mor L, Ravid D, Amrami N, et al. Usefulness of global longitudinal strain for early identification of subclinical left ventricular dysfunction in patients with active cancer. *Am J Cardiol.* (2018) 122:1784–9. doi: 10.1016/j.amjcard.2018.08.019
109. Mercurio V, Cuomo A, Della Pepa R, Ciervo D, Cella L, Pirozzi F, et al. What is the cardiac impact of chemotherapy and subsequent radiotherapy in lymphoma patients? *Antioxid Redox Signal.* (2019) 31:1166–74. doi: 10.1089/ars.2019.7842
110. Plana JC, Thavendiranathan P, Bucciarelli-Ducci C, Lancellotti P. Multi-modality imaging in the assessment of cardiovascular toxicity in the cancer patient. *JACC Cardiovasc Imaging.* (2018) 11:1173–86. doi: 10.1016/j.jcmg.2018.06.003
111. Layoun ME, Yang EH, Herrmann J, Iliescu CA, Lopez-Mattei JC, Marmagkiolis K, et al. Applications of cardiac computed tomography in the cardio-oncology population. *Curr Treat Options Oncol.* (2019) 20:47. doi: 10.1007/s11864-019-0645-2
112. Pitekova B, Ravi S, Shah SV, Mladosievicova B, Heitner S, Ferencik M. The role of imaging with cardiac computed tomography in cardio-oncology patients. *Curr Cardiol Rep.* (2016) 18:87. doi: 10.1007/s11886-016-0768-z
113. Yang Y, Yam Y, Chen L, Aljizeeri A, Aliyary Ghraboghly S, Al-Harbi I, et al. Assessment of left ventricular ejection fraction using low radiation dose computed tomography. *J Nucl Cardiol.* (2016) 23:414–21. doi: 10.1007/s12350-015-0123-6
114. Jordan JH, Hundley WG. MRI of cardiotoxicity. *Cardiol Clin.* (2019) 37:429–39. doi: 10.1016/j.ccl.2019.07.007
115. Jeong D, Gladish G, Chitiboi T, Fradley MG, Gage KL, Schiebler ML. MRI in cardio-oncology: a review of cardiac complications in oncologic care. *J Magn Reson Imaging.* (2019) 50:1349–66. doi: 10.1002/jmri.26895
116. Loffler AI, Salerno M. Cardiac MRI for the evaluation of oncologic cardiotoxicity. *J Nucl Cardiol.* (2018) 25:2148–58. doi: 10.1007/s12350-018-1293-9
117. Dreyfuss AD, Bravo PE, Koumenis C, Ky B. Precision cardio-oncology. *J Nucl Med.* (2019) 60:443–50. doi: 10.2967/jnumed.118.220137
118. Zhang P, Hu X, Yue J, Meng X, Han D, Sun X, et al. Early detection of radiation-induced heart disease using (99m)Tc-MIBI SPECT gated myocardial perfusion imaging in patients with oesophageal cancer during radiotherapy. *Radiother Oncol.* (2015) 115:171–8. doi: 10.1016/j.radonc.2015.04.009
119. Zyromska A, Malkowski B, Wisniewski T, Majewska K, Reszke J, Makarewicz R. ^{15}O-H_2O PET/CT as a tool for the quantitative assessment of early post-radiotherapy changes of heart perfusion in breast carcinoma patients. *Br J Radiol.* (2018) 91:20170653. doi: 10.1259/bjr.20170653
120. Tong D, Zaha VG. Metabolic imaging in cardio-oncology. *J Cardiovasc Transl Res.* (2019). doi: 10.1007/s12265-019-09927-9
121. Soufer A, Liu C, Henry ML, Baldassarre LA. Nuclear cardiology in the context of multimodality imaging to detect cardiac toxicity from cancer therapeutics: established and emerging methods. *J Nucl Cardiol.* (2019). doi: 10.1007/s12350-019-01671-6
122. Kahanda MG, Hanson CA, Patterson B, Bourque JM. Nuclear cardio-oncology: from its foundation to its future. *J Nucl Cardiol.* (2019). doi: 10.1007/s12350-019-01655-6
123. Raucci A, Di Maggio S, Scavello F, D'Ambrosio A, Bianchi ME, Capogrossi MC. The Janus face of HMGB1 in heart disease: a necessary update. *Cell Mol Life Sci.* (2019) 76:211–29. doi: 10.1007/s00018-018-2930-9
124. Sarkozy M, Gaspar R, Zvara A, Kiscsatari L, Varga Z, Kovari B, et al. Selective heart irradiation induces cardiac overexpression of the pro-hypertrophic miR-212. *Front Oncol.* (2019) 9:598. doi: 10.3389/fonc.2019.00598
125. Rousseau M, Gaugler MH, Rodallec A, Bonnaud S, Paris F, Corre I. RhoA GTPase regulates radiation-induced alterations in endothelial cell adhesion and migration. *Biochem Biophys Res Commun.* (2011) 414:750–5. doi: 10.1016/j.bbrc.2011.09.150
126. Oesterle A, Liao JK. The pleiotropic effects of statins - from coronary artery disease and stroke to atrial fibrillation and ventricular tachyarrhythmia. *Curr Vasc Pharmacol.* (2019) 17:222–32. doi: 10.2174/1570161111666180817155058
127. Tanaka S, Fukumoto Y, Nochioka K, Minami T, Kudo S, Shiba N, et al. Statins exert the pleiotropic effects through small GTP-binding protein dissociation stimulator upregulation with a resultant Rac1 degradation. *Arterioscler Thromb Vasc Biol.* (2013) 33:1591–600. doi: 10.1161/ATVBAHA.112.300922
128. Liao JK, Laufs U. Pleiotropic effects of statins. *Annu Rev Pharmacol Toxicol.* (2005) 45:89–118. doi: 10.1146/annurev.pharmtox.45.120403.095748
129. Wilkinson EL, Sidaway JE, Cross MJ. Statin regulated ERK5 stimulates tight junction formation and reduces permeability in human cardiac endothelial cells. *J Cell Physiol.* (2018) 233:186–200. doi: 10.1002/jcp.26064
130. Cerda A, Fajardo CM, Basso RG, Hirata MH, Hirata RD. Role of microRNAs 221/222 on statin induced nitric oxide release in human endothelial cells. *Arq Bras Cardiol.* (2015) 104:195–201. doi: 10.5935/abc.20140192
131. Taylor C, Correa C, Duane FK, Aznar MC, Anderson SJ, Bergh J, et al. Estimating the risks of breast cancer radiotherapy: evidence from modern radiation doses to the lungs and heart and from previous randomized trials. *J Clin Oncol.* (2017) 35:1641–9. doi: 10.1200/JCO.2016.72.0722
132. Dlugosz-Danecka M, Gruszka AM, Szmit S, Olszanecka A, Ogorka T, Sobocinski M, et al. Primary cardioprotection reduces mortality in lymphoma patients with increased risk of anthracycline cardiotoxicity, treated by R-CHOP regimen. *Chemotherapy.* (2018) 63:238–45. doi: 10.1159/000492942

133. Gulati G, Heck SL, Rosjo H, Ree AH, Hoffmann P, Hagve TA, et al. Neurohormonal Blockade and Circulating cardiovascular biomarkers during anthracycline therapy in breast cancer patients: results from the PRADA (prevention of cardiac dysfunction during adjuvant breast cancer therapy) study. *J Am Heart Assoc.* (2017) 6:e006513. doi: 10.1161/JAHA.117.006513
134. Bentzen SM. Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. *Nat Rev Cancer.* (2006) 6:702–13. doi: 10.1038/nrc1950
135. Hahn AW, Resink TJ, Bernhardt J, Ferracin F, Buhler FR. Stimulation of autocrine platelet–derived growth factor AA-homodimer and transforming growth factor beta in vascular smooth muscle cells. *Biochem Biophys Res Commun.* (1991) 178:1451–8. doi: 10.1016/0006-291X(91)91056-I
136. Armanious MA, Mishra S, Fradley MG. Electrophysiologic toxicity of chemoradiation. *Curr Oncol Rep.* (2018) 20:45. doi: 10.1007/s11912-018-0691-0
137. Subramanian V, Seemann I, Merl-Pham J, Hauck SM, Stewart FA, Atkinson MJ, et al. Role of TGF beta and PPAR alpha signaling pathways in radiation response of locally exposed heart: integrated global transcriptomics and proteomics analysis. *J Proteome Res.* (2017) 16:307–18. doi: 10.1021/acs.jproteome.6b00795
138. Slezak J, Kura B, Babal P, Barancik M, Ferko M, Frimmel K, et al. Potential markers and metabolic processes involved in the mechanism of radiation-induced heart injury. *Can J Physiol Pharmacol.* (2017) 95:1190–203. doi: 10.1139/cjpp-2017-0121
139. Klee NS, McCarthy CG, Martinez-Quinones P, Webb RC. Out of the frying pan and into the fire: damage-associated molecular patterns and cardiovascular toxicity following cancer therapy. *Ther Adv Cardiovasc Dis.* (2017) 11:297–317. doi: 10.1177/1753944717729141
140. Peng Y, Croce CM. The role of MicroRNAs in human cancer. *Signal Transduct Target Ther.* (2016) 1:15004. doi: 10.1038/sigtrans.2015.4
141. Lamichhane SR, Thachil T, De Ieso P, Gee H, Moss SA, Milic N. Prognostic role of microRNAs in human non-small-cell lung cancer: a systematic review and meta-analysis. *Dis Markers.* (2018) 2018:8309015. doi: 10.1155/2018/8309015
142. Zheng Q, Chen C, Guan H, Kang W, Yu C. Prognostic role of microRNAs in human gastrointestinal cancer: a systematic review and meta-analysis. *Oncotarget.* (2017) 8:46611–23. doi: 10.18632/oncotarget.16679
143. Asiaf A, Ahmad ST, Arjumand W, Zargar MA. MicroRNAs in breast cancer: diagnostic and therapeutic potential. *Methods Mol Biol.* (2018) 1699:23–43. doi: 10.1007/978-1-4939-7435-1_2
144. Aghdam AM, Amiri A, Salarinia R, Masoudifar A, Ghasemi F, Mirzaei H. MicroRNAs as diagnostic, prognostic, and therapeutic biomarkers in prostate cancer. *Crit Rev Eukaryot Gene Expr.* (2019) 29:127–39. doi: 10.1615/CritRevEukaryotGeneExpr.2019025273
145. Wan TM, Iyer DN, Ng L. Roles of microRNAs as non-invasive biomarker and therapeutic target in colorectal cancer. *Histol Histopathol.* (2020) 35, 225–237. doi: 10.14670/HH-18-171
146. Movahedpour A, Ahmadi N, Ghasemi Y, Savardashtaki A, Shabaninejad Z. Circulating microRNAs as potential diagnostic biomarkers and therapeutic targets in prostate cancer: current status and future perspectives. *J Cell Biochem.* (2019) 120:16316–29. doi: 10.1002/jcb.29053
147. Eken SM, Christersdottir T, Winski G, Sangsuwan T, Jin H, Chernogubova E, et al. miR-29b mediates the chronic inflammatory response in radiotherapy-induced vascular disease. *JACC Basic Transl Sci.* (2019) 4:72–82. doi: 10.1016/j.jacbts.2018.10.006
148. Zhang C, Zhang K, Huang F, Feng W, Chen J, Zhang H, et al. Exosomes, the message transporters in vascular calcification. *J Cell Mol Med.* (2018) 22:4024–33. doi: 10.1111/jcmm.13692
149. Hu Y, Xia W, Hou M. Macrophage migration inhibitory factor serves a pivotal role in the regulation of radiation-induced cardiac senescencethrough rebalancing the microRNA-34a/sirtuin 1 signaling pathway. *Int J Mol Med.* (2018) 42:2849–58. doi: 10.3892/ijmm.2018.3838
150. Esplugas R, Belles M, Serra N, Arenas M, Hernandez V, Vallve JC, et al. Effect of radiation on the expression of CVD-related miRNAs, inflammation and endothelial dysfunction of HUVECs. *Anticancer Res.* (2019) 39:771–80. doi: 10.21873/anticanres.13174
151. Kim EJ, Lee J, Jung YR, Park JJ, Park MJ, Lee JS, et al. Involvement of corin downregulation in ionizing radiation-induced senescence of myocardial cells. *Int J Mol Med.* (2015) 35:731–8. doi: 10.3892/ijmm.2014.2048
152. Halle M, Christersdottir T, Back M. Chronic adventitial inflammation, vasa vasorum expansion, and 5-lipoxygenase up-regulation in irradiated arteries from cancer survivors. *FASEB J.* (2016) 30:3845–52. doi: 10.1096/fj.201600620R
153. Park H, Kim CH, Jeong JH, Park M, Kim KS. GDF15 contributes to radiation-induced senescence through the ROS-mediated p16 pathway in human endothelial cells. *Oncotarget.* (2016) 7:9634–44. doi: 10.18632/oncotarget.7457
154. Chen ZY, Hu YY, Hu XF, Cheng LX. The conditioned medium of human mesenchymal stromal cells reduces irradiation-induced damage in cardiac fibroblast cells. *J Radiat Res.* (2018) 59:555–64. doi: 10.1093/jrr/rry048
155. Gao S, Wu R, Zeng Y. Up-regulation of peroxisome proliferator-activated receptor gamma in radiation-induced heart injury in rats. *Radiat Environ Biophys.* (2012) 51:53–9. doi: 10.1007/s00411-011-0390-9
156. Schlaak RA, Frei A, Schottstaedt AM, Tsaih SW, Fish BL, Harmann L, et al. Mapping genetic modifiers of radiation-induced cardiotoxicity to rat chromosome 3. *Am J Physiol Heart Circ Physiol.* (2019) 316:H1267–80. doi: 10.1152/ajpheart.00482.2018
157. Ricciotti E, Sarantopoulou D, Grant GR, Sanzari JK, Krigsfeld GS, Kiliti AJ, et al. Distinct vascular genomic response of proton and gamma radiation-A pilot investigation. *PLoS ONE.* (2019) 14:e0207503. doi: 10.1371/journal.pone.0207503
158. Shang S, Wang J, Chen S, Tian R, Zeng H, Wang L, et al. Exosomal miRNA-1231 derived from bone marrow mesenchymal stem cells inhibits the activity of pancreatic cancer. *Cancer Med.* (2019) 8:7728–40. doi: 10.1002/cam4.2633
159. Chen B, Xu P, Wang J, Zhang C. The role of MiRNA in polycystic ovary syndrome (PCOS). *Gene.* (2019) 706:91–6. doi: 10.1016/j.gene.2019.04.082
160. Bascunan KA, Perez-Bravo F, Gaudioso G, Vaira V, Roncoroni L, Elli L, et al. A miRNA-based blood and mucosal approach for detecting and monitoring celiac disease. *Dig Dis Sci.* (2019). doi: 10.1007/s10620-019-05966-z
161. Wang H, Wang TT, Lv XP. Expression and prognostic value of miRNA-29b in peripheral blood for endometrial cancer. *Future Oncol.* (2018) 14:1365–76. doi: 10.2217/fon-2017-0594
162. Chen PC, Yu CC, Huang WY, Huang WH, Chuang YM, Lin RI, et al. c-Myc acts as a competing endogenous RNA to sponge miR-34a, in the upregulation of CD44, in urothelial carcinoma. *Cancers.* (2019) 11:E1457. doi: 10.3390/cancers11101457
163. Soyocak A, Kurt H, Ozgen M, Turgut Cosan D, Colak E, Gunes HV. miRNA-146a, miRNA-155 and JNK expression levels in peripheral blood mononuclear cells according to grade of knee osteoarthritis. *Gene.* (2017) 627:207–11. doi: 10.1016/j.gene.2017.06.027
164. Freitas LS, Silveira AC, Martins FC, Costa-Hong V, Lebkuchen A, Cardozo KHM, et al. Severe obstructive sleep apnea is associated with circulating microRNAs related to heart failure, myocardial ischemia, and cancer proliferation. *Sleep Breath.* (2020). doi: 10.1007/s11325-019-02003-1
165. Gao J, Liu J, Zhang Y, Guan B, Qu H, Chai H, et al. PBMCs-Derived microRNA signature as a prethrombotic status discriminator in stable coronary artery disease. *Thromb Haemost.* (2020) 120:121–31. doi: 10.1055/s-0039-1700518
166. Zhu GF, Chu T, Ruan Z, Zhang M, Zhou M, Zhang Q, et al. Inflammation-related microRNAs are associated with plaque stability calculated by IVUS in coronary heart disease patients. *J Interv Cardiol.* (2019) 2019:9723129. doi: 10.1155/2019/9723129
167. Vogel J, Niederer D, Engeroff T, Vogt L, Troidl C, Schmitz-Rixen T, et al. Effects on the profile of circulating miRNAs after single bouts of resistance training with and without blood flow restriction-a three-arm, randomized crossover trial. *Int J Mol Sci.* (2019) 20:E3249. doi: 10.3390/ijms20133249
168. Rizzacasa B, Morini E, Mango R, Vancheri C, Budassi S, Massaro G, et al. MiR-423 is differentially expressed in patients with stable and unstable coronary artery disease: a pilot study. *PLoS ONE.* (2019) 14:e0216363. doi: 10.1371/journal.pone.0216363
169. Cheng M, Yang J, Zhao X, Zhang E, Zeng Q, Yu Y, et al. Circulating myocardial microRNAs from infarcted hearts are carried in exosomes and mobilise bone marrow progenitor cells. *Nat Commun.* (2019) 10:959. doi: 10.1038/s41467-019-08895-7

170. Viczenczova C, Szeiffova Bacova B, Egan Benova T, Kura B, Yin C, Weismann P, et al. Myocardial connexin-43 and PKC signalling are involved in adaptation of the heart to irradiation-induced injury: implication of miR-1 and miR-21. *Gen Physiol Biophys.* (2016) 35:215–22. doi: 10.4149/gpb_2015038
171. Palayoor ST, John-Aryankalayil M, Makinde AY, Falduto MT, Magnuson SR, Coleman CN. Differential expression of stress and immune response pathway transcripts and miRNAs in normal human endothelial cells subjected to fractionated or single-dose radiation. *Mol Cancer Res.* (2014) 12:1002–15. doi: 10.1158/1541-7786.MCR-13-0623
172. Babini G, Tanno B, De Stefano I, Giardullo P, Leonardi S, Pasquali E, et al. Bioinformatic analysis of dose- and time-dependent miRNome responses. *Radiat Prot Dosimetry.* (2019) 183:151–5. doi: 10.1093/rpd/ncy215
173. Wang L, Meng X, Li G, Zhou Q, Xiao J. Circular RNAs in cardiovascular diseases. *Adv Exp Med Biol.* (2018) 1087:191–204. doi: 10.1007/978-981-13-1426-1_15
174. Zhou Q, Zhang Z, Bei Y, Li G, Wang T. Circular RNAs as novel biomarkers for cardiovascular diseases. *Adv Exp Med Biol.* (2018) 1087:159–70. doi: 10.1007/978-981-13-1426-1_13
175. ClinicalTrials.gov. Availabe online: https://clinicaltrials.gov/ct2/home (accessed on January 15, 2020).
176. Van Taunay JS, Albelda MT, Frias JC, Lipinski MJ. Biologics and cardiovascular disease. *J Cardiovasc Pharmacol.* (2018) 72:77–85. doi: 10.1097/FJC.0000000000000595
177. Costa WS, Ribeiro MN, Cardoso LE, Dornas MC, Ramos CF, Gallo CB, et al. Nutritional supplementation with L-arginine prevents pelvic radiation-induced changes in morphology, density, and regulating factors of blood vessels in the wall of rat bladder. *World J Urol.* (2013) 31:653–8. doi: 10.1007/s00345-012-0938-6
178. Parent S, Pituskin E, Paterson DI. The cardio-oncology program: a multidisciplinary approach to the care of cancer patients with cardiovascular disease. *Can J Cardiol.* (2016) 32:847–51. doi: 10.1016/j.cjca.2016.04.014
179. Rose-Felker K, Effinger K, Kelleman MS, Sachdeva R, Meacham LR, Border WL. Improving paediatric cardiologists' awareness about the needs of childhood cancer survivors: results of a single-centre directed educational initiative. *Cardiol Young.* (2019) 29:808–12. doi: 10.1017/S104795111900088X
180. Montazeri K, Unitt C, Foody JM, Harris JR, Partridge AH, Moslehi J. ABCDE steps to prevent heart disease in breast cancer survivors. *Circulation.* (2014) 130:e157–9. doi: 10.1161/CIRCULATIONAHA.114.008820
181. Scott JM, Nilsen TS, Gupta D, Jones LW. Exercise therapy and cardiovascular toxicity in cancer. *Circulation.* (2018) 137:1176–91. doi: 10.1161/CIRCULATIONAHA.117.024671
182. Kosalka P, Johnson C, Turek M, Sulpher J, Law A, Botros J, et al. Effect of obesity, dyslipidemia, and diabetes on trastuzumab-related cardiotoxicity in breast cancer. *Curr Oncol.* (2019) 26:e314–21. doi: 10.3747/co.26.4823
183. Michel L, Rassaf T. Cardio-oncology: need for novel structures. *Eur J Med Res.* (2019) 24:1. doi: 10.1186/s40001-018-0359-0
184. Lancellotti P, Suter TM, Lopez-Fernandez T, Galderisi M, Lyon AR, Van der Meer P, et al. Cardio-Oncology Services: rationale, organization, and implementation. *Eur Heart J.* (2019) 40:1756–63. doi: 10.1093/eurheartj/ehy453
185. Kounis NG, Koniari I, Plotas P, Soufras GD, Tsigkas G, Davlouros P, et al. Emergence, development, and future of cardio-oncology in China: cardiohypersensitivity, cardiotoxicity and the Kounis syndrome. *Chin Med J.* (2019) 132:753–4. doi: 10.1097/CM9.0000000000000130
186. Kostakou PM, Kouris NT, Kostopoulos VS, Damaskos DS, Olympios CD. Cardio-oncology: a new and developing sector of research and therapy in the field of cardiology. *Heart Fail Rev.* (2019) 24:91–100. doi: 10.1007/s10741-018-9731-y
187. Gilchrist SC, Barac A, Ades PA, Alfano CM, Franklin BA, Jones LW, et al. Cardio-oncology rehabilitation to manage cardiovascular outcomes in cancer patients and survivors: a scientific statement from the American Heart Association. *Circulation.* (2019) 139:e997–1012. doi: 10.1161/CIR.0000000000000679
188. Barish R, Lynce F, Unger K, Barac A. Management of cardiovascular disease in women with breast cancer. *Circulation.* (2019) 139:1110–20. doi: 10.1161/CIRCULATIONAHA.118.039371
189. Francolini G, Desideri I, Meattini I, Becherini C, Terziani F, Olmetto E, et al. Assessment of a guideline-based heart substructures delineation in left-sided breast cancer patients undergoing adjuvant radiotherapy: quality assessment within a randomized phase III trial testing a cardioprotective treatment strategy (SAFE-2014). *Strahlenther Onkol.* (2019) 195:43–51. doi: 10.1007/s00066-018-1388-x
190. Lorenzen EL, Taylor CW, Maraldo M, Nielsen MH, Offersen BV, Andersen MR, et al. Inter-observer variation in delineation of the heart and left anterior descending coronary artery in radiotherapy for breast cancer: a multi-centre study from Denmark and the UK. *Radiother Oncol.* (2013) 108:254–8. doi: 10.1016/j.radonc.2013.06.025

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BRIEF RESEARCH REPORT
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Differences in Expression of Mitochondrial Complexes Due to Genetic Variants May Alter Sensitivity to Radiation-Induced Cardiac Dysfunction

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Radiation therapy is received by over half of all cancer patients. However, radiation doses may be constricted due to normal tissue side effects. In thoracic cancers, including breast and lung cancers, cardiac radiation is a major concern in treatment planning. There are currently no biomarkers of radiation-induced cardiotoxicity. Complex genetic modifiers can contribute to the risk of radiation-induced cardiotoxicities, yet these modifiers are largely unknown and poorly understood. We have previously reported the SS (Dahl salt-sensitive/Mcwi) rat strain is a highly sensitized model of radiation-induced cardiotoxicity compared to the more resistant Brown Norway (BN) rat strain. When rat chromosome 3 from the resistant BN rat strain is substituted into the SS background (SS.BN3 consomic), it significantly attenuates radiation-induced cardiotoxicity, demonstrating inherited genetic variants on rat chromosome 3 modify radiation sensitivity. Genes involved with mitochondrial function were differentially expressed in the hearts of SS and SS.BN3 rats 1 week after radiation. Here we further assessed differences in mitochondria-related genes between the sensitive SS and resistant SS.BN3 rats. We found mitochondrial-related gene expression differed in untreated hearts, while no differences in mitochondrial morphology were seen 1 week after localized heart radiation. At 12 weeks after localized cardiac radiation, differences in mitochondrial complex protein expression in the left ventricles were seen between the SS and SS.BN3 rats. These studies suggest that differences in mitochondrial gene expression caused by inherited genetic variants may contribute to differences in sensitivity to cardiac radiation.

Keywords: radiation, radiation-induced heart damage, mitochondria, consomic rats, oxidative phosphorylation, echocardiogram, cardiotoxicity

INTRODUCTION

Radiation therapy (RT) is used in over half of all cancer patients to treat malignancies and improve patient survival (1). RT can be administered to the thoracic region in treating chest tumors including Hodgkin lymphoma and breast and lung cancers. Despite advances in planning and delivering techniques (2–5), these techniques are not universally available and/or utilized by all providers (6), and RT to the thoracic region even with these techniques can still result in some exposure of the heart that can lead to cardiotoxicity (7, 8). Irradiation to the heart and surrounding vasculature may lead to toxicities including pericarditis, ischemic heart disease, myocardial fibrosis, cardiomyopathy, arrhythmias, and/or valvular abnormalities, collectively referred to as radiation-induced heart dysfunction (RIHD) (9–11). These normal tissue side effects may arise months to decades after RT, potentially leading to increased morbidity and mortality (12–14).

Cardiomyocytes are the most abundant cell type in the heart occupying roughly 70–85% total volume, and ∼30% of the heart volume consists of cardiomyocyte mitochondria (15–17). The heart demands very high levels of adenosine triphosphate (ATP) for healthy function (18), and therefore mitochondrial function is crucial in maintaining heart health by coupling respiration with oxidative phosphorylation to generate ATP (7, 19, 20). Mitochondria are known to have roles in metabolism, cell death, and stress responses including combating reactive oxygen species (ROS). In addition to causing direct effects to DNA that may lead to cell death, radiation also causes indirect effects including the production of ROS. The mitochondria function to protect against ROS-induced cellular damage, and therefore, mitochondria play a role in protecting the normal heart tissue against radiation induced toxicity (21).

We previously reported that the inbred Dahl salt-sensitive/Mcwi (SS) rat strain was more sensitive to localized image-guided cardiac radiation than the Brown Norway (BN) strain, and that substitution of chromosome 3 from the BN strain into the SS background (SS.BN3 consomic rats) confers dramatic resistance to radiation-induced cardiac dysfunction when compared to the SS strain (22). Consomic chromosome substitution studies can be used to map complex genetic modifiers of pathophysiologic phenotypes (23–26). In our previous consomic rat study with the SS strain that was relatively sensitive to localized cardiac radiation when compared to the SS.BN3 consomic strain, the top genetic pathways differentially expressed between SS and SS.BN3 consomic rat ventricles 1 week after radiation included mitochondrial-related genes (22). However, expression of mitochondrial genes was not measured in unirradiated SS and SS.BN3 rat hearts, and protein expression of mitochondrial complexes was not examined. There is a need to better understand the mechanisms of mitochondrial dysfunction that may lead to RIHD. Here we examined changes in gene expression of all mitochondria-encoded genes and nuclear-encoded mitochondria oxidative phosphorylation complex genes between the sensitive SS and comparatively resistant SS.BN3 rat hearts that were not treated with radiation (sham treated). We also examined mitochondrial morphology using transmission electron microscopy, as well as the protein levels of mitochondrial oxidative phosphorylation complexes in isolated mitochondria from the left ventricles of SS and SS.BN3 rats after localized cardiac radiation. These results suggest that genetic changes can lead to altered expression of mitochondrial oxidative phosphorylation complexes that may contribute to differences in responses to localized cardiac irradiation. Better understanding of the role of mitochondrial dysfunction in RIHD may lead to targeted therapeutics to protect and/or mitigate RIHD while maintaining therapeutic effects of radiation therapy.

MATERIALS AND METHODS

Rats and Irradiation Procedure

The rat cardiac irradiation procedure has been reported elsewhere (22). In brief, female SS and SS.BN3 rats [Medical College of Wisconsin (23)] aged 10–12 weeks were randomized into different treatment groups. Animals were anesthetized with 3% isoflurane and given localized heart irradiation using a the high-precision image-guided X-RAD SmART irradiator (Precision X-Ray, North Branford, CT). A 24 Gy × 1 fraction was given to the isocenter of the heart, with equally weighted anterior-posterior and 2 lateral beams (1:1:1, 225 kVp, 13 mA, 0.32 mm Cu, 2.69 Gy/min) using a 1.5 cm collimator. Pilot V1.8 Imaging Software (University Health Network, Toronto, Canada) was used to create two-dimensional projections over 360° to provide CT scans in sagittal, coronal, and axial views, with each projection on the heart centered to fit into the collimator. Monte Carlo-based treatment planning was utilized to calculate radiation dose (MAASTRO Radiotherapy Clinic, Netherlands). Age-matched sham-irradiated animals were included in the study. Animals were irradiated and housed in pathogen-free conditions with a 12:12 light:dark cycle and access to a standard diet (0.4% salt) and water. All procedures were performed according to the American Guidelines for the Ethical Care of Animals and approved by our Institutional Animal Care and Use Committee.

Echocardiography

The echocardiogram procedure for rats has been reported elsewhere (22). In brief, echocardiography with M-mode was used to assess cardiac function on irradiated and sham treated rats at baseline, 3- and 5- months post-RT. An echocardiograph Vivid 7 with an 11-MHz M12L linear-array transducer and EchoPac software (General Electric, Wauwatosa, WI) was used to perform the examinations. Imaging was conducted in the short-axis view at mid-level of the left ventricle, by a sonographer with three consecutive heartbeats measured where the average was utilized for analyses (27, 28). For strain analysis, images were processed with EchoPac Q analysis software (General Electric, Wauwatosa, WI). A cardiac cycle was defined from peak one R wave to the peak of the following wave. The endocardial border was traced during an end-systolic frame in the short-axis view at mid-ventricle to calculate radial and circumferential strain. The computer produced a profile of radial (myocardial deformation toward the center) and circumferential (myocardial deformation along the curvature) strain percentage over time.

RNA-Sequencing

The RNA-sequencing protocol was previously reported (22, 23). Briefly, total RNA was extracted by TRIzol (Thermo Fisher Scientific, Waltham, MA) from the left ventricle tissue of 11–13 weeks old female mock-treated SS and SS.BN3 rats (N = 4–5/group) from a group of rats matched to 1 week post-radiation rats (not reported here, but previously reported). For RNA-seq, a library preparation was made for each sample, indexed for multiplexing, and sequenced using an Illumina HiSeq2500 (Illumina, San Diego, CA). The Trim Galore program (v0.4.1) was used to trim bases with a Phred quality score <20 [https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/]. The RSEM program function "rsem-prepare-reference" (v1.3.0) was used to extract the transcript sequences from the Rat genome (Rnor6.0, Ensembl release 98) (30) and to generate Bowtie2 indices (Bowtie2 v2.2.8) (31), followed by read alignment and expression quantification using the "rsem-calculate-expression" function. Differential expression (DE) analysis was performed using the Bioconductor package DESeq2 version 1.12.4 (29) to compute log2 fold changes and FDR-adjusted p-values. Statistical significance was determined at an FDR threshold of 0.05. Data were analyzed for molecular and functional pathway enrichment using the IPA tool (Qiagen). All raw sequencing data can be accessed from the Sequence Read Archive, BioProject ID PRJNA525087 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA525087).

Transmission Electron Microscopy (TEM)

Rat left ventricle was harvested 1 week after 1 × 24 Gy cardiac RT or sham from adult female SS and SS.BN3 rats (N = 2–5/group) and fixed in 2.5% glutaraldehyde in 100 mM sodium cacodylate buffer pH 7.2. The samples were then post-fixed in 1% OsO_4 on ice for 1 h, followed by dehydration in a graded methanol series, and an embedding in EPON 812 (EMS, Hatfield, PA). Ultra-thin sections (60 nm) were cut, stained with uranyl acetate and Reynolds lead citrate, and examined with a Hitachi H600 Transmission Electron Microscope (TEM) (Hitachi High Technologies America Inc., Pleasanton, CA). Representative images to assess cardiac mitochondrial morphology were captured at 20,000X magnification.

Mitochondrial Isolation and Western Blot Analyses

Rat hearts were harvested 12 weeks after either 1 × 24 Gy localized cardiac radiation or sham treatment (22). Heart mitochondria isolation has previously been reported (32, 33). In brief, fresh heart tissue was minced in ice cold isolation buffer [200 mM mannitol, 50 mM sucrose, 5 mM KH_2PO_4, 5 mM 3-(N-morpholino) propanesulfonic acid, and 1 mM EGTA, with 0.1% bovine serum albumin, pH 7.15]. The minced tissue was homogenized in the presence of 5 U/ml protease (P5459, Sigma Life Science, St. Louis, MO) followed by differential centrifugation at 4°C. The final pellet was resuspended in isolation buffer and protein concentration was determined by the Bradford method. For Western blot analysis, isolated mitochondria were lysed using a RIPA buffer containing protease and phosphatase inhibitors, centrifugated, and the supernatant was collected. Total protein was assessed using a BCA Protein Assay Kit (23225, Thermo Scientific, Rockford, IL). Mitochondrial protein lysates were loaded and separated using SDS-PAGE and then transferred onto a PVDF membrane. The following antibodies were used in the present study using mitochondrial lysates: total OXPHOS rodent WB antibody cocktail (1:2500; ab110413; Abcam) and anti-COX IV antibody Mitochondrial Loading Control (1:5000; ab16056; Abcam).

Statistical Analysis

Analyses of the western blotting were evaluated by a Student's t-test. Blots were imaged on ImageQuant LAS 4000 (GE Healthcare Life Sciences, Marlborough, MA), and analyzed using ImageQuant TL software (version 8.1.0.0). All western blotting results reported are representative of 3 technical replicates. The criterion for significance was $P < 0.05$. Data are reported as means ± SE. For our RNA-sequencing studies (22), power analysis was determined using a combination of simulated and experimental data approach previously described (34). We performed 100 simulations based on a RNAseq count data from our previous study (35). This analysis suggested that 4 replicates per group in a 2-group comparison would provide more than 90% power to detect genes differentially expressed at FDR 0.05 level. All power calculations and animal numbers for our studies were also performed by a non-biased statistician (S.-W.T.).

RESULTS AND DISCUSSION

Previously, we have demonstrated that the SS rat strain is more sensitive to localized image-guided cardiac radiation that the SS.BN3 consomic rat strain, which differs only in substitution of chromosome 3 from the BN strain, as measured by pleural effusions, echocardiogram indices of left-sided heart failure and strain, as well as mortality. We also demonstrated that the SS and SS.BN3 strains had differentially expressed mitochondria-related genes in the left ventricle 1 week after radiation, as measured with RNA-sequencing (22). In this study, we examined the differential expression of oxidative phosphorylation genes from both the mitochondrial and nuclear genomes between SS and SS.BN3 left ventricles in rats 1 week after sham radiation treatment, at 11–13 weeks of age (N = 4/condition), which is the same time period reported previously after radiation (22). Of the mitochondrial-encoded genes coding for oxidative phosphorylation complexes, 13 of 13 genes are differentially expressed between SS and SS.BN3 rats at FDR < 0.05 (**Figure 1A**, **Supplemental Table 1B**). Expression of these genes was significantly higher in the protected SS.BN3 rats in comparison to the more sensitive SS rats. In addition, of the 80 nuclear encoded rat genes involved in encoding mitochondrial complexes I–V, 74 of 80 genes were differentially expressed between SS and SS.BN3 rats at FDR < 0.05 (**Figure 1B**, **Supplemental Table 1A**). Interestingly, these genes had higher expression in SS rats compared to the SS.BN3 rats. We subsequently examined whether there were changes in mitochondrial morphology between the SS and SS.BN3 rat left ventricles. TEM was performed on mitochondria isolated from rat hearts at 1 week post-radiation or sham treatment. Longitudinal views of tissue were examined for each condition,

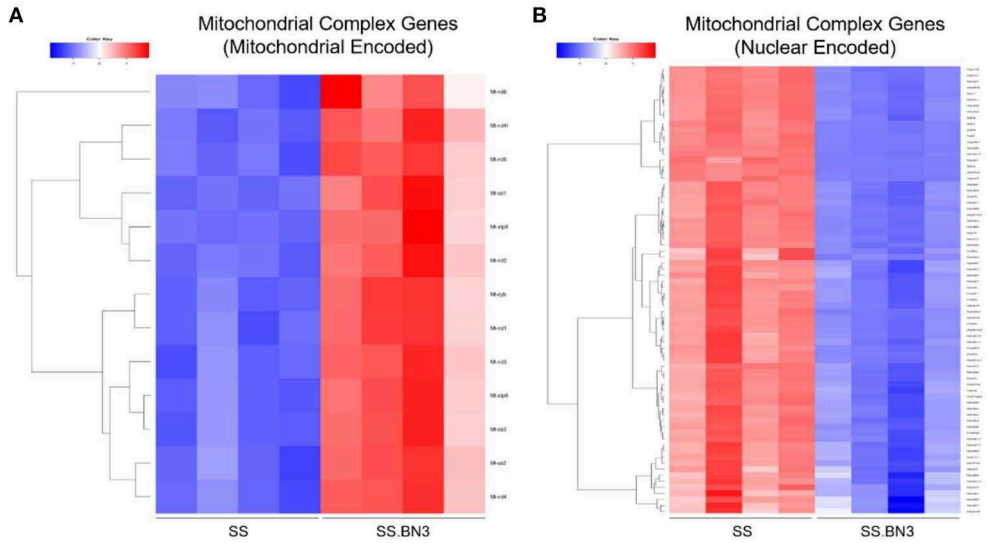

FIGURE 1 | RNA-seq analysis of control SS and SS.BN3 hearts. Total RNA was extracted and RNA-seq was performed on RNA from the left ventricle tissue of adult 10–12 week old female SS and SS.BN3 rats harvested 1 week after mock treatment ($N = 4$/group). Differential expression analysis was performed, followed by generation of heat maps of **(A)** 13 mitochondrial encoded genes and **(B)** 74 nuclear encoded genes differentially expressed at FDR < 0.05 and involved in the mitochondrial complexes that drive oxidative phosphorylation.

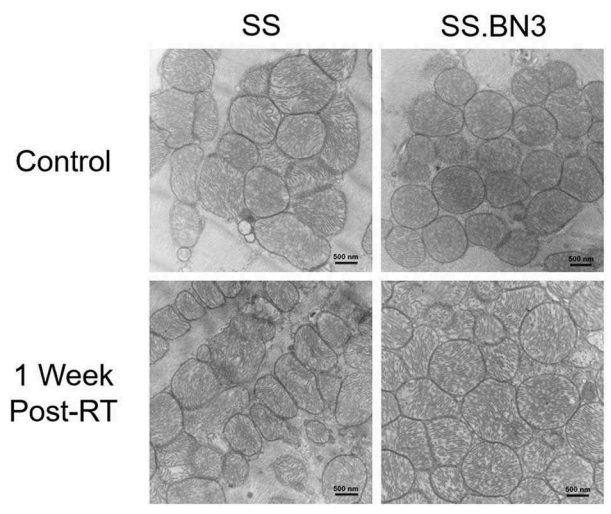

FIGURE 2 | Representative TEM images revealed SS and SS.BN3 have no observed changes in mitochondrial morphology at 1 week post-radiation therapy (RT). Transmission electron microscopy (TEM) was performed on SS and SS.BN3 rat left ventricle tissue harvested 1 week after either 24 Gy RT or mock treatment ($N = 2$–5/group). No gross changes in mitochondria were seen between groups. Representative images from each condition are shown. Scale bar = 500 nm.

where total mitochondria and irregular shaped mitochondria were counted. This revealed no morphological differences between SS and SS.BN3, with representative images shown in **Figure 2**.

Our data in **Figure 1**, along with previously published data (22), demonstrate that changes in gene expression of oxidative phosphorylation complex genes are differentially expressed in the left ventricles of both the non-irradiated rats and rats irradiated with a single dose of 24 Gy to the whole heart. However, the functional consequences of these changes at later time points had not been examined. We isolated mitochondria from SS and SS.BN3 rats (N of 3–4 per group) 12 weeks post-treatment with 24 Gy of localized heart radiation or sham (no radiation). We then performed Western blotting on the isolated mitochondria to examine protein expression of mitochondrial complexes I–V. This revealed no significantly significant changes between complex I–V in the unirradiated SS vs. SS.BN3 heart, but significant increases were seen in complexes I, III, and V in the SS.BN3 vs. SS hearts (**Figures 3A–F**, **Supplemental Figure 1**). **Figure 3** shows representative results from 3 technical replicates of each Western blot. Protein expression levels were assess using NADH: Ubiquinone Oxidoreductase Subunit B8 (NDUFB8; complex I) (**Figure 3A**), Succinate dehydrogenase ubiquinone iron-sulfur subunit (SDHB; complex II) (**Figure 3B**), Ubiquinol Cytochrome C Reductase Core Protein 2 (UQCRC2; complex III) (**Figure 3C**), Mitochondrial Cytochrome C Oxidase I (MT-CO1; complex IV) (**Figure 3D**), and Mitochondrial ATP Synthase 5A (ATP5A; complex V) (**Figure 3E**). Representative Western blots of the mitochondrial lysates are shown in **Figure 3F**, $N = 3$–4/group. complexes I, III, and V showed increased expressions in the SS.BN3 vs. SS with RT lysates (complex I: $P = 0.004$, 2/3 blots significant; complex III: $P = 0.02$, all 3 blots were significant; CV: $P = 0.004$, all 3 blots were significant). There was also a trend in complex IV with increased expression in SS.BN3 vs. SS with RT (1/3 blots significant). Although the OXPHOS antibody cocktail consists of a mixture of five antibodies to detect the five different complex subunits, different subunits were quantified at different exposure times to be in the linear

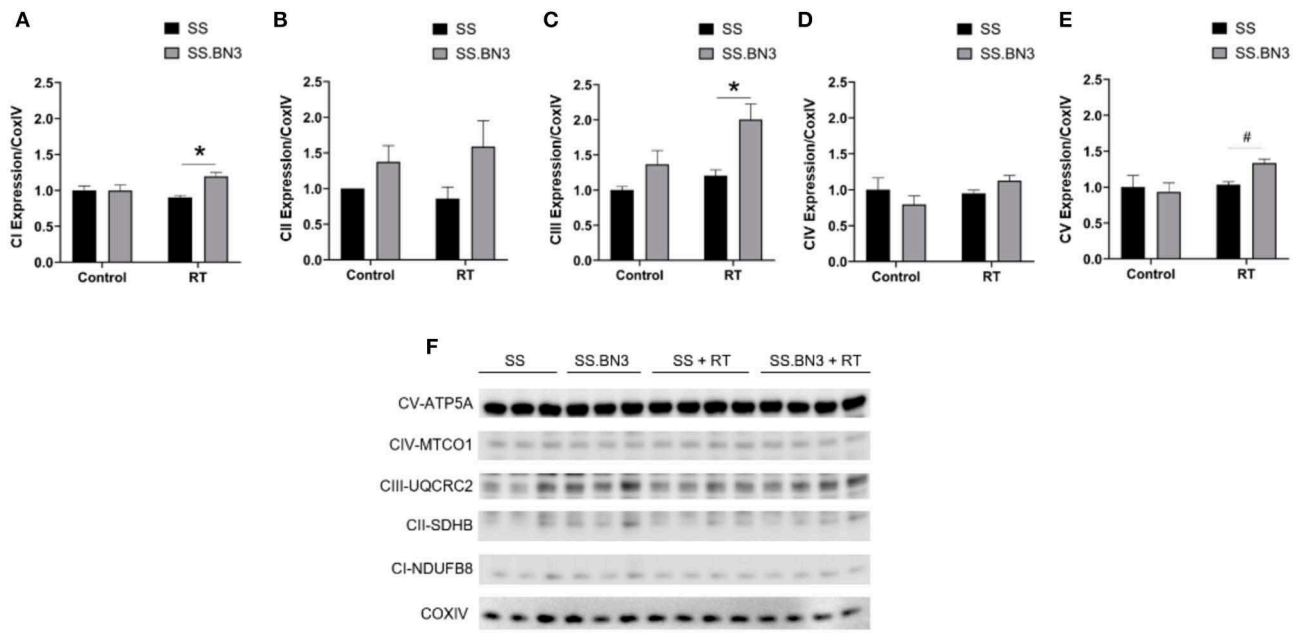

FIGURE 3 | Oxidative phosphorylation complex expression in rat cardiac mitochondria. Subunits of the mitochondrial complex expression was visualized via Western blotting and quantified. These included **(A)** Nuclear-coded NDUFB8 Complex I-subunit, **(B)** Nuclear-coded SDHB Complex II-subunit, **(C)** Nuclear-coded UQCR2 Complex III-subunit, **(D)** Mitochondrial-coded MTCO1 Complex IV-subunit, and **(E)** Nuclear-coded ATP5A Complex V-subunit. All were measured and quantified from a Western blot from mitochondria lysates of rat hearts 12 weeks post-RT or sham treatment **(F)**. Representative blots are originated from different exposure times of the same blot using an antibody cocktail, and technical replicates of the Western blot were run 3 times total, with a representative blot and quantifications from one experiment shown. Values are expressed as means ± SEM normalized to their respective COX IV loading control, and then expressed as fold change relative to the SS sham treated; $n = 3$–4/ group; $^{*}P < 0.05$, $^{\#}P < 0.01$. A Student's *t*-test was used to determine significance in SS vs. SS.BN3 control (lanes 1–3 and 4–6, respectively) and SS vs. SS.BN3 with RT (lanes 7–10 and 11–14, respectively).

detection range, as shown in **Figure 3F**. The full blots at different exposure times are shown in **Supplemental Figure 1**. These results indicate that genetic changes in rat chromosome 3 can lead to significant changes in mitochondrial complex expression several weeks after high-dose cardiac radiation exposure, at a time when echocardiogram changes are seen demonstrating differences in left ventricular heart function between the SS and SS.BN3 rats (22). M-mode echocardiogram imaging, performed previously (22) demonstrated cardiac dysfunction in SS rats compared to SS.BN3 rats displaying hyperdynamic systolic function (**Figure 4A**). Analysis of both radial and circumferential strain at 3 and 5 months post-RT revealed the SS rat hearts had significantly decreased myocardium deformation, consistent with decreased systolic dysfunction (**Figures 4B,C**).

A number of studies have implicated mitochondrial changes in the development of cardiac dysfunction following radiation, both in pre-clinical models and in human studies (7, 36–39). In C57BL/6N mice that received sham, 0.2 Gy, or 2 Gy of heart radiation, functional and proteomic alterations were seen 4 weeks following irradiation. This included changes in proteins related to oxidative phosphorylation (36, 40–42). Functionally, partial deactivation of complexes I and III were observed in mice receiving 2 Gy of cardiac radiation. In a separate publication, this group also examined the long-term effects of cardiac radiation, finding that respiratory capacity was still reduced 40 weeks after 2 Gy of cardiac radiation (38). In separate studies, C57BL/6 mice treated with 8 or 16 Gy of cardiac radiation demonstrated increased free fatty acids and reduced levels of complexes I, III, and V (39). Studies of mitochondrial-related proteins in the left ventricles of decreased nuclear workers exposed to varying levels of radiation (external exposure ranges from 100 mcGy to >5 Gy) revealed dose-dependent reductions in complexes I, III, and V, and changes in complexes II and IV in those with the highest radiation exposures (42).

There are limitations from this study that should be acknowledged. We examined the effects of RIHD with the treatment dose of 1 × 24 Gy cardiac RT. We have previously reported similar cardiac trends by using a fractionated regimen of 9 Gy × 5 (22). The dosing regimen was determined based on previous studies of studying RIHD from cardiac RT in rats (7, 43–47). To better mimic cancer patient thoracic RT, future studies are needed with both partial heart irradiation and increased fractions of smaller daily radiation dose to more closely resemble the radiation exposure observed. In addition to cancer patients receiving thoracic RT, recent studies report the using 25 Gy cardiac RT in a single fraction to treat ventricular tachycardia (48, 49). Our rat model of cardiac RT is very relevant to this clinical model of treatment and could be further used to study side effects and biologic changes that occur from this high dose cardiac RT. Additional considerations include how

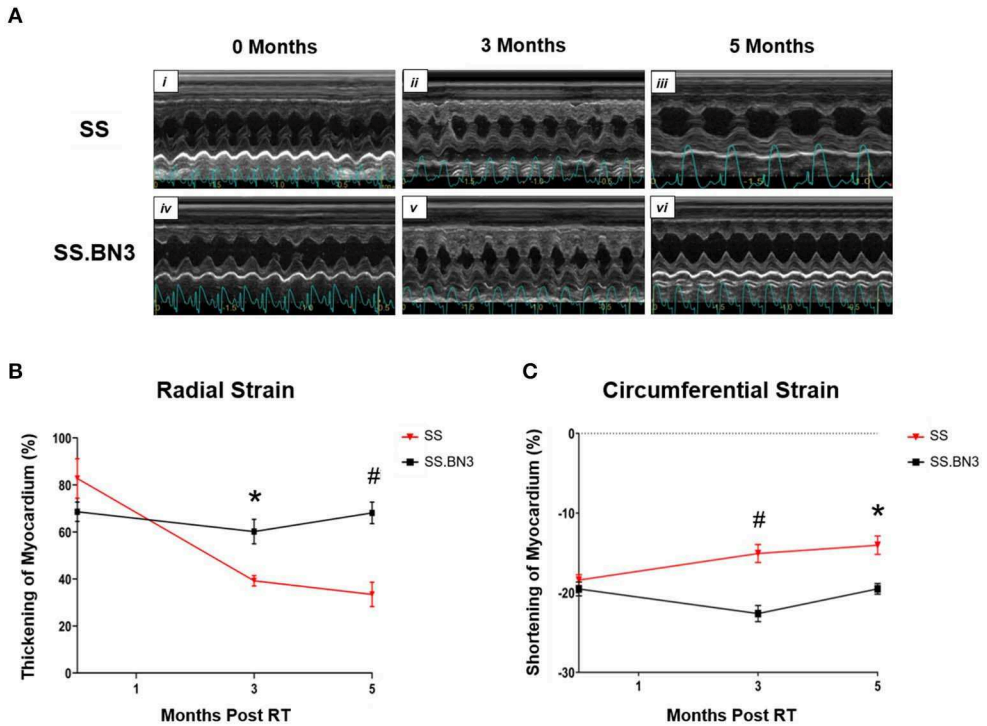

FIGURE 4 | Echocardiograms indicated SS rats have decreased heart function compared with SS.BN3 rats after 24 Gy localized heart RT. **(A)** M-mode echocardiogram images of SS and SS.BN3 rats that received 24 Gy RT at baseline, 3 months, and 5 months post -RT. **(B)** Radial strain was lower in the SS rats at 3 and 5 months post-RT shown via decreased thickening of myocardium. **(C)** Circumferential strain also showed decreased function in SS vs. SS.BN3 at 3 and 5 months post-RT via decreased ability to contract, indicated by a smaller negative percentage. Values are means ± SEM. $^{*}P < 0.01$, $^{\#}P < 0.001$.

these findings can be translated into future applications. Other than the 13 mitochondrial encoded genes, many genes involved in mitochondrial dysfunction, sirtuin signaling and cardiac hypertrophy were also found to be differentially expressed between SS and SS.BN3 rats (22). Candidates involved in these pathways as well as mitochondrial gene transcription, translation, and regulation could be further tested to investigate their roles in radiation-induced cardiotoxicity. The use of pharmacologic modulators of these pathways and transgenic models could also be pursued to further elucidate mechanisms of RIHD to prevent and/or mitigate effects observed in patients receiving radiation therapy.

In this current study, we demonstrate changes in the levels of oxidative phosphorylation complexes between genetically similar rats, differing only in the single nucleotide polymorphisms on chromosome 3, that demonstrate dramatic differences in the development of radiation-induced cardiotoxicity after localized radiation exposure to the heart (22). These results demonstrate that there are differences in gene expression of both mitochondrial-encoded and nuclear-encoded genes for the oxidative phosphorylation complexes in the left ventricles of unirradiated SS and SS.BN3 rats (**Figure 1**), as well as the left ventricles of SS and SS.BN3 rats 1 week after 24 Gy of localized cardiac irradiation (22). It is unclear why there are differences in the direction of differential expression of oxidative phosphorylation complex genes encoded by mitochondrial vs. nuclear genomes. In general, the mitochondrial genome is more likely to experience DNA damage than nuclear DNA following radiation due to the lack of protective effect from histones (50), as well as less efficient DNA repair (51, 52). However, as our results here demonstrate, differences in mitochondrial-encoded genes are seen between SS and SS.BN3 left ventricles even without radiation treatment (**Figure 1**). Although large numbers of mitochondrial genes are differentially expressed in SS vs. SS.BN3 rats in unirradiated and irradiated left ventricles, no gross changes in mitochondrial morphology were seen in the left ventricles 1 week after radiation or sham treatments (**Figure 2**). However, at a later timepoint of 12 weeks following 24 Gy of localized cardiac radiation, differences in expression of complex I, III, and V proteins were seen in isolated mitochondrial in the SS vs. SS.BN3 samples. Taken together, these results indicate that inherited genetic variants can lead to differences in oxidative phosphorylation gene expression that may contribute to differences in radiation-induced cardiac dysfunction.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in https://www.ncbi.nlm.nih.gov/sra, NCBI Accession No. PRJNA525087.

ETHICS STATEMENT

The animal study was reviewed and approved by Medical College of Wisconsin Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

RS, MF, AC, JM, and CB conceived and designed the research. RS, AF, GS, and CW performed the experiments. RS, AF, CW, S-WT, and CB analyzed data and interpreted results. RS, AF, and S-WT prepared figures. RS and CB drafted the manuscript. RS, GS, JM, AC, and CB edited and revised manuscript. All authors approved the final version of manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.00023/full#supplementary-material

Supplemental Figure 1 | Full immunoblots against OXPHOS antibody cocktail in heart mitochondrial lysates from SS and SS.BN3 rats with either 24 Gy RT or sham treatment at different exposure times of **(A)** 4 s for C-V, **(B)** 8 s for C-III, **(C)** 30 s for C-IV, and **(D)** 5 min for C-I and C-II.

Supplemental Table 1A | Expression of Nuclear-Encoded Mitochondrial Complex Genes in SS.BN3 vs. SS Left Ventricles 1 Week After 24 Gy of Localized Heart Radiation.

Supplemental Table 1B | Expression of Mitochondrial-Encoded Genes in SS.BN3 vs. SS Left Ventricles 1 Week After 24 Gy of Localized Heart Radiation and Differentially Expressed Mitochondrial Genes in SS versus SS-BN3 Left Ventricles Mitochondrial-Encoded Genes.

REFERENCES

1. Cutter DJ, Darby SC, Yusuf SW. Risks of heart disease after radiotherapy. *Texas Heart Inst J.* (2011) 38:257–8.
2. Bergom C, Currey A, Desai N, Tai A, Strauss JB. Deep inspiration breath hold: techniques and advantages for cardiac sparing during breast cancer irradiation. *Front Oncol.* (2018) 8:87. doi: 10.3389/fonc.2018.00087
3. Bergom C, Prior P, Kainz K, Morrow NV, Ahunbay EE, Walker A, et al. A phase I/II study piloting accelerated partial breast irradiation using CT-guided intensity modulated radiation Therapy in the Prone Position. *Radiother Oncol.* (2013) 108:215–9. doi: 10.1016/j.radonc.2013.05.039
4. Ehrbar S, Perrin R, Peroni M, Bernatowicz K, Parkel T, Pytko I, et al. Respiratory motion-management in stereotactic body radiation therapy for lung cancer - a dosimetric comparison in an anthropomorphic lung phantom (LuCa). *Radiother Oncol.* (2016) 121:328–34. doi: 10.1016/j.radonc.2016.10.011
5. Modiri A, Sabouri P, Gu X, Timmerman R, Sawant A. Inversed-planned respiratory phase gating in lung conformal radiation therapy. *Int J Radiation Oncol Biol Phys.* (2017) 99:317–24. doi: 10.1016/j.ijrobp.2017.05.039
6. Desai N, Currey A, Kelly T, Bergom C. Nationwide trends in heart-sparing techniques utilized in radiation therapy for breast cancer. *Adv Radiation Oncol.* (2019) 4:246–52. doi: 10.1016/j.adro.2019.01.001
7. Sridharan V, Aykin-Burns N, Tripathi P, Krager KJ, Sharma SK, Moros EG, et al. Radiation-induced alterations in mitochondria of the rat heart. *Radiation Res.* (2014) 181:324–34. doi: 10.1667/RR13452.1
8. Stewart FA. Mechanisms and dose-response relationships for radiation-induced cardiovascular disease. *Ann ICRP.* (2012) 41:72–9. doi: 10.1016/j.icrp.2012.06.031
9. Boerma M. Experimental radiation-induced heart disease: past, present, and future. *Radiation Res.* (2012) 178:1–6. doi: 10.1667/RR2933.1
10. Darby SC, Ewertz M, McGale P, Bennet AM, Blom-Goldman U, Brønnum D, et al. Risk of ischemic heart disease in women after radiotherapy for breast cancer. *N Engl J Med.* (2013) 368:987–98. doi: 10.1056/NEJMoa1209825
11. Donnellan E, Phelan D, McCarthy CP, Collier P, Desai M, Griffin B. Radiation-induced heart disease: a practical guide to diagnosis and management. *Cleveland Clin J Med.* (2016) 83:914–22. doi: 10.3949/ccjm.83a.15104
12. Gagliardi G, Lax I, Rutqvist LE. Partial irradiation of the heart. *Semin Radiation Oncol.* (2001) 11:224–33. doi: 10.1053/srao.2001.23483
13. Lin R, Tripuraneni P. Radiation therapy in early-stage invasive breast cancer. *Indian J Surg Oncol.* (2011) 2:101–11. doi: 10.1007/s13193-011-0048-8
14. Clarke M, Collins R, Darby S, Davies C, Elphinstone P, Evans V, et al. Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. *Lancet.* (2005) 366:2087–106. doi: 10.1016/S0140-6736(05)67887-7
15. Zhou P, Pu WT. Recounting cardiac cellular composition. *Circul Res.* (2016) 118:368–70. doi: 10.1161/CIRCRESAHA.116.308139
16. Dämmrich J, Pfeifer U. Cardiac hypertrophy in rats after supravalvular aortic constriction. I size and number of cardiomyocytes, endothelial and interstitial cells. *Virchows Arch B Cell Pathol Incl Mol Pathol.* (1983) 43:265–86. doi: 10.1007/BF02932961
17. Tang Y, Nyengaard JR, Andersen JB, Baandrup U, Gundersen HJ. The application of stereological methods for estimating structural parameters in the human heart. *Anatomical Rec.* (2009) 292:1630–47. doi: 10.1002/ar.20952
18. Finck BN, Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) regulatory cascade in cardiac physiology and disease. *Circulation.* (2007) 115:2540–8. doi: 10.1161/CIRCULATIONAHA.107.670588
19. Fernie AR, Carrari F, Sweetlove LJ. Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. *Curr Opin Plant Biol.* (2004) 7:254–61. doi: 10.1016/j.pbi.2004.03.007
20. Reichert AS, Neupert W. Mitochondriomics or what makes us breathe. *Trends Genet.* (2004) 20:555–62. doi: 10.1016/j.tig.2004.08.012
21. Kam WW, Banati RB. Effects of ionizing radiation on mitochondria. *Free Radical Biol Med.* (2013) 65:607–19. doi: 10.1016/j.freeradbiomed.2013.07.024
22. Schlaak RA, Frei A, Schottstaedt AM, Tsaih SW, Fish BL, Harmann L, et al. Mapping genetic modifiers of radiation-induced cardiotoxicity to rat chromosome 3. *Am J Physiol Heart Circul Physiol.* (2019) 316:H1267–80. doi: 10.1152/ajpheart.00482.2018
23. Flister MJ, Tsaih SW, Stoddard A, Plasterer C, Jagtap J, Parchur AK, et al. Host genetic modifiers of nonproductive angiogenesis inhibit breast cancer. *Breast Cancer Res Treat.* (2017) 165:53–64. doi: 10.1007/s10549-017-4311-8
24. Cowley AW, Roman RJ, Kaldunski ML, Dumas P, Dickhout JG, Greene AS, et al. Brown norway chromosome 13 confers protection from high salt to consomic dahl S rat. *Hypertension.* (2001) 37:456–61. doi: 10.1161/01.HYP.37.2.456
25. Mattson DL, Dwinell MR, Greene AS, Kwitek AE, Roman RJ, Jacob HJ, et al. Chromosome substitution reveals the genetic basis of dahl salt-sensitive hypertension and renal disease. *Am J Physiol Renal Physiol.* (2008) 295:F837–42. doi: 10.1152/ajprenal.90341.2008
26. Jagtap J, Sharma G, Parchur AK, Gogineni V, Bergom C, White S, et al. Methods for detecting host genetic modifiers of tumor vascular function using

dynamic near-infrared fluorescence imaging. *Biomed Optics Express.* (2018) 9:543–56. doi: 10.1364/BOE.9.000543
27. Migrino RQ, Zhu X, Pajewski N, Brahmbhatt T, Hoffmann R, Zhao M. Assessment of segmental myocardial viability using regional 2-dimensional strain echocardiography. *J Am Soc Echocardiogr.* (2007) 20:342–51. doi: 10.1016/j.echo.2006.09.011
28. Schattke S, Xing Y, Lock J, Brechtel L, Schroeckh S, Spethmann S, et al. Increased longitudinal contractility and diastolic function at rest in well-trained amateur marathon runners: a speckle tracking echocardiography study. *Cardiovasc Ultrasound.* (2014) 12:11. doi: 10.1186/1476-7120-12-11
29. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. *Genome Biol.* (2014) 15:550. doi: 10.1186/s13059-014-0550-8
30. Li B, Dewey CN. RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics.* (2011) 12:323. doi: 10.1186/1471-2105-12-323
31. Langmead B, Salzberg SL. Fast gapped-read alignment with bowtie 2. *Nat Methods.* (2012) 9:357–9. doi: 10.1038/nmeth.1923
32. Ait-Aissa K, Blaszak SC, Beutner G, Tsaih SW, Morgan G, Santos JH, et al. Mitochondrial oxidative phosphorylation defect in the heart of subjects with coronary artery disease. *Sci Rep.* (2019) 9:7623. doi: 10.1038/s41598-019-43761-y
33. Mishra J, Davani AJ, Natarajan GK, Kwok WM, Stowe DF, Camara AKS, et al. Cyclosporin A increases mitochondrial buffering of calcium: an additional mechanism in delaying mitochondrial permeability transition pore opening. *Cells.* (2019) 8:E1052. doi: 10.3390/cells8091052
34. Ching T, Huang S, Garmire LX. Power analysis and sample size estimation for *RNA*-Seq differential expression. *RNA.* (2014) 20:1684–96. doi: 10.1261/rna.046011.114
35. Flister MJ, Endres BT, Rudemiller N, Sarkis AB, Santarriaga S, Roy I, et al. CXM: a new tool for mapping breast cancer risk in the tumor microenvironment. *Cancer Res.* (2014) 74:6419–29. doi: 10.1158/0008-5472.CAN-13-3212
36. Barjaktarovic Z, Schmaltz D, Shyla A, Azimzadeh O, Schulz S, Haagen J, et al. Radiation-induced signaling results in mitochondrial impairment in mouse heart at 4 weeks after exposure to X-rays. *PLoS ONE.* (2011) 6:e27811. doi: 10.1371/journal.pone.0027811
37. Khan MY. Radiation-induced cardiomyopathy. I an electron microscopic study of cardiac muscle cells. *Am J Pathol.* (1973) 73:131–46.
38. Barjaktarovic Z, Shyla A, Azimzadeh O, Schulz S, Haagen J, Dörr W, et al. Ionising radiation induces persistent alterations in the cardiac mitochondrial function of C57BL/6 mice 40 weeks after local heart exposure. *Radiother Oncol.* (2013) 106:404–10. doi: 10.1016/j.radonc.2013.01.017
39. Azimzadeh O, Sievert W, Sarioglu H, Yentrapalli R, Barjaktarovic Z, Sriharshan A, et al. PPAR alpha: a novel radiation target in locally exposed mus musculus heart revealed by quantitative proteomics. *J Proteome Res.* (2013) 12:2700–14. doi: 10.1021/pr400071g
40. Burch GE, Sohal RS, Sun SC, Miller GC, Colcolough HL. Effects of radiation on the human heart. An electron microscopic study. *Arch Int Med.* (1968) 121:230–4. doi: 10.1001/archinte.1968.03640030022003
41. Baselet B, Rombouts C, Benotmane AM, Baatout S, Aerts A. Cardiovascular diseases related to ionizing radiation: the risk of low-dose exposure (review). *Int J Mol Med.* (2016) 38:1623–41. doi: 10.3892/ijmm.2016.2777
42. Azimzadeh O, Azizova T, Merl-Pham J, Subramanian V, Bakshi MV, Moseeva M, et al. A dose-dependent perturbation in cardiac energy metabolism is linked to radiation-induced ischemic heart disease in mayak nuclear workers. *Oncotarget.* (2016) 8:9067–78. doi: 10.18632/oncotarget.10424
43. Sridharan V, Tripathi P, Sharma S, Corry PM, Moros EG, Singh A, et al. Effects of late administration of pentoxifylline and tocotrienols in an image-guided rat model of localized heart irradiation. *PLoS ONE.* (2013) 8:e68762. doi: 10.1371/journal.pone.0068762
44. Sridharan V, Thomas CJ, Cao M, Melnyk SB, Pavliv O, Joseph J, et al. Effects of local irradiation combined with sunitinib on early remodeling, mitochondria, and oxidative stress in the rat heart. *Radiother Oncol.* (2016) 119:259–64. doi: 10.1016/j.radonc.2016.03.027
45. Boerma M, Roberto KA, Hauer-Jensen M. Prevention and treatment of functional and structural radiation injury in the rat heart by pentoxifylline and alpha-tocopherol. *Int J Radiation Oncol Biol Phys.* (2008) 72:170–7. doi: 10.1016/j.ijrobp.2008.04.042
46. Sridharan V, Sharma SK, Moros EG, Corry PM, Tripathi P, Lieblong BJ, et al. Effects of radiation on the epidermal growth factor receptor pathway in the heart. *Int J Radiation Biol.* (2013) 89:539–47. doi: 10.3109/09553002.2013.782110
47. Sridharan V, Seawright JW, Antonawich FJ, Garnett M, Cao M, Singh P, et al. Late administration of a palladium lipoic acid complex (POLY-MVA) modifies cardiac mitochondria but not functional or structural manifestations of radiation-induced heart disease in a rat model. *Radiation Res.* (2017) 187:361–6. doi: 10.1667/RR14643.1
48. Cuculich PS, Schill MR, Kashani R, Mutic S, Lang A, Cooper D, et al. Noninvasive cardiac radiation for ablation of ventricular tachycardia. *N Engl J Med.* (2017) 377:2325–36. doi: 10.1056/NEJMoa1613773
49. Robinson CG, Samson PP, Moore KMS, Hugo GD, Knutson N, Mutic S, et al. Phase I/II trial of electrophysiology-guided noninvasive cardiac radioablation for ventricular tachycardia. *Circulation.* (2019) 139:313–21. doi: 10.1161/CIRCULATIONAHA.118.038261
50. Ljungman M, Hanawalt PC. Efficient protection against oxidative DNA damage in chromatin. *Mol Carcinogenesis.* (1992) 5:264–9. doi: 10.1002/mc.2940050406
51. Prithivirajsingh S, Story MD, Bergh SA, Geara FB, Ang KK, Ismail SM, et al. Accumulation of the common mitochondrial DNA deletion induced by ionizing radiation. *FEBS Lett.* (2004) 571:227–32. doi: 10.1016/j.febslet.2004.06.078
52. Richard SM, Bailliet G, Páez GL, Bianchi MS, Peltomäki P, Bianchi NO. Nuclear and mitochondrial genome instability in human breast cancer. *Cancer Res.* (2000) 60:4231–7.

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REVIEW
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Therapeutic Targets for the Treatment of Cardiac Fibrosis and Cancer: Focusing on TGF-β Signaling

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Transforming growth factor-β (TGF-β) is a common mediator of cancer progression and fibrosis. Fibrosis can be a significant pathology in multiple organs, including the heart. In this review, we explain how inhibitors of TGF-β signaling can work as antifibrotic therapy. After cardiac injury, profibrotic mediators such as TGF-β, angiotensin II, and endothelin-1 simultaneously activate cardiac fibroblasts, resulting in fibroblast proliferation and migration, deposition of extracellular matrix proteins, and myofibroblast differentiation, which ultimately lead to the development of cardiac fibrosis. The consequences of fibrosis include a wide range of cardiac disorders, including contractile dysfunction, distortion of the cardiac structure, cardiac remodeling, and heart failure. Among various molecular contributors, TGF-β and its signaling pathways which play a major role in carcinogenesis are considered master fibrotic mediators. In fact, recently the inhibition of TGF-β signaling pathways using small molecule inhibitors, antibodies, and gene deletion has shown that the progression of several cancer types was suppressed. Therefore, inhibitors of TGF-β signaling are promising targets for the treatment of tissue fibrosis and cancers. In this review, we discuss the molecular mechanisms of TGF-β in the pathogenesis of cardiac fibrosis and cancer. We will review recent *in vitro* and *in vivo* evidence regarding antifibrotic and anticancer actions of TGF-β inhibitors. In addition, we also present available clinical data on therapy based on inhibiting TGF-β signaling for the treatment of cancers and cardiac fibrosis.

Keywords: anticancer, antifibrotic, cancer, cardiac fibrosis, inhibitors of TGF-β signaling, transforming growth factor-β (TGF-β)

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INTRODUCTION

Transforming growth factor-β (TGF-β) is a crucial member of the TGF-β superfamily and its sophisticated signaling pathways have pleiotropic effects that regulate several systems throughout the body such as cell growth, cell differentiation, apoptosis, motility and invasion, tissue remodeling, angiogenesis, and the immune response (1–6). TGF-β signaling dysfunctions are frequently found in tumors and these dysfunctions play critical roles in tumor progression (e.g., development and metastasis) (7–9). In addition, TGF-β is a major profibrotic mediator that plays an important role in the development of fibrosis (10). Due to the significant implication of TGF-β signaling in cancer as well as in fibrosis (**Figure 1**), drug research into treatments for cancer and

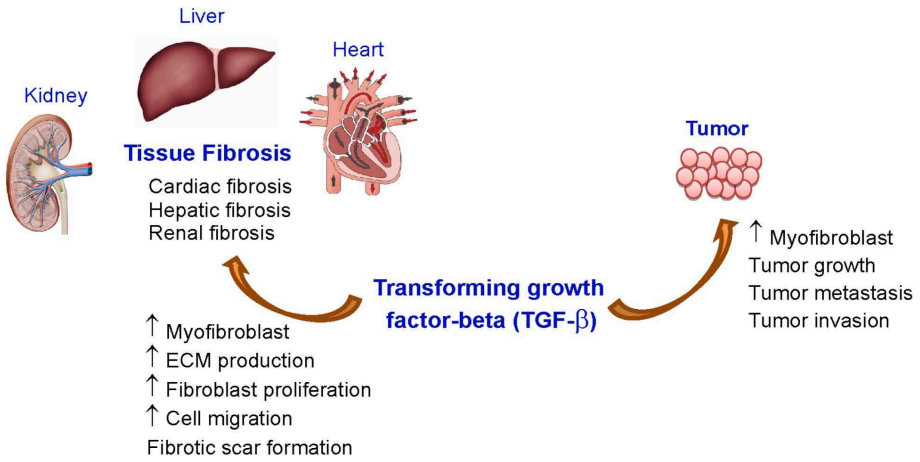

FIGURE 1 | Effects of TGF-β on tissue fibrosis and cancer. ECM, extracellular matrix; TGF-β, transforming growth factor-beta-β.

fibrosis has aimed to develop various approaches to inhibit TGF-β signaling. Thus, the number of lead compounds used either in animal models or in clinical studies related to cancer and fibrosis is currently growing. Targeting TGF-β signaling pathways could be a novel therapeutic strategy to treat a variety of fibrotic disorders and cancers.

The synthesis and secretion of TGF-β, including its activity, is markedly increased in experimental models of fibrosis and in patients with tissue fibrosis (e.g., liver, lung, kidney, and heart). Fibrosis is an important pathophysiological phenomenon in many tissues. It is characterized by fibroblast activation and accumulation, an imbalance of extracellular matrix (ECM) production and degradation, and myofibroblast differentiation, which results in the accumulation of fibrotic scar and tissue stiffness, leading to distortions of organ architecture and function [Reviewed in (11, 12)].

Among fibrotic conditions in various organs, cardiac fibrosis is a major pathologic disorder associated with a great number of cardiovascular diseases resulting from an excessive ECM protein deposition in the heart [Reviewed in (11, 12)]. The etiologies of cardiac fibrosis and myocardial stiffness are multifactorially developed in response to multiple risk factors (13, 14) include myocardial infarction (MI), hypertension (15), diabetes (16, 17), aging (16), and excessive alcohol consumptions (18, 19) leading to the excessive deposition of ECM. After cardiac injury, alterations in ECM homeostasis, the upregulation and release of growth factors and cytokines, and differentiation of fibroblasts into myofibroblasts dynamically modulate cardiac fibroblast characteristics and functions, leading to myocardial fibrosis. Myocardial fibrosis is associated with fibrotic scar formation, myocardial stiffness, and the progression of heart failure (HF) (20–23). Treatment of HF and cardiac fibrosis still has limited efficacy and currently there is no drug approved for the treatment of cardiac fibrosis. The main reason is that the underlying mechanism of fibrosis is still unclear. However, cardiovascular diseases remain the leading global cause of death (22, 23) and understanding the pathogenesis of fibrotic myocardial remodeling is crucial to identifying innovative treatment strategies for patients with cardiac fibrosis.

In the heart, activation of cardiac fibroblasts mainly by TGF-β leads to alterations in cardiac ECM and cardiac remodeling that play a major role in the development and progression of heart diseases (10, 22). A significant number of preclinical and clinical studies have reported that inhibition of TGF-β signaling pathways by various strategies exhibited potential effectiveness for the treatment of cardiac fibrosis. Cancers and fibrotic diseases share the most common pathologies associated with the activity of TGF-β (1, 2). Here, we review the molecular mechanisms and signaling pathways of TGF-β and their effect on cancer and cardiac fibrosis, and we also summarize the role of inhibition of TGF-β for anticancer and antifibrotic therapies.

Introduction of Cancer

Cancer is defined as a collection of diseases relating to atypical cell growth. In physiological process, new cells can grow, divide, and replace senescent or damaged cells. However, this systemically process fails when cancer develops as aged or injured cells remain survive, together with a proliferation of unneeded new cells. These unnecessary cells can divide, spread, and invade nearby tissues without stopping. Also, the harm cells can possibly travel through the blood or lymph system to invade remote tissues. This atypical cell growth and spreading is known as carcinogenesis (24). Widespread and recognized theory of carcinogenesis is the DNA mutations that disrupt the normal balance between proliferation and cell death. Variants of inherited genes and environmental factors might play a pivotal role in DNA mutations. In addition, viruses containing oncogenes are recently known as a trigger of cancer cell growth (24).

Therapeutic Targets for Treatment of Cancers

Treatment of cancers can be achieved using several strategies such as surgery, radiation, and especially drugs. Chemotherapy is a conventional treatment by using toxic drugs to kill cancer cells. Beyond fast-growing cancer cells, traditional anticancer drugs using for chemotherapy damage healthy cells that rapidly grow and divide, leading to multiple adverse effects (25). Newer drugs for the treatment of cancers were subsequently developed for a preferable safety issue and prevailing therapeutic efficacy (25). Hormonal therapy is another strategy to cease the growth of cancer which required certain hormones. Due to the blockade, undesired effects of anti-hormone drugs can be seen depending on types of interfered hormone (26, 27). Targeted therapy is a type of cancer treatment using drugs targeting particular molecules required for the pathogenesis of individual cancer. Nevertheless, treated cancer cells can gradually resist to targeted therapy, and conventional chemotherapy might be needed to be co-administered in the regimen for a better outcome (28). Immunotherapy is a novel treatment method by enhancing immune system for eradicating cancer cells. Despite solely activated self-immune cells, overactive immunity against cancer also influences healthy cells and tissues resulting in various adverse effects (29). Described anticancer drug classes and representative drugs among each class are demonstrated in **Table 1**. However, in-depth review regarding mechanism of drug action, clinical effectiveness, and safety profile of these anticancer drugs are beyond our scope. Furthermore, it should be noted that although anticancer drugs appears to be diverse and abundant, we still need distinct agents to deal with innumerable types of advanced cancers in clinical practice, especially multi-drug resistant cancers (30). Therefore, in this review, we focus on the role of TGF-β and its signaling on the treatment of cancer.

TABLE 1 | Available anticancer drug classes and representative drugs among each class.

Classes	Example sub-classes	Representative drugs
Chemotherapy (25)	Alkylating agents	Cyclophosphamide, cisplatin
	Topoisomerase inhibitors	Irinotecan, etoposide, doxorubicin
	Mitotic inhibitors	Vincristine, paclitaxel
	Anti-metabolites	Methotrexate, cytarabine, hydroxyurea
	Others	Bleomycin, L-asparaginase
Hormonal therapy (26, 27)	GnRH analogs	Buserelin, degarelix
	Anti-androgens	Cyproterone, flutamide
	Aromatase inhibitors	Aminoglutethimide, anastrozole
	SERMs	Tamoxifen
Targeted therapy (28)	Receptor tyrosine kinase inhibitors	Erlotinib, gefitinib, lapatinib
	Intracellular tyrosine kinase inhibitors	Imatinib, nilotinib, everolimus
	Phenotype-directed inhibitors	Rituximab, alemtuzumab
	Ligand-receptor binding inhibitors	Bevacizumab, cetuximab, trastuzumab
	Proteasome inhibitors	Bortezomib
Immunotherapy (29)	PRR agonists	Imiquimod, mifamurtide
	Checkpoint inhibitors	Ipilimumab, nivolumab
	Cytokines	IFN-α, IFN-β
	Cell-based immunotherapies	Sipuleucel-T

GnRH, gonadotropin releasing hormone; IFN, interferon; PRR, pattern recognition receptor; SERMs, selective estrogen receptor modulators.

Introduction of Cardiac Fibrosis

Cardiac fibrosis is a pathological remodeling process following cardiac injury, MI, and other heart diseases. Cardiac fibrosis disrupts the communication and function of myocytes and non-myocyte cells in the heart, leading to contractile dysfunction and arrhythmia. Fibrosis also accelerates the remodeling processes that exhibit detrimental effects on the heart (23, 31).

The imbalance between production and degradation of interstitial ECM proteins leads to progressively increased cardiac stiffness and diastolic dysfunction (23). Lines of existed evidence demonstrates that the pathogenesis of diastolic dysfunction caused by cardiac fibrosis (32, 33). In the fibrotic heart, collagens mainly from activated myofibroblasts undergoes cross-linking process contributing to the progression of diastolic dysfunction and the restricted cardiac chamber compliance (34, 35). In addition, ECM overproduction and deposition between the layers of cardiac myocytes results in the disruption of myocardial electrophysiological functions, which leads to contractile dysfunction and an increased risk of cardiac arrhythmia (36, 37). In fact, TGF-β induced cardiac fibrosis is seriously involved in the pathogenesis of arrhythmia by disturbing electrical signal conduction, leading to the generation of re-entry circuits (10).

Myofibroblasts

In the heart, cardiac fibroblasts can be transdifferentiated into myofibroblasts with contractile, migratory, and secretory properties (**Figure 2**). Myofibroblast is a key regulator that accelerates the fibrotic response in many conditions associated with HF. Regardless of the etiology of cardiac fibrosis, myofibroblast transdifferentiation is a hallmark of the fibrotic response in the heart [Reviewed in (20, 23)].

Myofibroblasts are the activated form of fibroblasts. They overexpress α-smooth muscle actin (α-SMA) and contain contractile bundles of actin filaments resembling the myofibrils of smooth muscle cells and associated proteins organized into prominent stress fibers (38). The incorporation of α-SMA into contractile bundles is a major characteristic of differentiated myofibroblasts and significantly increases contractile function. Thus, α-SMA has been suggested to be the most significant marker of myofibroblasts (39). Although α-SMA is found in human myocardial scars, the other structural ECM proteins such as collagens, vimentin, and desmin are also present in fibrotic scars (40). Fibroblast differentiation into myofibroblast is controlled by a variety of growth factors and cytokines. Among them, TGF-β is

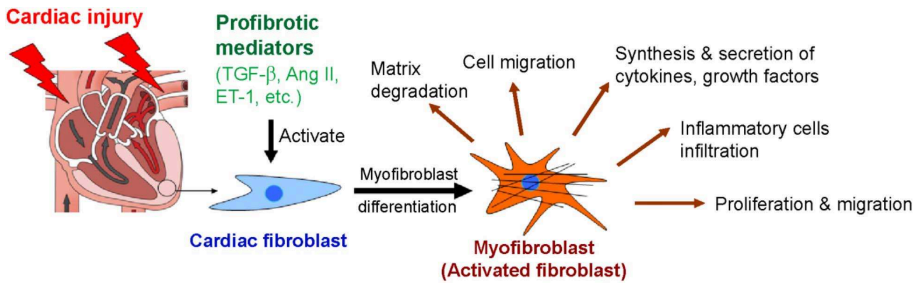

FIGURE 2 | Myofibroblast differentiation and functions of myofibroblasts after cardiac injury. Ang II, angiotensin II; ET-1, endothelin-1; TGF-β, transforming growth factor-β.

a strong inducer that stimulates myofibroblast formation (**Figure 2**).

Fibroblasts are abundant in normal hearts and can differentiate into myofibroblasts via profibrotic mediators such as TGF-β (41, 42). This process suggests that the activation of resident fibroblasts represents a major source of myofibroblasts in hearts with fibrosis. In addition, proliferating myofibroblasts are commonly found in high numbers in the infracted area of the heart (41, 42).

Following cardiac fibroblast activation, inflammatory cells (e.g., macrophages, monocytes, and mast cells) infiltrate the site of remodeling myocardium and secrete various types of profibrotic mediators, including growth factors and cytokines [Reviewed in (43)]. These mediators have been found to promote myofibroblast formation, but the most significant and common inducer is TGF-β (44). TGF-β accelerates the differentiation of resident fibroblasts, epithelial cells, and endothelial cells into myofibroblasts (44). Thus, agents that inhibit myofibroblast differentiation might provide a tool to prevent the maladaptive myocardial remodeling that occurs in response to profibrotic stimuli and for fibrosis prevention.

Overproduction of ECM Proteins

Alterations in ECM homeostasis, especially in terms of ECM overproduction, lead to cardiac dysfunction. Several mediators, including angiotensin II (Ang II), and TGF-β, regulate ECM production by cardiac fibroblasts (45). In response to cardiac injury, myocardial fibrosis results from an imbalance of both ECM synthesis and degradation, leading to an accumulation of collagen type I and III in the heart (20, 23). Deposition of ECM proteins is significantly increased in the hearts of patients with cardiac diseases (46). In addition, the levels of cardiac fibrosis are associated with cardiac dysfunction (46). Moreover, ECM deposition and fibroblast activation contribute to the impairment of ventricular compliance and filling due to increased ventricular stiffness (20, 23). Furthermore, overproduction of ECM interrupts the electrophysiological functions in the heart, leading to arrhythmias (10).

Therapeutic Targets for Treatment of Cardiac Fibrosis

According to cardiac fibrosis is associated with cardiac remodeling and is involved in the pathogenesis of HF, the prevention and reversal of cardiac fibrosis is an important therapeutic target for the treatment of HF. Numerous signaling pathways, through a variety of profibrotic mediators (e.g., Ang II, endothelin-1 [ET-1], and TGF-β), have been implicated in the activation of cardiac fibroblasts and the development of cardiac fibrosis. Modulation of these signaling pathways using inhibitors is of great interest for the treatment and prevention of cardiac fibrosis. Below, we summarize the update and important roles of several agents that act against cardiac fibrosis (**Table 2**). Although, both angiotensin converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) have already demonstrated significant efficacy in reducing cardiac fibrosis in human and animal models of HF, neither ACEIs nor ARBs have been approved for the treatment of cardiac fibrosis. Further studies are required to establish the molecular mechanisms of ACEIs and ARBs not only for treatment but also for reversal of fibrotic remodeling in HF.

TGF-β SIGNAL TRANSDUCTION

TGF-β is a member of the TGF-β superfamily, which is comprised of TGF-β, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), activin and inhibin (65). Members of this diversify superfamily are the pleiotropic multifunctional polypeptides that play a role in a wide range of physiological cellular activities such as growth, proliferation, differentiation, and apoptosis (65). Among these polypeptides, TGF-β has been proven to be one of the major factors driving the fibrotic response in most organs (2). In mammals, there are 3 isoforms of TGF-β: TGF-β1, TGF-β2, and TGF-β3. These highly homologous polypeptides, encoded by various genes, are synthesized, processed and regulated in a similar fashion. However, these 3 isoforms are secreted by various types of cells and signals through the same receptors, but they exhibit distinct patterns of distribution in different tissues (3, 66). Even though any isoform can be found in fibrotic tissues, the progression of organ fibrosis, in particular cardiac fibrosis, is predominantly attributed to TGF-β1 (67). To date, information on isoform-specific activities of various isoforms of TGF-β in a specific

TABLE 2 | Therapeutic targets/strategies for treatment of cardiac fibrosis.

Targets/Strategies	Results	References
Inhibitors of TGF-β and its signaling pathway	Anti-TGF-β neutralizing antibody prevents myocardial fibrosis in pressure-overloaded hearts	(47)
	Blockade of TGF-β-activated kinase 1 (TAK1) inhibits TGF-β-mediated extracellular matrix (ECM) overproduction in cardiac fibroblasts	(48)
	Inhibition of p38-MAPK suppresses TGF-β-induced myofibroblast activation and ECM production	(49)
TβRI (ALK5) inhibitors	ALK5 inhibition attenuates cardiac dysfunction and remodeling after myocardial infarction (MI)	(50)
	SM16 (ALK5 inhibitor) attenuates progression of cardiac fibrosis in left ventricular (LV) pressure overload	(51)
TβRII inhibitors	Dominant negative mutant of TβRII inhibits interstitial fibrosis in pressure-overload hearts	(52)
Smad inhibitors	Halofuginone (Smad3 inhibitor) attenuates radiation-induced fibrosis	(53)
Angiotensin converting enzyme inhibitors/angiotensin II receptor blockers (ACEIs/ARBs)	Losartan inhibits the progression of cardiac hypertrophy and fibrosis	(54)
	Lisinopril improves cardiac function and attenuates fibrosis in patients with hypertension and hypertrophy	(55)
	Losartan reduces angiotensin II (Ang II)-induced collagen synthesis and fibroblast activation	(56)
Endothelin receptor (ETR) antagonists	Bosentan improves cardiac function and reduces infarct size in a rat model of ischemia/reperfusion injury	(57)
	ET_AR antagonists prevented cardiac fibrosis in hypertensive-induced rats	(58)
Adenosine receptor (AR) agonists	Stimulation of $A_{2B}R$ attenuates fibrosis and remodeling in a rat model of MI	(59)
	Stimulation of $A_{2B}R$ inhibits ET-1-induced fibroblast proliferation and α-SMA synthesis	(60)
	Stimulation of $A_{2B}R$ inhibits Ang II-induced collagen synthesis and myofibroblast differentiation	(61)
β-Adrenergic receptor (βAR) signaling	Blockade of βAR attenuates cardiac fibrosis in an animal model of heart failure (HF)	(62)
	Gene deletion of GRK2 enhances survival, improves contractility, and inhibits cardiac remodeling in a mouse model of post-MI	(63)
	Treatment with β-blockers (e.g., atenolol, metoprolol, and propranolol) blocked the effects of βAR-mediated fibroblast activation	(64)

pathology is lacking and needs further investigation. Next, the signaling of TGF-β, excluding conclusions regarding specific isoforms, is discussed in detail.

The synthesis, release, and activation of TGF-β is a complex process (**Figure 3**). Following intracellular biosynthesis, a dimer of TGF-β is secreted as an inactive protein complex (latent TGF-β), which is retained in the ECM. Active TGF-β1 can be liberated from ECM by multiple activators such as reactive oxygen species (ROS), plasmin, thrombospondin-1, and αvβ6 integrin (68). Once active TGF-β is released from ECM, it binds to transmembrane TGF-β receptor type II (TβRII) of a target cell. This receptor-ligand interaction induces serine/threonine kinase activity of TβRII for autophosphorylation (69). The canonical pathway of TGF-β signaling is initiated after phosphorylated TβRII forms a stable heteromeric complex with TGF-β receptor type I (TβRI), also known as activin receptor-like kinase 5 (ALK5), for the transphosphorylation of residual phosphate to TβRI (70). This receptor binding complex, which is a heterotetrameric combination between two molecules of TβRII and another two of TβRI, recruits and phosphorylates the downstream signaling proteins Smad2 or Smad3, which are called receptor-activated Smads. After phosphorylation, Smad2 or Smad3 is released and forms an intracellular complex with Smad4, the mediator Smad. This intracellular complex between Smad2/4 or Smad3/4 moves from the cytoplasm into the nucleus, where it binds to promoter regions of the genes involved in physiological process of induction of specific gene expression (71). For an example of fibrogenesis, gene encoding α-SMA, collagens, and fibronectin are significantly upregulated via the Smad3-dependent pathway (72). The expression of these fibrosis-related genes plays a pivotal role in the cellular transdifferentiation that generates myofibroblasts and the production/deposition of ECM by myofibroblasts in fibrotic tissue (72). In addition to fibrogenesis, the Smad-mediated signaling pathway is also a significant intracellular process activated by TGF-β that increases genes associated with carcinogenesis (73). Furthermore, the activation of TGF-β signaling results in the expression of Smad7, an inhibitory SMAD, which acts as a negative regulator by interacting with Smad2 or Smad3, thereby mitigating signaling through receptor-activated Smads and further decreasing TGF-β actions (74).

Beyond canonical pathways or Smad-mediated signaling, TGF-β might mediate signaling directly by activating kinase enzymes via non-Smad signaling pathways, which are also known as non-canonical pathways (**Figure 4**). The non-Smad signaling pathways are initially propagated by either or both phosphorylated TβRI and TβRII for modulating downstream cellular responses. It has been reported that crosstalk between canonical and non-canonical pathways appeared to occur in most TGF-β-mediated effects (75). Epithelial-to-mesenchymal transition (EMT) plays a significant role in the pathogenesis of cancer. In part, this process requires an activation of ERK by TGF-β to upregulate the genes involving in remodeling of cell-matrix adhesion, thereby promoting the motility of the transformed cells (76). Also, EMT might be induced by TGF-β via both TβRI and TβRII through the activation

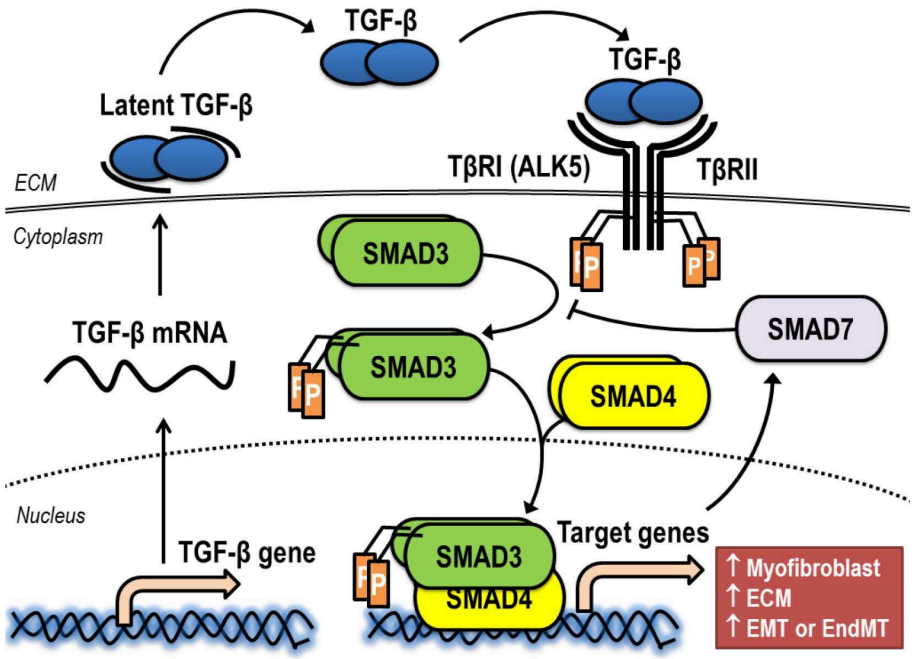

FIGURE 3 | Synthesis, release, and activation of TGF-β signaling via the canonical pathway. ALK5, activin receptor-like kinase 5; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; EndMT, endothelial-to-mesenchymal transition; TβRI, TGF-β receptor type I; TβRII, TGF-β receptor type II.

of TNF receptor-associated factor 6 (TRAF6). TRAF6 is capable of recruiting TGF-β-activated kinase 1 (TAK1) to subsequently allow the activation of c-Jun amino terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38-MAPK) (77). In addition, the TRAF6-TAK1-JNK/p38 pathway is believed to be an essential pathway for TGF-β-induced apoptosis (78). Similar to the ERK and JNK/p38-MAPK pathway, the Ras homolog gene family member A (RhoA) is also a signaling mediator of EMT. TGF-β-induce RhoA degradation by phosphorylating partitioning-defective 6 (Par6), which subsequently recruits Smad-specific E3 ubiquitin protein ligase (Smurf1) to loosen tight junctions and rearrange the actin cytoskeleton, a prerequisite step for EMT (79). Another non-Smad signaling pathway contributing to TGF-β-promoted EMT is the phosphoinositide 3-kinase (PI3K)/Akt (protein kinase B) pathway, which subsequently activates the mammalian target of rapamycin (mTOR) and phosphorylation of S6 kinase (S6K) (80, 81). In addition, TGF-β1 signaling can be regulated at the post-transcriptional level via the expression of microRNAs (miRNAs), and the expression of miRNAs might play a role in TGF-β1-mediated EMT also (82).

TGF-β Signaling in the Development of Cancers

For the ultimate outcome of TGF-β-mediated responses in any pathological condition, it is apparent that a combination of canonical and non-canonical pathways are coordinated (1). Cancers and fibrotic diseases are the most common pathologies associated with the activity of TGF-β. Currently, most putative drugs affecting TGF-β for the treatment of cardiac fibrosis were initially developed for the management of cancer; therefore, we next discuss the signaling of TGF-β in carcinogenesis.

In the pathogenesis of cancer, TGF-β acts as a tumor suppressor in early stages of the disease. However, in later stages, TGF-β turns into a tumor promoter. This paradoxical role of TGF-β is due to a bypass of the cytostatic effect of TGF-β in tumor cells (4). The tumor suppressive effect of TGF-β is derived from various cellular effects. TGF-β stabilizes the cell cycle of epithelial cells by upregulating multiple cyclin-dependent kinases: p15, p21, and p27, via the canonical pathway (83). Also, via the Smad-dependent pathway, TGF-β downregulates genes associated with cell proliferation, such as c-Myc (84). In addition, the canonical pathway contributes to the tumor suppressive effects of TGF-β by inducing gene encoding B-cell lymphoma 2 (BCL2) and subsequently activating BIM for apoptotic processes in human B cells (85). Conversely, non-canonical pathways might mediate the apoptotic effect of TGF-β by inducing caspase-8 expression and activating BID in human gastric carcinoma cells (86). The difference in signaling of TGF-β-mediated apoptosis indicates that the cellular context is essential for controlling the main pathway in the tumor suppressive effects of TGF-β. The tumor promoting effects of TGF-β such as EMT, invasion, metastasis, and angiogenesis emerge when cancer progresses to a later stage (5, 87). The upregulation of miR-106b-25 cluster targets Smad7 to ameliorate the TGF-β signaling that is not generally found in normal tissues is an excellent example of this phenomenon. In human breast cancer, increased miR-106b-25 leads to the inhibition of tumor suppressive protein p21 and BIM, thereby allowing tumor cells to grow via the

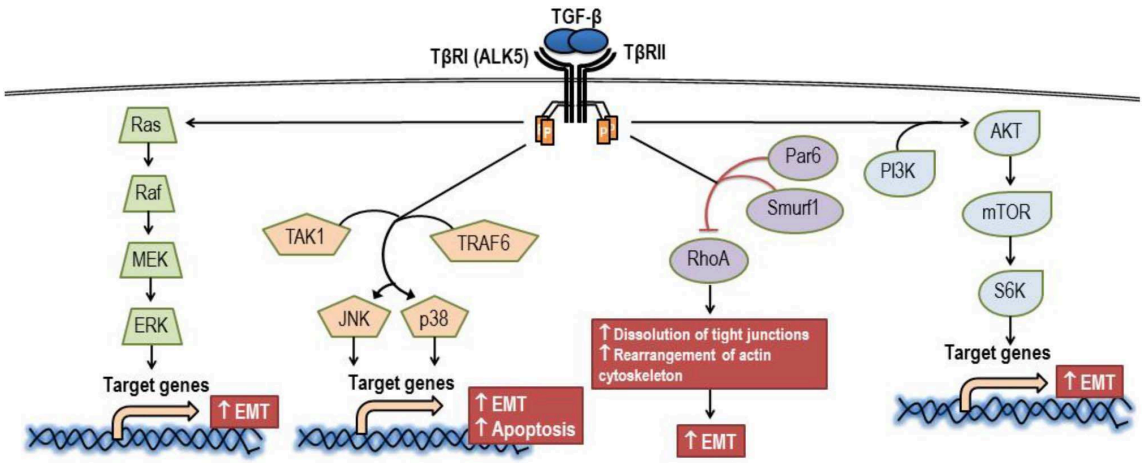

FIGURE 4 | Signaling via the non-canonical pathway of TGF-β. AKT, protein kinase B; ALK5, activin receptor-like kinase 5; EMT, epithelial to mesenchymal transition; ERK, extracellular signal-regulated kinase; JNK, c-Jun amino terminal kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; Par6, partitioning-defective 6; PI3K, phosphoinositide 3-kinase; p38, p38 mitogen-activated protein kinase; Raf, Raf proto-oncogene serine/threonine-protein kinase; Ras, Ras GTPase; RhoA, Ras homolog gene family member A; Smurf1, SMAD specific E3 ubiquitin protein ligase; S6K, phosphorylation of S6 kinase; TAK1, TGF-β-activated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6; TβRI, TGF-β receptor type I; TβRII, TGF-β receptor type II.

activation of TGF-β (88). Interestingly, TGF-β also regulates the functions of various immune cells, including the modulation of cytokines released from these cells. Impairment of TGF-β signaling pathways leads to immune dysregulation, fibrosis, and cancer [Reviewed in (7)]. TGF-β is produced as a complex with latency associated peptide (LAP). This complex associates with ECM by binding to latent TGF-β binding protein (LTBP) or glycoprotein A repetitions predominant (GARP) expressed on T cells, especially on Tregs, or platelets. Integrins bind to the complex and stimulate the release of TGF-β from the complex. The release of active TGF-β promotes oncogenesis and immune tolerance in breast cancer (89). Inhibition of αvβ8 integrins potentiates cytotoxic T cell responses and recruitment of immune cells to tumor centers. Cancer cells can evade host immunity by mobilizing active TGF-β1 through αvβ8 integrins (90). Thus, TGF-β acts as a significant suppressor of immune responses during tumor progression.

In general, tissue fibrosis is considered a main step in triggering cancer development. An apparent example is hepatocellular carcinoma, the most common form of liver cancer. Cirrhosis, which is known as the end-stage of liver fibrosis, occurs in most patients who ultimately develop hepatocellular carcinoma (91). Interestingly, the progression of fibrosis to cancer in the heart is rare. The low incidence of cardiac cancer might be due to the fact that cardiac cells, in particular cardiomyocytes, are fully differentiated cells. Moreover, the regenerative capacity of cardiomyocytes is considered to be negligibly low. Thus, cardiomyocytes appear to resist further transformation and proliferation processes such as EMT in the development of cancer (92). Accordingly, signaling of TGF-β in fibrogenesis of the heart might not be identical to that occurring in other organs where progressive fibrosis ultimately develops cancers.

TGF-β Signaling in the Development of Cardiac Fibrosis

During tissue injury, TGF-β expression is increased to play a role in the tissue repair process and scar formation. In the heart tissue following MI, TGF-β signaling plays an important role in reparative, angiogenetic, and fibrotic responses by modulating inflammation (93). Studies on mice and dogs have revealed that TGF-β1 and TGF-β2 were upregulated in the early phase after MI, and then TGF-β3 was increased in a later stage post-infarction myocardium (94). Among various cells that release TGF-β, a significant amount of TGF-β might be released from infiltrated macrophages that migrate to the injured area to engulf the damaged cardiomyocytes, as shown in a mouse model (95). On the other hand, a study using a porcine model of chronic coronary constriction revealed that cardiomyocytes were a significant source of TGF-β (96). Another study suggested that TGF-β was found in the extracellular fluid of ischemic canine myocardium tissue (97). Multiple pathways involving integrins and thrombospondin-1 were found to be associated with the release of TGF-β from the cardiac ECM-bound TGF-β (98, 99). Following the release of active TGF-β, TGF-β binds to the receptors, as described earlier, to activate intracellular responses in the infarcted tissue. The TGF-β-mediated effects can be classified into 4 actions in the following order: cardiomyocyte survival, immune cell-related action, formation of myofibroblasts, and production/deposition of ECM, all of which modulate the effects on myocardial endothelial cells.

TGF-β-mediated effects on cardiomyocyte survival in MI appear to be dependent on the time period after MI. In the early phase, exogenous TGF-β administered before or immediately after ischemic injury to an isolated perfused heart showed cardioprotective effects by reducing the amount

of superoxide anions, maintaining coronary relaxation, and reducing injurious responses of exogenous TNF-α (100). Similarly, a study has shown that the infarct size of intact rat hearts receiving TGF-β during early reperfusion was reduced, and this reduction was due to activation of MAPK (101). However, the mechanism underlying cardioprotection remains poorly understood. Conversely, a proapoptotic effect of TGF-β via interplay with Ang II was demonstrated in a study using rat cardiomyocytes (102). The findings showed that the actions of exogenous TGF-β are likely dependent on the timing of administration.

Immune cells play a pivotal role in fibrogenesis, and TGF-β regulates both the phenotype and function of the immune cells. It is worth noting that TGF-β can be either a pro- or anti-inflammatory mediator of the immune response in *in vitro* studies [Reviewed in (93)]. Factors that determine the effects of TGF-β include the types of cytokines and the origin of the tissue (103). In an *in vivo* study, TGF-β suppressed T cell-mediated inflammation in genetically modified mice with T cell-specific loss of TβRII. Thus, the results from this *in vivo* study implicate an immunosuppressive effect of TGF-β (104). Nevertheless, the specific TGF-β-mediated effects on the phenotype of immune cells, together with its signaling and significance in the regulation of fibrosis, in the infarcted tissue remain unknown in the infarcted tissue.

TGF-β-mediated effects on the formation of myofibroblasts and on the induction of transformed myofibroblasts to further produce/deposit ECM are currently recognized central to the role of TGF-β in the pathogenesis of fibrosis. In cardiac fibrosis, Smad3-deficient mice that underwent reperfused MI showed significantly less fibroblast proliferation and ECM when compared to those of wild-type mice (105, 106). Even though the origin of the cells that underwent transformation has been debated (107), a recent study using fibroblast-specific, TGF-β signaling pathway knockout mice demonstrated that myofibroblasts in cardiac fibrosis are derived from resident fibroblasts, which activated via the TGF-β-Smad2/3 signaling pathway (72). These results suggest that the canonical pathway of TGF-β is principally involved in the pathogenesis of cardiac fibrosis. Interestingly, it was found that the Smad3-dependent pathway is essential for the upregulation of connective tissue growth factor (CTGF), which in turn acts as a mediator to stimulate fibroblast differentiation and collagen synthesis (108). Beyond the formation of myofibroblasts, genes encoding collagen type I and III were upregulated in cardiac fibroblasts isolated from rabbit hearts following treatment with TGF-β (109). The TAK1/p38-MAPK pathway in the cardiomyocytes of non-infarcted myocardium was found to be activated in rats after acute MI, suggesting a role for this non-canonical pathway in ventricular hypertrophy and remodeling (110). Nevertheless, the significance of Smad-independent pathways in the transformation of cardiac fibroblasts appears to be less proven than that of renal and pulmonary fibrosis (111, 112). Finally, a study on TGF-β-overexpressed mice showed increase expression of tissue inhibitors of matrix metalloproteinases (TIMPs), which regulate the remodeling of ECM in the cardiac tissue. However, the signaling of TGF-β was not evaluated in this study (113).

In addition to cardiomyocytes, immune cells, and transformed myofibroblasts, vascular endothelial cells might also play an important role in cardiac fibrosis. It has been found that endothelial cells served as a source of chemokines and played a role in recruiting neutrophils and monocytes to the heart after MI (114). Interestingly, although TGF-β plays a role in angiogenesis in cancers (8), information on the effects of TGF-β on angiogenesis in infarcted myocardium is limited at present. Moreover, although most cardiac myofibroblasts originate from resident fibroblasts, a study has shown that endothelial cells might be activated by the TGF-β via Smad3-dependent pathway and transform into myofibroblasts, thereby inducing cardiac fibrosis (115).

TGF-β INHIBITORS FOR THE TREATMENT OF CANCERS AND CARDIAC FIBROSIS

Inhibitors of TGF-β Signaling for the Treatment of Cancers

TGF-β suppresses cell proliferation leading to apoptosis in the early phase of tumor development, whereas it aggravates tumor invasion and metastasis via boosting immune escape, angiogenesis, and EMT of tumors at an advanced stage (116). The paradoxical impact of TGF-β signaling in various tumors raises concerns that anti-TGF-β signaling might lead to a poor prognosis due to its tumor suppressor role. This concern has delayed progression in the development of TGF-β inhibitors as therapeutic agents. In addition, some experimental models have revealed that TβRI inhibitors aggravated the potential for cardiotoxicity (117).

However, several potential approaches to interfering with TGF-β signaling to prevent TGF-β production and block its signaling pathway have emerged. Next, we summarize the results of TGF-β inhibitors that have been studied in preclinical or clinical trials on carcinogenesis. The studies can be mainly categorized into 3 levels: (1) The ligand level: Direct blockage of TGF-β ligand synthesis by antisense molecules; (2) The ligand-receptor level: Inhibition of TGF-β ligand-receptor interaction using monoclonal antibodies or soluble TGF-β decoy receptors (traps); and (3) The intracellular level: Suppression of the TGF-β signaling pathway by tyrosine kinase inhibitors that disturb the downstream signaling of TGF-β related proteins (9, 118). The examples of current therapeutic agents in preclinical and clinical development in oncology are summarized in **Tables 3**, **4**.

Trabedersen (AP12009)

Preclinical data

Trabedersen (AP12009, Antisense Pharma) is a synthetic, 18-oligomer phosphorothioate antisense oligonucleotide (ASO). It was developed as an ASO specifically targeting human TGF-β2 mRNA, which leads to a reduction in TGF-β2 expression, cellular proliferation, and cellular migration in various types of tumors *in vitro* and *in vivo*, including gliomas (119), melanoma (120), pancreatic carcinomas (121, 122), and colorectal cancer (123). Trabedersen has been shown to reduce cell proliferation, tumor growth, cell migration or metastasis, and vascularization

TABLE 3 | Preclinical studies of TGF-β inhibitors for cancer treatment.

Agents	Target	Experiments/Models	References
1. THE LIGAND LEVEL			
Trabedersen (AP12009)	TGF-β2 mRNA	*In vivo*: patient-derived gliomas	(119)
		In vivo: induced melanoma tumor in mice	(120)
		In vitro: pancreatic carcinomas	(121)
		In vivo: human metastatic pancreatic cancer	(122)
		In vivo: human colon carcinomas	(123)
2. THE LIGAND-RECEPTOR LEVEL			
Soluble TβRII	TβRII	*In vitro*: human metastatic pancreatic cancer cells	(124)
		In vivo: patient-derived endometrial cancer	(125)
Soluble TβRIII (βglycan)	TβRIII	*In vivo*: patient-derived tissue from renal cancer	(126)
		In vivo: patient-derived tissue non-small-cell lung carcinoma	(127)
		In vivo: human xenograft model of breast cancer	(128)
3. THE INTRACELLULAR LEVEL			
Galunisertib (LY2157299)	TβRI	*In vivo*: patient-derived pancreatic, lung, colorectal cancer	(129)
		In vivo: human ovarian cancer in nude mice	(130)
		In vitro: hepatocellular carcinoma cells	(131–133)
Vactosertib (EW-7197)	TβRI	*In vivo*: lung metastases from breast cancer mice or transgenic MMTV/cNeu mice	(134)
EW-7195	TβRI	*In vivo*: lung metastases from breast cancer mice	(135)
LY2109761	TβRI/II	*In vivo*: metastatic colorectal cancer	(136)
		In vivo: metastatic hepatocellular carcinoma	(137)
SD208	TβRI	*In vivo*: metastatic breast cancer	(138)
		In vivo: metastatic pancreatic cancer	(139)

in human pancreatic cancer cells and in mouse model of human metastatic pancreatic cancer (122).

Clinical data

After several preclinical studies provided evidence of potential clinical efficacy, trabedersen was moved to phase I/II trials in patients with recurrent high-grade gliomas (119, 140, 149). Trabedersen was initially assessed for its safety and efficacy in phase I/II dose escalation studies in patients with high-grade gliomas and found a significant increase of median survival time after recurrence, exceeding that of standard chemotherapy (149). Similarly, prolonged survival and high response rates after treatment with trabedersen were observed in phase I/II studies in patients with recurrent or refractory malignant glioma, WHO grade III or IV (119). However, trabedersen was further compared with standard chemotherapy (temozolomide or procarbazine/lomustine/vincristine) in patients with recurrent or refractory malignant glioma (WHO grade III or IV) in a phase IIb trial. The results revealed that trabedersen did not control tumor growth, but delayed responses were observed after discontinuation of treatment (140).

Belagenpumatucel-L Vaccine

The principle of anti-TGF-β cancer vaccines is to deliver antisense molecules of TGF-β into cancer cells and overturn the effects of immunosuppression in host cells, as well as to enhance antitumor immunity (9). Belagenpneumatucel–L (Lucanix, NovaRx) is a TGF-β2, antisense, gene-modified non-viral based allogenic tumor cell vaccine. It was developed from non-small cell lung cancer (NSCLC) and modified to express ASO, which leads to suppression of the immunosuppressive activity implicit in TGF-β2 overexpressing cancer cells (141).

Clinical data

Currently, an anti-TGF-β cancer vaccine, belagenpumatucel-L, has entered a phase III study to determine whether it improves overall survival (OS) and might be useful for stimulating immune reactions. A dose-related survival difference was achieved in patients who received belagenpumatucel-L at least 2.5×10^7 cells/injection in a phase II trial involving patients with stages II, III, and IV NSCLC. Moreover, immune function measurements revealed an increase in cytokine production, including IFN-γ, IL-6, and IL-4, among clinical responders, who also displayed an elevated antibody-mediated response to the vaccine human leukocyte antigens (HLAs) (141). Likewise, a further study to evaluate its safety and response at the previously defined optimal dose found the median survival of patients with fewer than 2 circulating tumor cells (CTCs) at baseline was longer than patients with 2 or more CTCs. Thus, plasma levels of CTCs are associated with the OS of patients with stage IV NSCLC (142). Nevertheless, in a phase III trial with 532 patients with stage III/IV NSCLC who did not progress after platinum-based induction chemotherapy with or without irradiation,

TABLE 4 | Clinical studies of TGF-β inhibitors for cancer treatment.

Agents	Target	Phase	Study design	Main findings	References
1. THE LIGAND LEVEL					
Trabedersen (AP12009)	TGF-β2 mRNA	IIb	A randomized controlled trial compared to standard chemotherapy in refractory malignant (high-grade) glioma ($N = 145$)	Unchanged tumor growth Delayed responses after treatment discontinuation	(140)
2. THE LIGAND-RECEPTOR LEVEL					
Belagenpumatucel-L	TGF-β2	II	A randomized, dose-variable trial in stages II, IIIA, IIIB, and IV non-small-cell lung carcinoma (NSCLC) ($N = 75$)	Improved overall survival (OS) Increased IFN-γ, IL-4, and IL-6 production	(141)
Belagenpumatucel-L	TGF-β2	II	A randomized trial in advanced NSCLC ($N = 21$)	Increased OS	(142)
Belagenpumatucel-L	TGF-β2	III	A randomized trial in stage III/IV NSCLC after platinum-based therapy ($N = 532$)	Unchanged OS	(143)
Fresolimumab (GC-1008)	Pan TGF-β	II	An open-label trial in malignant pleural mesothelioma ($N = 13$)	Increased OS in patients who produced antitumor antibodies	(144)
Fresolimumab (GC-1008)	Pan TGF-β	II	An open label randomized trial in metastatic breast cancer with radiotherapy ($N = 23$)	Increased OS Well-tolerated Higher dose improved CD8	(145)
3. THE INTRACELLULAR LEVEL					
Galunisertib (LY2157299)	TβRI	II	A randomized study in metastatic pancreatic adenocarcinoma used gemcitabine for first-line therapy ($N = 156$)	Improved OS	(146)
Galunisertib (LY2157299)	TβRI	II	A randomized trial in hepatocellular carcinoma treated with galunisertib as monotherapy after sorafenib failure ($N = 109$)	Median OS of 8.3 months	(147)
Tasisulam (LY573636)	TGF-β	II	A randomized study as second-line or third-line treatment for metastatic soft tissue sarcoma ($N = 101$)	Modest activity as second-/third-line treatment (Median OS = 8.71 months)	(148)

belagenpumatucel-L did not increase survival compared with placebo (143).

Fresolimumab (GC1008)

Clinical Data

Fresolimumab (GC1008, Genzyme/Sanofi) is a fully human monoclonal antibody blocking pan-TGF-β (TGF-β1, TGF-β2, and TGF-β3) [Reviewed in (150)]. Fresolimumab demonstrated acceptable safety and preliminary evidence of antitumor activity in a phase I trial on patients with previously treated malignant melanoma or renal cell carcinoma (151). In a phase II trial on 13 patients with malignant pleural mesothelioma, 3 patients showed stable disease for at least 3 months, and those who produced antitumor antibodies had an increased median OS. However, treatment with fresolimumab had no effect on the expression of NK, $CD4^+$, or $CD8^+$ T cell activating and inhibitory markers, other than a decrease in the expression of CD244 (also known as 2B4) and CD266 (best known as DNAM1) on NK cells (144). A phase II trial on 23 patients with metastatic breast cancer undergoing radiotherapy has reported that fresolimumab in combination with focal radiotherapy significantly increased OS and was well-tolerated in a dose-dependent manner. Higher doses of fresolimumab correlated with an improved $CD8^+$ pool, leading to a favorable systemic immune response and longer median OS (145).

Galunisertib (LY2157299)

Preclinical Data

Galunisertib monohydrate (LY2157299, Eli Lilly) is a small-molecule inhibitor of TβRI that robustly downregulate the phosphorylation of Smad2 in pancreatic, lung, colorectal (129), and ovarian cancer (130). Galunisertib effectively demonstrated potent inhibition of both canonical and non-canonical pathways in a variety of *in vitro* hepatocellular carcinoma cells regardless of TGF-β pathway protein expression (131, 132). Nevertheless, the antiproliferative activity of TGF-β pathway inhibitors is quite limited. It has been reported that TGF-β inhibited cell proliferation while inducing apoptosis in cell lines with low endogenous levels of TGF-β and Smad7 and strong transcriptional Smad3 activity (PLC/PRF/5, HepG2, Hep3B, HuH7). However, cancer cells were sensitive to TGF-β-dependent growth inhibition and displayed limited sensitivity to galunisertib in another group of cell lines expressing high quantities of TGF-β and Smad7 and showing significantly reduced Smad3 signaling (SK-HEP1, SK-Suni, SK-Sora, JHH6, HLE, HLF, and FLC-4) (132, 133). Despite limited antiproliferative activity *in vitro*, galunisertib exhibited antiproliferative effects in *ex vivo* models, indicating that inhibition of TGF-β can exert anticancer properties (131, 133). Nevertheless, from the reports on several preclinical studies, treatment with TGF-β inhibitors as monotherapy might display limited efficacy. However, the immunological effects of galunisertib are strongly

augmented in combination with other checkpoint inhibitors (152, 153).

Clinical data

Among small molecule inhibitors, galunisertib is one of the most advanced. It has shown promising results in clinical trials due to its safety profile, with no cardiac potential toxicity in humans, which was a primary concern with first-generation TGF-β inhibitors (154). A phase I study on 28 patients with Grade IV glioma showed galunisertib was well-tolerated. The dose limiting toxicities included pulmonary embolism and thrombocytopenia, but no cardiotoxicities were observed (155). In addition, the safety of galunisertib was confirmed by a first-in-human dose study with 79 cancer patients with glioma and solid tumors treated with galunisertib as monotherapy or in combination with lomustine. No medically relevant cardiac toxicity or signs of cardiovascular injury were found, including increased blood pressure, troponin I, BNP, or hs-CRP or reductions in cystatin C levels (156). Likewise, no safety concerns or dose limiting toxicities was observed after treatment with galunisertib in patients with glioblastoma based on a pharmacokinetic/pharmacodynamic (PK/PD) model (157). Galunisertib as monotherapy and as second-line therapy after sorafenib failure in a subset of 109 patients with hepatocellular carcinoma yielded a median OS of 8.3 months in a phase II trial (147). Interestingly, patients who had decreased expression levels of specified blood biomarkers [e.g., alpha-fetoprotein (AFP), TGF-β1, and CDH1] had improved clinical outcomes, indicating that the effects of galunisertib might be more pronounced in patients with a poor prognosis due to elevated AFP at baseline (147). Similarly, galunisertib in combination with gemcitabine improved OS with minimal added toxicity in a phase II study on patients with locally advanced or metastatic pancreatic adenocarcinoma who were considered candidates for first-line chemotherapy with gemcitabine (146).

Vactosertib (EW-7197) and EW-7195

Preclinical Data

Vactosertib (EW-7197 or TEW-7197), a novel small molecule inhibitor of ALK5, has been recently developed as a more potent and specific antitumoral compound than galunisertib. Vactosertib and EW-7195 expressed potent antimetastatic activity *in vivo* via an inhibition of TGF-β1-induced Smad/TGFβ signaling, cell migration, invasion, EMT, and breast tumor metastasis to the lung in xenografted nude mice and transgenic MMTV/cNeu mice (134, 135). In addition, vactosertib expressed the potential to boost cytotoxic T lymphocyte function in 4T1 orthotopic-grafted mice and prolonged the lifespan of 4T1 breast tumor-bearing mice (134).

Clinical data

Vactosertib is currently being tested in phase I/II clinical trials for several cancer types in combination with chemotherapy or antibodies against immune checkpoints. A phase I study is evaluating the safety and tolerability of the drug in combination with paclitaxel in 12 metastatic gastric cancer patients (NCT03698825). The phase Ib/IIa trials include a study of vactosertib in combination with durvalumab in patients with advanced NSCLC who progressed following platinum-based chemotherapy ($N = 63$) (NCT03732274). A combination with pembrolizumab is being employed for metastatic or locally advanced colorectal or gastric/gastroesophageal junction adenocarcinoma ($N = 67$) (NCT03724851), and a combination with imatinib is being employed for patients with advanced desmoid tumors ($N = 24$) (NCT03802084). The latest phase II trial aims to determine whether administration of vactosertib with durvalumab will provide meaningful increases in the overall response rate in patients with urothelial cancers that fail to achieve a CR with anti-PD-1/PD-L1 based regimens ($N = 48$) (NCT04064190).

Remarkably, given TGF-β signaling plays a crucial role in fibrotic states, vactosertib has recently been investigated as an antifibrotic agent to delay the development of fibrosis in primary organs including the liver, kidney, and lung. Vactosertib was found to suppress fibrosis-induced accumulation of ROS and ECM proteins (collagen, α-SMA, fibronectin, and integrins) in the liver, lungs, and kidneys of mice due to its antifibrotic mechanism via inhibition of both TGF-β1/Smad2/3 and ROS signaling (158). A study on a rat model of Peyronie's disease showed that vactosertib suppressed phospho-Smad2 expression and recruitment of inflammatory cells, leading to a decline in fibrotic plaques (159). Thus, vactosertib and EW-7195 could be a promising antifibrotic compound for the treatment of fibrotic diseases.

Tasisulam (LY573636)

Clinical Data

Tasisulam has completed many trials in various oncologic diseases, including phase I studies on patients with essential thrombocythemia and acute myeloid leukemia (NCT00718159) and solid tumors (NCT01214668) and phase II trials on patients with ovarian cancer (NCT00428610), metastatic breast cancer (NCT00992225), NSCL cancer (NCT00363766), and malignant melanoma (NCT00383292). A phase II study on tasisulam as second- or third-line treatment for 101 patients with unresectable or metastatic soft tissue sarcoma reported that tasisulam demonstrated modest activity with a median OS of 8.71 months (148). Consequently, the synergistic and additive effects of tasisulam combined with other anticancer agents are currently of interest. Currently there is an ongoing phase I trial of tasisulam in combination with sunitinib, a multiple tyrosine kinase, in renal cancer patients (NCT01258348), and with pemetrexed, an inhibitor of purine synthesis, in patients with solid tumors (NCT01215916).

M7824 (MSB0011359C)

Interestingly, recent preclinical study has been reported that M7824 (MSB0011359C) which is a dual inhibitor of programmed death ligand 1 (PD-L1) and TGF-β inhibited tumor growth and metastasis more effectively than treatment with TGF-β inhibitor alone. Thus, M7824 (an inhibitor of PD-L1 and TGF-β) exhibits potent and superior antitumor effects compared to that of TGF-β inhibitor monotherapy and is likely to help minimize potential side effects (160).

Inhibitors of TGF-β Signaling for the Treatment of Cardiac Fibrosis

The renin-angiotensin system (RAS) inhibitors are currently used as standard therapy for HF and have been shown to inhibit activation of fibroblast and differentiation into myofibroblast. However, cardiac fibrosis persists in patients with HF even when treated with these conventional RAS inhibitors, indicating a need to develop novel and effective antifibrotic therapies for heart disease (161). Currently, due to its established role in cardiac fibrosis, there is great interest in inhibiting the TGF-β signaling pathway (6, 161). TGF-β is considered a mediator of cancer and fibrosis. Thus, blockades of TGF-β signaling activity using receptor antagonists, inhibition via antibody or antisense oligonucleotide, or even using gene deletion of TGF-β signaling molecules are potential therapeutic strategies.

Anti-TGF-β1 neutralizing antibodies have also been under investigation as potential antifibrotic agents by interfering with TGF-β signaling. Administration with anti-TGF-β1 antibody attenuated cardiac fibrosis and diastolic abnormalities in a rat model of pressure overload (47) (**Table 2**). Although these antibodies attenuated fibroblast activation and collagen synthesis, no improvements in overall cardiac functions were found in pressure-overloaded rats (47). Furthermore, anti-TGF-β neutralizing antibody inhibited ECM proteins synthesis and reduced cardiac fibrosis in a rat model induced by a chronic blockade of nitric oxide synthesis (162). However, in a mouse model of MI, a neutralizing anti-TGF antibody administered before or after coronary artery ligation resulted in increased mortality rates and left ventricular (LV) dilation after MI (163).

Alternative approaches have included inhibition of the expression of TGF-β using antisense oligonucleotides (164), and the use of a soluble TβRII, which either acts by adsorbing TGF-β or acting as a dominant negative receptor (165). Inhibitors of ALK5 (TβRI) are under investigation for antifibrotic effects in the heart. Inhibitor of ALK5 which decrease TGF-β activity can rescue cardiac dysfunction and ameliorate cardiac remodeling in post-MI hearts (50). Moreover, ALK5 inhibitors can also suppress the collagen synthesis and attenuate the progression of fibrosis in animal model of pressure overload induced by transverse aortic constriction, and inhibit TGF-β-mediated collagen synthesis in cardiac fibroblasts (51) (**Table 2**).

In addition to the canonical Smad-mediated signaling pathway, TGF-β also stimulates the non-canonical MAPK signaling pathways such as JNK-dependent and p38-MAPK-dependent pathways (166–168). These MAPK signaling pathways are involved in TGF-β-mediated activation of TAK1 which is thought to play a role in cardiac fibrosis and remodeling. Cardiac specific overexpression of the active form of TAK1 induced myocardial hypertrophy and HF (166–168), suggesting that TAK1 is a major effector of TGF-β signaling. Blockade of TAK1 activity attenuated TGF-β-mediated ECM protein overproduction in cardiac fibroblasts (48) (**Table 2**). In addition to inhibition of TAK1, inhibition of p38-MAPK is being investigated for its efficacy in the treatment of cardiac fibrosis. Inhibitors of p38-MAPK suppress myofibroblast activation and expression of ECM proteins and α-SMA induced by TGF-β, while overexpression of p38-MAPK induces myofibroblast differentiation in cardiac fibroblasts (49).

Two promising antifibrotic agents include tranilast and pirfenidone, which inhibit the actions of TGF-β as well as other pathogenic growth factors by unclear mechanisms (169). Current agents and therapeutic targets in preclinical and clinical development for the treatment of cardiac fibrosis and heart-related diseases are summarized in **Tables 5, 6**.

GW788388

Preclinical data

GW788388 is a potent inhibitor of both ALK5 and TGβRII with an improved pharmacokinetic profile (184) and minimal toxic effects (185). Several studies have been demonstrated that GW788388 pre-clinically reduces cardiac fibrosis in various models. GW788388 inhibited the development of cardiac fibrosis by suppression of collagen I and fibronectin synthesis, increased survival, and improved cardiac function in an experimental murine model of Chagas heart disease (170). Deletion of SCN5A, a gene encoding the main cardiac sodium channel $NaV_{1.5}$, has been associated with inherited progressive cardiac conduction disease. GW788388 chronically inhibited TGF-β receptors and prevented fibrosis in a Scn5a heterozygous knockout ($Scn5a^{+/-}$) mouse model of progressive cardiac conduction disease (171). Furthermore, treatment with GW788388 attenuated systolic dysfunction and delayed LV remodeling by reducing the phosphorylated Smad2, α-SMA, and collagen I in a rat model of HF following MI (50). Taken together, GW788388 appears to be a promising antifibrotic agent, although further studies are warranted.

Pirfenidone

Preclinical data

Pirfenidone is an oral antifibrotic drug initially approved for the treatment of idiopathic pulmonary fibrosis (186). Pirfenidone inhibited TGF-β expression and also inhibited the profibrotic effects of TGF-β signaling (187). Thus, pirfenidone might be a promising agent for the treatment of cardiac fibrosis. A reduction in ventricular hypertrophy without lowering systolic blood pressure has been detected in the deoxycorticosterone acetate (DOCA)-salt hypertensive rats after pirfenidone treatment (172). Moreover, pirfenidone decreased total and non-scar myocardial fibrosis, which has been associated with decreased infarct scarring, improved LV function, and decreased ventricular tachycardia in rat MI model (173). Administration of pirfenidone reversed cardiac fibrosis, including renal fibrosis, and attenuated myocardial stiffness in streptozotocin (STZ)-diabetic rats (176).

Given pirfenidone has significant antifibrotic and anti-inflammatory properties, the anti-inflammatory effects of pirfenidone have been investigated. Pirfenidone inhibited NLRP3 expression and formation, contributing to a reduction in IL-1β synthesis, and attenuation of IL-1β-induced inflammatory and profibrotic responses in a mouse model with transverse aortic constriction (TAC)-induced LV remodeling (174). Similar effects were observed in murine pressure-overload injury; pirfenidone increased survival and attenuated fibrosis through suppression

TABLE 5 | Preclinical studies of TGF-β inhibitors for treatment of cardiac fibrosis.

Agents	Targets	Experiments/Models	References
GW788388	ALK5 and TβRII	*In vivo*: murine Chagas disease	(170)
		In vivo: *Scn5a*$^{+/-}$ mouse model of cardiac conduction disease	(171)
		In vivo: rat model of heart failure (HF) following myocardial infarction (MI)	(50)
Pirfenidone	TGF-β	*In vivo*: Deoxycorticosterone acetate (DOCA)-salt hypertensive rats	(172)
		In vivo: rat MI model	(173)
		In vivo: Transverse aortic constriction (TAC)-induced left ventricular (LV) remodeling mouse model	(174)
		In vivo: TAC-induced pressure-overloaded HF model	(175)
		In vivo: Streptozotocin (STZ)-induced diabetic rats	(176)
Tranilast	TGF-β	*In vivo*: STZ-induced diabetic (mRen2)27 rats	(177, 178)
		In vivo: DOCA/salt and renovascular hypertensive rats	(179, 180)
		In vivo: LV remodeling post-MI rats	(181)
		In vivo: hypertensive (mRen2)27 rats	(182)

TABLE 6 | Clinical studies of TGF-β inhibitors for treatment of cardiac fibrosis.

Agents	Phase	Study design	Main findings	References
Pirfenidone	II	A double-blind placebo-controlled phase II study in hypertrophic cardiomyopathy associated with left ventricular diastolic function patients ($N = 50$)	Not available	NCT00011076
Pirfenidone	II	A double-blind, randomized, placebo-controlled phase II trial in patients with chronic heart failure with preserved ejection fraction (HFpEF) and myocardial fibrosis ($N = 129$)	Not available	NCT02932566
Tranilast	III	A double-blind, randomized, placebo-controlled phase III trial in 11,484 patients after percutaneous coronary intervention (PCI) (PRESTO)	Tranilast did not improve the quantitative measures of restenosis	(183)

of myocardial fibrosis and vascular permeability in pressure-overloaded hearts (175). Therefore, pirfenidone might be a potential treatment for cardiac fibrosis.

Clinical data

Although pirfenidone has shown efficacy in the treatment of idiopathic pulmonary fibrosis in humans (186), clinical trials for the treatment of cardiac fibrosis are ongoing and the results have not yet been published. A phase II study of pirfenidone in patients with hypertrophic cardiomyopathy associated with LV diastolic function aims to examine the effectiveness of pirfenidone in improving heart function and reducing of myocardial fibrosis. The study was completed with unpublished data (NCT00011076). Another phase II trial is ongoing and will finish in Jan 2020. This trial is exploring the antifibrotic effects of pirfenidone on patients with chronic heart failure with preserved ejection fraction (HFpEF) and cardiac fibrosis by determining changes in myocardial ECM volume and investigating the relationship between myocardial fibrosis and myocardial energetics (PIROUETTE study, NCT02932566) (188).

Tranilast

Preclinical data

Tranilast has been used to treat allergic disorders (e.g., allergic rhinitis, asthma, and atopic dermatitis); however, tranilast might also be useful for other medical conditions due to its ability to suppress TGF-β expression and activity. The molecular mechanisms underlying its antifibrotic actions are not completely understood, but tranilast might inhibit several profibrotic growth factors such as TGF-β and platelet-derived growth factor (PDGF) (22). The effects of tranilast on inhibition of cardiac fibrosis have also been supported by multiple animal models of cardiomyopathy. In STZ-induced (mRen-2)27 diabetic rats, tranilast treatment attenuated cardiac matrix deposition in association with reductions in phospho-Smad2 of the heart (177). In a similar model, administration of tranilast attenuated cardiac dysfunction and structural abnormalities in diabetic cardiomyopathy with improved LV systolic and diastolic function, while tranilast did not affect Smad phosphorylation but it significantly attenuated TGF-β-induced p44/42 MAPK phosphorylation (178).

The underlying mechanisms of the antifibrotic effects of tranilast have been attributed to its regulation of TGF-β signaling and to suppression of the infiltration of inflammatory cells, including monocytes and macrophages. The mRNA levels of TGF-β1, plasminogen activator inhibitor 1 (PAI-1), monocyte chemotactic protein-1 (MCP-1), IL-6, procollagens were attenuated, and myocardial fibrosis and collagen accumulation were suppressed in DOCA/salt hypertensive rats receiving tranilast (179). Similar findings were observed in other animal models of renovascular hypertensive rats (180) and hypertensive

(mRen-2)27 rats (182). Interestingly, tranilast-mediated inhibition of cardiac fibrosis is independent of changes in blood pressure in these studies, suggesting that tranilast directly targeted cardiac fibrosis and might be beneficial for HF treatment in addition to current therapeutic strategies (181).

Clinical data

Restenosis after percutaneous coronary intervention (PCI) is a major adverse outcome following stent placement. In limited trials, administration of tranilast reduced the frequency of angiographic restenosis after PCI (189). Accordingly, the Prevention of Restenosis With Tranilast and Its Outcomes (PRESTO) trial was designed as a phase III trial with a large group of patients after PCI to investigate major adverse cardiovascular events of tranilast. It was found that tranilast did not improve restenosis or its clinical sequelae in patients receiving successful PCI (183). However, the number of events of MI was significantly reduced with tranilast treatment. The most commonly reported adverse events were laboratory test abnormalities consisting of hyperbilirubinemia, elevations in hepatic enzymes, and increased serum creatinine (183).

CONCLUSION

TGF-β is a multifunctional cytokine regulator acting through transmembrane serine/threonine kinase receptors and intracellular Smad transcriptional regulators. Once TGF-β is activated, it regulates ECM remodeling and promotes a fibroblast to myofibroblast transition, which is essential for fibrotic processes. Given TGF-β plays a major role in various stages of cancer progression and in the development of cardiac fibrosis, TGF-β and its signaling pathway offer opportunities for novel treatment strategies in patients with cancer and cardiac fibrosis. Research on the underlying mechanisms and the therapeutic targets of TGF-β inhibitors for cancer and cardiac fibrosis has advanced significantly in recent decades. The inhibitors of TGF-β signaling for cancer and fibrosis have been extensively studied in animal models and clinical studies; however, translation of these findings into human pathologic conditions has been limited due to the broad range of responses to TGF-β and its role in tissue homeostasis. Currently, various types of TGF-β inhibitors are challenged and tested their efficacies in patients with cancers. A few of TGF-β inhibitors are subjected into the clinical studies for treatment of cardiac fibrosis. The development of more specific agents targeting TGF-β signaling pathways such as M7824, a bifunctional fusion protein composed of TGF-β trap, and a monoclonal antibody against programmed death ligand 1 (PD-L1) are likely to help minimize potential side effects and enhances efficacy for treatment of cancers. Furthermore, the combination of anti-TGF-β therapies with various mechanisms of action might have greater efficacy against cancer and cardiac fibrosis.

AUTHOR CONTRIBUTIONS

WP, TL, and SM wrote the manuscript. HK reviewed and edited. All authors agree to submit the manuscript.

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REFERENCES

1. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor-β in human disease. *N Engl J Med*. (2000) 342:1350–8. doi: 10.1056/NEJM200005043421807
2. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-β: the master regulator of fibrosis. *Nat Rev Nephrol*. (2016) 12:325–8. doi: 10.1038/nrneph.2016.48
3. Roberts AB. Molecular and cell biology of TGF-beta. *Miner Electrolyte Metab*. (1998) 24:111–9. doi: 10.1159/000057358
4. Smith AL, Robin TP, Ford HL. Molecular pathways: targeting the TGF-β pathway for cancer therapy. *Clin Cancer Res*. (2012) 18:4514–21. doi: 10.1158/1078-0432.CCR-11-3224
5. Xie F, Ling L, van Dam H, Zhou F, Zhang L. TGF-β signaling in cancer metastasis. *Acta Biochim Biophys Sin*. (2018) 50:121–32. doi: 10.1093/abbs/gmx123
6. Walton KL, Johnson KE, Harrison CA. Targeting TGF-β mediated SMAD signaling for the prevention of fibrosis. *Front Pharmacol*. (2017) 8:461. doi: 10.3389/fphar.2017.00461
7. Batlle E, Massagué J. Transforming growth factor-β signaling in immunity and cancer. *Immunity*. (2019) 50:924–40. doi: 10.1016/j.immuni.2019.03.024
8. Pardali E, ten Dijke P. Transforming growth factor-β signaling and tumor angiogenesis. *Front Biosci*. (2009) 14:4848–61. doi: 10.2741/3573
9. Haque S, Morris JC. Transforming growth factor-β: a therapeutic target for cancer. *Hum Vaccin Immunother*. (2017) 13:1741–50. doi: 10.1080/21645515.2017.1327107
10. Khan R, Sheppard R. Fibrosis in heart disease: understanding the role of transforming growth factor-β in cardiomyopathy, valvular disease and arrhythmia. *Immunology*. (2006) 118:10–24. doi: 10.1111/j.1365-2567.2006.02336.x
11. Biernacka A, Dobaczewski M, Frangogiannis NG. TGF-β signaling in fibrosis. *Growth Fact*. (2011) 29:196–202. doi: 10.3109/08977194.2011.595714
12. Pohlers D, Brenmoehl J, Löffler I, Müller CK, Leipner C, Schultze-Mosgau S, et al. TGF-beta and fibrosis in different organs- molecular pathway imprints. *Biochim Biophys Acta*. (2009) 1792:746–56. doi: 10.1016/j.bbadis.2009.06.004
13. Najafi F, Jamrozik K, Dobson AJ. Understanding the 'epidemic of heart failure: a systematic review of trends in determinants of heart failure. *Eur J Heart Fail*. (2009) 11:472–9. doi: 10.1093/eurjhf/hfp029
14. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling- concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. *J Am Coll Cardiol*. (2000) 35:569–82. doi: 10.1016/S0735-1097(99)00630-0
15. Norton GR, Tsotetsi J, Trifunovic B, Hartford C, Candy GP, Woodiwiss AJ. Myocardial stiffness is attributed to alterations in cross-linked collagen rather than total collagen or phenotypes in spontaneously hypertensive rats. *Circulation*. (1997) 96:1991–8. doi: 10.1161/01.CIR.96.6.1991
16. Liu J, Masurekar MR, Vatner DE, Jyothirmayi GN, Regan TJ, Vatner SF, et al. Glycation end-product cross-link breaker reduces collagen and improves cardiac function in aging diabetic heart. *Am J Physiol Heart Circ Physiol*. (2003) 285:H2587–91. doi: 10.1152/ajpheart.00516.2003

17. Yue Y, Meng K, Pu Y, Zhang X. Transforming growth factor-β (TGF-β) mediates cardiac fibrosis and induces diabetic cardiomyopathy. *Diabetes Res Clin Pract*. (2017) 133:124–30. doi: 10.1016/j.diabres.2017.08.018
18. Voskoboinik A, Costello BT, La Gerche A, Prabhu S, Wong G, Flannery MD, et al. Relation of alcohol consumption to left ventricular fibrosis using cardiac magnetic resonance imaging. *Am J Cardiol*. (2019) 123:460–5. doi: 10.1016/j.amjcard.2018.10.026
19. El Hajj EC, El Hajj MC, Voloshenyuk TG, Mouton AJ, Khoutorova E, Molina PE, et al. Alcohol modulation of cardiac matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs favors collagen accumulation. *Alcohol Clin Exp Res*. (2014) 38:448–56. doi: 10.1111/acer.12239
20. Ma ZG, Yuan YP, Wu HM, Zhang X, Tang QZ. Cardiac fibrosis: new insights into the pathogenesis. *Int J Biol Sci*. (2018) 14:1645–57. doi: 10.7150/ijbs.28103
21. Russo I, Frangogiannis NG. Diabetes-associated cardiac fibrosis: cellular effectors, molecular mechanisms and therapeutic opportunities. *J Mol Cell Cardiol*. (2016) 90:84–93. doi: 10.1016/j.yjmcc.2015.12.011
22. Edgley AJ, Krum H, Kelly DJ. Targeting fibrosis for the treatment of heart failure: a role for transforming growth factor-β. *Cardiovasc Ther*. (2012) 30:e30–40. doi: 10.1111/j.1755-5922.2010.00228.x
23. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. *Cell Mol Life Sci*. (2014) 71:549–74. doi: 10.1007/s00018-013-1349-6
24. DeVita VT Jr, Rosenberg SA. Two hundred years of cancer research. *N Engl J Med*. (2012) 366:2207–14. doi: 10.1056/NEJMra1204479
25. Shewach DS, Kuchta RD. Introduction to cancer chemotherapeutics. *Chem Rev*. (2009) 109:2859–61. doi: 10.1021/cr900208x
26. Lumachi F, Luisetto G, Basso SM, Basso U, Brunello A, Camozzi V. Endocrine therapy of breast cancer. *Curr Med Chem*. (2011) 18:513–22. doi: 10.2174/092986711794480177
27. Tammela TA. Endocrine treatment of prostate cancer. *J Steroid Biochem Mol Biol*. (2004) 92:287–95. doi: 10.1016/j.jsbmb.2004.10.005
28. Baudino TA. Targeted cancer therapy: the next generation of cancer treatment. *Curr Drug Discov Technol*. (2015) 12:3–20. doi: 10.2174/1570163812666150602144310
29. Zhang H, Chen J. Current status and future directions of cancer immunotherapy. *J Cancer*. (2018) 9:1773–81. doi: 10.7150/jca.24577
30. Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B. The different mechanisms of cancer drug resistance: a brief review. *Adv Pharm Bull*. (2017) 7:339–48. doi: 10.15171/apb.2017.041
31. Frangogiannis NG. Pathophysiology of myocardial infarction. *Compr Physiol*. (2015) 5:1841–75. doi: 10.1002/cphy.c150006
32. Kass DA, Bronzwaer JG, Paulus WJ. What mechanisms underlie diastolic dysfunction in heart failure? *Circ Res*. (2004) 94:1533–42. doi: 10.1161/01.RES.0000129254.25507.d6
33. Burlew BS, Weber KT. Cardiac fibrosis as a cause of diastolic dysfunction. *Herz*. (2002) 27:92–8. doi: 10.1007/s00059-002-2354-y
34. López B, Querejeta R, González A, Larman M, Díez J. Collagen cross-linking but not collagen amount associates with elevated filling pressures in hypertensive patients with stage C heart failure: potential role of lysyl oxidase. *Hypertension*. (2012) 60:677–83. doi: 10.1161/HYPERTENSIONAHA.112.196113
35. Woodiwiss AJ, Tsotetsi OJ, Sprott S, Lancaster EJ, Mela T, Chung ES, et al. Reduction in myocardial collagen cross-linking parallels left ventricular dilatation in rat models of systolic chamber dysfunction. *Circulation*. (2001) 103:155–60. doi: 10.1161/01.CIR.103.1.155
36. Spach MS, Boineau JP. Microfibrosis produces electrical load variations due to loss of side-to-side cell connections: a major mechanism of structural heart disease arrhythmias. *Pacing Clin Electrophysiol*. (1997) 20:397–413. doi: 10.1111/j.1540-8159.1997.tb06199.x
37. de Bakker JM, van Capelle FJ, Janse MJ, Tasseron S, Vermeulen JT, de Jonge N, et al. Fractionated electrograms in dilated cardiomyopathy: origin and relation to abnormal conduction. *J Am Coll Cardiol*. (1996) 27:1071–8. doi: 10.1016/0735-1097(95)00612-5
38. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol*. (2002) 3:349–63. doi: 10.1038/nrm809
39. Sappino AP, Schürch W, Gabbiani G. Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. *Lab Invest*. (1990) 63:144–61.
40. Willems IE, Havenith MG, De Mey JG, Daemen MJ. The alpha-smooth muscle actin-positive cells in healing human myocardial scars. *Am J Pathol*. (1994) 145:868–75.
41. van Putten S, Shafieyan Y, Hinz B. Mechanical control of cardiac myofibroblasts. *J Mol Cell Cardiol*. (2016) 93:133–42. doi: 10.1016/j.yjmcc.2015.11.025
42. Travers JG, Kamal FA, Robbins J, Yutzey KE, Blaxall BC. Cardiac fibrosis: the fibroblast awakens. *Circ Res*. (2016) 118:1021–40. doi: 10.1161/CIRCRESAHA.115.306565
43. Frangogiannis NG. Cardiac fibrosis: cell biological mechanisms, molecular pathways and therapeutic opportunities. *Mol Aspects Med*. (2019) 65:70–99. doi: 10.1016/j.mam.2018.07.001
44. Kurose H, Mangmool S. Myofibroblasts and inflammatory cells as players of cardiac fibrosis. *Arch Pharm Res*. (2016) 39:1100–13. doi: 10.1007/s12272-016-0809-6
45. Baudino TA, Carver W, Giles W, Borg TK. Cardiac fibroblasts: friend or foe? *Am J Physiol Heart Circ Physiol*. (2006) 291:H1015–26. doi: 10.1152/ajpheart.00023.2006
46. Fan D, Takawale A, Lee J, Kassiri Z. Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. *Fibrogene Tissue Repair*. (2012) 5:15. doi: 10.1186/1755-1536-5-15
47. Kuwahara F, Kai H, Tokuda K, Kai M, Takeshita A, Egashira K, et al. Transforming growth factor-β function blocking prevents myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. *Circulation*. (2002) 106:130–5. doi: 10.1161/01.CIR.0000020689.12472.E0
48. Ono K, Ohtomo T, Ninomiya-Tsuji J, Tsuchiya M. A dominant negative TAK1 inhibits cellular fibrotic responses induced by TGF-β. *Biochem Biophys Res Commun*. (2003) 307:332–7. doi: 10.1016/S0006-291X(03)01207-5
49. See F, Thomas W, Way K, Tzanidis A, Kompa A, Lewis D, et al. p38 mitogen-activated protein kinase inhibition improves cardiac function and attenuates left ventricular remodeling following myocardial infarction in the rat. *J Am Coll Cardiol*. (2004) 44:1679–89. doi: 10.1016/j.jacc.2004.07.038
50. Tan SM, Zhang Y, Connelly KA, Gilbert RE, Kelly DJ. Targeted inhibition of activin receptor-like kinase 5 signaling attenuates cardiac dysfunction following myocardial infarction. *Am J Physiol Heart Circ Physiol*. (2010) 298:H1415–25. doi: 10.1152/ajpheart.01048.2009
51. Engebretsen KV, Skårdal K, Bjørnstad S, Marstein HS, Skrbic B, Sjaastad I, et al. Attenuated development of cardiac fibrosis in left ventricular pressure overload by SM16, an orally active inhibitor of ALK5. *J Mol Cell Cardiol*. (2014) 76:148–57. doi: 10.1016/j.yjmcc.2014.08.008
52. Lucas JA, Zhang Y, Li P, Gong K, Miller AP, Hassan E, et al. Inhibition of transforming growth factor-β signaling induces left ventricular dilation and dysfunction in the pressure-overloaded heart. *Am J Physiol Heart Circ Physiol*. (2010) 298:H424–32. doi: 10.1152/ajpheart.00529.2009
53. Xavier S, Piek E, Fujii M, Javelaud D, Mauviel A, Flanders KC, et al. Amelioration of radiation-induced fibrosis: inhibition of transforming growth factor-β signaling by halofuginone. *J Biol Chem*. (2004) 279:15167–76. doi: 10.1074/jbc.M309798200
54. Shimada YJ, Passeri JJ, Baggish AL, O'Callaghan C, Lowry PA, Yannekis G, et al. Effects of losartan on left ventricular hypertrophy and fibrosis in patients with nonobstructive hypertrophic cardiomyopathy. *JACC Heart Fail*. (2013) 1:480–7. doi: 10.1016/j.jchf.2013.09.001
55. Brilla CG, Funck RC, Rupp H. Lisinopril-mediated regression of myocardial fibrosis in patients with hypertensive heart disease. *Circulation*. (2000) 102:1388–93. doi: 10.1161/01.CIR.102.12.1388
56. Galie PA, Russell MW, Westfall MV, Stegemann JP. Interstitial fluid flow and cyclic strain differentially regulate cardiac fibroblast activation via AT1R and TGF-β1. *Exp Cell Res*. (2012) 318:75–84. doi: 10.1016/j.yexcr.2011.10.008
57. Singh AD, Amit S, Kumar OS, Rajan M, Mukesh N. Cardioprotective effects of bosentan, a mixed endothelin type A and B receptor antagonist, during myocardial ischaemia and reperfusion in rats. *Basic Clin Pharmacol Toxicol*. (2006) 98:604–10. doi: 10.1111/j.1742-7843.2006.pto_405.x
58. Ammarguellat F, Larouche I, Schiffrin EL. Myocardial fibrosis in DOCA-salt hypertensive rats: effect of endothelin ET_A receptor antagonism. *Circulation*. (2001) 103:319–24. doi: 10.1161/01.CIR.103.2.319

59. Wakeno M, Minamino T, Seguchi O, Okazaki H, Tsukamoto O, Okada K, et al. Long-term stimulation of adenosine A_2B receptors begun after myocardial infarction prevents cardiac remodeling in rats. *Circulation*. (2006) 114:1923–32. doi: 10.1161/CIRCULATIONAHA.106.630087
60. Phosri S, Arieyawong A, Bunrukchai K, Parichatikanond W, Nishimura A, Nishida M, et al. Stimulation of adenosine A_2B receptor inhibits endothelin-1-induced cardiac fibroblast proliferation and α-smooth muscle actin synthesis through the cAMP/Epac/PI3K/Akt-signaling pathway. *Front Pharmacol*. (2017) 8:428. doi: 10.3389/fphar.2017.00428
61. Phosri S, Bunrukchai K, Parichatikanond W, Sato VH, Mangmool S. Epac is required for exogenous and endogenous stimulation of adenosine A_2B receptor for inhibition of angiotensin II-induced collagen synthesis and myofibroblast differentiation. *Purinergic Signal*. (2018) 14:141–56. doi: 10.1007/s11302-017-9600-5
62. Le Elizabeth D, Pascotto M, Leong-Poi H, Sari I, Micari A, Kaul S. Anti-inflammatory and pro-angiogenic effects of beta blockers in a canine model of chronic ischemic cardiomyopathy: comparison between carvedilol and metoprolol. *Basic Res Cardiol*. (2013) 108:384. doi: 10.1007/s00395-013-0384-7
63. Raake PW, Vinge LE, Gao E, Boucher M, Rengo G, Chen X, et al. G protein-coupled receptor kinase 2 ablation in cardiac myocytes before or after myocardial infarction prevents heart failure. *Circ Res*. (2008) 103:413–22. doi: 10.1161/CIRCRESAHA.107.168336
64. Nuamnaichati N, Sato VH, Moongkarndi P, Parichatikanond W, Mangmool S. Sustained β-AR stimulation induces synthesis and secretion of growth factors in cardiac myocytes that affect on cardiac fibroblast activation. *Life Sci*. (2018) 193:257–69. doi: 10.1016/j.lfs.2017.10.034
65. Heldin CH, Miyazono K, ten Dijke P. TGF-β signalling from cell membrane to nucleus through SMAD proteins. *Nature*. (1997) 390:465–71. doi: 10.1038/37284
66. Roth DA, Gold LI, Han VK, McCarthy JG, Sung JJ, Wisoff JH, et al. Immunolocalization of transforming growth factor-β1, -β2, and -β3 and insulin-like growth factor I in premature cranial suture fusion. *Plast Reconstr Surg*. (1997) 99:300–9. doi: 10.1097/00006534-199702000-00002
67. Dobaczewski M, Chen W, Frangogiannis NG. Transforming growth factor (TGF)-β signaling in cardiac remodeling. *J Mol Cell Cardiol*. (2011) 51:600–6. doi: 10.1016/j.yjmcc.2010.10.033
68. Shi M, Zhu J, Wang R, Chen X, Mi L, Walz T, et al. Latent TGF-β structure and activation. *Nature*. (2011) 474:343–9. doi: 10.1038/nature10152
69. Wrana JL, Attisano L, Wieser R, Ventura F, Massagué J. Mechanism of activation of the TGF-β receptor. *Nature*. (1994) 370:341–7. doi: 10.1038/370341a0
70. Loomans HA, Andl CD. Activin receptor-like kinases: a diverse family playing an important role in cancer. *Am J Cancer Res*. (2016) 6:2431–47.
71. Euler-Taimor G, Heger J. The complex pattern of SMAD signaling in the cardiovascular system. *Cardiovasc Res*. (2006) 69:15–25. doi: 10.1016/j.cardiores.2005.07.007
72. Khalil H, Kanisicak O, Prasad V, Correll RN, Fu X, Schips T, et al. Fibroblast-specific TGF-β-Smad2/3 signaling underlies cardiac fibrosis. *J Clin Invest*. (2017) 127:3770–83. doi: 10.1172/JCI94753
73. Zhao M, Mishra L, Deng CX. The role of TGF-β/SMAD4 signaling in cancer. *Int J Biol Sci*. (2018) 14:111–23. doi: 10.7150/ijbs.23230
74. Yan X, Liao H, Cheng M, Shi X, Lin X, Feng XH, et al. Smad7 protein interacts with receptor-regulated Smads (R-Smads) to inhibit transforming growth factor-β (TGF-β)/Smad signaling. *J Biol Chem*. (2016) 291:382–92. doi: 10.1074/jbc.M115.694281
75. Zhang YE. Non-Smad signaling pathways of the TGF-β family. *Cold Spring Harb Perspect Biol*. (2017) 9:a022129. doi: 10.1101/cshperspect.a022129
76. Xie L, Law BK, Chytil AM, Brown KA, Aakre ME, Moses HL. Activation of the Erk pathway is required for TGF-β1-induced EMT *in vitro*. *Neoplasia*. (2004) 6:603–10. doi: 10.1593/neo.04241
77. Bakin AV, Rinehart C, Tomlinson AK, Arteaga CL. p38 mitogen-activated protein kinase is required for TGFβ-mediated fibroblastic transdifferentiation and cell migration. *J Cell Sci*. (2002) 115:3193–206. Retrived from: https://jcs.biologists.org/content/115/15/3193
78. Yu L, Hébert MC, Zhang YE. TGF-β receptor-activated p38 MAP kinase mediates Smad-independent TGF-β responses. *EMBO J*. (2002) 21:3749–59. doi: 10.1093/emboj/cdf366
79. Ozdamar B, Bose R, Barrios-Rodiles M, Wang HR, Zhang Y, Wrana JL. Regulation of the polarity protein Par6 by TGFβ receptors controls epithelial cell plasticity. *Science*. (2005) 307:1603–9. doi: 10.1126/science.1105718
80. Bakin AV, Tomlinson AK, Bhowmick NA, Moses HL, Arteaga CL. Phosphatidylinositol 3-kinase function is required for transforming growth factor-β-mediated epithelial to mesenchymal transition and cell migration. *J Biol Chem*. (2000) 275:36803–10. doi: 10.1074/jbc.M005912200
81. Lamouille S, Derynck R. Cell size and invasion in TGF-β-induced epithelial to mesenchymal transition is regulated by activation of the mTOR pathway. *J Cell Biol*. (2007) 178:437–51. doi: 10.1083/jcb.200611146
82. Suzuki HI. MicroRNA control of TGF-β signaling. *Int J Mol Sci*. (2018) 19:1901. doi: 10.3390/ijms19071901
83. Robson CN, Gnanapragasam V, Byrne RL, Collins AT, Neal DE. Transforming growth factor-β1 up-regulates p15, p21 and p27 and blocks cell cycling in G1 in human prostate epithelium. *J Endocrinol*. (1999) 160:257–66. doi: 10.1677/joe.0.1600257
84. Yagi K, Furuhashi M, Aoki H, Goto D, Kuwano H, Sugamura K, et al. C-myc is a downstream target of the smad pathway. *J Biol Chem*. (2002) 277:854–61. doi: 10.1074/jbc.M104170200
85. Spender LC, O'Brien DI, Simpson D, Dutt D, Gregory CD, Allday MJ, et al. TGF-β induces apoptosis in human B cells by transcriptional regulation of BIK and BCL-XL. *Cell Death Differ*. (2009) 16:593–602. doi: 10.1038/cdd.2008.183
86. Kim SG, Jong HS, Kim TY, Lee JW, Kim NK, Hong SH, et al. Transforming growth factor-β1 induces apoptosis through Fas ligand-independent activation of the Fas death pathway in human gastric SNU-620 carcinoma cells. *Mol Biol Cell*. (2004) 15:420–34. doi: 10.1091/mbc.e03-04-0201
87. Zonneville J, Safina A, Truskinovsky AM, Arteaga CL, Bakin AV. TGF-β signaling promotes tumor vasculature by enhancing the pericyte-endothelium association. *BMC Cancer*. (2018) 18:670. doi: 10.1186/s12885-018-4587-z
88. Smith AL, Iwanaga R, Drasin DJ, Micalizzi DS, Vartuli RL, Tan AC, et al. The miR-106b-25 cluster targets Smad7, activates TGF-β signaling, and induces EMT and tumor initiating cell characteristics downstream of six1 in human breast cancer. *Oncogene*. (2012) 31:5162–71. doi: 10.1038/onc.2012.11
89. Metelli A, Wu BX, Fugle CW, Rachidi S, Sun S, Zhang Y, et al. Surface expression of TGFβ docking receptor GARP promotes oncogenesis and immune tolerance in breast cancer. *Cancer Res*. (2016) 76:7106–17. doi: 10.1158/0008-5472.CAN-16-1456
90. Takasaka N, Seed RI, Cormier A, Bondesson AJ, Lou J, Elattma A, et al. Integrin αvβ8-expressing tumor cells evade host immunity by regulating TGF-β activation in immune cells. *JCI Insight*. (2018) 3:e122591. doi: 10.1172/jci.insight.122591
91. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology*. (2004) 127:S35–50. doi: 10.1053/j.gastro.2004.09.014
92. Taguchi S. Comprehensive review of the epidemiology and treatments for malignant adult cardiac tumors. *Gen Thorac Cardiovasc Surg*. (2018) 66:257–62. doi: 10.1007/s11748-018-0912-3
93. Frangogiannis NG. The role of transforming growth factor (TGF)-β in the infarcted myocardium. *J Thorac Dis*. (2017) 9(Suppl. 1):S52–63. doi: 10.21037/jtd.2016.11.19
94. Dewald O, Ren G, Duerr GD, Zoerlein M, Klemm C, Gersch C, et al. Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. *Am J Pathol*. (2004) 164:665–77. doi: 10.1016/S0002-9440(10)63154-9
95. van Amerongen MJ, Harmsen MC, van Rooijen N, Petersen AH, van Luyn MJ. Macrophage depletion impairs wound healing and increases left ventricular remodeling after myocardial injury in mice. *Am J Pathol*. (2007) 170:818–29. doi: 10.2353/ajpath.2007.060547
96. Wünsch M, Sharma HS, Markert T, Bernotat-Danielowski S, Schott RJ, Kremer P, et al. *In situ* localization of transforming growth factor-β1 in porcine heart: enhanced expression after chronic coronary artery constriction. *J Mol Cell Cardiol*. (1991) 23:1051–62. doi: 10.1016/0022-2828(91)91640-D
97. Birdsall HH, Green DM, Trial J, Youker KA, Burns AR, MacKay CR, et al. Complement C5a, TGF-β1, and MCP-1, in sequence, induce migration of monocytes into ischemic canine myocardium within the

first one to five hours after reperfusion. *Circulation*. (1997) 95:684–92. doi: 10.1161/01.CIR.95.3.684
98. Sarrazy V, Koehler A, Chow ML, Zimina E, Li CX, Kato H, et al. Integrins αvβ5 and αvβ3 promote latent TGF-β1 activation by human cardiac fibroblast contraction. *Cardiovasc Res*. (2014) 102:407–17. doi: 10.1093/cvr/cvu053
99. Frangogiannis NG, Ren G, Dewald O, Zymek P, Haudek S, Koerting A, et al. Critical role of endogenous thrombospondin-1 in preventing expansion of healing myocardial infarcts. *Circulation*. (2005) 111:2935–42. doi: 10.1161/CIRCULATIONAHA.104.510354
100. Lefer AM, Tsao P, Aoki N, Palladino MA Jr. Mediation of cardioprotection by transforming growth factor-β. *Science*. (1990) 249:61–4. doi: 10.1126/science.2164258
101. Baxter GF, Mocanu MM, Brar BK, Latchman DS, Yellon DM. Cardioprotective effects of transforming growth factor-β1 during early reoxygenation or reperfusion are mediated by p42/p44 MAPK. *J Cardiovasc Pharmacol*. (2001) 38:930–9. doi: 10.1097/00005344-200112000-00015
102. Schröder D, Heger J, Piper HM, Euler G. Angiotensin II stimulates apoptosis via TGF-β1 signaling in ventricular cardiomyocytes of rat. *J Mol Med*. (2006) 84:975–83. doi: 10.1007/s00109-006-0090-0
103. Celada A, Maki RA. Transforming growth factor-β enhances the M-CSF and GM-CSF-stimulated proliferation of macrophages. *J Immunol*. (1992) 148:1102–5.
104. Gorelik L, Flavell RA. Abrogation of TGFβ signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity*. (2000) 12:171–81. doi: 10.1016/S1074-7613(00)80170-3
105. Dobaczewski M, Bujak M, Li N, Gonzalez-Quesada C, Mendoza LH, Wang XF, et al. Smad3 signaling critically regulates fibroblast phenotype and function in healing myocardial infarction. *Circ Res*. (2010) 107:418–28. doi: 10.1161/CIRCRESAHA.109.216101
106. Bujak M, Ren G, Kweon HJ, Dobaczewski M, Reddy A, Taffet G, et al. Essential role of Smad3 in infarct healing and in the pathogenesis of cardiac remodeling. *Circulation*. (2007) 116:2127–38. doi: 10.1161/CIRCULATIONAHA.107.704197
107. Krenning G, Zeisberg EM, Kalluri R. The origin of fibroblasts and mechanism of cardiac fibrosis. *J Cell Physiol*. (2010) 225:631–7. doi: 10.1002/jcp.22322
108. Duncan MR, Frazier KS, Abramson S, Williams S, Klapper H, Huang X, et al. Connective tissue growth factor mediates transforming growth factor-β-induced collagen synthesis: down-regulation by cAMP. *FASEB J*. (1999) 13:1774–86. doi: 10.1096/fasebj.13.13.1774
109. Eghbali M, Tomek R, Sukhatme VP, Woods C, Bhambi B. Differential effects of transforming growth factor-β1 and phorbol myristate acetate on cardiac fibroblasts. regulation of fibrillar collagen mRNAs and expression of early transcription factors. *Circ Res*. (1991) 69:483–90. doi: 10.1161/01.RES.69.2.483
110. Matsumoto-Ida M, Takimoto Y, Aoyama T, Akao M, Takeda T, Kita T. Activation of TGF-β1-TAK1-p38 MAPK pathway in spared cardiomyocytes is involved in left ventricular remodeling after myocardial infarction in rats. *Am J Physiol Heart Circ Physiol*. (2006) 290:H709–15. doi: 10.1152/ajpheart.00186.2005
111. Wang S, Wilkes MC, Leof EB, Hirschberg R. Imatinib mesylate blocks a non-Smad TGF-β pathway and reduces renal fibrogenesis *in vivo*. *FASEB J*. (2005) 19:1–11. doi: 10.1096/fj.04-2370com
112. Daniels CE, Wilkes MC, Edens M, Kottom TJ, Murphy SJ, Limper AH, et al. Imatinib mesylate inhibits the profibrogenic activity of TGF-β and prevents bleomycin-mediated lung fibrosis. *J Clin Invest*. (2004) 114:1308–16. doi: 10.1172/JCI200419603
113. Seeland U, Haeuseler C, Hinrichs R, Rosenkranz S, Pfitzner T, Scharffetter-Kochanek K, et al. Myocardial fibrosis in transforming growth factor-β1 (TGF-β1) transgenic mice is associated with inhibition of interstitial collagenase. *Eur J Clin Invest*. (2002) 32:295–303. doi: 10.1046/j.1365-2362.2002.00985.x
114. Frangogiannis NG. The immune system and the remodeling infarcted heart: cell biological insights and therapeutic opportunities. *J Cardiovasc Pharmacol*. (2014) 63:185–95. doi: 10.1097/FJC.0000000000000003
115. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med*. (2007) 13:952–61. doi: 10.1038/nm1613
116. Chen Y, Di C, Zhang X, Wang J, Wang F, Yan JF, et al. Transforming growth factor β signaling pathway: a promising therapeutic target for cancer. *J Cell Physiol*. (2020) 253:1903–14. doi: 10.1002/jcp.29108
117. Anderton MJ, Mellor HR, Bell A, Sadler C, Pass M, Powell S, et al. Induction of heart valve lesions by small-molecule ALK5 inhibitors. *Toxicol Pathol*. (2011) 39:916–24. doi: 10.1177/0192623311416259
118. Neuzillet C, Tijeras-Raballand A, Cohen R, Cros J, Faivre S, Raymond E, et al. Targeting the TGFβ pathway for cancer therapy. *Pharmacol Ther*. (2015) 147:22–31. doi: 10.1016/j.pharmthera.2014.11.001
119. Hau P, Jachimczak P, Schlingensiepen R, Schulmeyer F, Jauch T, Steinbrecher A, et al. Inhibition of TGF-β2 with AP 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies. *Oligonucleotides*. (2007) 17:201–12. doi: 10.1089/oli.2006.0053
120. Zhang C, Zhang F, Tsan R, Fidler IJ. Transforming growth factor-β2 is a molecular determinant for site-specific melanoma metastasis in the brain. *Cancer Res*. (2009) 69:828–35. doi: 10.1158/0008-5472.CAN-08-2588
121. Jonson T, Albrechtsson E, Axelson J, Heidenblad M, Gorunova L, Johansson B, et al. Altered expression of TGFβ receptors and mitogenic effects of TGFβ in pancreatic carcinomas. *Int J Oncol*. (2001) 19:71–81. doi: 10.3892/ijo.19.1.71
122. Schlingensiepen KH, Jaschinski F, Lang SA, Moser C, Geissler EK, Schlitt HJ, et al. Transforming growth factor-β2 gene silencing with trabedersen (AP 12009) in pancreatic cancer. *Cancer Sci*. (2011) 102:1193–200. doi: 10.1111/j.1349-7006.2011.01917.x
123. Bellone G, Carbone A, Tibaudi D, Mauri F, Ferrero I, Smirne C, et al. Differential expression of transforming growth factors-β1, -β2 and -β3 in human colon carcinoma. *Eur J Cancer*. (2001) 37:224–33. doi: 10.1016/S0959-8049(00)00391-9
124. Rowland-Goldsmith MA, Maruyama H, Kusama T, Ralli S, Korc M. Soluble type II transforming growth factor-beta (TGF-β) receptor inhibits TGF-β signaling in COLO-357 pancreatic cancer cells *in vitro* and attenuates tumor formation. *Clin Cancer Res*. (2001) 7:2931–40. Retrived from: https://clincancerres.aacrjournals.org/content/7/9/2931.article-info
125. Sakaguchi J, Kyo S, Kanaya T, Maida Y, Hashimoto M, Nakamura M, et al. Aberrant expression and mutations of TGF-β receptor type II gene in endometrial cancer. *Gynecol Oncol*. (2005) 98:427–33. doi: 10.1016/j.ygyno.2005.04.031
126. Copland JA, Luxon BA, Ajani L, Maity T, Campagnaro E, Guo H, et al. Genomic profiling identifies alterations in TGFβ signaling through loss of TGFβ receptor expression in human renal cell carcinogenesis and progression. *Oncogene*. (2003) 22:8053–62. doi: 10.1038/sj.onc.1206835
127. Finger EC, Turley RS, Dong M, How T, Fields TA, Blobe GC. TβRIII suppresses non-small cell lung cancer invasiveness and tumorigenicity. *Carcinogenesis*. (2008) 29:528–35. doi: 10.1093/carcin/bgm289
128. Bandyopadhyay A, López-Casillas F, Malik SN, Montiel JL, Mendoza V, Yang J, et al. Antitumor activity of a recombinant soluble betaglycan in human breast cancer xenograft. *Cancer Res*. (2002) 62:4690–5. Retrived from: https://cancerres.aacrjournals.org/content/62/16/4690
129. Calon A, Lonardo E, Berenguer-Llergo A, Espinet E, Hernando-Momblona X, Iglesias M, et al. Stromal gene expression defines poor-prognosis subtypes in colorectal cancer. *Nat Genet*. (2015) 47:320–9. doi: 10.1038/ng.3225
130. Alsina-Sanchis E, Figueras A, Lahiguera Á, Vidal A, Casanovas O, Graupera M, et al. The TGFβ pathway stimulates ovarian cancer cell proliferation by increasing IGF1R levels. *Int J Cancer*. (2016) 139:1894–903. doi: 10.1002/ijc.30233
131. Dituri F, Mazzocca A, Fernando J, Peidrò FJ, Papappicco P, Fabregat I, et al. Differential inhibition of the TGF-β signaling pathway in HCC cells using the small molecule inhibitor LY2157299 and the D10 monoclonal antibody against TGF-β receptor type II. *PLoS ONE*. (2013) 8:e67109. doi: 10.1371/journal.pone.0067109
132. Dzieran J, Fabian J, Feng T, Coulouarn C, Ilkavets I, Kyselova A, et al. Comparative analysis of TGF-β/Smad signaling dependent cytostasis in human hepatocellular carcinoma cell lines. *PLoS ONE*. (2013) 8:e72252. doi: 10.1371/journal.pone.0072252

133. Serova M, Tijeras-Raballand A, Dos Santos C, Albuquerque M, Paradis V, Neuzillet C, et al. Effects of TGF-β signalling inhibition with galunisertib (LY2157299) in hepatocellular carcinoma models and in *ex vivo* whole tumor tissue samples from patients. *Oncotarget*. (2015) 6:21614–27. doi: 10.18632/oncotarget.4308
134. Son JY, Park SY, Kim SJ, Lee SJ, Park SA, Kim MJ, et al. EW-7197, a novel ALK-5 kinase inhibitor, potently inhibits breast to lung metastasis. *Mol Cancer Ther*. (2014) 13:1704–16. doi: 10.1158/1535-7163.MCT-13-0903
135. Park CY, Son JY, Jin CH, Nam JS, Kim DK, Sheen YY. EW-7195, a novel inhibitor of ALK5 kinase inhibits EMT and breast cancer metastasis to lung. *Eur J Cancer*. (2011) 47:2642–53. doi: 10.1016/j.ejca.2011.07.007
136. Zhang B, Halder SK, Zhang S, Datta PK. Targeting transforming growth factor-β signaling in liver metastasis of colon cancer. *Cancer Lett*. (2009) 277:114–20. doi: 10.1016/j.canlet.2008.11.035
137. Mazzocca A, Fransvea E, Lavezzari G, Antonaci S, Giannelli G. Inhibition of transforming growth factor-β receptor I kinase blocks hepatocellular carcinoma growth through neo-angiogenesis regulation. *Hepatology*. (2009) 50:1140–51. doi: 10.1002/hep.23118
138. Ge R, Rajeev V, Ray P, Lattime E, Rittling S, Medicherla S, et al. Inhibition of growth and metastasis of mouse mammary carcinoma by selective inhibitor of transforming growth factor-β type I receptor kinase *in vivo*. *Clin Cancer Res*. (2006) 12:4315–30. doi: 10.1158/1078-0432.CCR-06-0162
139. Gaspar NJ, Li L, Kapoun AM, Medicherla S, Reddy M, Li G, et al. Inhibition of transforming growth factor-β signaling reduces pancreatic adenocarcinoma growth and invasiveness. *Mol Pharmacol*. (2007) 72:152–61. doi: 10.1124/mol.106.029025
140. Bogdahn U, Hau P, Stockhammer G, Venkataramana NK, Mahapatra AK, Suri A, et al. Targeted therapy for high-grade glioma with the TGF-β2 inhibitor trabedersen: results of a randomized and controlled phase IIb study. *Neuro Oncol*. (2011) 13:132–42. doi: 10.1093/neuonc/noq142
141. Nemunaitis J, Dillman, RO, Schwarzenberger, PO, Senzer, et al. Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. *J Clin Oncol*. (2006) 24:4721–30. doi: 10.1200/JCO.2005.05.5335
142. Nemunaitis J, Nemunaitis M, Senzer N, Snitz P, Bedell C, Kumar P, et al. Phase II trial of Belagenpumatucel-L, a TGF-β2 antisense gene modified allogeneic tumor vaccine in advanced non small cell lung cancer (NSCLC) patients. *Cancer Gene Ther*. (2009) 16:620–4. doi: 10.1038/cgt.2009.15
143. Giaccone G, Bazhenova LA, Nemunaitis J, Tan M, Juhász E, Ramlau R, et al. A phase III study of belagenpumatucel-L, an allogeneic tumour cell vaccine, as maintenance therapy for non-small cell lung cancer. *Eur J Cancer*. (2015) 51:2321–9. doi: 10.1016/j.ejca.2015.07.035
144. Stevenson JP, Kindler HL, Papasavvas E, Sun J, Jacobs-Small M, Hull J, et al. Immunological effects of the TGFβ-blocking antibody GC1008 in malignant pleural mesothelioma patients. *Oncoimmunology*. (2013) 2:e26218. doi: 10.4161/onci.26218
145. Formenti SC, Lee P, Adams S, Goldberg JD, Li X, Xie MW, et al. Focal irradiation and systemic TGFβ blockade in metastatic breast cancer. *Clin Cancer Res*. (2018) 24:2493–504. doi: 10.1158/1078-0432.CCR-17-3322
146. Melisi D, Garcia-Carbonero R, Macarulla T, Pezet D, Deplanque G, Fuchs M, et al. Galunisertib plus gemcitabine vs. gemcitabine for first-line treatment of patients with unresectable pancreatic cancer. *Br J Cancer*. (2018) 119:1208–14. doi: 10.1038/s41416-018-0246-z
147. Faivre SJ, Santoro A, Kelley RK, Merle P, Gane E, Douillard JY, et al. A phase 2 study of a novel transforming growth factor-β (TGF-β) receptor I kinase inhibitor, LY2157299 monohydrate (LY), in patients with advanced hepatocellular carcinoma (HCC). *J Clin Oncol*. (2014) 32:173. doi: 10.1200/jco.2014.32.3_suppl.lba173
148. Ryan CW, Matias C, Agulnik M, Lopez-Pousa A, Williams C, de Alwis DP, et al. A phase II study of tasisulam sodium (LY573636 sodium) as second-line or third-line treatment for patients with unresectable or metastatic soft tissue sarcoma. *Invest New Drugs*. (2013) 31:145–51. doi: 10.1007/s10637-012-9819-5
149. Schlingensiepen KH, Schlingensiepen R, Steinbrecher A, Hau P, Bogdahn U, Fischer-Blass B, et al. Targeted tumor therapy with the TGF-β2 antisense compound AP 12009. *Cytokine Growth Factor Rev*. (2006) 17:129–39. doi: 10.1016/j.cytogfr.2005.09.002
150. David JM, Dominguez C, Palena C. Pharmacological and immunological targeting of tumor mesenchymalization. *Pharmacol Ther*. (2017) 170:212–25. doi: 10.1016/j.pharmthera.2016.11.011
151. Morris JC, Tan AR, Olencki TE, Shapiro GI, Dezube BJ, Reiss M, et al. Phase I study of GC1008 (fresolimumab): a human anti-transforming growth factor-β (TGFβ) monoclonal antibody in patients with advanced malignant melanoma or renal cell carcinoma. *PLoS ONE*. (2014) 9:e90353. doi: 10.1371/journal.pone.0090353
152. Holmgaard RB, Schaer DA, Li Y, Castaneda SP, Murphy MY, Xu X, et al. Targeting the TGFβ pathway with galunisertib, a TGFβRI small molecule inhibitor, promotes anti-tumor immunity leading to durable, complete responses, as monotherapy and in combination with checkpoint blockade. *J Immunother Cancer*. (2018) 6:47. doi: 10.1186/s40425-018-0356-4
153. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, et al. TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature*. (2018) 554:544–8. doi: 10.1038/nature25501
154. de Gramont A, Faivre S, Raymond E. Novel TGF-β inhibitors ready for prime time in onco-immunology. *Oncoimmunology*. (2017) 6:e1257453. doi: 10.1080/2162402X.2016.1257453
155. Rodon J, Carducci MA, Sepulveda-Sánchez JM, Azaro A, Calvo E, Seoane J, et al. First-in-human dose study of the novel transforming growth factor-β receptor I kinase inhibitor LY2157299 monohydrate in patients with advanced cancer and glioma. *Clin Cancer Res*. (2015) 21:553–60. doi: 10.1158/1078-0432.CCR-14-1380
156. Kovacs RJ, Maldonado G, Azaro A, Fernández MS, Romero FL, Sepulveda-Sánchez JM, et al. Cardiac safety of TGF-β receptor I kinase inhibitor LY2157299 monohydrate in cancer patients in a first-in-human dose study. *Cardiovasc Toxicol*. (2015) 15:309–23. doi: 10.1007/s12012-014-9297-4
157. Gueorguieva I, Cleverly AL, Stauber A, Sada Pillay N, Rodon JA, Miles CP, et al. Defining a therapeutic window for the novel TGF-β inhibitor LY2157299 monohydrate based on a pharmacokinetic/pharmacodynamic model. *Br J Clin Pharmacol*. (2014) 77:796–807. doi: 10.1111/bcp.12256
158. Park SA, Kim MJ, Park SY, Kim JS, Lee SJ, Woo HA, et al. EW-7197 inhibits hepatic, renal, and pulmonary fibrosis by blocking TGF-β/Smad and ROS signaling. *Cell Mol Life Sci*. (2015) 72:2023–39. doi: 10.1007/s00018-014-1798-6
159. Song KM, Chung DY, Choi MJ, Ghatak K, Nguyen NM, Limanjaya A, et al. Vactosertib, a novel, orally bioavailable activin receptor-like kinase 5 inhibitor, promotes regression of fibrotic plaques in a rat model of Peyronie's disease. *World J Mens Health*. (2019). doi: 10.5534/wjmh.190071. [Epub ahead of print].
160. Lan Y, Zhang D, Xu C, Hance KW, Marelli B, Qi J, et al. Enhanced preclinical antitumor activity of M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-β. *Sci Transl Med*. (2018) 10:eaan5488. doi: 10.1126/scitranslmed.aan5488
161. Fang L, Murphy AJ, Dart AM. A clinical perspective of anti-fibrotic therapies for cardiovascular disease. *Front Pharmacol*. (2017) 8:186. doi: 10.3389/fphar.2017.00186
162. Tomita H, Egashira K, Ohara Y, Takemoto M, Koyanagi M, Katoh M, et al. Early induction of transforming growth factor-β via angiotensin II type 1 receptors contributes to cardiac fibrosis induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension*. (1998) 32:273–9. doi: 10.1161/01.HYP.32.2.273
163. Frantz S, Hu K, Adamek A, Wolf J, Sallam A, Maier SK, et al. Transforming growth factor-β inhibition increases mortality and left ventricular dilatation after myocardial infarction. *Basic Res Cardiol*. (2008) 103:485–92. doi: 10.1007/s00395-008-0739-7
164. Ihn H, Yamane K, Kubo M, Tamaki K. Blockade of endogenous transforming growth factor-β signaling prevents up-regulated collagen synthesis in scleroderma fibroblasts: association with increased expression of transforming growth factor-β receptors. *Arthritis Rheum*. (2001) 44:474–80. doi: 10.1002/1529-0131(200102)44:2<474::AID-ANR67>3.0.CO;2-#
165. Okada H, Takemura G, Kosai K, Li Y, Takahashi T, Esaki M, et al. Postinfarction gene therapy against transforming growth factor-β signal modulates infarct tissue dynamics and attenuates left ventricular remodeling and heart failure. *Circulation*. (2005) 111:2430–7. doi: 10.1161/01.CIR.0000165066.71481.8E

166. Liao P, Georgakopoulos D, Kovacs A, Zheng M, Lerner D, Pu H, et al. The *in vivo* role of p38 MAP kinases in cardiac remodeling and restrictive cardiomyopathy. *Proc Natl Acad Sci USA*. (2001) 98:12283–8. doi: 10.1073/pnas.211086598
167. Yan W, Wang P, Zhao CX, Tang J, Xiao X, Wang DW. Decorin gene delivery inhibits cardiac fibrosis in spontaneously hypertensive rats by modulation of transforming growth factor-β/Smad and p38 mitogen-activated protein kinase signaling pathways. *Hum Gene Ther*. (2009) 20:1190–200. doi: 10.1089/hum.2008.204
168. Zhang D, Gaussin V, Taffet GE, Belaguli NS, Yamada M, Schwartz RJ, et al. TAK1 is activated in the myocardium after pressure overload and is sufficient to provoke heart failure in transgenic mice. *Nat Med*. (2000) 6:556–63. doi: 10.1038/75037
169. Park S, Nguyen NB, Pezhouman A, Ardehali R. Cardiac fibrosis: potential therapeutic targets. *Transl Res*. (2019) 209:121–37. doi: 10.1016/j.trsl.2019.03.001
170. de Oliveira FL, Araújo-Jorge TC, de Souza EM, de Oliveira GM, Degrave WM, Feige JJ, et al. Oral administration of GW788388, an inhibitor of transforming growth factor-β signaling, prevents heart fibrosis in Chagas disease. *PLoS Negl Trop Dis*. (2012) 6:e1696. doi: 10.1371/journal.pntd.0001696
171. Derangeon M, Montnach J, Cerpa CO, Jagu B, Patin J, Toumaniantz G, et al. Transforming growth factor-β receptor inhibition prevents ventricular fibrosis in a mouse model of progressive cardiac conduction disease. *Cardiovasc Res*. (2017) 113:464–74. doi: 10.1093/cvr/cvx026
172. Mirkovic S, Seymour AM, Fenning A, Strachan A, Margolin SB, Taylor SM, et al. Attenuation of cardiac fibrosis by pirfenidone and amiloride in DOCA-salt hypertensive rats. *Br J Pharmacol*. (2002) 135:961–8. doi: 10.1038/sj.bjp.0704539
173. Nguyen DT, Ding C, Wilson E, Marcus GM, Olgin JE. Pirfenidone mitigates left ventricular fibrosis and dysfunction after myocardial infarction and reduces arrhythmias. *Heart Rhythm*. (2010) 7:1438–45. doi: 10.1016/j.hrthm.2010.04.030
174. Wang Y, Wu Y, Chen J, Zhao S, Li H. Pirfenidone attenuates cardiac fibrosis in a mouse model of TAC-induced left ventricular remodeling by suppressing NLRP3 inflammasome formation. *Cardiology*. (2013) 126:1–11. doi: 10.1159/000351179
175. Yamagami K, Oka T, Wang Q, Ishizu T, Lee JK, Miwa K, et al. Pirfenidone exhibits cardioprotective effects by regulating myocardial fibrosis and vascular permeability in pressure-overloaded hearts. *Am J Physiol Heart Circ Physiol*. (2015) 309:H512–22. doi: 10.1152/ajpheart.00137.2015
176. Miric G, Dallemagne C, Endre Z, Margolin S, Taylor SM, Brown L. Reversal of cardiac and renal fibrosis by pirfenidone and spironolactone in streptozotocin-diabetic rats. *Br J Pharmacol*. (2001) 133:687–94. doi: 10.1038/sj.bjp.0704131
177. Martin J, Kelly DJ, Mifsud SA, Zhang Y, Cox AJ, See F, et al. Tranilast attenuates cardiac matrix deposition in experimental diabetes: role of transforming growth factor-β. *Cardiovasc Res*. (2005) 65:694–701. doi: 10.1016/j.cardiores.2004.10.041
178. Kelly DJ, Zhang Y, Connelly K, Cox AJ, Martin J, Krum H, et al. Tranilast attenuates diastolic dysfunction and structural injury in experimental diabetic cardiomyopathy. *Am J Physiol Heart Circ Physiol*. (2007) 293:H2860–9. doi: 10.1152/ajpheart.01167.2006
179. Kagitani S, Ueno H, Hirade S, Takahashi T, Takata M, Inoue H. Tranilast attenuates myocardial fibrosis in association with suppression of monocyte/macrophage infiltration in DOCA/salt hypertensive rats. *J Hypertens*. (2004) 22:1007–15. doi: 10.1097/00004872-200405000-00024
180. Hocher B, Godes M, Olivier J, Weil J, Eschenhagen T, Slowinski T, et al. Inhibition of left ventricular fibrosis by tranilast in rats with renovascular hypertension. *J Hypertens*. (2002) 20:745–51. doi: 10.1097/00004872-200204000-00034
181. See F, Watanabe M, Kompa AR, Wang BH, Boyle AJ, Kelly DJ, et al. Early and delayed tranilast treatment reduces pathological fibrosis following myocardial infarction. *Heart Lung Circ*. (2013) 22:122–32. doi: 10.1016/j.hlc.2012.08.054
182. Pinto YM, Pinto-Sietsma SJ, Philipp T, Engler S, Kossamehl P, Hocher B, et al. Reduction in left ventricular messenger RNA for transforming growth factor-β1 attenuates left ventricular fibrosis and improves survival without lowering blood pressure in the hypertensive TGR(mRen2)27 rat. *Hypertension*. (2000) 36:747–54. doi: 10.1161/01.HYP.36.5.747
183. Holmes DJ, Savage M, LaBlanche JM, Grip L, Serruys PW, Fitzgerald P, et al. Results of prevention of REStenosis with tranilast and its outcomes (PRESTO) trial. *Circulation*. (2002) 106:1243–50. doi: 10.1161/01.CIR.0000028335.31300.DA
184. Gellibert F, de Gouville AC, Woolven J, Mathews N, Nguyen VL, Bertho-Ruault C, et al. Discovery of 4-{4-[3-(pyridin-2-yl)-1H-pyrazol-4-yl]pyridin-2-yl}-N-(tetrahydro-2H- pyran-4-yl)benzamide (GW788388): a potent, selective, and orally active transforming growth factor-β type I receptor inhibitor. *J Med Chem*. (2006) 49:2210–21. doi: 10.1021/jm0509905
185. Petersen M, Thorikay M, Deckers M, van Dinther M, Grygielko ET, Gellibert F, et al. Oral administration of GW788388, an inhibitor of TGF-β type I and II receptor kinases, decreases renal fibrosis. *Kidney Int*. (2008) 73:705–15. doi: 10.1038/sj.ki.5002717
186. King TE, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med*. (2014) 370:2083–92. doi: 10.1056/NEJMoa1402582
187. Iyer SN, Gurujeyalakshmi G, Giri SN. Effects of pirfenidone on transforming growth factor-β gene expression at the transcriptional level in bleomycin hamster model of lung fibrosis. *J Pharmacol Exp Ther*. (1999) 291:367–73.
188. Lewis GA, Schelbert EB, Naish JH, Bedson E, Dodd S, Eccleson H, et al. Pirfenidone in heart failure with preserved ejection fraction-rationale and design of the PIROUETTE trial. *Cardiovasc Drugs Ther*. (2019) 33:461–70. doi: 10.1007/s10557-019-06876-y
189. Tamai H, Katoh O, Suzuki S, Fujii K, Aizawa T, Takase S, et al. Impact of tranilast on restenosis after coronary angioplasty: tranilast restenosis following angioplasty trial (TREAT). *Am Heart J*. (1999) 138:968–75. doi: 10.1016/S0002-8703(99)70025-6

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REVIEW
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Cardiotoxicity of Anthracyclines

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Cardiotoxicity is a feared side effect that may limit the clinical use of anthracyclines. It may indeed affect the quality of life and survival of patients with cancer, regardless of oncological prognosis. This paper provides an overview of anthracycline-induced cardiotoxicity in terms of definition, classification, incidence, risk factors, possible mechanisms, diagnosis, and treatment. We also report effective strategies for preventing cardiotoxicity. In addition, we discuss limiting current approaches, the need for a new classification, and early cardiotoxicity detection and treatment. Probably, anthracycline-induced cardiotoxicity is a continuous phenomenon that starts from myocardial cell injury; it is followed by left ventricular ejection fraction (LVEF) and, if not diagnosed and cured early, progressively leads to symptomatic heart failure. Anthracycline-induced cardiotoxicity can be detected at a preclinical phase. The role of biomarkers, in particular troponins, in identifying subclinical cardiotoxicity and its therapy with angiotensin-converting enzyme inhibitors (mainly enalapril) to prevent LVEF reduction is a recognized and effective strategy. If cardiac dysfunction has already occurred, partial or complete LVEF recovery may still be obtained in case of early detection of cardiotoxicity and prompt heart failure treatment.

Keywords: cardiotoxicity, anthracyclines, early detection, troponin, prevention, reversibility, ACE-inhibitors, beta-blockers

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INTRODUCTION

Anthracyclines are cytostatic antibiotics (1), introduced into the clinical field in the 1960s. As of 2012, anthracyclines were among the most diffused chemotherapeutic agents, and they still represent the base of treatment in many solid cancers and hematological malignancies (1, 2).

Unfortunately, anthracyclines are considered the principal culprit drugs behind chemotherapy-induced cardiotoxicity (1–5). The pathognomonic manifestation of anthracycline-induced cardiotoxicity is a hypokinetic cardiomyopathy progressively leading to heart failure, first described in 1967 (6). The onset of anthracycline-cardiomyopathy, also at the pre-clinical stage, may negatively affect the cardiovascular outcome of patients as also limit the chemotherapeutic strategies (4, 5).

INCIDENCE AND RISK FACTORS

The risk of anthracycline-induced heart failure increases as the cumulative dose administered increases: 3–5% with 400 mg/m^2 and as high as 18–48% at 700 mg/m^2 (4). However, there is a different level of risk for each patient scheduled for anthracycline therapy: patients less than 5 years old or more than 65 years old, with prior or concurrent chest irradiation, pre-existing heart disease, or already known cardiovascular risk factors, have an increased risk for cardiotoxicity

TABLE 1 | Baseline risk factors for anthracycline-induced cardiotoxicity (4, 7).

Current myocardial disease	Demographic and other CV risk factors
• Heart failure • Asymptomatic LV dysfunction (LVEF <50%) • Evidence of CAD (previous myocardial infarction, angina, PCI or CABG, myocardial ischemia) • Moderate and severe VHD with LVH or LV impairment • Hypertensive heart disease with LV hypertrophy • Hypertrophic cardiomyopathy • Dilated cardiomyopathy • Restrictive cardiomyopathy • Cardiac sarcoidosis with myocardial involvement • Significant cardia arrhythmias (AF, ventricular tachyarrhythmias)	• Age (<5 or >65 years) • Family history of premature CV disease (<50 years) • Arterial hypertension • Diabetes mellitus • Hypercholesterolemia
Previous cardiotoxic cancer treatment	**Lifestyle risk factors**
• Prior anthracycline use • Prior radiotherapy to chest or mediastinum	• Smoking • High alcohol intake • Obesity • Sedentary habit

AF, atrial fibrillation; CABG, coronary artery bypass graft; CAD, coronary artery disease; CV, cardiovascular; LV, left ventricular; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; PCI, percutaneous coronary intervention; VHD, valvular heart disease.

(**Table 1**) (4, 7). Moreover, anthracycline-induced cardiotoxicity risk increases with the use of other agents that may increase its incidence. In particular, trastuzumab, while very effective in treating breast cancer, interferes with myocyte survival pathways, crucial in countering the toxic effects of anthracyclines (5, 7, 8).

MECHANISMS

The specific mechanisms of anthracycline cardiotoxicity still remain unclear. A potential mechanism is the generation of reactive oxygen species (ROS), changes in iron metabolism, and Ca2þ signaling. In 2014, topoisomerase (Top) 2β was indicated as the critical mediator of anthracycline's cardiac toxic effect (9). Top2 can uncoil deoxyribonucleic acid (DNA) filaments during DNA replication, transcription, or recombination. The anthracycline inhibition of Top2β causes mitochondrial dysfunction and leads to activation of cell death pathways and ROS deposit (2, 3, 10).

The cardiomyocyte has always been considered the main cellular target of anthracycline toxic effect in the heart, as their destruction results in the progressive development of cardiac dysfunction. More recently, however, other cell types—such as cardiac progenitor cells, cardiac fibroblasts, and endothelial cells—have been identified as potential additional targets, creating a more complex and intriguing scenario in the pathogenesis of anthracycline-induced cardiomyopathy (**Figure 1**) (11). So far, the principal mechanisms, with potential differential impact and grade of involvement in different cell types, are oxidative stress, DNA damage, senescence, and cell death.

CLASSIFICATION

A previous and more dated classification identified three distinct types of anthracycline-induced cardiotoxicity (**Table 2**): acute, occurring after a single dose, or a single course, with the onset of symptoms within 14 days from the end of treatment, which is usually reversible; early-onset chronic, occurring within 1 year, the principal form of cardiotoxicity, from a clinical and epidemiological stand-point, presenting as a dilated-hypokinetic cardiomyopathy, with progressive evolution toward heart failure; and late-onset chronic, developing years, possibly decades, after the end of anthracycline therapy. The two chronic forms are considered irreversible, with a poor prognosis and a limited to heart failure therapy. This classification stems back to early 1980s, and it is mainly based on small retrospective studies reporting the occurrence of heart failure symptoms in childhood cancer survivors (12–14). In particular, in a milestone study, Steinherz et al. reported cases of heart failure occurrence many years after the end of anthracycline-chemotherapy, and the percentage of patients with cardiac dysfunction, as well as the severity of the dysfunction itself, increased in parallel with time elapsed from the end of anthracycline administration (14). However, the clinical relevance of such a classification at present is uncertain, especially when referred to adult populations.

In particular, recent findings challenge this old classification, suggesting that anthracycline-induced cardiotoxicity is potentially a continuous phenomenon, starting at the myocardial cell level, followed by progressive functional decline, progressively leading to overt heart failure. (**Figure 2**) (5, 8, 15). To be practical, anthracycline-associated cardiotoxicity is now thought to occur at the time of first exposure, a hypothesis supported by the finding of troponin release after anthracycline administration (16). Clinical presentation may occur years later the initial damage (16–18). Looking at symptoms, the diagnosis may take years ("late" cardiotoxicity). Considering LVEF reduction, it may take months ("early" cardiotoxicity). With the use of circulating biomarkers, such as troponin (pre-clinical myocardial cell damage), prompt identification of cardiotoxicity is possible, allowing for an "acute" form. So far, we are probably observing the evolving stages of the same phenomenon and not three distinct diseases (15, 17, 18).

DIAGNOSIS AND DEFINITION

The diagnosis of anthracycline-induced cardiotoxicity has remained the same over the last 60 years. It has always been based on heart failure symptoms, and, later, also on evidence of LVEF drop (echocardiography or multi-gated acquisition scans) (4, 18). A former definition adopted was an LVEF absolute decrease higher than 10% points, associated with a decline <50% (5). More recently, the consensus [Plana et al. (19)] defined it

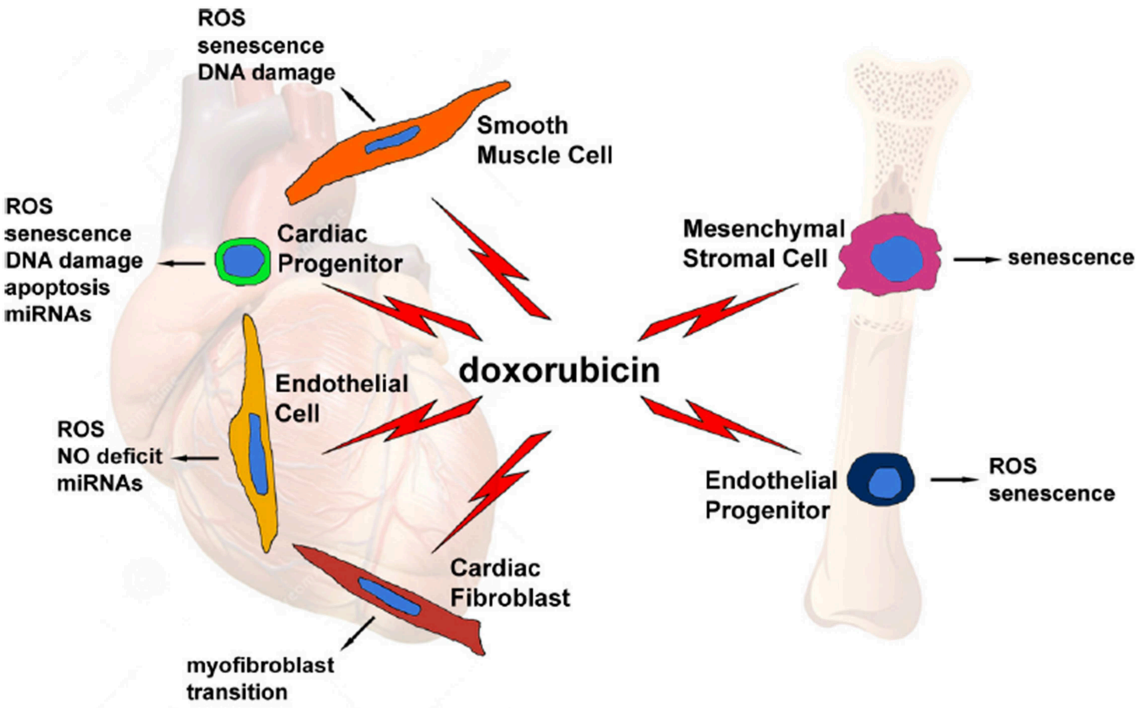

FIGURE 1 | Graphical representation of several doxorubicin-targeted cell types, with potential side effects and cellular and molecular events evoked by the drug. From Cappetta et al. (11).

as an LVEF decrying >10% points, with a final value <53% (19). In patients at low risk—i.e., without risk factors or a negative cardiovascular history, with an indication to receive a low dose of anthracyclines (total cumulative dose ≤240 mg/m²) or standard dose followed by trastuzumab-based regimens—cardiac monitoring is not suggested by the American Society of Clinical Oncology guidelines. Moreover, they suggest a diagnosis of cardiotoxicity based on clinical symptoms (20). Reasons comprise "medicalization, the possibility of causing stress and anxiety, and costs" to be incurred (20, 21). Otherwise, the international cardiological guidelines recommend monitoring of cardiac function by serial LVEF measurements, but do not provide an accurate indication on timing, frequency, modalities, and long-term schedule (7). Moreover, a diagnosis based on symptoms or asymptomatic decrease of LVEF is not only delayed, but also potentially prevents any form of effective prevention, as the cardiac damage may be no longer reversible (17, 18).

A recent study evaluating a significant (n = 2,625) population scheduled for anthracycline therapy showed that close monitoring of LVEF after chemotherapy allowed nearly all (98%) cases of cardiotoxicity to be identified within the first 12 months of follow-up (15). In addition, early treatment with angiotensin-converting enzyme (ACE)-inhibitors (enalapril) and beta-blockers (carvedilol or bisoprolol) enabled normalization of cardiac function in most cases (82%), but only 11% of patients who had renormalized LVEF had full recovery—i.e., the same LVEF value as before the start of anthracyclines—while the final LVEF value in 71% of patients remained below the baseline value (**Figure 3**).

TABLE 2 | Old classification of anthracycline-induced cardiotoxicity (7, 12–14).

Characteristics	Acute cardiotoxicity	Early-onset chronic cardiotoxicity	Late-onset chronic cardiotoxicity
Onset	During or within 2 weeks after AC treatment	Within 1 year after the completion of AC treatment	>1 year after the completion of AC treatment
Dose dependent	Unknown	Yes	Yes
Clinical features	Depression of myocardial contractility	Dilated/Hypokinetic cardiomyopathy	Dilated/Hypokinetic cardiomyopathy
Course	Usually reversible	Usually irreversible	Usually irreversible
		Refractory to traditional heart failure therapy	Refractory to traditional heart failure therapy
		Poor prognosis	Poor prognosis

These findings confirm that this approach is limited in identifying reversible cardiotoxicity, probably because left ventricular compensation mechanisms have been exhausted (8). Of great importance, the evidence of a normal LVEF does not exclude the risk of future deterioration of cardiac function.

TREATMENT

The historical concept that anthracycline-induced cardiotoxicity is irreversible, with a reported mortality rate up to 60% within 2

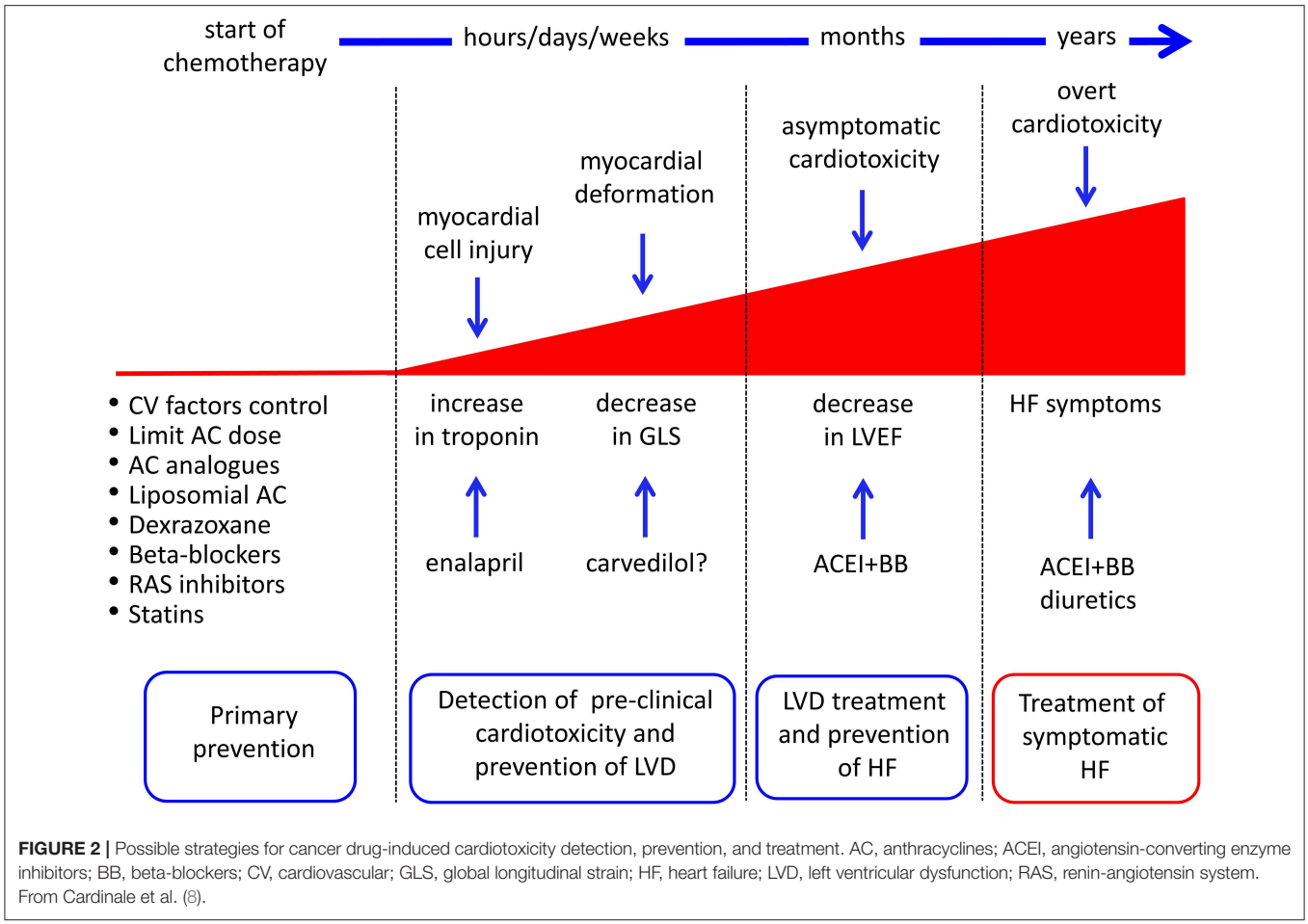

FIGURE 2 | Possible strategies for cancer drug-induced cardiotoxicity detection, prevention, and treatment. AC, anthracyclines; ACEI, angiotensin-converting enzyme inhibitors; BB, beta-blockers; CV, cardiovascular; GLS, global longitudinal strain; HF, heart failure; LVD, left ventricular dysfunction; RAS, renin-angiotensin system. From Cardinale et al. (8).

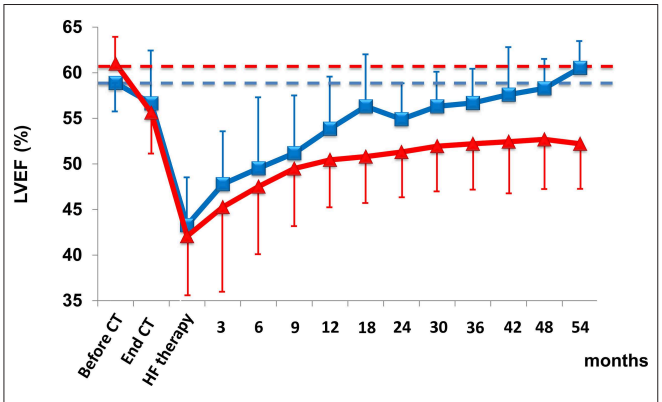

FIGURE 3 | LVEF in patients with cardiotoxicity and with partial (triangle) or full (square) recovery with heart failure therapy. Data are mean ± SD. CT, chemotherapy; HF, heart failure. From Cardinale et al. (15).

years of diagnosis, is now reconsidered. In particular, this belief is based on seminal studies in which heart failure therapeutic strategies were limited (i.e., digoxin, diuretics), or on studies with small populations, retrospective design, short follow-up, or on case reports (22–30).

Up until 2010, the response to heart failure therapy of patients with anthracycline-induced cardiotoxicity hadn't been thoroughly investigated. Moreover, these kind of patients have been excluded from large randomized trials evaluating the impact of current heart failure therapies (8).

The effectiveness of ACE-inhibitors and beta-blockers has been prospectively assessed in two extensive papers (15, 31). In 201 patients with anthracycline-induced cardiotoxicity, an inverse relationship in terms of LVEF improvement has been found between the time interval from the end of chemotherapy and the beginning of heart failure therapy (**Figure 4A**) (31). LVEF recovery rate was 64% in those treated early (i.e., within 2 months after the end of chemotherapy); later on, however, this percentage rapidly decreased, with no complete recovery after 6 months. After 12 months, obtaining even partial LVEF improvement was almost impossible (**Figure 4B**) (31). It emerges that cardiotoxicity is not irreversible, but that reversibility is a matter of time, depending on early diagnosis, allowing prompt treatment. Furthermore, these findings, based on standard cardiac symptoms surveillance, might miss this change (8).

On the contrary, close monitoring and timely treatment with HF therapies have reported that they are critical for functional recovery in a non-selected population treated with anthracycline, allowing early detection of cardiotoxicity in the

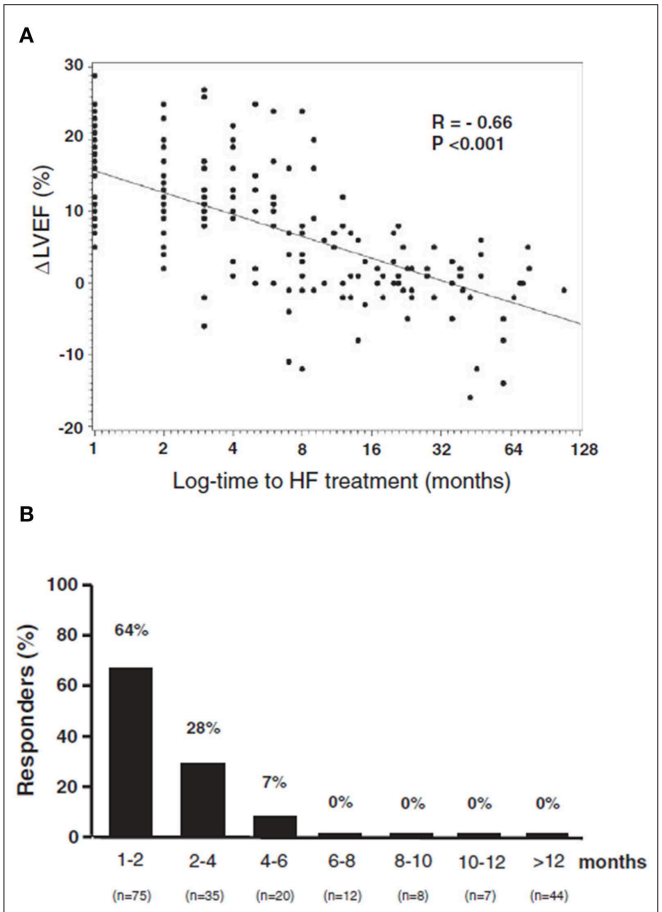

FIGURE 4 | (A) Percentage of patients who recovered (Responders), according to the time elapsed from anthracycline administration and the start of heart failure therapy. **(B)** Relationship between maximal LVEF during the follow-up period and log time elapsed from chemotherapy and the start of treatment [time-to-heart failure (HF) treatment]. From Cardinale et al. (31).

vast majority of cases during the first year after chemotherapy, with normalization of LVEF (final value of LVEF >50%) in 82% of cases (15). However, only 11% of patients had a complete restoration (i.e., final LVEF equal to baseline). This highlights the need for detection methods able to identify early cardiotoxicity and for strategies aimed at preventing the development and the progression of left ventricular dysfunction.

PRECLINICAL EARLY DETECTION

Today, at an early preclinical stage, we can detect cardiotoxicity long before symptoms of heart failure occur and before an asymptomatic drop in LVEF. Most data relate to cardiac biochemical markers: mainly troponins and echocardiography of tissue Doppler and strain (5, 7, 8).

Troponin Assessment in Anthracycline-Treated Patients

Troponin may be considered the gold standard biomarker for myocardial injury and cardiotoxicity from different causes/etiologies (32). Troponin has many advantages: elevated cardiac specificity, high sensitivity, availability, and costs respective to imaging methods. Moreover, there are limited variability issues. In this field, several studies have demonstrated that troponins may detect cardiotoxicity in patients treated with anthracyclines (**Table 3**) (33–56).

The most extensive study included 703 cancer patients, in whom Troponin I (TnI) was assessed before and during the first 72 h after chemotherapy (early TnI), and after 1 month (late TnI) (38). Three different troponin release patterns were recognized: Troponin I remained within the normal interval in 72% of patients, rose at only early evaluation in 21%, and increased at early and late assessments in 9%. Patients with no rise in troponin showed little difference in LVEF and had a good prognosis, with a low incidence of significant adverse heart events (MACE) (1%) during follow-up. Alternatively, TnI-positive patients had a higher rate of MACE: In particular, severe cardiac dysfunction and a higher rate of MACE were associated with a persistent TnI elevation compared to patients with only a temporary rise ($p < 0.001$). Based on the high negative predictive value (99%), TnI has been able to safely identify low-risk patients, limiting the need and subsequent costs of close long-term cardiac monitoring (34, 35, 38). Conversely, TnI-positive patients deserve more stringent monitoring, mainly those showing a persistent TnI increase.

In summary, we can assert that troponin evaluation in patients treated with anthracyclines allows for:

1. Prediction of the development of future left ventricular dysfunction;
2. Prediction of left ventricular dysfunction severity, because the peak value of troponin is closely related to the extent of LVEF reduction;
3. Stratification of cardiac risk after anthracyclines and tailoring of the schedule of post-chemotherapy monitoring of cardiac function;
4. Identification of cardiotoxicity prone patients, in whom a cardioprotective therapy can be considered; and
5. Exclusion of most patients from prolonged cardiologic surveillance.

On the other hand, the identified weakness points are:

1. Repeated assessments of troponins are needed to detect positivity;
2. The ideal timing for troponin detection must still be defined;
3. Standardization of routine troponin use in this clinical setting is a current need; and
4. Timing in which a single sampling of troponin could be obtained.

Other Circulating Biomarkers

Although patients with pre-treatment levels of natriuretic peptides (BNP and N-terminal prohormone) tend to experience cardiac events (including cardiac dysfunction), the results are sparse (44, 57, 58).

More recently (57), BNP levels were shown to be significantly higher after every anthracycline cycle in

TABLE 3 | Clinical studies demonstrating Troponins as predictor of anticancer drug-induced left ventricular dysfunction (33–56).

Study (year)	Patients (n.)	Cancer type	Drugs	Troponin type	Cut off	Timing of assessment
Lipshultz et al. (33)	15*	ALL	AC	T	0.03 ng/mL	Before CT; 1–3 days after each dose
Cardinale et al. (34)	201	Various	HD CT	I	0.04 ng/ml	0–12–24–36–72 h after CT
Cardinale et al. (35)	232	Breast cancer	HD CT	I	0.04 ng/ml	0–12–24–36–72 h after CT
Auner et al. (36)	30	Hematological	HD Cycl	T	0.03 ng/ml	Before CT; 1–14 days after CT
Sandri et al. (37)	179	Various	HD CT	I	0.04 ng/ml	0–12–24–36–72 h after CT
Cardinale et al. (38)	703	Various	HD CT	I	0.04 ng/ml	0–12–24–36–72 h after CT
Specchia et al. (39)	79	Hematological	AC	I	0.15 ng/ml	Before CT; weekly x 4 times
Kilickap et al. (40)	41	Various	AC	T	0.10 ng/ml	Before CT; 3–5 days after 1st and last dose
Lee et al. (41)	86	Hematological	AC	I	0.20 ng/ml	Before each dose
Schmidinger et al. (42)	74	Renal cancer	Sunitinib/sorafenib	T	0.02	Before CT, bimonthly, symptoms occurrence
Cardinale et al. (43)	251	Breast cancer	AC, TRZ	I	0.04 ng/ml	Before and after each cycle
Sawaya et al. (44)	43	Breast cancer	AC+taxanes+TRZ	HS-I	0.015 ng/ml	Before CT; after 3 and 6 months during CT
Lipshultz et al. (45)	205*	ALL	AC/AC+dexrazoxane	I/T	Any detectable amount	Before CT; 1–7 days after each dose; end CT
Sawaya et al. (46)	81	Breast cancer	AC+taxanes+TRZ	HS-I	30 pg/mL	Before CT; after 3 and 6 months during CT
Draft et al. (47)	53	Various	AC	I	0.06 ng/ml	Before CT; after 1, 3, 6 months
Mornos et al. (48)	74	Various	AC	HS-T	NA	Before CT; after 6, 12, 24, 52 weeks
Mavinkurve-Groothuis et al. (49)	60*	ALL	AC	HS-T	0.01 ng/mL	Before CT; after 3 and 12 months
Ky et al. (50)	78	Breast cancer	AC+taxanes+TRZ	HS-I	NA	Before CT; after 3 and 6 months during CT
Mornos et al. (51)	92	Various	AC	HS-T	NA	Before CT; after 12 and 36 weeks
Putt et al. (52)	78	Breast cancer	AC+taxanes+TRZ	HS-I	NA	Before CT; every 3 months (max 15 months)
Zardavas et al. (54)	412	Breast cancer	AC+taxanes+TRZ	HS-T/US-I	14 ng/L/40 ng/L	Before CT; week 13, 25, 52; month 18, 24, 30, 36
Olivieri et al. (54)	99	Lymphoma	AC/lipoAC	US-I	0.08 ng/ml	Before CT; 1, 24–72 h after each cycle
Kitayama et al. (55)	40	Breast cancer	AC/AC+TRZ/TRZ	HS-T	NA	Before CT; every 3 months during CT
Shafi et al. (56)	82	Breast cancer	AC	US-I	NA	1, 24 h after each cycle

*AC, anthracycline-containing chemotherapy; ALL, acute lymphoblastic leukemia; CT, chemotherapy; Cycl, cyclophosphamide; HD, high-dose; LAP, lapatinib; lipoAC, liposomial anthracycline; NA, not available; I, troponin I; T, troponin T; TRZ, trastuzumab; HS, high-sensitive; US, ultra-sensitive *, pediatric population.*

subjects following cardiac events, while another study demonstrated an association between an increased BNP at 72 h after chemotherapy and a decrease of LVEF at 1 year (59).

Actually, studies of other biomarkers, including microRNAs (miRNAs), C-reactive protein (CRP), growth differentiation factor-15 (GDF-15), myeloperoxidase (MPO), and galectin-3 (Gal-3) have not demonstrated an association between pretreatment biomarker levels and cardiovascular outcomes (60, 61).

Regarding monitoring for cardiovascular toxicity during therapy, CRP has shown conflicting findings (50).

More recently, some reports emerged in the field of microRNAs, in particular for miR-1, showing a trend to earlier detection of cardiotoxicity respective to troponin (62, 63).

Another study of patients during a period of 10 years after anthracycline therapy did not find an association with Gal-3 and LV dysfunction.

A separate study that included Gal-3 and ST2 found no association with these biomarkers and LVEF 1 year after therapy (64, 65).

Tissue Doppler and Strain Echocardiography

Novel echocardiographic methods have emerged as sensitive parameters in the early identification of cardiotoxicity. In particular, introduction of tissue Doppler and strain imaging techniques can detect early subclinical changes in cardiac function, before LVEF falls (4, 7, 19, 51). In this respect, myocardial deformation (strain imaging) has emerged as a sensitive marker for earlier detection of myocardial dysfunction. In particular, 2D (and more recently, 3D) speckle tracking imaging, allowing the evaluation of global myocardial deformation in the longitudinal axis (global longitudinal strain, GLS, %), has become a clinical standard. Several papers demonstrated the value of GLS in detecting subclinical myocardial dysfunction, with prognostic relevance in terms of overt LV dysfunction in cancer patients (66–69).

The recent ASE/EACVI consensus defined a relative decrease in GLS of >15% from baseline as an indicator of subclinical LV dysfunction and appropriate use criteria for multi-modality imaging include strain for the evaluation of patient candidates for chemotherapy (19, 70). Finally, the SUCCOUR trial (first randomized controlled trial of GLS-guided therapy introduction)

will better define the role of GLS for surveillance for chemotherapy-related cardiac dysfunction (71).

However, these methodologies are not always readily available in all laboratories and seldom used in the routine evaluation of patients receiving anthracyclines (8).

An Integrated Approach to Biomarkers and Cardiac Imaging

Breakthroughs in laboratory technology have allowed for the introduction of more specific and sensitive troponin assay methods (55), which are able to measure minimal amounts (high-sensitivity [HS] dosing systems) of a biomarker that were not detectable with previous methods. This is of pivotal importance, since troponin release as a consequence of anthracycline cardiotoxicity may be minimal, and it is essential to use high-precision dosing systems (72).

The first HS troponin trial enrolled 45 breast cancer patients who were treated with anthracyclines, taxanes, and trastuzumab (44). International and regional myocardial function was assessed at baseline, every 3 months, with tissue Doppler and strain imaging, combined with troponin. A reduction in the longitudinal strain and an increase in HS troponin were predictive of late left ventricular dysfunction after the end of anthracyclines. Notably, the combined assessment of imaging methods and changes in troponin resulted in an increased specificity (93% combined vs. 73% for each single method). Ky et al. tested a multi-marker approach in a similar population of breast cancer patients receiving the same anti-cancer therapy regimen (50). All levels of the markers increased significantly from baseline (except for NT-proBNP and Galectin-3). However, at the end of anthracycline therapy, only HS troponin absolute values and changes in troponin and myeloperoxidase levels resulted as predictors of further development of left ventricular dysfunctions.

PRIMARY PREVENTION: REDUCTION OF THE DIRECT CARDIOTOXIC EFFECT (FIGURE 2)

Limitation of the Maximum Dose of Anthracyclines

Present oncologic guidelines recommend limiting the total cumulative dose of anthracyclines to 450–550 mg/ml (4, 8). However, this may limit the effectiveness of anti-cancer treatment. Moreover, significant variability exists in terms of proneness to anthracycline cardiotoxicity, suggesting that genetic variation might modulate the risk (5, 7, 8).

Use of Less Cardiotoxic Anthracycline Analogs

Epirubicin, idarubicin, and mitoxantrone are analogs of anthracyclines that are less cardiotoxic than conventional anthracyclines. Epirubicin cardiotoxicity occurs after higher doses of doxorubicin. However, to obtain the same clinical response, higher doses must be given. In preclinical studies and animal models, idarubicin and mitoxantrone also showed a less cardiotoxic profile than doxorubicin (5, 7).

Use of Liposomal Anthracyclines

In the heart, liposomes cannot get out from the vascular space because capillaries have tight junctions. As the tendency to accumulate in the heart cells is limited, this may reduce the risk of cardiotoxicity. On the contrary, the liposomes reach high concentrations in the tumor site, leaving the circulatory system where tumor growth damages the capillaries itself (73, 74).

PRIMARY PREVENTION: PHARMACOLOGIC PREVENTION (FIGURE 2)

Lifestyle Measures

Before pharmacologic strategies, primary prevention starts indeed with lifestyle corrective measures.

Since a strong link exists between cancer and cardiovascular risk factor, addressing smoking and sedentary habits (potentially leading to obesity, with a detrimental role especially in the post-menopausal women), as well as high alcohol intake, is pivotal. A healthy diet has been associated with a protective effect in terms of cancer relapses and cardiovascular disease, while smoking has an ominously detrimental effect. While light to moderate alcohol intake has shown a protective impact in terms of cardiovascular disease, the results in terms of risk of developing cardiotoxicity are conflicting (75–77).

Of notice, several pieces of evidence emerged on the protective role of exercise training (and eventually, cardiac rehabilitation) against cardiotoxicity (78).

The Use of Cardioprotection

The use of cardioprotective drugs to reduce the direct cardiotoxic effect is a potential alternative to anthracycline treatment modifications, dosage limitations, or interruptions (4, 5, 8).

The hypothesis that iron chelators may reduce the cardiotoxicity induced by anthracyclines suggests that dexrazoxane may be a clinically useful cardioprotective agent (9, 79). Doxorubicin is a potent Top2 inhibitor. In the clinical scenario, many studies demonstrated that dexrazoxane significantly reduces cardiotoxicity in adults and pediatric populations: Patients treated with dexrazoxane had a significantly lower incidence of heart failure than untreated patients. Apart from patients with metastatic breast cancer treated with doses of doxorubicin >300 mg/mq and despite previous findings, dexrazoxane is not routinely used in clinical practice, because suspected of interfering with the anti-tumor effects and by the occurrence of secondary malignancies. In September 2011, the outcome of a referral (80) that recommended several restrictions on dexrazoxane use in both children and adults with cancer was published. However, several new trials on the benefit-risk of dexrazoxane have been published from then (81–83). So far, dexrazoxane results an effective cardioprotector when administered with anthracycline chemotherapy being not associated with a reduction in anti-tumor efficacy or survival or a relevant increased risk of second primary malignancies, and can

be recommended as a cardioprotector particularly for children and adolescents for whom the development of anthracycline-induced cardiotoxicity could have a crucial prognostic impact. These studies contributed to the CHMP's decision to remove the contraindication on Cardioxane (84).

Macedo et al. recently published a systematic review and meta-analysis of nine trials (seven randomized and two retrospective non-randomized trials) on the efficacy of dexrazoxane in patients with breast cancer treated with anthracyclines (with or without trastuzumab). Despite the quality of available evidence remaining low, dexrazoxane was shown to reduce the risk of heart failure and cardiac events, independently from previous exposure to anthracyclines. The oncological response and survival rates were not affected by dexrazoxane (85).

Other potentially cardioprotective agents have been studied in animal models and small clinical studies. Preliminary data are promising, but they need to be ratified by further extensive studies (2, 5, 7, 8).

The Use of Cardiovascular Agents

Several heart failure drugs have been shown to be effective in terms of cardioprotection against anthracyclines (**Table 4**) (86–98).

Overall, a recent meta-analysis of randomized clinical trials of adult patients that underwent chemotherapy and cardiovascular therapies vs. placebo with follow-up (17 trials, 1,984 patients) showed higher (although with small changes) LVEF values at follow-up in cancer patients receiving neurohormonal therapies (99).

Beta-Blockers

The non-cardioselective beta-blocker carvedilol is cardioprotective against anthracyclines toxicity. *In vitro* studies and a small randomized clinical trial, the drug was able to prevent the development of ventricular dysfunction (86). In breast cancer patients, carvedilol blunted strain abnormalities and the increase in troponin, preserving diastolic function, after anthracycline use (100). However, the drug failed to prevent an LVEF reduction >10% (101). It appears that carvedilol's efficacy is linked to its antioxidant activity rather than its beta-blocking action. Indeed, a comparative study of carvedilol and atenolol, a selective β1 antagonist with no antioxidant properties, showed that carvedilol—but not atenolol—prevented mitochondrial damage and mitigated the ultrastructural changes associated with doxorubicin (8, 102).

Nebivolol, a selective β1 antagonist with vasodilatory properties, started 7 days before anthracyclines and continued for 6 months in 27 patients with breast cancer prevented a significant decrease of LVEF and an increase of NT-proBNP (87). In a retrospective study including 106 breast cancer patients, a reduced incidence of heart failure over a 5-year follow-up period was associated with the continuation of beta-blocker therapy during oncology treatment—including anthracyclines (88). Existing data indicate, from preclinical studies, that

TABLE 4 | Cardiovascular drugs showing a prophylactic effect against anticancer therapy-induced LVD in adult cancer populations.

Study (year)	Study design/follow-up	*N*	Cancer type	Drugs	Intervention	Results
BETA-BLOCKERS						
Kalay et al. (86)	RCT/6 months	50	Various	AC	Carvedilol	No LVEF↓
Kaya et al. (87)	RCT/6 months	45	Breast cancer	AC	Nebivolol	No LVEF and NT-proBNP↑
Seicean et al. (88)	Retrospective/5 years	318	Breast cancer	AC,TRZ	Beta-blockers	HF ↓
Pituskin et al. (89)	RCT/12 months	99	Breast cancer	CT+TRZ	Bisoprolol	No LVEF ↓
ACEI						
Cardinale et al. (90)	RCT/12 months	114	Various	HD CT	Enalapril	No LVEF ↓; MACE incidence ↓
Pituskin et al. (89)	RCT/12 months	99	Breast cancer	CT+TRZ	Perindopril	No LVEF ↓
ARB						
Nakamae et al. (91)	RCT/7 days	40	NHL	AC	Valsartan	No LVEDD↑; no BNP and ANP↑; no QT↑
Cadeddu et al. (92)	RCT/18 months	49	Various	AC	Telmisartan	No peak strain rate ↓; no interleukin-6↑
Gulati et al. (93)	RCT/1.5–16 months	120	Breast cancer	AC+Tx+TRZ	Candesartan	No LVEF ↓
ALDOSTERONE ANTAGONISTS						
Akpek et al. (94)	RCT/6 months	83	Breast cancer	AC	Spironolactone	No LVEF↓; no TNI and BNP↑;
ACEI + BETA-BLOCKERS						
Bosh et al. (95)	RCT/6 months	90	Hematological	AC	Enalapril + carvedilol	No LVEF↓; death↓; HF ↓
STATINS						
Acar et al. (96)	RCT/6 months	40	Hematological	AC	Atorvastatin	No LVEF↓
Seicean et al. (97)	Retrospective/5 years	67	Breast cancer	AC	Statins	No HF ↓
Chotenimitkhun et al. (98)	PO	51	Various	AC	Atorvastatin/simvastatin	No LVEF↓

ACEI, angiotensin-converting enzyme inhibitor; ANP, atrial natriuretic peptide; ARB, angiotensin receptor blocker; BNP, brain natriuretic peptide; HD CT, high-dose chemotherapy; LVD, left ventricular dysfunction; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; HF, heart failure; MACE, major adverse cardiac events; NHL, non Hodgkin lymphoma; NT-proBNP, N-terminal-proBNP; QT, QT interval; PO, prospective observational; RCT, randomized controlled trial; Tx, taxanes; TNI, troponin I; TRZ, trastuzumab.

cardio-specific beta blockers offer superior protection against anthracycline damage than non-cardioselective ones (8).

ACE-Inhibitors and Sartans

Experimental data demonstrated a crucial role of the renin-angiotensin system (RAS) in the development and progression of cardiomyopathy induced by anthracyclines (90). Valsartan, administered in combination with anthracyclines, blunted natriuretic peptides increase, the increase in chamber size in patients with non-Hodgkin's lymphoma treated doxorubicin (91). The authors hypothesized a direct inhibition of the drug, independent from hemodynamic effects (8, 90).

Telmisartan, started before epirubicin, was able to prevent strain reduction and inflammatory markers increase because of its RAS blocking action, but also because of its anti-inflammatory and anti-oxidant properties (92).

In the PRADA (Prevention of Cardiac Dysfunction during Adjuvant Breast Cancer Therapy) trial candesartan—but not metoprolol—administrated with adjuvant chemotherapy including anthracyclines, with or without trastuzumab, can protect against an early decline in LVEF, assessed with cardiac MRI (93).

The MANTICORE-101 study (Multi-disciplinary Approach to Novel Therapies in Cardiology Oncology Research) tested the use of perindopril vs. bisoprolol in the prevention of left ventricular remodeling, defined as an increase in end-diastolic diameters and primary study point, and of left ventricular dysfunction in HER2+ breast cancer patients treated with trastuzumab prior to anthracycline (89). Neither drug prevent left ventricular remodeling; however, the use of both drugs was associated with a preserved left-ventricular function in multi-variate analysis.

The combination of enalapril and carvedilol have been tested in the OVERCOME study (preventiOn of left-ventricular dysfunction with enalapril and carvedilol). The study involved 90 patients treated with anthracyclines, with malignant hemopathies. LVEF didn't change in the intervention group after 6 months, but decreased significantly in controls. In addition, the intervention group had a lower rate of combined death or heart failure or death, heart failure, and a final LVEF of <45 % (95).

Aldosterone Antagonists

A recent randomized trial, including 43 breast cancer patients, evaluated the use of spironolactone vs. placebo. Spironolactone was started 1 week before anthracyclines. Three weeks after the end of chemotherapy, the treated group did not show relevant variations in LVEF and rise in troponin I and NT-proBNP (94). In ELEVATE (Effect of Eplerenone on Left Ventricular Diastolic Function in Women Receiving Anthracyclines for Breast Cancer), a recent randomized placebo-controlled trial, administration of eplerenone for 6 months was not associated with significant differences in ventricular function compared with placebo in patients with breast cancer treated with anthracyclines (103).

Statins

The effect of statins on cardiotoxicity of anthracyclines is most likely due to their pleiotropic effect, and in particular, to their antioxidant properties (8). Forty hematologic cancer patients with no history of heart disease were randomized to receive atorvastatin or placebo before the onset of anthracyclines (95). The dosage was 40 mg/day, regardless of the levels of cholesterol, and lasted for 6 months. During the follow-up, a reduction of the high-sensitivity reactive C protein level and no significant changes in LVEF were observed in the statin group.

Conversely, the LVEF value in the control group resulted in a significant reduction from the baseline. The protective effect of statins also emerged when chemotherapy was started in patients already receiving statins for the prevention of cardiovascular disease (96). In a retrospective observational study of 67 breast cancer patients treated with anthracycline, statin therapy continued to be associated with significant reduction in the risk of heart failure and cardiac-related mortality during follow-up. More recently, patients on statin therapy for the prevention of cardiovascular disease reported a smaller drop in LVEF at 6 months in a retrospective observational study, including 51 patients with breast cancer or hematological malignancies treated with anthracyclines (97).

Perspectives

A recent study identified the molecular and cellular signature of dose-dependent, doxorubicin-mediated cardiotoxicity and provided evidence that prokineticin receptor (PKR-1)-1, acting at myocardial and vascular level, is a promising target to combat cardiotoxicity of cancer treatments (104).

Since G protein-coupled receptors (GPCRs) are a target of 40% of clinically used drugs and newly identified cardioprotective agents that bind GPCRs of adrenalin, adenosine, melatonin, ghrelin, galanin, gpelin, prokineticin, and cannabidiol may further aid in the cardioprotective task (105).

PREVENTION IN SELECTED HIGH-RISK PATIENTS

Prevention may be an option for all patients who are candidates for cardiotoxic therapy (primary prevention) or restricted to patients with preclinical symptoms of cardiotoxicity, with the advantage of limiting prophylactic therapy to a small number of patients (also reducing the side effects of preventive therapy, i.e., hemodynamic effects) (**Figure 2**).

A randomized trial has tested the cardioprotective capacity of enalapril, involving 473 patients with different types of cancers treated with high-dose chemotherapy (90). 114 patients showed an increase in troponin and were randomized for treatment with or without enalapril. After the end of chemotherapy, enalapril was begun, titrated as tolerated, and continued for 1 year. No patients in the enalapril-treated group showed a decrease in LVEF by 10 absolute points below the value of 50%—the study's primary endpoint—and the incidence of major cardiac events was remarkably small (**Figure 5**). Of note, in the enalapril community, the LVEF value was still the same as the baseline

value in 80% of cases after a follow-up duration of 12 months, showing that enalapril can be a very effective drug in the complete preservation of systolic function in this population.

Two studies are currently evaluating the efficacy of carvedilol as a preventive therapy in a selected patient with a deterioration in the strain parameter. For Research NCT02177175 (Carvedilol for the Prevention of Anthracyclines/Anti-HER2 Therapy-Associated Cardiotoxicity between Women with HER2+ breast cancer Using Myocardial Strain), the primary endpoint is the identification of a reduced LVEF value during the 1-year follow-up. At Northwestern University of Chicago, the research is still hiring.

PRIMARY VS. SECONDARY PREVENTION

Enalapril, which began early after the increase in troponin during anthracycline chemotherapy and continued for 12 months, is an effective therapy to avoid left ventricular dysfunction and subsequent heart events (90). Repeated assessment, however, is needed to detect an increase in troponin, as the marker may increase at different times after infusion with therapy (dose of anthracycline and schedules). Primary prevention, applied to all anthracycline-treated patients, does not pose this downside. The ICOSONE (International CardioOncology Society-One) randomized trial prospectively compared the efficacy of two different approaches, to test whether enalapril, initiated in all patients before chemotherapy (Prevention Group), was able to prevent troponin rise and further the development of left ventricular dysfunction, and to test whether this strategy was more successful than enalapril initiated only after troponin elevation during chemotherapy (Troponin-triggered Group) (106). The study included 273 patients from 21 different Centers of Oncology. The most-often administered anthracyclines were epirubicin and doxorubicin. During chemotherapy and the 12-month follow-up, no significant reduction in LVEF and a minimal incidence of cardiovascular events were detected in both groups. Only three patients experienced cardiotoxicity defined as a 10% reduction in LVEF, below 50% value.

In brief, the main result of the study was that the two approaches appear to be similarly effective in preventing left ventricular dysfunction and adverse cardiac events, endorsing the use of enalapril in averting anthracycline-induced cardiotoxicity, irrespectively from the strategy used.

Which strategy is best? Secondary prevention (i.e., troponin driven) has the limitation of repeated blood samplings. Nevertheless, considering the high negative predictive value of troponin (34, 35, 37, 38), this strategy appears warranted and cost-effective, as it permits the exclusion of low-risk patients (patients without troponin rise, the vast majority) from long-term monitoring programs based on imaging techniques with a relevant cost–benefit ratio by reducing "medicalization, distress,

TABLE 5 | Pros and Cons of primary prevention vs. secondary prevention with enalapril (83).

Primary prevention with enalapril	Enalapril in troponin + patients
PROS: • Very low incidence LVD & MACE • Troponin assessment not required	PROS: • Very low incidence LVD & MACE • Monitoring during up-titration in about 20% pts • Only pts at high-risk exposed to side effects • FU monitoring not required in troponin negative patients • Low cost-benefit ratio
CONS: • Monitoring during up-titration in 100% • All pts exposed to side effects • FU monitoring required in all pts • High cost-benefit ratio	CONS: • Repeated TNI assessment

LVD, left ventricular dysfunction; MACE, major adverse cardiac events; FU, follow-up; TNI, troponin I.

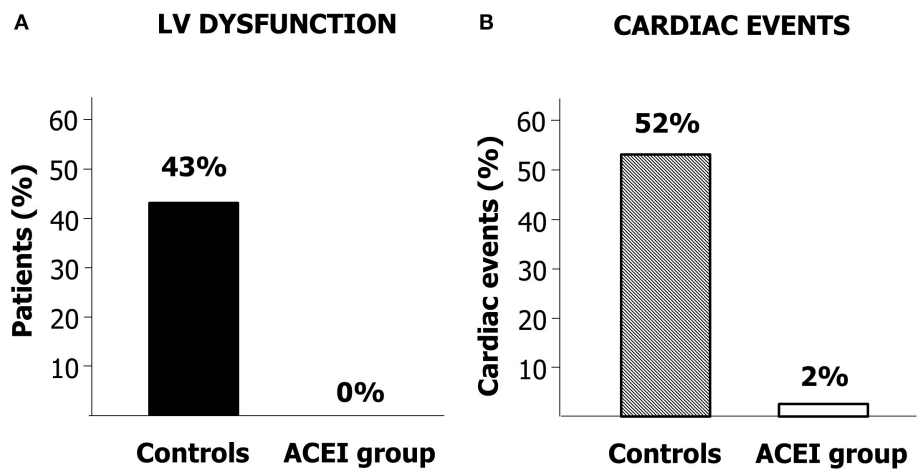

FIGURE 5 | (A) Percentage of patients developing cardiac dysfunction in the enalapril-treated group (ACEI Group) and controls. **(B)** Incidence of cardiac events in patients treated with ACEI Group and in Controls. Modified from Cardinale et al. (90).

anxiety, and costs" (21). Primary prevention, although not needing a repeated evaluation of troponin during chemotherapy, can be hard in terms of clinical surveillance during the drug up-titration to include 100% of patients. Finally, it may expose to potential side effects all those low-risk subjects for cardiotoxicity (**Table 5**) (106).

CONCLUSION

Anthracycline-induced cardiotoxicity is still a significant problem that compromises the quality of life and overall survival of cancer patients. However, recent findings demonstrate that this form of cardiomyopathy is mostly reversible with early detection and prompt therapeutic introduction strategy. Probably, anthracycline-induced cardiotoxicity is a single and continuous phenomenon, from cellular to clinical stage, starting with myocardial cell injury, followed by progressive LVEF decline and, potentially, overt heart failure. The current standard for monitoring cardiac function (periodic assessment of LVEF), detects cardiotoxicity at a late stage when a significant impairment has already occurred, precluding the chance of effectively prevent and treat its development.

The use of troponins to identify patients with subclinical cardiotoxicity combined with early treatment with ACE-inhibitors occurrence appears to be an effective method to prevent anthracycline-related left ventricular dysfunction and cardiac events.

Finally, adoption of internal procedures, shared in a multi-disciplinary team, may actively aid in optimizing patient management. In this respect, a direct relationship with the laboratory medicine service for the assessment of troponin values during chemotherapy and the availability of a cardiologist and a dedicated nurse staff should always mix with an active collaboration with the referral oncologist/hematologist (possibly, surgeon) for updates and remains of pivotal importance (107, 108).

AUTHOR CONTRIBUTIONS

DC, FI, and CC contributed conception, design of the review, and wrote the first draft. All authors contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. *J Mol Cell Cardiol*. (2012) 52:1213–25. doi: 10.1016/j.yjmcc.2012.03.006
2. Henriksen PA. Anthracycline cardiotoxicity: an update on mechanisms, monitoring and prevention. *Heart*. (2018) 104:971–77. doi: 10.1136/heartjnl-2017-312103
3. Yeh ET, Chang HM. Oncocardiology-past, present, and future: a review. *JAMA Cardiol*. (2016) 1:1066–72. doi: 10.1001/jamacardio.2016.2132
4. Curigliano G, Cardinale D, Dent S, Criscitiello C, Aseyev O, Lenihan D, et al. Cardiotoxicity of anticancer treatments: epidemiology, detection, and management. *CA Cancer J Clin*. (2016) 66:309–25. doi: 10.3322/caac.21341
5. Bloom MW, Hamo CE, Cardinale D, Ky B, Nohria A, Baer L, et al. Cancer therapy-related cardiac dysfunction and heart failure: part 1: definitions, pathophysiology, risk factors, and imaging. *Circ Heart Fail*. (2016) 9:e002661. doi: 10.1161/CIRCHEARTFAILURE.115.002661
6. Tan C, Tasaka H, Yu KP, Murphy ML, Karnofsky DA. Daunomycin, an antitumor antibiotic, in the treatment of neoplastic disease. Clinical evaluation with special reference to childhood leukemia. *Cancer*. (1967) 20:333–53. doi: 10.1002/1097-0142(1967)20:3333::aid-cncr28202003023.0.co;2-k
7. Zamorano JL, Lancellotti P, Rodriguez Munoz D, Aboyans V, Asteggiano R, Galderisi M, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC committee for practice guidelines: the task force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). *Eur J Heart Fail*. (2017) 19:9–42. doi: 10.1002/ejhf.654
8. Cardinale D, Biasillo G, Cipolla CM. Curing cancer, saving the heart: a challenge that cardioncology should not miss. *Curr Cardiol Rep*. (2016) 18:51. doi: 10.1007/s11886-016-0731-z
9. Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med*. (2012) 18:1639–42. doi: 10.1038/nm.2919
10. Vejpongsa P, Yeh ET. Prevention of anthracycline-induced cardiotoxicity: challenges and opportunities. *J Am Coll Cardiol*. (2014) 64:938–45. doi: 10.1016/j.jacc.2014.06.1167
11. Cappetta D, Rossi F, Piegari E, Quaini F, Berrino L, Urbanek K, et al. Doxorubicin targets multiple players: a new view of an old problem. *Pharmacol Res*. (2018) 127:4–14. doi: 10.1016/j.phrs.2017.03.016
12. Giantris A, Abdurrahman L, Hinkle A, Asselin B, Lipshultz SE. Anthracycline-induced cardiotoxicity in children and young adults. *Crit Rev Oncol Hematol*. (1998) 27:53–68. doi: 10.1016/S1040-8428(97)10007-5
13. Grenier MA, Lipshultz SE. Epidemiology of anthracycline cardiotoxicity in children and adults. *Semin Oncol*. (1998) 25(4 Suppl. 10):72–85.
14. Steinherz LJ, Steinherz PG, Tan CT, Heller G, Murphy ML. Cardiac toxicity 4 to 20 years after completing anthracycline therapy. *JAMA*. (1991) 266:1672–7. doi: 10.1001/jama.266.12.1672
15. Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, et al. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. *Circulation*. (2015) 131:1981–8. doi: 10.1161/CIRCULATIONAHA.114.013777
16. Eschenhagen T, Force T, Ewer MS, de Keulenaer GW, Suter TM, Anker SD, et al. Cardiovascular side effects of cancer therapies: a position statement from the heart failure association of the European society of cardiology. *Eur J Heart Fail*. (2011) 13:1–10. doi: 10.1093/eurjhf/hfq213
17. Cardinale D, Biasillo G, Salvatici M, Sandri MT, Cipolla CM. Using biomarkers to predict and to prevent cardiotoxicity of cancer therapy. *Expert Rev Mol Diagn*. (2017) 17:245–56. doi: 10.1080/14737159.2017.1283219
18. Cardinale D, Cipolla CM. Chemotherapy-induced cardiotoxicity: importance of early detection. *Expert Rev Cardiovasc Ther*. (2016) 14:1297–9. doi: 10.1080/14779072.2016.1239528
19. Plana JC, Galderisi M, Barac A, Ewer MS, Ky B, Scherrer-Crosbie M, et al. Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American society of echocardiography and the European association of cardiovascular imaging. *Eur Heart J Cardiovasc Imaging*. (2014) 15:1063–93. doi: 10.1093/ehjci/jeu192

20. Armenian SH, Lacchetti C, Barac A, Carver J, Constine LS, Denduluri N, et al. Prevention and monitoring of cardiac dysfunction in survivors of adult cancers: American society of clinical oncology clinical practice guideline. *J Clin Oncol*. (2017) 35:893–911. doi: 10.1200/JCO.2016.70.5400
21. Levis BE, Binkley PF, Shapiro CL. Cardiotoxic effects of anthracycline-based therapy: what is the evidence and what are the potential harms? *Lancet Oncol*. (2017) 18:e445–56. doi: 10.1016/S1470-2045(17)30535-1
22. Felker GM, Thompson RE, Hare JM, Hruban RH, Clemetson DE, Howard DL, et al. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. *N Engl J Med*. (2000) 342:1077–84. doi: 10.1056/NEJM200004133421502
23. Saini J, Rich MW, Lyss AP. Reversibility of severe left ventricular dysfunction due to doxorubicin cardiotoxicity. Report of three cases. *Ann Intern Med*. (1987) 106:814–6. doi: 10.7326/0003-4819-106-6-814
24. Jensen BV, Nielsen SL, Skovsgaard T. Treatment with angiotensin-converting-enzyme inhibitor for epirubicin-induced dilated cardiomyopathy. *Lancet*. (1996) 347:297–9. doi: 10.1016/S0140-6736(96)90469-9
25. Fazio S, Palmieri EA, Ferravante B, Bone F, Biondi B, Sacca L. Doxorubicin-induced cardiomyopathy treated with carvedilol. *Clin Cardiol*. (1998) 21:777–9. doi: 10.1002/clc.4960211017
26. Noori A, Lindenfeld J, Wolfel E, Ferguson D, Bristow MR, Lowes BD. Beta-blockade in adriamycin-induced cardiomyopathy. *J Card Fail*. (2000) 6:115–9. doi: 10.1054/jcaf.2000.7505
27. Jensen BV, Skovsgaard T, Nielsen SL. Functional monitoring of anthracycline cardiotoxicity: a prospective, blinded, long-term observational study of outcome in 120 patients. *Ann Oncol*. (2002) 13:699–709. doi: 10.1093/annonc/mdf132
28. Mukai Y, Yoshida T, Nakaike R, Mukai N, Iwato K, Kyo T, et al. Five cases of anthracycline-induced cardiomyopathy effectively treated with carvedilol. *Intern Med*. (2004) 43:1087–8. doi: 10.2169/internalmedicine.43.1087
29. Tallaj JA, Franco V, Rayburn BK, Pinderski L, Benza RL, Pamboukian S, et al. Response of doxorubicin-induced cardiomyopathy to the current management strategy of heart failure. *J Heart Lung Transplant*. (2005) 24:2196–201. doi: 10.1016/j.healun.2004.12.108
30. Tabet JY, Meurin P, Ben Driss A, Berthaux X, Weber H, Renaud N, et al. Beta-blockade intolerance in anthracycline-induced cardiomyopathy. *Int J Cardiol*. (2006) 106:132–4. doi: 10.1016/j.ijcard.2004.12.052
31. Cardinale D, Colombo A, Lamantia G, Colombo N, Civelli M, de Giacomi G, et al. Anthracycline-induced cardiomyopathy: clinical relevance and response to pharmacologic therapy. *J Am Coll Cardiol*. (2010) 55:213–20. doi: 10.1016/j.jacc.2009.03.095
32. O'Brien PJ. Cardiac troponin is the most effective translational safety biomarker for myocardial injury in cardiotoxicity. *Toxicology*. (2008) 245:206–18. doi: 10.1016/j.tox.2007.12.006
33. Lipshultz SE, Rifai N, Sallan SE, Lipsitz SR, Dalton V, Sacks DB, et al. Predictive value of cardiac troponin T in pediatric patients at risk for myocardial injury. *Circulation*. (1997) 96:2641–8. doi: 10.1161/01.CIR.96.8.2641
34. Cardinale D, Sandri MT, Martinoni A, Tricca A, Civelli M, Lamantia G, et al. Left ventricular dysfunction predicted by early troponin I release after high-dose chemotherapy. *J Am Coll Cardiol*. (2000) 36:517–22. doi: 10.1016/S0735-1097(00)00748-8
35. Cardinale D, Sandri MT, Martinoni A, Borghini E, Civelli M, Lamantia G, et al. Myocardial injury revealed by plasma troponin I in breast cancer treated with high-dose chemotherapy. *Ann Oncol*. (2002) 13:710–5. doi: 10.1093/annonc/mdf170
36. Auner HW, Tinchon C, Linkesch W, Tiran A, Quehenberger F, Link H, et al. Prolonged monitoring of troponin T for the detection of anthracycline cardiotoxicity in adults with hematological malignancies. *Ann Hematol*. (2003) 82:218–22. doi: 10.1007/s00277-003-0615-3
37. Sandri MT, Cardinale D, Zorzino L, Passerini R, Lentati P, Martinoni A, et al. Minor increases in plasma troponin I predict decreased left ventricular ejection fraction after high-dose chemotherapy. *Clin Chem*. (2003) 49:248–52. doi: 10.1373/49.2.248
38. Cardinale D, Sandri MT, Colombo A, Colombo N, Boeri M, Lamantia G, et al. Prognostic value of troponin I in cardiac risk stratification of cancer patients undergoing high-dose chemotherapy. *Circulation*. (2004) 109:2749–54. doi: 10.1161/01.CIR.0000130926.51766.CC
39. Specchia G, Buquicchio C, Pansini N, Di Serio F, Liso V, Pastore D, et al. Monitoring of cardiac function on the basis of serum troponin I levels in patients with acute leukemia treated with anthracyclines. *J Lab Clin Med*. (2005) 145:212–20. doi: 10.1016/j.lab.2005.02.003
40. Kilickap S, Barista I, Akgul E, Aytemir K, Aksoyek S, Aksoy S, et al. cTnT can be a useful marker for early detection of anthracycline cardiotoxicity. *Ann Oncol*. (2005) 16:798–804. doi: 10.1093/annonc/mdi152
41. Lee HS, Son CB, Shin SH, Kim YS. Clinical correlation between brain natriutetic peptide and anthracyclin-induced cardiac toxicity. *Cancer Res Treat*. (2008) 40:121–6. doi: 10.4143/crt.2008.40.3.121
42. Schmidinger M, Zielinski CC, Vogl UM, Bojic A, Bojic M, Schukro C, et al. Cardiac toxicity of sunitinib and sorafenib in patients with metastatic renal cell carcinoma. *J Clin Oncol*. (2008) 26:5204–12. doi: 10.1200/JCO.2007.15.6331
43. Cardinale D, Colombo A, Torrisi R, Sandri MT, Civelli M, Salvatici M, et al. Trastuzumab-induced cardiotoxicity: clinical and prognostic implications of troponin I evaluation. *J Clin Oncol*. (2010) 28:3910–6. doi: 10.1200/JCO.2009.27.3615
44. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Cohen V, et al. Early detection and prediction of cardiotoxicity in chemotherapy-treated patients. *Am J Cardiol*. (2011) 107:1375–80. doi: 10.1016/j.amjcard.2011.01.006
45. Lipshultz SE, Scully RE, Lipsitz SR, Sallan SE, Silverman LB, Miller TL, et al. Assessment of dexrazoxane as a cardioprotectant in doxorubicin-treated children with high-risk acute lymphoblastic leukaemia: long-term follow-up of a prospective, randomised, multicentre trial. *Lancet Oncol*. (2010) 11:950–61. doi: 10.1016/S1470-2045(10)70204-7
46. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Tan TC, et al. Assessment of echocardiography and biomarkers for the extended prediction of cardiotoxicity in patients treated with anthracyclines, taxanes, and trastuzumab. *Circ Cardiovasc Imaging*. (2012) 5:596–603. doi: 10.1161/CIRCIMAGING.112.973321
47. Drafts BC, Twomley KM, D'Agostino R Jr, Lawrence J, Avis N, et al. Low to moderate dose anthracycline-based chemotherapy is associated with early noninvasive imaging evidence of subclinical cardiovascular disease. *JACC Cardiovasc Imaging*. (2013) 6:877–85. doi: 10.1016/j.jcmg.2012.11.017
48. Mornos C, Petrescu L. Early detection of anthracycline-mediated cardiotoxicity: the value of considering both global longitudinal left ventricular strain and twist. *Can J Physiol Pharmacol*. (2013) 91:601–7. doi: 10.1139/cjpp-2012-0398
49. Mavinkurve-Groothuis AM, Marcus KA, Pourier M, Loonen J, Feuth T, Hoogerbrugge PM, et al. Myocardial 2D strain echocardiography and cardiac biomarkers in children during and shortly after anthracycline therapy for acute lymphoblastic leukaemia (ALL): a prospective study. *Eur Heart J Cardiovasc Imaging*. (2013) 14:562–9. doi: 10.1093/ehjci/jes217
50. Ky B, Putt M, Sawaya H, French B, Januzzi JL Jr, Sebag IA, et al. Early increases in multiple biomarkers predict subsequent cardiotoxicity in patients with breast cancer treated with doxorubicin, taxanes, and trastuzumab. *J Am Coll Cardiol*. (2014) 63:809–16. doi: 10.1016/j.jacc.2013.10.061
51. Mornos C, Manolis AJ, Cozma D, Kouremenos N, Zacharopoulou I, Ionac A. The value of left ventricular global longitudinal strain assessed by three-dimensional strain imaging in the early detection of anthracyclinemediated cardiotoxicity. *Hellenic J Cardiol*. (2014) 55:235–44.
52. Putt M, Hahn VS, Januzzi JL, Sawaya H, Sebag IA, Plana JC, et al. Longitudinal changes in multiple biomarkers are associated with cardiotoxicity in breast cancer patients treated with doxorubicin, taxanes, and trastuzumab. *Clin Chem*. (2015) 61:1164–72. doi: 10.1373/clinchem.2015.241232
53. Zardavas D, Suter TM, van Veldhuisen DJ, Steinseifer J, Noe J, Lauer S, et al. Role of troponins I and T and N-terminal prohormone of brain natriuretic peptide in monitoring cardiac safety of patients with early-stage human epidermal growth factor receptor 2-positive breast cancer receiving trastuzumab: a herceptin adjuvant study cardiac marker substudy. *J Clin Oncol*. (2017) 35:878–84. doi: 10.1200/JCO.2015.65.7916
54. Olivieri J, Perna GP, Bocci C, Montevecchi C, Olivieri A, Leoni P, et al. Modern management of anthracycline-induced cardiotoxicity in lymphoma

patients: low occurrence of cardiotoxicity with comprehensive assessment and tailored substitution by nonpegylated liposomal doxorubicin. *Oncologist*. (2017) 22:422–31. doi: 10.1634/theoncologist.2016-0289
55. Kitayama H, Kondo T, Sugiyama J, Kurimoto K, Nishino Y, Kawada M, et al. High-sensitive troponin T assay can predict anthracycline- and trastuzumab-induced cardiotoxicity in breast cancer patients. *Breast Cancer*. (2017) 24:774–82. doi: 10.1007/s12282-017-0778-8
56. Shafi A, Siddiqui N, Imtiaz S, Din Sajid MU. Left ventricular systolic dysfunction predicted by early troponin I release after anthracycline based chemotherapy in breast cancer patients. *J Ayub Med Coll Abbottabad*. (2017) 29:266–9.
57. Lenihan DJ, Stevens PL, Massey M, Plana JC, Araujo DM, Fanale MA, et al. The utility of point-of-care biomarkers to detect cardiotoxicity during anthracycline chemotherapy: a feasibility study. *J Card Fail*. (2016) 22:433–8. doi: 10.1016/j.cardfail.2016.04.003
58. Feola M, Garrone O, Occelli M, Francini A, Biggi A, Visconti G, et al. Cardiotoxicity after anthracycline chemotherapy in breast carcinoma: effects on left ventricular ejection fraction, troponin I and brain natriuretic peptide. *Int J Cardiol*. (2011) 148:194–8. doi: 10.1016/j.ijcard.2009.09.564
59. Sandri MT, Salvatici M, Cardinale D, Zorzino L, Passerini R, Lentati P, et al. N-terminal pro-B-type natriuretic peptide after high-dose chemotherapy: a marker predictive of cardiac dysfunction? *Clin Chem*. (2005) 51:1405–10. doi: 10.1373/clinchem.2005.050153
60. Hoeger CW, Hayek SS. Role of cardiovascular biomarkers in the risk stratification, monitoring, and management of patients with cancer. *Cardiol Clin*. (2019) 37:505–23. doi: 10.1016/j.ccl.2019.07.015
61. Weir RA, Petrie CJ, Murphy CA, Clements S, Steedman T, Miller AM, et al. Galectin-3 and cardiac function in survivors of acute myocardial infarction. *Circ Heart Fail*. (2013) 6:492–8. doi: 10.1161/CIRCHEARTFAILURE.112.000146
62. Freres P, Bouznad N, Servais L, Josse C, Wenric S, Poncin A, et al. Variations of circulating cardiac biomarkers during and after anthracycline-containing chemotherapy in breast cancer patients. *BMC Cancer*. (2018) 18:102. doi: 10.1186/s12885-018-4015-4
63. Oliveira-Carvalho V, Ferreira LR, Bocchi EA. Circulating mir-208a fails as a biomarker of doxorubicin-induced cardiotoxicity in breast cancer patients. *J Appl Toxicol*. (2015) 35:1071–2. doi: 10.1002/jat.3185
64. Armenian SH, Gelehrter SK, Vase T, Venkatramani R, Landier W, Wilson KD, et al. Screening for cardiac dysfunction in anthracycline-exposed childhood cancer survivors. *Clin Cancer Res*. (2014) 20:6314–23. doi: 10.1158/1078-0432.CCR-13-3490
65. van Boxtel W, Bulten BF, Mavinkurve-Groothuis AM, Bellersen L, Mandigers CM, Joosten LA, et al.F. New biomarkers for early detection of cardiotoxicity after treatment with docetaxel, doxorubicin and cyclophosphamide. *Biomarkers*. (2015) 20:143–8. doi: 10.3109/1354750X.2015.1040839
66. Narayan HK, Finkelman B, French B, Plappert T, Hyman D, Smith AM, et al. Detailed echocardiographic phenotyping in breast cancer patients: associations with ejection fraction decline, recovery, and heart failure symptoms over 3 years of follow-up. *Circulation*. (2017) 135:1397–12. doi: 10.1161/CIRCULATIONAHA.116.023463
67. Thavendiranathan P, Poulin F, Lim KD, Plana JC, Woo A, Marwick TH. Use of myocardial strain imaging by echocardiography for the early detection of cardiotoxicity in patients during and after cancer chemotherapy: a systematic review. *J Am Coll Cardiol*. (2014) 63(25 Pt A):2751–68. doi: 10.1016/j.jacc.2014.01.073
68. Brown J, Jenkins C, Marwick TH. Use of myocardial strain to assess global left ventricular function: a comparison with cardiac magnetic resonance and 3-dimensional echocardiography. *Am Heart J*. (2009) 157:102.e1–5. doi: 10.1016/j.ahj.2008.08.032
69. Liu J, Banchs J, Mousavi N, Plana JC, Scherrer-Crosbie M, Thavendiranathan P, et al. Contemporary role of echocardiography for clinical decision making in patients during and after cancer therapy. *JACC Cardiovasc Imaging*. (2018) 11:1122–131. doi: 10.1016/j.jcmg.2018.03.025
70. Doherty JU, Kort S, Mehran R, Schoenhagen P, Soman P, Dehmer GJ, et al. ACC/AATS/AHA/ASE/ASNC/HRS/SCAI/SCCT/SCMR/STS 2019 Appropriate use criteria for multimodality imaging in the assessment of cardiac structure and function in nonvalvular heart disease: a report of the American college of cardiology appropriate use criteria task force, American association for thoracic surgery, American heart association, American society of echocardiography, American society of nuclear cardiology, heart rhythm society, society for cardiovascular angiography and interventions, society of cardiovascular computed tomography, society for cardiovascular magnetic resonance, and the society of thoracic surgeons. *J Am Coll Cardiol*. (2019) 73:488–516. doi: 10.1016/j.jacc.2018.10.038.
71. Negishi T, Thavendiranathan P, Negishi K, Marwick TH, SUCCOUR Investigators. Rationale and design of the strain surveillance of chemotherapy for improving cardiovascular outcomes: the SUCCOUR trial. *JACC Cardiovasc Imaging*. (2018) 11:1098–105.
72. Smith LA, Cornelius VR, Plummer CJ, Levitt G, Verrill M, Canney P, et al. Cardiotoxicity of anthracycline agents for the treatment of cancer: systematic review and meta-analysis of randomised controlled trials. *BMC Cancer*. (2010) 10:337. doi: 10.1186/1471-2407-10-337
73. van Dalen EC, van der Pal HJ, Kremer LC. Different dosage schedules for reducing cardiotoxicity in people with cancer receiving anthracycline chemotherapy. *Cochrane Database Syst Rev*. (2016) 3:CD005008. doi: 10.1002/14651858.CD005008.pub4
74. Rafiyath SM, Rasul M, Lee B, Wei G, Lamba G, Liu D. Comparison of safety and toxicity of liposomal doxorubicin vs. conventional anthracyclines: a meta-analysis. *Exp Hematol Oncol*. (2012) 1:10. doi: 10.1186/2162-3619-1-10
75. Mehta LS, Watson KE, Barac A, Beckie TM, Bittner V, Cruz-Flores S, et al. American heart association cardiovascular disease in, C. Special populations committee of the council on clinical, C. council on, Stroke N, C. council on quality of, and outcomes R. cardiovascular disease and breast cancer: where these entities intersect: a scientific statement from the American heart association. *Circulation*. (2018) 137:e30–e66. doi: 10.1161/CIR.0000000000000556
76. Brown S. Preventive cardio-oncology: the time has come. *Front. Cardiovasc. Med*. (2020). 6:187. doi: 10.3389/fcvm.2019.00187
77. Yin BA, Brewster AM, Barac A, Thoman W, Oeffinger KC, Gilchrist SC. Cardiovascular prevention strategies in breast cancer. *J Am Coll Cardiol CardioOnc*. (2019) 2:322–325. doi: 10.1016/j.jaccao.2019.09.001
78. Barish R, Lynce F, Unger K, Barac A. Management of cardiovascular disease in women with breast cancer. *Circulation*. (2019) 139:1110–20. doi: 10.1161/CIRCULATIONAHA.118.039371
79. Nitiss KC, Nitiss JL. Twisting and ironing: doxorubicin cardiotoxicity by mitochondrial DNA damage. *Clin Cancer Res*. (2014) 20:4737–9. doi: 10.1158/1078-0432.CCR-14-0821
80. EMA. *European Medicines Agency*. Assessment Report. Dexrazoxane-Containing Medicinal Products. EMA (2011).
81. van Dalen EC, Caron HN, Dickinson HO, Kremer LC. Cardioprotective interventions for cancer patients receiving anthracyclines. *Cochrane Database Syst Rev*. (2011) CD003917. doi: 10.1002/14651858.CD003917.pub4
82. Reichardt P, Tabone MD, Mora J, Morland B, Jones RL. Risk-benefit of dexrazoxane for preventing anthracycline-related cardiotoxicity: re-evaluating the European labeling. *Future Oncol*. (2018) 14:2663–676. doi: 10.2217/fon-2018-0210
83. Asselin BL, Devidas M, Chen L, Franco VI, Pullen J, Borowitz MJ, et al. Cardioprotection and safety of dexrazoxane in patients treated for newly diagnosed T-cell acute lymphoblastic leukemia or advanced-stage lymphoblastic non-hodgkin lymphoma: a report of the children's oncology group randomized trial pediatric oncology group 9404. *J Clin Oncol*. (2016) 34:854–62. doi: 10.1200/JCO.2015.60.8851
84. EMA. European Medicines Agency. *Questions and Answers on Cardioxane (Dexrazoxane: Powder for Solution for Injection: 500 mg). Outcome of a Procedure Under Article 13 of Regulation (EC) No 1234/2008*. EMA (2017).
85. Macedo AVS, Lyon AR, Nascimento BR, Putzu A, Rossi L, Costa RB, et al. Efficacy of dexrazoxane in preventing anthracycline cardiotoxicity in breast cancer. *J Am Coll Cardiol CardioOnc*. (2019) 1:68–79. doi: 10.1016/j.jaccao.2019.08.003
86. Kalay N, Basar E, Ozdogru I, Er O, Cetinkaya Y, Dogan A, et al. Protective effects of carvedilol against anthracycline-induced cardiomyopathy. *J Am Coll Cardiol*. (2006) 48:2258–62. doi: 10.1016/j.jacc.2006.07.052
87. Kaya MG, Ozkan M, Gunebakmaz O, Akkaya H, Kaya EG, Akpek M, et al. Protective effects of nebivolol against anthracycline-induced

cardiomyopathy: a randomized control study. *Int J Cardiol*. (2013) 167:2306–10. doi: 10.1016/j.ijcard.2012.06.023
88. Seicean S, Seicean A, Alan N, Plana JC, Budd GT, Marwick TH. Cardioprotective effect of beta-adrenoceptor blockade in patients with breast cancer undergoing chemotherapy: follow-up study of heart failure. *Circ Heart Fail*. (2013) 6:420–6. doi: 10.1161/CIRCHEARTFAILURE.112.000055
89. Pituskin E, Mackey JR, Koshman S, Jassal D, Pitz M, Haykowsky MJ, et al. Multidisciplinary approach to novel therapies in cardio-oncology research (MANTICORE 101-Breast): a randomized trial for the prevention of trastuzumab-associated cardiotoxicity. *J Clin Oncol*. (2017) 35:870–77. doi: 10.1200/JCO.2016.68.7830
90. Cardinale D, Colombo A, Sandri MT, Lamantia G, Colombo N, Civelli M, et al. Prevention of high-dose chemotherapy-induced cardiotoxicity in high-risk patients by angiotensin-converting enzyme inhibition. *Circulation*. (2006) 114:2474–81. doi: 10.1161/CIRCULATIONAHA.106.635144
91. Nakamae H, Tsumura K, Terada Y, Nakane T, Nakamae M, Ohta K, et al. Notable effects of angiotensin II receptor blocker, valsartan, on acute cardiotoxic changes after standard chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisolone. *Cancer*. (2005) 104:2492–8. doi: 10.1002/cncr.21478
92. Cadeddu C, Piras A, Mantovani G, Deidda M, Dessi M, Madeddu C, et al. Protective effects of the angiotensin II receptor blocker telmisartan on epirubicin-induced inflammation, oxidative stress, and early ventricular impairment. *Am Heart J*. (2010) 160:487 e1–7. doi: 10.1016/j.ahj.2010.05.037
93. Gulati G, Heck SL, Rosjo H, Ree AH, Hoffmann P, Hagve TA, et al. Neurohormonal blockade and circulating cardiovascular biomarkers during anthracycline therapy in breast cancer patients: results from the PRADA (prevention of cardiac dysfunction during adjuvant breast cancer therapy) study. *J Am Heart Assoc*. (2017) 6:006513. doi: 10.1161/jaha.117.006513
94. Akpek M, Ozdogru I, Sahin O, Inanc M, Dogan A, Yazici C, et al. Protective effects of spironolactone against anthracycline-induced cardiomyopathy. *Eur J Heart Fail*. (2015) 17:81–9. doi: 10.1002/ejhf.196
95. Bosch X, Rovira M, Sitges M, Domenech A, Ortiz-Perez JT, de Caralt TM, et al. Enalapril and carvedilol for preventing chemotherapy-induced left ventricular systolic dysfunction in patients with malignant hemopathies: the OVERCOME trial (prevention of left ventricular dysfunction with enalapril and carvedilol in patients submitted to intensive chemotherapy for the treatment of malignant hEmopathies). *J Am Coll Cardiol*. (2013) 61:2355–62. doi: 10.1016/j.jacc.2013.02.072
96. Acar Z, Kale A, Turgut M, Demircan S, Durna K, Demir S, et al. Efficiency of atorvastatin in the protection of anthracycline-induced cardiomyopathy. *J Am Coll Cardiol*. (2011) 58:988–9. doi: 10.1016/j.jacc.2011.05.025
97. Seicean S, Seicean A, Plana JC, Budd GT, Marwick TH. Effect of statin therapy on the risk for incident heart failure in patients with breast cancer receiving anthracycline chemotherapy: an observational clinical cohort study. *J Am Coll Cardiol*. (2012) 60:2384–90. doi: 10.1016/j.jacc.2012.07.067
98. Chotenimitkhun R, D'Agostino R Jr, Lawrence JA, Hamilton CA, Jordan JH, Vasu S, et al. Chronic statin administration may attenuate early anthracycline-associated declines in left ventricular ejection function. *Can J Cardiol*. (2015) 31:302–7. doi: 10.1016/j.cjca.2014.11.020
99. Vaduganathan M, Hirji SA, Qamar A, Bajaj N, Gupta A, Zaha V, et al. Efficacy of neurohormonal therapies in preventing cardiotoxicity in patients with cancer undergoing chemotherapy. *J Am Coll Cardiol CardioOnc*. (2019) 1:54–65. doi: 10.1016/j.jaccao.2019.08.006
100. Elitok A, Oz F, Cizgici AY, Kilic L, Ciftci R, Sen F, et al. Effect of carvedilol on silent anthracycline-induced cardiotoxicity assessed by strain imaging: a prospective randomized controlled study with six-month follow-up. *Cardiol J*. (2014) 21:509–15. doi: 10.5603/CJ.a2013.0150
101. Avila MS, Ayub-Ferreira SM, de Barros Wanderley MR Jr, das Dores Cruz F, Gonçalves Brandão SM, Rigaud VOC, et al. Carvedilol for prevention of chemotherapy-related cardiotoxicity: the CECCY trial. *J Am Coll Cardiol*. (2018) 71:2281–90. doi: 10.1016/j.jacc.2018.02.049
102. Oliveira PJ, Bjork JA, Santos MS, Leino RL, Froberg MK, Moreno AJ, et al. Carvedilol-mediated antioxidant protection against doxorubicin-induced cardiac mitochondrial toxicity. *Toxicol Appl Pharmacol*. (2004) 200:159–68. doi: 10.1016/j.taap.2004.04.005
103. Davis MK, Villa D, Tsang TSM, Starovoytov A, Gelmon K, Sean A, et al. Effect of eplerenone on diastolic function in women receiving anthracycline-based chemotherapy for breast cancer. *J Am Coll Cardiol CardioOnc*. (2019) 2:295–8. doi: 10.1016/j.jaccao.2019.10.001
104. Gasser CA, Audebrand A, Daglayan A, Charavin M, B.Escoubet, Karpov P, et al. Prokineticin receptor-1 signaling inhibits dose- and time-dependent anthracycline-induced cardiovascular toxicity via myocardial and vascular protection. *J Am Coll Cardiol CardioOnc*. (2019) 1:84–102. doi: 10.1016/j.jaccao.2019.06.003
105. Audebrand A, Désaubry L, Nebigil CG. Targeting GPCRs against cardiotoxicity induced by anticancer treatments. *Front. Cardiovasc. Med*. 6:194. (2019). doi: 10.3389/fcvm.2019.00194
106. Cardinale D, Ciceri F, Latini R, Franzosi MG, Sandri MT, Civelli M, et al. Anthracycline-induced cardiotoxicity: a multicenter randomised trial comparing two strategies for guiding prevention with enalapril: the international cardiooncology society-one trial. *Eur J Cancer*. (2018) 94:126–37. doi: 10.1016/j.ejca.2018.02.005
107. Cardinale D, Caruso V, Cipolla CM. The breast cancer patient in the cardioncology unit. *J Thorac Dis*. (2018) 10:S4306–22. doi: 10.21037/jtd.2018.10.06
108. Ky B. JACC: CardioOncology: poised to serve a maturing, collaborative field. *J Am Coll Cardiol CardioOnc*. (2019) 1:131–132. doi: 10.1016/j.jaccao.2019.06.001

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Cardiovascular Damage Associated With Chest Irradiation

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The improvement of anticancer-therapies results in a greater amount of long-term survivors after radiotherapy. Therefore, the understanding of cardiotoxicity after irradiation is of increasing importance. Cardiovascular adverse events after chest irradiation have been acknowledged for a long time but remain difficult to diagnose. Long-term cardiovascular adverse events may become evident years or decades after radiotherapy and the spectrum of potential cardiovascular side effects is large. Recent experimental and clinical data indicate that cardiovascular symptoms may be caused especially by heart failure with preserved ejection fraction, which remains incompletely understood in patients after radiation therapy. Heart radiation dose and co-existing cardiovascular risk factors represent some of the most important contributors for incidence and severity of radiation-induced cardiovascular side effects. In this review, we aim to elucidate the underlying patho-mechanisms and to characterize the development of radiation-induced cardiovascular damage. Additionally, approaches for clinical management and treatment options are presented.

Keywords: radiation therapy, irradiation, cardio-oncology, cardiotoxicity, cardiovascular damage, cancer therapy

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INTRODUCTION

Radiation therapy is an important part of multimodal treatment strategies in cancer therapy. Fifty to sixty percent of all patients with advanced cancer undergo irradiation (1, 2). The increasing number of cancer survivors also leads to an increase occurrence of late-time adverse events following radiation therapy (3, 4). Although strategies to spare surrounding tissue have been developed in modern radiation therapy techniques, damage of healthy tissue/organs cannot totally be avoided by performing an effective cancer treatment using ionized radiation. Exposure of the heart during chest/ thoracic irradiation occurs in particular during treatment of breast and lung cancer (especially left sided) as well as mediastinal lymphomas (3, 5, 6). With increasing number of long-term survivors of esophageal cancer resulting from the addition of chemotherapy to radiotherapy, the risk for radiation-induced cardiovascular toxicity is now recognized as an issue of major concern also in this patient category (7).

Radiation-induced cardiovascular diseases typically manifest years or decades after cancer therapy. Therefore, a causal relation is often difficult to diagnose. Overall incidence and severity correlates with higher radiation dose, larger exposed volumes, younger age at time of exposure, and greater time elapsed since treatment (8, 9). But it has been shown that even little doses of 0.5 Gray (Gy) can significantly enhance cardiovascular risk for the patients (10) and that not total radiation dose but the "volume of the left ventricle receiving 5 Gy" (LV V5Gy) was an important prognostic dose-volume parameter (11). Moreover, concomitant or sequential treatment with

cardiotoxic chemotherapy (e.g., anthracyclines) poses an additional risk for the development of radiation-induced cardiovascular damage (6, 12, 13). Early diagnosis seems to be important to decrease long-term damage, reduce incidence of fatal cardiovascular adverse events and improve quality of life in cancer survivors.

But not only late-time effects are important. An association between higher values of heart dosimetric variables and a worse overall survival at a median follow-up of 2 years was described, suggesting that radiation to the heart could contribute to early mortality in a non-small cell lung cancer population (14). Especially lung cancer patients are also more likely to have pre-existing risk factors such as known cardiac diseases (15) and smoking history that may predispose them to cardiovascular events occurring at earlier time points than would be seen in a healthier patient population treated with thoracic radiation therapy (16, 17).

Myocardial tissue was found to be very sensible to cancer therapy due to high metabolic activity (12). Underlying patho-mechanisms as well as clinical management of radiation-induced cardiovascular diseases are still incompletely characterized. In this review, we discuss different approaches and cardio-oncological strategies after chest irradiation- from bench to bedside.

SPECTRUM OF CARDIOVASCULAR DISEASES FOLLOWING CHEST IRRADIATION

The relative risk of fatal cardiovascular events in survivors after Hodgkin's lymphoma is 2.2–12.7 (median follow-up 18.7 years) and 2–2.2 after breast cancer (median follow-up 12 years) (18, 19). In survivors of childhood-cancer and single therapy with radiation, over 22% show signs of diastolic dysfunction in echocardiography studies (20). The spectrum of cardiovascular diseases associated with chest irradiation is in its occurrence and appearance manifold. **Figure 1** gives an overview and illustrates the relation between different levels of cardiovascular damage.

Radiation-induced pericarditis has been feared because the acute form often led to a life-threatening constrictive pericarditis. Due to advances in radiation protocols (improved techniques, lower dosages and less volume exposed) the occurrence has become rare nowadays (21). In contrast, chronic pericarditis is still one of the most frequent radiation-induced cardiotoxicities and is characterized by exudation of a protein-rich secretion (10). After chemo- and radiation therapy for locally advanced non-small cell lung cancer an incidence of pericardial effusion of nearly 50% is described with the existing risk for cardiac tamponade (17, 22). The underlying patho-mechanism mainly includes inflammatory processes and fibrin-deposition due to microvascular damage (23).

Radiation-induced vascular damage can be divided into a micro- and marcovascular injury, but both can cause a significant myocardial perfusion deficit. Endothelial cells are describe to be very radiation-sensible and seem to form the initial point for patho-mechanistic changes after heart irradiation. Capillaries have only one layer of endothelial cells and are therefore especially challenged. Reduction in capillary density and a disturbed vascular network contribute particularly to the development of radiation-induced myocardial dysfunction (1). The macrovascular damage of the coronary arteries results in an enhanced development of arteriosclerosis. Possible radiation-exposed coronary segments such as the left main coronary artery and the ostial left anterior descending artery and ostial right coronary artery are mainly affected (8). The occurrence of a vascular inflammatory reaction, additional microvascular dysfunction, and subendothelial fibrosis leads to the development of unstable plaques in the large vessels and at the vascular

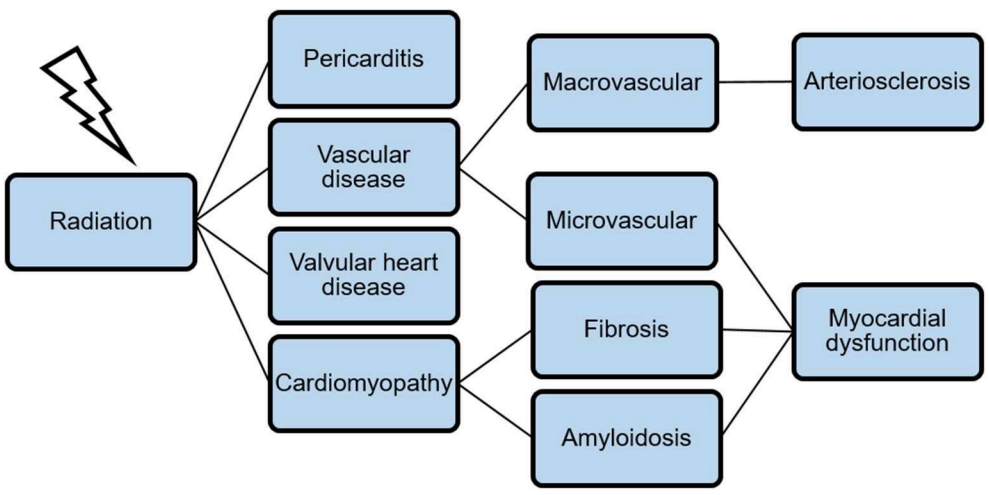

FIGURE 1 | A broad spectrum of cardiovascular diseases is associated with chest irradiation. The combination of microvascular dysfunction, fibrosis, and amyloidosis leads to myocardial dysfunction as a late-time adverse event.

bifurcations (23, 24). This results in an increased incidence of acute myocardial infarction, coronary heart disease, and the development of ischemic cardiomyopathy (25). In women who underwent radiotherapy for breast cancer an increase of major coronary events started within the first 5 years after radiotherapy and continued into the third decade after radiotherapy (26). A four- to seven-fold increased risk of highgrade coronary artery stenosis in mid and distal left anterior descending artery was investigated when comparing women with irradiated left sided with those with right-sided breast cancer (27, 28). The precise signaling pathways are not fully understood. Also a difference in radiosensitivity due to different structure and subregions of the heart is discussed.

The development of radiation-induced cardiomyopathy is based on a combination of structural changes in myocardial tissue as well as a perfusion deficit resulting from micro- and macrovascular changes. Clinically, patients usually have a characteristic, diastolic functional impairment and heart failure with preserved systolic ejection function (3). Diffuse, interstitial fibrosis and amyloid deposition have been forwarded as underlying causes (29–31). Arrhythmias can occur as a result of these structural changes and further conduction system abnormalities. Direct damage to critical structures such as the sinoatrial or atrio-ventricular nodes may lead to bradycardia or all types of heart block (23).

In the area of the heart valves, fibrotic processes are most common on aortic and mitral valves and are similar to degenerative changes. Often these changes are hemodynamically irrelevant, however, in patients with radiation in childhood, higher-grade stenosis, or insufficiency of the heart valve can manifest itself clinically in early adulthood and require surgical treatment (21).

PATHO-MECHANISM AND DEVELOPMENT OF RADIATION-INDUCED CARDIOVASCULAR DAMAGE

Ionizing radiation induces cell death mainly through induction of deoxyribonucleic acid (DNA) single- and double-strand breaks (32). During cancer therapy, ionizing radiation is also associated with increased risk of damage to healthy, cancer surrounding tissue. Cardiomyocytes are described to be radio-resistant (30) but endothelial cells are particularly sensitive to radiation and are suspected to be the initial point for cardiovascular radiation-induced damage due to changes in the surrounding milieu (1, 33). In addition to direct vascular damage, there is also a causal relationship between endothelial dysfunction and the development of muscular, valvular, and arrhythmogenic complications, since the resulting pro-inflammatory environment is a strong initiator of cardiac fibrosis (1).

The mechanisms of endothelial damage primarily base on the induction of apoptosis (acute process) and increased senescence (cell aging, chronic process) (1). As a result, an inflammatory reaction with increased leukocyte recruitment and increased oxidative stress develops through the release of cytokines (1). Depending on the context, radiation-induced DNA damage in endothelial cells can be repaired or trigger apoptosis, which can be p53-mediated or induced by sphingomyelin-produced ceramides (34). In p53-mediated apoptosis, mediation via cytochrome C-induced mitochondrial initiation of apoptotic cell death is leading (intrinsic signaling pathway) (1, 34). Senescence is also triggered by radiation-induced DNA damage. It leads to a change in the cellular phenotype of the endothelial cells and thus to a secretion of cytokines, proteins and other factors (1).

Apoptosis and senescence of endothelial cells together lead to an imbalance between pro- and anticoagulatory as well as pro- and anti-inflammatory factors in the vascular milieu. This leads to an increased adhesion of leukocytes and macrophages, chronic inflammation, a pro-thrombotic status and the increased occurrence of reactive oxygen species (1).

Radiation-induced senescence leads to an inactivation of the phosphoinositide-3-kinase/protein kinase B (PI3k/Akt) signaling pathway and downregulates the serine/threonine kinase mTor (mechanistic target of rapamycin). As a regulator of actin polymerization and the interaction of cell adhesion molecules such as integrins, mTor has a direct influence on the contractility of smooth muscle cells (35, 36). Furthermore, an increased expression of cell surface-located cluster of differentiation 44 (CD44) on endothelial cells has been described. This leads to an increased adhesion of monocytes and ultimately to an increased formation of arteriosclerosis (37).

In addition to adult endothelial cells, endothelial progenitor cells can also be damaged by radiation. This can lead to disturbed vascular remodeling and thus contribute to the development of vascular dysfunction (38). Within the endothelial progenitor cells, ionizing radiation triggers a p53 stabilization, a p21-mediated cell cycle arrest and finally an apoptosis mediated by Bax (Bcl-2-associated X protein) (39).

The development of radiation-induced cardiomyopathy results from an interaction of myocardial remodeling, degeneration and cellular dysfunction. A close connection with endothelial dysfunction due to the creation of a pro-fibrotic and pro-inflammatory environment has been suggested (10). Similar to the mechanisms of cardiac damage caused by anthracyclines, oxidative stress and inflammation lead to structural and functional damage to the cardiomyocytes due to membrane-bound lipid peroxidation (12). The inactivation of peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1α), a key player in the regulation of lipid metabolism in the heart, plays a crucial role, too (10, 40). In contrast to endothelial cells, cardiomyocytes no longer undergo cell division postnatal, so they show no morphological changes (41).

The pro-inflammatory environment is furthermore a strong initiator of cardiac fibrosis (1). For example, interleukin-13 mediated fibroblasts are recruited from various sources such as mesenchymal cells and the bone marrow and ensure myocardial collagen storage (especially collagen types I and III) (23). Increased plasma levels of TGFβ (transforming growth factor-β), angiotensin II and aldosterone are also found after cardiac radiation, lead to increased myocardial fibrosis and thus represent possible therapeutic approaches for cardioprotection during and after radiation therapy (42).

EXPERIMENTAL MODELS FOR CHARACTERIZATION OF FUNCTIONAL CARDIAC IMPAIRMENT AFTER CHEST IRRADIATION

Multiple animal models have been used to characterize radiation-induced cardiomyopathy. Radiation protocols vary between whole thorax and localized heart irradiation as well as single dose and fractionated schedules. Dosages differ between 5 up to 25 Gy (42). For investigation of radiation-induced coronary artery disease, transgenic mouse models are used because wild-type rodents are usually not prone to atherosclerosis (42). In ApoE$^{-/-}$ mice, the development of fatty streaks in carotid arteries was detected 4 weeks after radiation with 14 Gy and a reduction in vascular cell adhesion protein 1 (VCAM-1) as an indication for the development of atherosclerosis was described (43).

While endothelial cell damage plays a major role in the development of radiation-induced cardiac damage, reduction of microvascular density and cardiac capillary damage was found in mice and rats using different protocols (44, 45). This could be shown by an immuno-histological reduction of CD31 positive cells 40 and 60 weeks after 8 or 16 Gy single whole heart irradiation (45). For detailed evaluation of myocardial microvascular damage *in vivo*, DE-microCT (computed tomography) scans 4 or 8 weeks after partial heart irradiation with 12 Gy could show a time-dependent increase in accumulation of gold nanoparticles in the myocardium as a sign for extravasation. Perfusion defects have also be visualized using microSPECT (46).

Beside myocardial perfusion deficits through marco- and microvascular damages, late-onset radiation-induced cardiac damages are characterized by development of myocardial fibrosis. Collagen-deposition within the myocardial interstitium was described using histopathological staining with Masson's trichrome (31) and picrosirius red (30). In addition, an amyloid deposition was detected using congo-red staining (29, 45). Also increases in mRNA expression levels of pro-fibrotic genes like fibronectin have been shown after irradiation of rats' hearts (47).

Pre-clinical *in vivo* models can be used for characterization of cardiac functional impairment after irradiation using echocardiography and pressure-volume catheterization. Normal or even increased left-ventricular systolic function at baseline has been documented (30, 31, 45). In contrast, cardiac radiation exposure caused a diastolic dysfunction expressed by an elevated left ventricular end-diastolic pressure (LVEDP/ filling pressure) and higher *Tau* (time constant of isovolumentric relaxation) in radiated rats compared to control rats (30). Moreover, a reduced contractile reserve was found using mouse stress transthoracic echocardiography with isoproterenol (31).

Experimental studies also indicate a relevant heart-lung-interaction through thoracic radiation. Studies with irradiated rats showed that heart damage was aggravated if also the lung was irradiated and vice versa (47, 48). In addition, this could be translated to a clinical setting, suggesting an importance of heart and lung irradiation in the prediction of radiation-related valve disease in Hodgkin lymphoma survivors (49, 50).

TABLE 1 | Incidence of cardiovascular disease and mortality following chest irradiation.

Cardiovascular disease	Incidence
Pericarditis	5% after 5 years and 40 Gy exposure (51)
Coronary artery disease	7.4% per Gy risk increase after 10–20 years (52)
Systolic LV dysfunction	Incidence 5.7% after 20 years (20)
Diastolic LV dysfunction	Incidence up to 22.4% after 20 years (20)
Valvular heart disease	2.5% per Gy (<30 Gy cumulative dosis) up to 24.3% per Gy (>40 Gy cumulative dosis) risk increase after 30 years (53)
Cardiovascular mortality	4.1% per Gy with a median follow-up of 10 years (54)

LV, left ventricular; Gy, gray.

CLINICAL IMPLICATIONS AND THERAPEUTIC STRATEGIES

The occurrence of cardiovascular side effects after radiation is primarily dependent on the radiation dose and the time interval after the cancer therapy. **Table 1** summarizes information from various clinical studies regarding incidence of cardiovascular diseases.

Mediastinal radiation was identified as an important cardiovascular risk factor, but previously, cardiovascular diagnostics were usually only initiated after clinical symptoms had occurred. This leads to the fact that for example coronary heart disease after radiation manifests in a high proportion as fatal myocardial infarction. Late diagnosis is favored due to damaged peripheral nerve endings after mediastinal radiation whereby patients often present with atypical angina pectoris or even no symptoms (9). Peri-interventional and operative management is also aggravated due to the pronounced pathology at the time of diagnosis and mediastinal adhesions after tumor resection and radiation. The early diagnosis and therapy of radiation-induced heart disease is therefore of great relevance.

Cardiotoxic chemotherapy (e.g., anthracyclines) and chest irradiation is a common combination during treatment of breast cancer (Hodgkin), lymphoma, and childhood cancer which led to a success in the fight against cancer but but also reproduces the occurrence of long-term cardiotoxic side effects (6, 13, 55, 56). This is especially true for the development of heart failure due to a synergistic damage on cardiomyocytes (see also section on patho-mechanism) (12, 57). While resulted cardiomyopathy in patients treated with radiotherapy alone is characterized by diastolic dysfunction, combination of anthracycline therapy and chest irradiation more often leads to an additional clinical relevant systolic dysfunction (57, 58). Beside a simultaneous/sequential cardiotoxic chemotherapy, also patients with existing cardiovascular risk factors have a significantly increased risk of developing radiation-induced heart disease. Therefor an assessment of the individual cardiovascular risk profile should be conducted before starting radiation therapy (55, 59). In case of abnormalities, a cardio-oncological presentation for further diagnostics and development of

an interdisciplinary treatment plan is recommended (60–62). Optimizing existing cardiovascular risk factors and pre-existing conditions is particularly important. After mediastinal radiation therapy, a preventive diagnostic approach using an electrocardiogram (ECG) and transthoracic echocardiography are currently recommended 5 years after therapy, and in the following every 2–5 years depending on the individual presentation and risk assessment (3, 9, 59). Patients with childhood cancer are classified as high-risk collective from an average cardiac radiation dose of $\geq$35 Gy, adults from $>$30 Gy or at $<$30 Gy with co-existing history of anthracycline chemotherapy. Patients classified as high-risk should receive cardiac diagnostics with an ECG and echocardiography early (children 2 years, adults 1 year after radiation) (63, 64). The determination of the *global longitudinal strain* has been shown to be particularly sensitive in the detection of left ventricular dysfunction after mediastinal radiation (20) especially in combination of radiation with anthracycline chemotherapy (13). Cardiac magnetic resonance imaging (MRI) should also be considered in poor echocardiographic conditions. A stress test (e.g., bicycle ergometry/stress ECG) or alternatively a coronary CT should be performed 10 years after radiation (59). These recommendations are currently based primarily on expert opinions and implementation in the guidelines is still pending (65, 66).

The relevance of cardiac biomarkers for prediction of cancer-therapy related cardiovascular toxicity is being discussed (61). Radiation-induced cardiac-cell damage and changes in the left ventricular loading conditions have been linked to several biomarkers including N-terminal pro-B–type natriuretic peptide (NT-proBNP) and troponins (67, 68), but the clinical applicability is still unclear.

Radiation therapy aims to maximize tumor control, while minimize the risk for radiation-induced adverse normal tissue effects (69). Therefore, strategies to reduce heart dose during radiation therapy are crucial. Technical improvements like deep inspiration breath hold gating and particle therapy (59, 70) as well as intensity modulated radiotherapy or volumetric modulated arc therapy, where delivered radiation dose varies between different treatment areas were developed (71). This helps to spare normal tissue but technical and physical strategies reach a natural limit while the main goal is still to perform an effective cancer therapy. Therefore, development of medical concepts to specifically protect normal tissue damage during and after radiation therapy represents an important research topic.

One promising therapeutic approach to reduce radiation-induced cardiovascular damage is the application of angiotensin converting enzyme (ACE) inhibitors (72–74). Studies indicate that for example the preventive administration of captopril in animal models can reduce radiation-induced cardiac damage (72). Additionally, the positive effects of an early initiated therapy with ACE inhibitors and beta-blockers are discussed in the context of other cancer therapies to help prevent heart failure from cancer therapy in general (74). Also lipid-lowering therapies with simvastatin has been observed to reduce radiation-induced cardiac damage (73, 75). Furthermore, medical therapy by interleukin-1 blockade (administration of anakinra) targeting radiation-induced vascular inflammation, has been evaluated recently (76). So far, however, none of the therapeutic approaches have been implemented in clinical practice and further studies are needed.

CONCLUSION

Cardiovascular disease is the leading cause of non-malignancy related death in cancer survivors (50). Minimizing the cardiac radiation dose is currently the only causal way to prevent radiation-induced heart diseases. Additional, assessment of cardiovascular risk before, during and, after irradiation and early diagnosis of radiation-induced cardiac damage is essential to further improve mortality and morbidity in cancer survivors. Further studies to characterize radiation-induced cardiovascular damage and to evaluate potential treatment option are needed.

AUTHOR CONTRIBUTIONS

SM was responsible for review analysis, synthesis, and manuscript preparation. TR was responsible for drafting and proofreading the manuscript. MT was responsible for the concept design, synthesis, analysis, and drafting of the manuscript. All authors approve the paper for submission.

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REFERENCES

1. Venkatesulu BP, Mahadevan LS, Aliru ML, Yang X, Bodd MH, Singh PK, et al. Radiation-induced endothelial vascular injury: a review of possible mechanisms. *JACC Basic Transl Sci.* (2018) 3:563–72. doi: 10.1016/j.jacbts.2018.01.014
2. Delaney G, Jacob S, Featherstone C, Barton M. The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines. *Cancer.* (2005) 104:1129–37. doi: 10.1002/cncr.21324
3. Totzeck M, Schuler M, Stuschke M, Heusch G, Rassaf T. Cardio-oncology - strategies for management of cancer-therapy related cardiovascular disease. *Int J Cardiol.* (2019) 280:163–75. doi: 10.1016/j.ijcard.2019.01.038
4. Zamorano J. An ESC position paper on cardio-oncology. *Eur Heart J.* (2016) 37:2739–40. doi: 10.1093/eurheartj/ehw359
5. Atkins KM, Rawal B, Chaunzwa TL, Lamba N, Bitterman DS, Williams CL, et al. Cardiac radiation dose, cardiac disease, and mortality in patients with lung cancer. *J Am Coll Cardiol.* (2019) 73:2976–87. doi: 10.1016/j.jacc.2019.03.500
6. van Nimwegen FA, Ntentas G, Darby SC, Schaapveld M, Hauptmann M, Lugtenburg PJ, et al. Risk of heart failure in survivors of Hodgkin lymphoma: effects of cardiac exposure to radiation and anthracyclines. *Blood.* (2017) 129:2257–65. doi: 10.1182/blood-2016-09-740332

7. Beukema JC, van Luijk P, Widder J, Langendijk JA, Muijs CT. Is cardiac toxicity a relevant issue in the radiation treatment of esophageal cancer? *Radiother Oncol.* (2015) 114:85–90. doi: 10.1016/j.radonc.2014.11.037
8. Boerma M. Experimental radiation-induced heart disease: past, present, and future. *Radiat Res.* (2012) 178:1–6. doi: 10.1667/RR2933.1
9. Lee Chuy K, Nahhas O, Dominic P, Lopez C, Tonorezos E, Sidlow R, et al. Cardiovascular complications associated with mediastinal radiation. *Curr Treat Options Cardiovasc Med.* (2019) 21:31. doi: 10.1007/s11936-019-0737-0
10. Tapio S. Pathology and biology of radiation-induced cardiac disease. *J Radiat Res.* (2016) 57:439–48. doi: 10.1093/jrr/rrw064
11. van den Bogaard VA, Ta BD, van der Schaaf A, Bouma AB, Middag AM, Bantema-Joppe EJ, et al. Validation and modification of a prediction model for acute cardiac events in patients with breast cancer treated with radiotherapy based on three-dimensional dose distributions to cardiac substructures. *J Clin Oncol.* (2017) 35:1171–8. doi: 10.1200/JCO.2016.69.8480
12. Dalloz F, Maingon P, Cottin Y, Briot F, Horiot JC, Rochette L. Effects of combined irradiation and doxorubicin treatment on cardiac function and antioxidant defenses in the rat. *Free Radic Biol Med.* (1999) 26:785–800. doi: 10.1016/s0891-5849(98)00259-7
13. Mercurio V, Cuomo A, Della Pepa R, Ciervo D, Cella L, Pirozzi F, et al. What is the cardiac impact of chemotherapy and subsequent radiotherapy in lymphoma patients? *Antioxid Redox Signal.* (2019) 31:1166–74. doi: 10.1089/ars.2019.7842
14. Bradley JD, Paulus R, Komaki R, Masters G, Blumenschein G, Schild S, et al. Standard-dose versus high-dose conformal radiotherapy with concurrent and consolidation carboplatin plus paclitaxel with or without cetuximab for patients with stage IIIA or IIIB non-small-cell lung cancer (RTOG 0617): a randomised, two-by-two factorial phase 3 study. *Lancet Oncol.* (2015) 16:187–99. doi: 10.1016/S1470-2045(14)71207-0
15. Janssen-Heijnen ML, Schipper RM, Razenberg PP, Crommelin MA, Coebergh JW. Prevalence of co-morbidity in lung cancer patients and its relationship with treatment: a population-based study. *Lung Cancer.* (1998) 21:105–13. doi: 10.1016/S0169-5002(98)00039-7
16. Simone CB II. Thoracic radiation normal tissue injury. *Semin Radiat Oncol.* (2017). 27:370–7. doi: 10.1016/j.semradonc.2017.04.009
17. Wang K, Eblan MJ, Deal AM, Lipner M, Zagar TM, Wang Y, et al. Cardiac toxicity after radiotherapy for stage III non-small-cell lung cancer: pooled analysis of dose-escalation trials delivering 70 to 90 Gy. *J Clin Oncol.* (2017) 35:1387–94. doi: 10.1200/JCO.2016.70.0229
18. Jaworski C, Mariani JA, Wheeler G, Kaye DM. Cardiac complications of thoracic irradiation. *J Am Coll Cardiol.* (2013) 61:2319–28. doi: 10.1016/j.jacc.2013.01.090
19. Aleman BM, van den Belt-Dusebout AW, De Bruin ML, van 't Veer MB, Baaijens MH, de Boer JP, et al. Late cardiotoxicity after treatment for Hodgkin lymphoma. *Blood.* (2007) 109:1878–86. doi: 10.1182/blood-2006-07-034405
20. Armstrong GT, Joshi VM, Ness KK, Marwick TH, Zhang N, Srivastava D, et al. Comprehensive echocardiographic detection of treatment-related cardiac dysfunction in adult survivors of childhood cancer: results from the St. Jude Lifetime Cohort Study. *J Am Coll Cardiol.* (2015) 65:2511–22. doi: 10.1016/j.jacc.2015.04.013
21. Groarke JD, Nguyen PL, Nohria A, Ferrari R, Cheng S, Moslehi J. Cardiovascular complications of radiation therapy for thoracic malignancies: the role for non-invasive imaging for detection of cardiovascular disease. *Eur Heart J.* (2014) 35:612–23. doi: 10.1093/eurheartj/eht114
22. Ning MS, Tang L, Gomez DR, Xu T, Luo Y, Huo J, et al. Incidence and predictors of pericardial effusion after chemoradiation therapy for locally advanced non-small cell lung cancer. *Int J Radiat Oncol Biol Phys.* (2017) 99:70–9. doi: 10.1016/j.ijrobp.2017.05.022
23. Taunk NK, Haffty BG, Kostis JB, Goyal S. Radiation-induced heart disease: pathologic abnormalities and putative mechanisms. *Front Oncol.* (2015) 5:39. doi: 10.3389/fonc.2015.00039
24. Fajardo LF, Stewart JR. Capillary injury preceding radiation-induced myocardial fibrosis. *Radiology.* (1971) 101:429–33. doi: 10.1148/101.2.429
25. Lenneman CG, Sawyer DB. Cardio-oncology: an update on cardiotoxicity of cancer-related treatment. *Circ Res.* (2016) 118:1008–20. doi: 10.1161/CIRCRESAHA.115.303633
26. Darby SC, Ewertz M, McGale P, Bennet AM, Blom-Goldman U, Bronnum D, et al. Risk of ischemic heart disease in women after radiotherapy for breast cancer. *N Engl J Med.* (2013) 368:987–98. doi: 10.1056/NEJMoa1209825
27. Nilsson G, Witt Nystrom P, Isacsson U, Garmo H, Duvernoy O, Sjogren I, et al. Radiation dose distribution in coronary arteries in breast cancer radiotherapy. *Acta Oncol.* (2016) 55:959–63. doi: 10.1080/0284186X.2016.1182209
28. Nilsson G, Holmberg L, Garmo H, Duvernoy O, Sjogren I, Lagerqvist B, et al. Distribution of coronary artery stenosis after radiation for breast cancer. *J Clin Oncol.* (2012) 30:380–6. doi: 10.1200/JCO.2011.34.5900
29. Sievert W, Stangl S, Steiger K, Multhoff G. Improved overall survival of mice by reducing lung side effects after high-precision heart irradiation using a small animal radiation research platform. *Int J Radiat Oncol Biol Phys.* (2018) 101:671–9. doi: 10.1016/j.ijrobp.2018.02.017
30. Saiki H, Moulay G, Guenzel AJ, Liu W, Decklever TD, Classic KL, et al. Experimental cardiac radiation exposure induces ventricular diastolic dysfunction with preserved ejection fraction. *Am J Physiol Heart Circ Physiol.* (2017) 313:H392–407. doi: 10.1152/ajpheart.00124.2017
31. Mezzaroma E, Di X, Graves P, Toldo S, Van Tassell BW, Kannan H, et al. A mouse model of radiation-induced cardiomyopathy. *Int J Cardiol.* (2012) 156:231–3. doi: 10.1016/j.ijcard.2012.01.038
32. Mladenov E, Magin S, Soni A, Iliakis G. DNA double-strand break repair as determinant of cellular radiosensitivity to killing and target in radiation therapy. *Front Oncol.* (2013) 3:113. doi: 10.3389/fonc.2013.00113
33. Wirsdorfer F, Jendrossek V. Modeling DNA damage-induced pneumopathy in mice: insight from danger signaling cascades. *Radiat Oncol.* (2017) 12:142. doi: 10.1186/s13014-017-0865-1
34. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol.* (2007) 35:495–516. doi: 10.1080/01926230701320337
35. Yentrapalli R, Azimzadeh O, Sriharshan A, Malinowsky K, Merl J, Wojcik A, et al. The PI3K/Akt/mTOR pathway is implicated in the premature senescence of primary human endothelial cells exposed to chronic radiation. *PLoS ONE.* (2013) 8:e70024. doi: 10.1371/journal.pone.0070024
36. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol.* (2004) 6:1122–8. doi: 10.1038/ncb1183
37. Lowe D, Raj K. Premature aging induced by radiation exhibits pro-atherosclerotic effects mediated by epigenetic activation of CD44 expression. *Aging Cell.* (2014) 13:900–10. doi: 10.1111/acel.12253
38. Doyle B, Metharom P, Caplice NM. Endothelial progenitor cells. *Endothelium.* (2006) 13:403–10. doi: 10.1080/10623320601061656
39. Mendonca MS, Chin-Sinex H, Dhaemers R, Mead LE, Yoder MC, Ingram DA. Differential mechanisms of x-ray-induced cell death in human endothelial progenitor cells isolated from cord blood and adults. *Radiat Res.* (2011) 176:208–16. doi: 10.1667/RR2427.1
40. Azimzadeh O, Sievert W, Sarioglu H, Yentrapalli R, Barjaktarovic Z, Sriharshan A, et al. PPAR alpha: a novel radiation target in locally exposed Mus musculus heart revealed by quantitative proteomics. *J Proteome Res.* (2013) 12:2700–14. doi: 10.1021/pr400071g
41. Baker JE, Fish BL, Su J, Haworth ST, Strande JL, Komorowski RA, et al. 10 Gy total body irradiation increases risk of coronary sclerosis, degeneration of heart structure and function in a rat model. *Int J Radiat Biol.* (2009) 85:1089–100. doi: 10.3109/09553000903264473
42. Boerma M, Hauer-Jensen M. Preclinical research into basic mechanisms of radiation-induced heart disease. *Cardiol Res Pract.* (2010) 2011:858262. doi: 10.4061/2011/858262
43. Hoving S, Heeneman S, Gijbels MJ, Te Poele JA, Visser N, Cleutjens J, et al. Irradiation induces different inflammatory and thrombotic responses in carotid arteries of wildtype C57BL/6J and atherosclerosis-prone ApoE(-/-) mice. *Radiother Oncol.* (2012) 105:365–70. doi: 10.1016/j.radonc.2012.11.001
44. Lauk S, Kiszel Z, Buschmann J, Trott KR. Radiation-induced heart disease in rats. *Int J Radiat Oncol Biol Phys.* (1985) 11:801–8. doi: 10.1016/0360-3016(85)90314-1
45. Seemann I, Gabriels K, Visser NL, Hoving S, te Poele JA, Pol JF, et al. Irradiation induced modest changes in murine cardiac function despite progressive structural damage to the myocardium and microvasculature. *Radiother Oncol.* (2012) 103:143–50. doi: 10.1016/j.radonc.2011.10.011

46. Lee CL, Min H, Befera N, Clark D, Qi Y, Das S, et al. Assessing cardiac injury in mice with dual energy-microCT, 4D-microCT, and microSPECT imaging after partial heart irradiation. *Int J Radiat Oncol Biol Phys.* (2014) 88:686–93. doi: 10.1016/j.ijrobp.2013.11.238
47. Ghobadi G, van der Veen S, Bartelds B, de Boer RA, Dickinson MG, de Jong JR, et al. Physiological interaction of heart and lung in thoracic irradiation. *Int J Radiat Oncol Biol Phys.* (2012) 84:e639–46. doi: 10.1016/j.ijrobp.2012.07.2362
48. van Luijk P, Faber H, Meertens H, Schippers JM, Langendijk JA, Brandenburg S, et al. The impact of heart irradiation on dose-volume effects in the rat lung. *Int J Radiat Oncol Biol Phys.* (2007) 69:552–9. doi: 10.1016/j.ijrobp.2007.05.065
49. Cella L, D'Avino V, Palma G, Conson M, Liuzzi R, Picardi M, et al. Modeling the risk of radiation-induced lung fibrosis: irradiated heart tissue is as important as irradiated lung. *Radiother Oncol.* (2015) 117:36–43. doi: 10.1016/j.radonc.2015.07.051
50. Cella L, Palma G, Deasy JO, Oh JH, Liuzzi R, D'Avino V, et al. Complication probability models for radiation-induced heart valvular dysfunction: do heart-lung interactions play a role? *PLoS ONE.* (2014) 9:e111753. doi: 10.1371/journal.pone.0111753
51. Emami B, Purdy JA, Manolis J, Barest G, Cheng E, Coia L, et al. Three-dimensional treatment planning for lung cancer. *Int J Radiat Oncol Biol Phys.* (1991) 21:217–27. doi: 10.1016/0360-3016(91)90180-C
52. van Nimwegen FA, Schaapveld M, Cutter DJ, Janus CP, Krol AD, Hauptmann M, et al. Radiation dose-response relationship for risk of coronary heart disease in survivors of Hodgkin lymphoma. *J Clin Oncol.* (2016) 34:235–43. doi: 10.1200/JCO.2015.63.4444
53. Cutter DJ, Schaapveld M, Darby SC, Hauptmann M, van Nimwegen FA, Krol AD, et al. Risk of valvular heart disease after treatment for Hodgkin lymphoma. *J Natl Cancer Inst.* (2015) 107:djv008. doi: 10.1093/jnci/djv008
54. Taylor C, Correa C, Duane FK, Aznar MC, Anderson SJ, Bergh J, et al. Estimating the risks of breast cancer radiotherapy: evidence from modern radiation doses to the lungs and heart and from previous randomized trials. *J Clin Oncol.* (2017) 35:1641–9. doi: 10.1200/JCO.2016.72.072
55. Armstrong GT, Oeffinger KC, Chen Y, Kawashima T, Yasui Y, Leisenring W, et al. Modifiable risk factors and major cardiac events among adult survivors of childhood cancer. *J Clin Oncol.* (2013) 31:3673–80. doi: 10.1200/JCO.2013.49.3205
56. Runowicz CD, Leach CR, Henry NL, Henry KS, Mackey HT, Cowens-Alvarado RL, et al. American Cancer Society/American Society of clinical oncology breast cancer survivorship care guideline. *J Clin Oncol.* (2016) 34:611–35. doi: 10.1200/JCO.2015.64.3809
57. Adams MJ, Lipshultz SE. Pathophysiology of anthracycline- and radiation-associated cardiomyopathies: implications for screening and prevention. *Pediatr Blood Cancer.* (2005) 44:600–6. doi: 10.1002/pbc.20352
58. Tolba KA, Deliargyris EN. Cardiotoxicity of cancer therapy. *Cancer Invest.* (1999) 17:408–22. doi: 10.3109/07357909909021433
59. Chang HM, Okwuosa TM, Scarabelli T, Moudgil R, Yeh ETH. Cardiovascular complications of cancer therapy: best practices in diagnosis, prevention, and management: part 2. *J Am Coll Cardiol.* (2017) 70:2552–65. doi: 10.1016/j.jacc.2017.09.1095
60. van Nimwegen FA, Schaapveld M, Janus CP, Krol AD, Petersen EJ, Raemaekers JM, et al. Cardiovascular disease after Hodgkin lymphoma treatment: 40-year disease risk. *JAMA Intern Med.* (2015) 175:1007–17. doi: 10.1001/jamainternmed.2015.1180
61. Michel L, Rassaf T, Totzeck M. Biomarkers for the detection of apparent and subclinical cancer therapy-related cardiotoxicity. *J Thorac Dis.* (2018) 10:S4282–S95. doi: 10.21037/jtd.2018.08.15
62. Michel L, Mincu RI, Mahabadi AA, Settelmeier S, Al-Rashid F, Rassaf T, et al. Troponins and brain natriuretic peptides for the prediction of cardiotoxicity in cancer patients: a meta-analysis. *Eur J Heart Fail.* (2019) 22:350–61. doi: 10.1093/eurheartj/ehz748.0328
63. Armenian SH, Hudson MM, Mulder RL, Chen MH, Constine LS, Dwyer M, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. *Lancet Oncol.* (2015) 16:e123–36. doi: 10.1016/S1470-2045(14)70409-7
64. Armenian SH, Lacchetti C, Lenihan D. Prevention and monitoring of cardiac dysfunction in survivors of adult cancers: American Society of Clinical Oncology Clinical Practice Guideline Summary. *J Oncol Pract.* (2017) 13:270–5. doi: 10.1200/JOP.2016.018770
65. Rassaf T, Totzeck M. Modern concepts in cardio-oncology. *J Thorac Dis.* (2018) 10:S4386–90. doi: 10.21037/jtd.2018.11.110
66. Michel L, Rassaf T. Cardio-oncology: need for novel structures. *Eur J Med Res.* (2019) 24:1. doi: 10.1186/s40001-018-0359-0
67. D'Errico MP, Petruzzelli MF, Gianicolo EA, Grimaldi L, Loliva F, Tramacere F, et al. Kinetics of B-type natriuretic peptide plasma levels in patients with left-sided breast cancer treated with radiation therapy: results after one-year follow-up. *Int J Radiat Biol.* (2015) 91:804–9. doi: 10.3109/09553002.2015.1027421
68. D'Errico MP, Grimaldi L, Petruzzelli MF, Gianicolo EA, Tramacere F, Monetti A, et al. N-terminal pro-B-type natriuretic peptide plasma levels as a potential biomarker for cardiac damage after radiotherapy in patients with left-sided breast cancer. *Int J Radiat Oncol Biol Phys.* (2012) 82:e239–46. doi: 10.1016/j.ijrobp.2011.03.058
69. de Leve S, Wirsdorfer F, Jendrossek V. The CD73/Ado system-a new player in RT induced adverse late effects. *Cancers.* (2019) 11:1578. doi: 10.3390/cancers11101578
70. Verma V, Shah C, Mehta MP. Clinical outcomes and toxicity of proton radiotherapy for breast cancer. *Clin Breast Cancer.* (2016) 16:145–54. doi: 10.1016/j.clbc.2016.02.006
71. Taylor A, Powell ME. Intensity-modulated radiotherapy–what is it? *Cancer Imaging.* (2004) 4:68–73. doi: 10.1102/1470-7330.2004.0003
72. Yarom R, Harper IS, Wynchank S, van Schalkwyk D, Madhoo J, Williams K, et al. Effect of captopril on changes in rats' hearts induced by long-term irradiation. *Radiat Res.* (1993) 133:187–97. doi: 10.2307/3578356
73. Menezes KM, Wang H, Hada M, Saganti PB. Radiation matters of the heart: a mini review. *Front Cardiovasc Med.* (2018) 5:83. doi: 10.3389/fcvm.2018.00083
74. Totzeck M, Mincu RI, Heusch G, Rassaf T. Heart failure from cancer therapy: can we prevent it? *ESC Heart Fail.* (2019) 6:856–62. doi: 10.1002/ehf2.12493
75. Inano H, Suzuki K, Onoda M, Wakabayashi K. Anti-carcinogenic activity of simvastatin during the promotion phase of radiation-induced mammary tumorigenesis of rats. *Carcinogenesis.* (1997) 18:1723–7. doi: 10.1093/carcin/18.9.1723
76. Christersdottir T, Pirault J, Gistera A, Bergman O, Gallina AL, Baumgartner R, et al. Prevention of radiotherapy-induced arterial inflammation by interleukin-1 blockade. *Eur Heart J.* (2019) 40:2495–503. doi: 10.1093/eurheartj/ehz206

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REVIEW
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Human Pluripotent Stem Cell-Derived Cardiomyocytes for Assessment of Anticancer Drug-Induced Cardiotoxicity

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Cardiotoxicity is a major cause of high attrition rates among newly developed drugs. Moreover, anti-cancer treatment-induced cardiotoxicity is one of the leading reasons of mortality in cancer survivors. Cardiotoxicity screening *in vitro* may improve predictivity of cardiotoxicity by novel drugs, using human pluripotent stem cell (hPSC)-derived-cardiomyocytes. Anthracyclines, including Doxorubicin, are widely used and highly effective chemotherapeutic agents for the treatment of different forms of malignancies. Unfortunately, anthracyclines cause many cardiac complications early or late after therapy. Anthracyclines exhibit their potent anti-cancer effect primarily via induction of DNA damage during the DNA replication phase in proliferative cells. In contrast, studies in animals and hPSC-cardiomyocytes have revealed that cardiotoxic effects particularly arise from (1) the generation of oxidative stress inducing mitochondrial dysfunction, (2) disruption of calcium homeostasis, and (3) changes in transcriptome and proteome, triggering apoptotic cell death. To increase the therapeutic index of chemotherapeutic Doxorubicin therapy several protective strategies have been developed or are under development, such as (1) reducing toxicity through modification of Doxorubicin (analogs), (2) targeted delivery of anthracyclines specifically to the tumor tissue or (3) cardioprotective agents that can be used in combination with Doxorubicin. Despite continuous progress in the field of cardio-oncology, cardiotoxicity is still one of the major complications of anti-cancer therapy. In this review, we focus on current hPSC-cardiomyocyte models for assessing anthracycline-induced cardiotoxicity and strategies for cardioprotection. In addition, we discuss latest developments toward personalized advanced pre-clinical models that are more closely recapitulating the human heart, which are necessary to support *in vitro* screening platforms with higher predictivity. These advanced models have the potential to reduce the time from bench-to-bedside of novel antineoplastic drugs with reduced cardiotoxicity.

Keywords: human pluripotent stem cells, cardiomyocytes, cardio-oncology, organ-on-chip, cardiotoxicity

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INTRODUCTION

Cardiovascular diseases and cancer are the two leading causes of death in industrialized countries. Although in recent years new anti-cancer therapies improved long-term survival rates, this is unfortunately also accompanied by an increased risk of cardiovascular complications because of adverse side effects of these anti-cancer drugs. Anthracyclines are a group of widely used anti-cancer drugs that are known to cause cardiac complications, either early or late after start of treatment (1–7). The history of anthracyclines originates in the 1950s when Daunorubicin was isolated from the bacteria *Streptomyces peucetius*. Ten years later Doxorubicin, a more effective derivative of Daunorubicin, was identified (8). However, Doxorubicin received the ominous nickname "Red Devil" because of the detrimental side effects in combination with its red color. In approximately 11% of patients, Doxorubicin-induced cardiotoxicity leads to an acute response within 2–3 days of administration (early onset of cardiotoxicity), manifested by chest pain resulting from different forms of arrhythmia (3). Chronic Doxorubicin-induced cardiomyopathy has a lower incidence (ranging from 4 to 36% dependent on the dose) and can occur as late as 10 years after the last dose (late onset of chronic cardiotoxicity), with only a 50% 1-year survival prognosis when cardiomyopathy further develops into congestive heart failure (3, 7). Importantly, anthracycline-induced cardiotoxicity endangers cancer patients since the early 70s (9, 10). At present, almost half a century later, success in preventing or counteracting anthracycline-induced cardiotoxicity has been very limited. Moreover, although novel anti-cancer therapeutic compounds to specific target molecules, such as tyrosine kinase inhibitors, have been developed, drug-induced cardiotoxicity remains a persistent problem. One major reason for this is that current *in vitro* and animal models fail to show sufficient predictive power and limit extrapolation to patients, which consequently leads to high attrition rates during the process of drug development.

Development of preclinical human cell-based models for drug discovery that mimic human physiology and pathophysiology, has the potential to improve the predictability of adverse drug events (11). In particular, human pluripotent stem cells (hPSCs) are excellent candidates for developing these models since they replicate indefinitely and have the capacity to differentiate to any cell type of the human body, including functional cardiomyocytes. This is especially embraced in the cardiac field, since isolated primary human cardiomyocytes are extremely difficult to obtain and maintain in culture. Advances in differentiation and purification of hPSC-derived cardiomyocytes (hPSC-cardiomyocytes) and their unlimited availability has promoted strategies to use these cells for cardiotoxicity assessment of new drugs (12). Previously, the Food and Drug Administration (FDA), the supreme body worldwide that checks the quality and safety of medical products, initiated the so-called "Comprehensive *in vitro* Proarrhythmia assay (CIPA)," which represents a paradigm shift and encompasses evaluation of life-threatening proarrhythmic risk for all new compounds in a preclinical assay using hPSC-cardiomyocytes (13). This CIPA initiative signifies the enormous potential of hPSC-derived cardiomyocytes for preclinical drug screening. Here, we describe underlying mechanisms of anthracycline-induced cardiotoxicity, as well as therapeutic approaches for cardioprotection. Furthermore, we will discuss the potential to use hPSC-derived cardiac models for improved safety assessment of anti-cancer drugs and strategies to overcome current limitations for developing *in vitro* drug testing platforms with a higher predictivity.

Mechanism of Anthracycline-Induced Cardiotoxicity in hPSC-Cardiomyocytes

The potent therapeutic anti-cancer effect of Doxorubicin is mediated primarily via inhibition of topoisomerase IIa, an enzyme responsible for unwinding DNA before replication or transcription. This inhibition leads to DNA damage and consequently death of highly proliferating cancer cells, but also affects healthy proliferating cells, such as hematopoietic precursors, epithelial lining of the intestine and hair follicle cells. Fully mature cardiomyocytes are typically quiescent and do not express topoisomerase IIα, however studies performed in hPSC-cardiomyocytes demonstrated that Doxorubicin may bind to the enriched topoisomerase IIβ in cardiomyocytes, and knock-out of this isotype improved cell viability significantly when exposed to Doxorubicin (14, 15). A comprehensive review on damage mechanisms of anthracycline-induced cardiotoxicity has been published elsewhere (16). The underlying mechanisms of Doxorubicin-induced cardiotoxicity is not yet completely understood. However, several studies in hPSC-cardiomyocytes have shown that mitochondrial dysfunction, disruption of calcium homeostasis, as well as altered gene and protein expression levels triggering apoptotic cell death, play important roles in anthracycline-induced cardiotoxicity (**Figure 1**). Increased oxidative stress by elevated production of reactive oxygen (ROS) and nitrogen species (NOS) in the mitochondria of cardiomyocytes are major factors of Doxorubicin-induced cardiotoxicity, since cardiomyocytes are more sensitive to these molecules because they possess lower levels of antioxidant enzymes than other cell types (1, 3, 4, 17–20). As a consequence, higher stress levels in cardiomyocytes under oxidative stress circumstances can lead to cardiotoxicity (20). An increase in intracellular levels of ROS and NOS cause mitochondrial dysregulation, lipid peroxidation, DNA damage, and protein carbonylation. Cardiac mitochondria are the major site of Doxorubicin-induced ROS/NOS levels due to the localization of the major redox cycling enzymes such as NAD(P)H. Additionally, Doxorubicin becomes nearly irreversibly bound to cardiolipin. Cardiolipin is an essential phospholipid, almost exclusively expressed on the inner mitochondrial membrane. It has been shown that pathological changes in cardiolipin trigger ROS production and impair mitochondrial function (21). In addition, Doxorubicin also increases mitochondrial iron accumulation which further increases ROS production in the mitochondria. Indeed, cellular and mitochondrial (as measured by cellular H_2O_2 production and intra-mitochondrial O^{2-} levels) ROS production in hPSC-cardiomyocytes increases already 24 h after exposure to Doxorubicin at concentrations as low as 0.01 μM (patient serum levels of Doxorubicin vary in

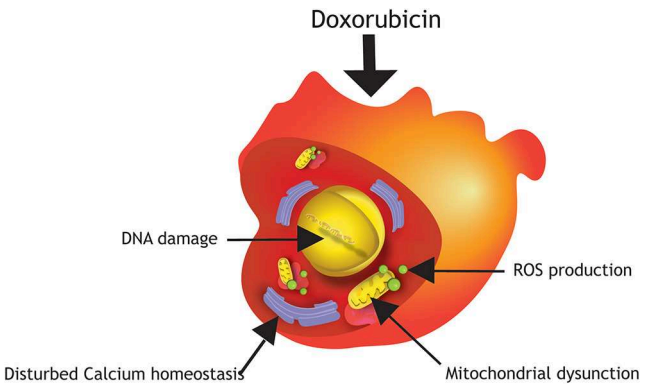

FIGURE 1 | Damage mechanisms of Doxorubicin in hPSC-cardiomyocytes. Major mechanisms of anthracycline-induced cardiotoxicity in hPSC-cardiomyocytes are DNA damage, production of reactive oxygen species (ROS), mitochondrial dysfunction and disturbed calcium homeostasis.

the range of 5–10 μM after single injection). Moreover, short term exposure to 5 μM Doxorubicin induces dramatic reduction of the mitochondrial transmembrane potential indicating mitochondrial dysfunction (14, 22). To meet the high energy demand required to sustain contractile function, cardiomyocytes rely on efficient mitochondrial oxidative metabolism for energy production instead of anaerobic glycolysis. Thus, a disturbed energy metabolism is a high risk for cardiomyocyte survival. Repeated Doxorubicin exposure reduces ATP levels which cannot be restored after Doxorubicin removal suggesting a prolonged effect of Doxorubicin on energy generation in hPSC-cardiomyocytes. Together these effects pinpoint to the fact that Doxorubicin induces mitochondrial dysfunction in hPSC-cardiomyocytes (22, 23). Importantly, structural and functional disturbances are more pronounced after repeated dosing of Doxorubicin (mimicking chronic exposure) (23–25).

Another mechanism by which anthracyclines fight cancer is the induction of apoptosis. Doxorubicin effectively induces apoptotic cell death via activation of so-called death receptors (DRs), such as TNF receptor 1 (TNFR1), Fas receptor, DR4 and DR5, in many cancer types. Activation of these DRs induces the assembly of death inducing signaling complex (DISC), which starts the caspase cascade to mediate cleavage of cellular proteins and ultimately apoptosis of the cell. This DR-mediated apoptosis machinery has been shown to be conserved in human cardiomyocytes. Indeed, a Doxorubicin concentration–dependent increase of caspase 3 and 7 and Annexin V, a marker of early apoptosis, and 7-aminoactinomycin D (7–AAD) or propidium iodide as markers of late apoptosis or necrosis, suggest a strong contribution of programmed cell death to reduced cell survival after exposure to Doxorubicin. Interestingly, in hPSC-cardiomyocytes Doxorubicin induces the expression of all four DRs in a dose-dependent fashion with p53 being the key upstream activator. This suggests a p53-regulated DR-mediated apoptotic pathway a key mechanism involved in early Doxorubicin-induced cardiotoxicity. In accordance with these findings, Doxorubicin-induced apoptosis was effectively blocked by pretreating hPSC-cardiomyocytes with a DR5 neutralizing antibody. This cardiotoxic effect was reversible since expression of DR4 and 5 proteins decreased after recovery for 7 days following washout of Doxorubicin (14, 15, 22, 26, 27). In contrast to these studies with acute exposure to Doxorubicin, Li et al. showed that p53 can protect against chronic Doxorubicin exposure by counteracting mitochondrial DNA depletion after chronic exposure of hPSC-cardiomyocytes (and mice) to low doses of Doxorubicin (28).

Disturbed calcium homeostasis in cardiomyocytes hampers proper cardiac contraction. Doxorubicin has been shown to induce accumulation of intracellular calcium release from the sarcoplasmic reticulum (SR) causing calcium overload with sarcomeric disarray and myofibril deterioration in hPSC-cardiomyocytes. Moreover, Doxorubicin exposure led to downregulation of several ion channel genes, including genes that encode for calcium channels CACNs (14, 22, 26).

In summary this data shows that mitochondrial dysfunction, apoptosis, as well as disturbed calcium homeostasis are the main mechanisms of anthracycline-induced cardiotoxicity. However, in 2018, a new mechanism of Doxorubicin-induced cardiotoxicity via downregulation of the RNA-binding-protein Quaking has been identified (29). Downregulation of Quaking regulates a set of circular RNAs involved in Doxorubicin-induced apoptosis suggesting that other mechanisms cannot be excluded.

Protective Mechanisms to Prevent or Reduce Anthracycline-Induced Cardiotoxicity

To increase the dose window of effective treatment without severe adverse side effects (therapeutic index) of conventional Doxorubicin therapy, several protective mechanisms have been developed, including modifications/analogs of Doxorubicin, targeted delivery or protective agents (**Figure 2**).

Analogs of Doxorubicin

The cardiotoxic nature of Doxorubicin pushed the development of Doxorubicin analogs into overdrive in the 1970s and 1980s, with over a 1000 analogs manufactured or discovered since (30). However, most of them failed to reach anti-tumor efficacy comparable to Doxorubicin and others were considered too toxic based on trials in rodents (30). Only a handful of analogs reached clinical trial phase, such as epirubicin, pirarubicin (also known as THP), idarubicin, mitoxanthrone, and others (31–42). The success of these analogs varies per clinical setting and malignancy, and a reduction in cardiotoxicity was not always achieved. In patients, cardiotoxic events are classically detected through loss of the left ventricular ejection fraction (LVEF), irregularities in ECG readings (in particular ST-T changes), changes in systolic time intervals (STI, indicating heart muscle failure) and in more recent work, the release of biomarkers in blood, such as cardiac Troponin-T and brain natriuretic peptide (BNP) (31, 33, 35–38, 41–45). Due to the year of discovery, early Doxorubicin analogs have not been tested *in vitro* using hPSC-cardiomyocytes to evaluate their cardiotoxicity, since these models were not available at the time. Whereas, biomarker release can also

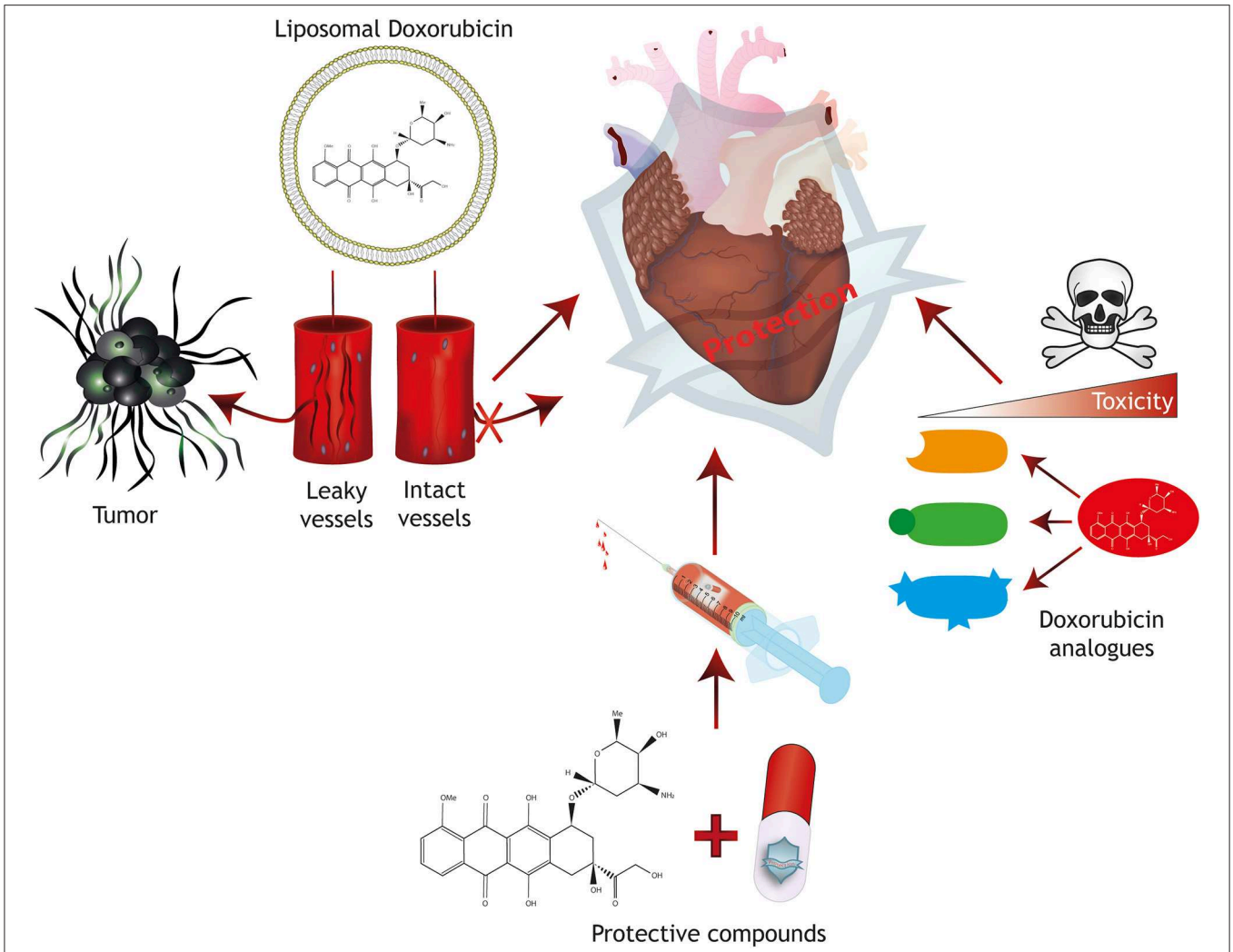

FIGURE 2 | Strategies for cardioprotection. Protective mechanisms for anthracycline-induced cardiotoxicity include the generation of Doxorubicin analogs with reduced toxicity, the combined treatment with protective compounds and targeted therapy approaches including liposomal packaging of Doxorubicin. Liposomal packaging prevents Doxorubicin from exiting the vasculature in tissues with tight endothelial barriers such as the heart, but allows targeting of a tumor with leaky capillaries.

be assessed *in vitro* using hPSC-cardiomyocytes, other clinical read-outs are more difficult to evaluate *in vitro*. Nevertheless, measurement of changes in action potential, contractility, and cell survival *in vitro* may be associated with ECG/STI changes and impaired LVEF and therefore it will be of interest to test Doxorubicin analogs in hPSC-cardiomyocytes for cardiac safety.

Targeted Delivery Strategies

An alternative to reducing cardiotoxic properties of anthracyclines is specifically delivering anthracyclines at the location where cancer cells reside. This can be achieved by carriers that encapsulate the compound and release it at the desired location. For example, liposomal encapsulation of anthracycline inhibits transfer from the circulation to tissues with a tight endothelial barrier (e.g., the heart), but allow transfer to tissues with leaky or fenestrated vasculature, such as in tumors, but also in organs such as lung, bone marrow, lymph nodes and liver (39, 46, 47). In the last two decades, numerous trials have been conducted to assess the safety and benefit of liposome-encapsulated Doxorubicin. Success of liposomal encapsulated anthracyclines is inconsistent. Liposomal encapsulation could abolish cardiotoxicity in some clinical trials, allowing a higher cumulative dose for treatment (39, 48–50). However, in some clinical settings, liposomal Doxorubicin did not reduce cardiotoxicity compared to free anthracyclines (51), suggesting that liposomal encapsulation only has limited benefit. Unmasked liposomes such as described thus far are relatively quickly cleared from the circulation by the reticulo-endothelial system (RES), which limits the therapeutic index of so-called non-PEGylated liposomal (NPL) (39).

PEGylated liposomal Doxorubicin (PLD) features an anchored polyethylene glycol (PEG) group on the exterior lipid surface that masks them from the RES, thus reducing uptake from the circulation (52). Caelyx (Doxil), a commercialized PLD, showed significant reduction in cardiotoxicity compared to free Doxorubicin in several studies, even when used for patients who have increased risk of cardiac complications (53–55). Unfortunately, PLDs are limited in their use because of increased incidence and severity of non-cardiac side effects such as palmar-plantar erythrodysesthesia (PPE), mucositis and haematogenic disorders (54, 56). Liposomes (PEGylated or not) cannot prevent severe adverse effects of Doxorubicin entering off-target tissues. The mechanism of specific delivery of classical liposomes depends greatly on the difference in endothelial barrier permeability, which is equally high in tumors and several healthy tissues alike, and not all malignancies cohere to the "tumor with a leaky vasculature" phenotype. In an attempt to increase specific targeting of cancer cells, liposomes were conjugated with antibodies that are directed against the human epidermal growth factor receptor 2 (HER2), which is highly expressed in breast cancer cells. *In vitro*, these liposomes were effective in killing HER2-positive cancer cells, but did not affect hPSC-cardiomyocytes, highlighting their specificity (47). The HERMIONE phase II trial is now being conducted to test the safety and efficacy in patients (57). Another recent development is the encapsulation of anthracyclines in nanocages, constructed of organic (virus-like particles (VLPs), protein, DNA or carbon-based particles) or inorganic particles (supramolecular nanosystems, hybrid metal-organic, gold, or silica-based systems). Advantages of this encapsulation method are: (1) a large carrier storage capacity, (2) a targeted release of the drug at the site of interest, (3) combined with a porous structure and (4) a low immunogenic surface (58). Nanocages have not been tested in clinical trials yet, however *in vitro* data suggests improved drug targeting of cancer cells and killing efficacy, and a lower cardiotoxicity, when administered in rodents (59–63). Unfortunately, nanocages have not been specifically tested *in vitro* on hPSC-cardiomyocytes to test their cardiotoxicity.

In addition to Doxorubicin encapsulation, latest modifications to Doxorubicin are being tested on cell cultures prior to animal testing to assess their safety for the heart, using currently available hPSC-cardiomyocyte *in vitro* models. These modifications of Doxorubicin are aimed at preventing the anthracycline from exerting its toxicity on cardiomyocytes by making the drug delivery more specific. For example, DTS-201, a Doxorubicin prodrug that is injected in a stable, cell-impermeable state, is cleaved by endopeptidases that are released by tumors, enabling entry into nearby cells and specific anti-tumor activity. In a phase I trial, DTS-201 could be administered at a cumulative dose equivalent to three times the recommended dose of free Doxorubicin, without triggering a significant drop in LVEF (64). Another promising technique is the development of Doxorubicin-dendrimer conjugates with acetylgalactosamine attached to the dendrimer surface. These conjugates are specifically taken up by hepatic cancer cells, increasing therapeutic index and reducing cardiac exposure and thus toxicity in mice. In addition, *in vitro*, these conjugates did not affect hPSC-cardiomyocyte viability and electrophysiology, or induce apoptosis, demonstrating the specificity for drug uptake by the target cell type (65).

Protective Agents to Alleviate Cardiotoxicity

Cardioprotection during anthracycline-based therapies can also be offered by combining the anthracycline with a protective compound. As the cardiotoxic mechanism of anthracyclines involves inducing oxidative stress and mitochondrial dysfunction, protective agents that are able to lower the production of ROS or lower the workload of the heart during anthracycline treatment may be beneficial to reduce anthracycline-induced cardiotoxicity. Factors that may reduce oxidative stress in the heart, such as statins and natural antioxidants, and compounds that reduce the workload of the heart, such as angiotensin-converting-enzyme (ACE) inhibitors and beta-blockers, have been combined with anthracyclines in clinical trials, with mixed success (66–77). As far as known, these compounds have never been tested in hPSC-cardiomyocytes for assessment of their direct cardioprotective potential, even though reducing oxidative stress in particular can be readily modeled *in vitro*. Since both ischemic heart disease and anthracycline-mediated cardiotoxicity share common pathological pathways, therapies for one disease could also be beneficial for the other. Trials with more original approaches, such as remote ischemic conditioning or physical exercise and caloric restriction, strategies that were beneficial in the setting of ischemic heart disease, have been started with the purpose to reduce cardiotoxicity caused by chemotherapy (78, 79). Results of these trials are not yet available.

In hPSC-cardiomyocytes it has been shown that Doxorubicin causes arrhythmic contractions by inducing the accumulation of calcium in the cell and thereby disturbing calcium homeostasis (14, 22). This would suggest that calcium antagonists can help to reduce Doxorubicin-induced cardiotoxicity. Interestingly, a calcium antagonistic compound called prenylamine, was able to reduce cardiotoxic effects when co-administered with Doxorubicin, before it was withdrawn from the market 1 year later (80). Reason for withdrawal was the pro-arrhythmic properties of prenylamine, inducing long QT syndrome (81). Other calcium agonists are currently primarily used at lower doses to reduce Doxorubicin resistance of cancer cells, by inhibiting their capacity to pump out drugs via the P-glycoprotein ATP-dependent efflux pumps (82). Low doses of these calcium antagonists could be repurposed to reduce Doxorubicin-induced cardiotoxicity.

Neuregulin-1β, an ErbB receptor (HER2) family ligand, has been proven effective against Doxorubicin-induced cardiotoxicity, but is also pro-neoplastic in many cancers via formation of ErbB2/3 interactions (83–86). Even if neuregulin, as key mediator of endothelial–cardiomyocyte crosstalk, is able to protect ventricular cardiomyocytes from anthracycline-induced apoptosis, its use might thus be controversial in the clinic. Nevertheless, phase I, II and III clinical trials have been completed or are ongoing (NCT01251406, NCT1214096, and NCT01541202) (84–86). Patients with symptomatic heart failure (HF) and left ventricular dysfunction showed

improved cardiac function with increased LVEF after treatment with recombinant human neuregulin 1. In contrast to native neuregulin-1β, engineered bivalent neuregulin-1β has reduced pro-neoplastic potential because of a shift toward ErbB3 homotypic interactions. Bivalent neuregulin-1β is anti-neoplastic or cytostatic in cancer cells. Importantly, bivalent neuregulin-1β showed similar cardioprotective properties as neuregulin in hPSC-cardiomyocytes and in mice with chronic cardiomyopathy (87). Especially the reduced pro-neoplastic potential of bivalent neuregulin-1β offers translational potential for cardioprotection after anthracycline therapy, however to date, bivalent neuregulin-1β has not been taken into clinical trial.

Dexrazoxane, a catalytic topoisomerase inhibitor and iron chelator, is the only marketed cardioprotective agent that is used specifically to counteract the adverse effects of anthracyclines (88). In children with acute lymphoblastic leukemia, leukemia or lymphoma, the combined treatment with Doxorubicin and Dexrazoxane significantly reduces release of troponin (as biomarker of cardiotoxicity) and Doxorubicin-induced cardiac remodeling. Importantly, the combination with Dexrazoxane improves left ventricular function compared to Doxorubicin alone (89, 90). Dexrazoxane is also effective in relapsed patients with leukemia when administered prior or after a high cumulative dose (91). In breast cancer patients with increased risk of cardiotoxicity because of an enhanced cumulative dose of anthracycline, Dexrazoxane also robustly reduced the incidence of cardiac events, such as decreased ejection fraction, and incidence and severity of CHF (92, 93). Even in a retrospective 2–20 year follow-up, Dexrazoxane demonstrated late-clinical and subclinical cardioprotective effect (94).

Treatment of cardiomyocytes with Dexrazoxane is therefore expected to prevent cardiotoxicity. However, pre– and co–treatment of hPSC-cardiomyocytes with Dexrazoxane could not alleviate Doxorubicin-induced cardiotoxicity and increased cardiotoxicity at concentrations as low as 0.1 μM (22, 95). This discrepancy between *in vivo* and *in vitro* may be related to the relative immature character of hPSC-cardiomyocytes. Early stage hPSC-cardiomyocytes may be more sensitive to Doxorubicin because of higher expression levels of topoisomerase IIα and are thus more prone to severe DNA damage (as mentioned earlier: Doxorubicin primarily affects topoisomerase IIα), whereas in mature cardiomyocytes topoisomerase IIα switches to the IIβ isoform. Importantly, Dexrazoxane might exert its protective effects via depletion of topoisomerase IIβ, the major topoisomerase isoform in more mature cardiomyocytes (95, 96). This suggests that more advanced models are needed to assess cardiotoxicity *in vitro*.

HPSC-Cardiomyocyte Models in Drug Screening of Tyrosine Kinase Inhibitors (TKis)

Not only anthracyclines, but also safety assessment of other classes of anti-cancer drugs may benefit from hPSC-based *in vitro* models. For example, malignancies caused by hyperactive receptor tyrosine kinases (RTKs), which drive proliferation and survival, are treated with FDA-approved tyrosine-kinase inhibitors (TKis). However, a number of TKis have been associated with adverse cardiac side effects following treatment of cancer patients, such as reduced LVEF, myocardial infarction, arrhythmias and heart failure (97). While animal models have often failed to predict cardiotoxic effects during safety assessment (98), TKis have shown to inhibit viability, contractility, electrophysiology, calcium handling and cardiac oxidative phosphorylation of hPSC-cardiomyocytes (99–101). Different TKis show cell type specific cytotoxicity, either in hPSC-derived cardiomyocytes, endothelial cells and fibroblasts or hPSCs, suggesting that TKis differently affect cardiovascular and non-cardiovascular cell types (99).

TKis can be subdivided into small molecules, including crizotinib, sunitinib, or sorafenib, and monoclonal antibodies. Importantly, different TKis have distinct toxicity profiles and not all TKIs induce cardiotoxicity (97). The broad-range TKi sunitinib, for example, decreased hPSC-cardiomyocyte viability, as well as AMP-activated protein kinase (AMPK), increased lipid accumulation and induced arrhythmic events in sunitinib-treated hPSC-cardiomyocytes. Crizotinib, an ALK/MET inhibitor, increased ROS production and caspase activation, induced cholesterol accumulation and an irregular beat pattern. Similarly, Nilotinib, a second generation Bcr-Abl inhibitor, increased ROS generation and caspase activation and induced arrhythmic beating. Interestingly, compared to the TKis sunitinib, crizotinib and nilotinib, the relatively cardiac-safe TKi erlotinib only displayed minor effects on hPSC-cardiomyocyte health in the same study (98) which suggests that hPSC-cardiomyocytes may represent a reliable *in vitro* model for predicting cardiotoxic side effects.

RTK activity may also be reduced by blocking the human epidermal growth factor receptor 2 (HER2), which activation is a common feature of a subset of malignant diseases, particularly breast cancer (102). While trastuzumab (TZM), a TKi monoclonal antibody blocking HER2, greatly improves treatment against HER2 positive malignancies, inhibition of HER2 signaling was found to be detrimental for cardiac function (102–104). In a subset of breast cancer patients, treatment with TZM led to mild to severe decrease in LVEF without apparent myocardial tissue damage or cell loss. Using patient-derived hPSC-cardiomyocyte 2D monolayer cultures it was shown that TZM affected metabolic and mitochondrial processes, of which the severity depended on genetic variation. Moreover, this disease phenotype could be rescued using metabolism-stimulating agents, such as AMPK (102). Additionally, HER2 activation triggered by exogenous neuregulin-1 could reduce hPSC-cardiomyocyte damage and cell loss during Doxorubicin exposure, an effect that was lost upon inhibition of the HER2 receptor by TZM (103, 104). These findings indicate that hPSC-cardiomyocyte 2D models may serve as a valuable tool to identify cardioprotective compounds in a high throughput system, which can be further validated in more advanced human-based 3D cardiac models.

Generation of Advanced hPSC-Derived *in vitro* Models for Accurate Assessment of Cardiac Safety

Although cardiotoxic effects could be observed in hPSC-cardiomyocyte based models, not all aspects of *in vivo* cardiotoxicity are recapitulated in current human *in vitro* models. One of the important shortcomings of hPSC-cardiomyocyte models is their relative immature phenotype. Reviews on the topic of the immaturity of hPSC-cardiomyocytes, including strategies to increase the level of maturity have already been described elsewhere (105–107). **Figure 3** shows a description of current *in vitro* models utilizing hPSC-cardiomyocytes, including their advantages and limitations. It is therefore crucial to develop innovative advanced human models that approximate the adult human heart. In order to achieve this, defined multicellular models, consisting of the main cell-types of the heart, such as atrial or ventricular cardiomyocytes, endothelial cells, smooth muscle cells and fibroblasts, need to be constructed.

Cardiac tissue formation in 3D tissues, such as cardiac microtissues, more closely mimics native heart tissue which might allow studying more physiologically relevant dosing profiles and deciphering acute vs. chronic cardiotoxicity (25). For generating other 3D advanced cardiac models, so-called engineered heart tissues (EHTs), hPSC-cardiomyocytes are mixed with fibroblasts (or other cardiac cells) in a hydrogel and poured into a casting mold around silicon posts, leading to beating structures within the first week under continuous mechanical strain. Previously, it has been shown that 3D EHTs display a higher degree of maturation based on sarcomeric organization, formation of T-tubules and functional characterization (e.g., contraction force, electrophysiology) (108–110). Cardiotoxicity of anti-cancer agents, other than anthracyclines, such as the TKi sunitinib, has already been assessed in EHTs, which resulted in triggered activation of apoptosis and loss of contractile force, spontaneous beating and mitochondrial membrane potential (111).

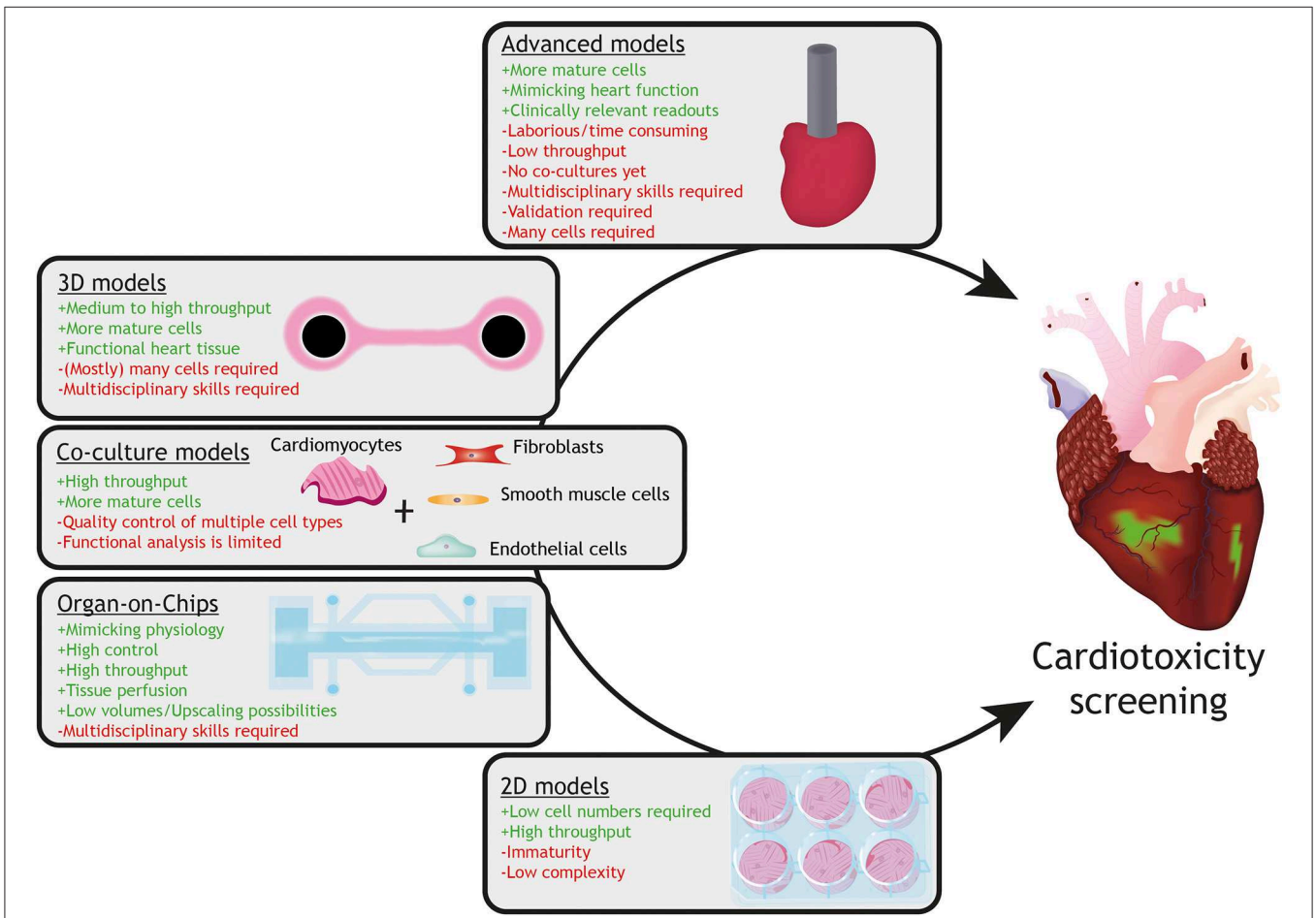

FIGURE 3 | Current *in vitro* models with hPSC-cardiomyocytes and their advantages (+ green) and limitations (– red). Models with increasing complexity (from 2D monolayers to 3D advanced models) are available for assessment of drug-induced cardiotoxicity utilizing hPSC-cardiomyocytes. Whereas 2D monolayer cardiomyocyte cultures can be used for high throughput-screening for specific cellular responses, they lack the physiology, multicellular interactions and tissue organization, which can be provided by organ-on-chip, co-culture or 3D tissue models (for instance engineered heart tissues). Advanced cardiac models, such as an engineered mini heart tube with the capacity to pump fluid, mimic clinically-relevant function(s) of the heart.

Until now studies did not consider subtype-specific cardiomyocytes for cardiotoxicity testing. This would be of high importance, as different cardiac subtypes may exhibit different susceptibility to Doxorubicin. Especially, because of the well-established arrhythmogenic potential of Doxorubicin (31, 112, 113), it is highly relevant to assess arrhythmogenic effects of Doxorubicin on hPSC-derived atrial and ventricular cardiomyocytes. Moreover, in a trial comparing Doxorubicin to Epirubicin in treatment of breast cancer, a quarter of all cardiac side effects of Doxorubicin was identified as sinus tachycardia, for Epirubicin this was half of the cardiotoxic responses in the trial population (31). In addition, a recent study showed that genetic loci are associated with both increased sensitivity to Doxorubicin-induced hPSC-pacemaker cell death and a higher risk of arrhythmia in patients (114), which justifies to perform cardiotoxicity testing on pacemaker cells. Previously, we and others have shown efficient production of atrial, ventricular and pacemaker cardiomyocytes from hPSCs (115–118).

In the cardiac microenvironment *in vivo*, each cardiomyocyte is surrounded by 3–4 capillaries with a distance between each cardiomyocyte and an endothelial cell of about 1 μm (119). This delicate build-up allows for defined cardiomyocyte-endothelium crosstalk via paracrine signaling and cell-cell contact (119–122). This data suggests that the crosstalk of human cardiomyocytes with highly metabolic active cardiac endothelial cells is very important. Recent advances in micro-engineering and stem cell technologies enabled development of microfluidic devices in which living cells (mostly several cell-types) are cultured in channels or small compartments and perfused in a controlled manner, mimicking the microenvironment and responses of tissues or organs (123–125). These so-called *organ-on-chips* can be combined with integrated sensors (for example for biochemical and electrical readouts) and are compatible with live imaging (for example using stem cell-based fluorescent reporter lines) (126) and human cell and tissue sampling. Very recently, Weng et al. (127) generated a multiple chambered tissue chip, a so-called organ-on-a-chip, which allowed simultaneous culturing of tumor tissue and hPSC-cardiomyocytes while being connected via a microfluidic channel, lined with endothelial cells. This design enables simultaneous testing of potential anti-tumor effects and cardiotoxicity of drugs administered via the endothelial layer. Another important aspect of drug efficacy and safety assessment is to predict pharmacokinetic parameters, determined by absorption, distribution, metabolism and excretion (ADME) of drugs, which are difficult to measure or model *in vitro*. As mentioned before, organ-on-chips mimic organ-like function and responses in perfusable mircophysiological systems and may offer an opportunity to overcome current limitations related to pharmacokinetic modeling. Multiple organ-on-chip devices with organ-level functionality could be connected to each other with vascularized, endothelium-lined channels mimicking blood circulation and recapitulating tissue-tissue interfaces, thus modeling organ crosstalk and metabolism of compounds in a single system (128, 129). For predictive physiologically based pharmacokinetic (PBPK) modeling, it is of paramount importance to combine defined hPSC culture methods and 3D tissue engineering or organoid formation with technical advances in the field of organ-on-chip technology, ultimately leading to interconnected multiple human organ-on-chip platforms (for example, combining heart-, liver- and gut-on-chip models).

FUTURE OUTLOOK

Because of the previously mentioned shortcomings, a full recapitulation of clinical cardiotoxic manifestations is beyond the capacity of current *in vitro* models. Interestingly, recent developments have shown that it is feasible to build human *in vitro* models that are more closely resembling functional human hearts for assessment of clinically relevant parameters, which may greatly enhance the predictability of these *in vitro* models for safety pharmacology and disease phenotypes. Recently, Li et al. created for the first time a human ventricular-like cardiac organoid chamber (hvCOC) with hPSC-cardiomyocytes and dermal fibroblasts (130). Similarly, Macqueen and colleagues generated a one cell layer thick ventricular-like tube from hPSC-cardiomyocytes (131). Both HvCOCs and ventricular tubes were able to pump fluid, which allowed measuring ejection fraction and pressure-volume loops as readouts for cardiac function. Although these developments are promising, combination with other cardiac cells, such as endothelial and smooth muscle cells and fibroblasts, may lead to further maturation and improvement of function. Future studies will be required in order to evaluate the predictive values of these human advanced 3D cardiac models in preclinical drug testing, their potential to reduce time for bringing new drugs from bench-to-bedside and repurposing of drugs.

Moreover, state-of-the-art multiple organ-on-chip platforms will also advance predictability of efficacy and toxicity of combinatorial drug (cardioprotective) treatment and specific target delivery.

It is evident that interpatient variability further complicates development of predictive *in vitro* models for drug screening. This is also true for anthracycline-induced cardiotoxicity, which varies tremendously from patient to patient. These variations may be caused due to environmental factors, life-style or genetic variance. Patient-derived (or genetically modified) hPSCs provide the possibility to include disease-associated genetic risk factors in these *in vitro* models, which can be directly compared to isogenic control hPSC lines. Interestingly, studies with hPSC-cardiomyocytes have shown that cardiotoxic effects of Doxorubicin were dependent on the genetic background of patient-derived hPSC-cardiomyocytes (22). Similarly, characterization of hPSC-cardiomyocytes from 45 individuals showed inter-individual variation in transcriptional response after 24 h exposure to different concentrations of Doxorubicin, which were predictive of *in vitro* cell damage as measured by cardiac troponin release of cardiomyocytes (132). These findings highlight the importance of personalized medicine and risk stratification, which will allow us to predict for which patients new therapies will be effective and safe and for which not. This will reduce the attrition rate, costs and time of drug development and facilitate repurposing of drugs that

have shown a lack of efficacy or risk of toxicity. Consequently, successful implementation of these advanced hPSC-based models will lead to better and safer drugs.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Šimunek T, Štěrba M, Popelová O, Adamcová M, Hrdina R, Gerši V. Anthracycline-induced cardiotoxicity: Overview of studies examining the roles of oxidative stress and free cellular iron. *Pharmacol Rep*. (2009) 61:154–71. doi: 10.1016/S1734-1140(09)70018-0
2. Albini A, Pennesi G, Donatelli F, Cammarota R, De Flora S, Noonan DM. Cardiotoxicity of anticancer drugs: The need for cardio-oncology and cardio-oncological prevention. *J Natl Cancer Inst*. (2010) 102:14–25. doi: 10.1093/jnci/djp440
3. Chatterjee K, Zhang J, Honbo N, Karliner JS. Doxorubicin cardiomyopathy. *Cardiology*. (2010) 115:155–62. doi: 10.1159/000265166
4. Štěrba M, Popelová O, Vávrová A, Jirkovský E, Kovaríková P, Geršl V, et al. Oxidative stress, redox signaling, and metal chelation in anthracycline cardiotoxicity and pharmacological cardioprotection. *Antioxid Redox Signal*. (2013) 18:899–929. doi: 10.1089/ars.2012.4795
5. Franco VI, Lipshultz SE. Cardiac complications in childhood cancer survivors treated with anthracyclines. *Cardiol Young*. (2015) 25:107–16. doi: 10.1017/S1047951115000906
6. Kankeu C, Clarke K, Passante E, Huber HJ. Doxorubicin-induced chronic dilated cardiomyopathy—the apoptosis hypothesis revisited. *J Mol Med*. (2017) 95:239–48. doi: 10.1007/s00109-016-1494-0
7. Dong J, Chen H. Cardiotoxicity of anticancer therapeutics. *Front Cardiovasc Med*. (2018) 5:9. doi: 10.3389/fcvm.2018.00009
8. Volkova M, Russell R. Anthracycline cardiotoxicity: prevalence, pathogenesis and treatment. *Curr Cardiol Rev*. (2012) 7:214–20. doi: 10.2174/157340311799960645
9. Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity. *Cancer*. (1973) 32:302–14. doi: 10.1002/1097-0142(197308)32:2<302::AID-CNCR2820320205>3.0.CO;2-2
10. Rinehart JJ, Lewis RP, Balcerzak SP, Riehart JJ, Lewis RP, Balcerzak SP. Adriamycin cardiotoxicity in man. *Ann Intern Med*. (1974) 81:475–8. doi: 10.7326/0003-4819-81-4-475
11. Moreno L, Pearson ADJ. How can attrition rates be reduced in cancer drug discovery? *Expert Opin Drug Discov*. (2013) 8:363–8. doi: 10.1517/17460441.2013.768984
12. Devalla HD, Passier R. Cardiac differentiation of pluripotent stem cells and implications for modeling the heart in health and disease. *Sci Transl Med*. (2018) 10:1–14. doi: 10.1126/scitranslmed.aah5457
13. Colatsky T, Fermini B, Gintant G, Pierson JB, Sager P, Sekino Y, et al. The Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative — Update on progress. *J Pharmacol Toxicol Methods*. (2016) 81:15–20. doi: 10.1016/j.vascn.2016.06.002
14. Maillet A, Tan K, Chai X, Sadananda SN, Mehta A, Ooi J, et al. Modeling doxorubicin-induced cardiotoxicity in human pluripotent stem cell derived-cardiomyocytes. *Sci Rep*. (2016) 6:25333. doi: 10.1038/srep25333
15. Zhao L, Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. *Sci Rep*. (2017) 7:44735. doi: 10.1038/srep44735
16. Nebigil CG, Désaubry L. Updates in anthracycline-mediated cardiotoxicity. *Front Pharmacol*. (2018) 9:1–13. doi: 10.3389/fphar.2018.01262
17. Arena E, D'Alessandro N, Dusonchet L, Geraci M, Rausa L, Sanguedolce R. Repair kinetics of DNA, RNA and proteins in the tissues of mice treated with doxorubicin. *Arzneimittelforschung*. (1979) 29:901-—2.
18. Abou El Hassan MAI, Verheul HMW, Jorna AS, Schalkwijk C, Van Bezu J, Van Der Vijgh WJF, et al. The new cardioprotector Monohydroxyethylrutoside protects against doxorubicin-induced inflammatory effects in vitro. *Br J Cancer*. (2003) 89:357–62. doi: 10.1038/sj.bjc.6601022
19. Zhang S, Liu X, Bawa-Khalfe T, Lu L-S, Lyu YL, Liu LF, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med*. (2012) 18:1639–42. doi: 10.1038/nm.2919
20. Cappetta D, De Angelis A, Sapio L, Prezioso L, Illiano M, Quaini F, et al. Oxidative stress and cellular response to doxorubicin: a common factor in the complex milieu of anthracycline cardiotoxicity. *Oxid Med Cell Longev*. (2017) 2017:1–13. doi: 10.1155/2017/1521020
21. Dudek J. Role of cardiolipin in mitochondrial signaling pathways. *Front Cell Dev Biol*. (2017) 5:90. doi: 10.3389/fcell.2017.00090
22. Burridge PW, Li YF, Matsa E, Wu H, Ong S-G, Sharma A, et al. Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. *Nat Med*. (2016) 22:547–56. doi: 10.1038/nm.4087
23. Chaudhari U, Ellis JK, Wagh V, Nemade H, Hescheler J, Keun HC, et al. Metabolite signatures of doxorubicin induced toxicity in human induced pluripotent stem cell-derived cardiomyocytes. *Amino Acids*. (2017) 49:1955–63. doi: 10.1007/s00726-017-2419-0
24. Louisse J, Wüst RCI, Pistollato F, Palosaari T, Barilari M, Macko P, et al. Assessment of acute and chronic toxicity of doxorubicin in human induced pluripotent stem cell-derived cardiomyocytes. *Toxicol Vitr*. (2017) 42:182–90. doi: 10.1016/j.tiv.2017.04.023
25. Verheijen M, Schrooders Y, Gmuender H, Nudischer R, Clayton O, Hynes J, et al. Bringing in vitro analysis closer to in vivo: Studying doxorubicin toxicity and associated mechanisms in 3D human microtissues with PBPK-based dose modelling. *Toxicol Lett*. (2018) 294:184–92. doi: 10.1016/j.toxlet.2018.05.029
26. Holmgren G, Sartipy P, Andersson CX, Lindahl A, Synnergren J. Expression profiling of human pluripotent stem cell-derived cardiomyocytes exposed to doxorubicin-Integration and visualization of multi-omics data. *Toxicol Sci*. (2018) 163:182–95. doi: 10.1093/toxsci/kfy012
27. McSweeney KM, Bozza WP, Alterovitz WL, Zhang B. Transcriptomic profiling reveals p53 as a key regulator of doxorubicin-induced cardiotoxicity. *Cell Death Discov*. (2019) 5:1–11. doi: 10.1038/s41420-019-0182-6
28. Li J, Wang PY, Long NA, Zhuang J, Springer DA, Zou J, et al. P53 prevents doxorubicin cardiotoxicity independently of its prototypical tumor suppressor activities. *Proc Natl Acad Sci USA*. (2019) 116:19626–34. doi: 10.1073/pnas.1904979116
29. Gupta SK, Garg A, Bär C, Chatterjee S, Foinquinos A, Milting H, et al. Quaking inhibits doxorubicin-mediated cardiotoxicity through regulation of cardiac circular RNA expression short communication. *Circ Res*. (2018) 122:246–54. doi: 10.1161/CIRCRESAHA.117.311335
30. Weiss RB, Sarosy G, Clagett-Carr K, Russo M, Leyland-Jones B. Anthracycline analogs: the past, present, and future. *Cancer Chemother Pharmacol*. (1986) 18:185–97. doi: 10.1007/bf00273384
31. Ambrosini G, Balli M, Garusi G, Demicheli R, Jirillo A, Bonciarelli G, et al. Phase III randomized study of fluorouracil, epirubicin, and cyclophosphamide v fluorouracil, doxorubicin, and cyclophosphamide in advanced breast cancer: an Italian multicentre trial. Italian

Multicentre Breast Study with Epirubicin. *J Clin Oncol.* (1988) 6:976–82. doi: 10.1200/JCO.1988.6.6.976
32. Dittrich C, Baur M, Mader R, Schlappack O, Dudczak R, Leitha T, et al. Phase I-II study on weekly administration of pirarubicin in patients with metastatic breast cancer. *Am J Clin Oncol Cancer Clin Trials*. (1990) 13:S29–39. doi: 10.1097/00000421-199012001-00008
33. Drings P, Günther IU, Gatzemeier U, Berdel W, Stahl M, Salewski E, et al. Pirarubicin in advanced non-small cell lung cancer: a trial of the phase i/ii study group of the association for medical oncology of the german cancer society. *Oncol Res Treat.* (1990) 13:180–4. doi: 10.1159/000216754
34. Dhingra K, Frye D, Newman RA, Walters R, Theriault R, Fraschini G, et al. Phase II clinical and pharmacological study of pirarubicin in combination with 5-fluorouracil and cyclophosphamide in metastatic breast cancer. *Clin Cancer Res.* (1995) 1:691–7.
35. Zinzani PL, Martelli M, Storti S, Musso M, Cantonetti M, Leone G, et al. Phase III comparative trial using CHOP vs CIOP in the treatment of advanced intermediate-grade non-Hodgkin's lymphoma. *Leuk Lymphoma*. (1995) 19:329–35. doi: 10.3109/10428199509107906
36. Llombart-Cussac A, Pivot X, Rhor-Alvarado A, Le Cesne A, Le Chevalier T, Tursz T, et al. First-line vinorelbine-mitoxantrone combination in metastatic breast cancer patients relapsing after an adjuvant anthracycline regimen: results of a phase II study. *Oncology*. (1998) 55:384–90. doi: 10.1159/000011883
37. Nielsen OS, Dombernowsky P, Mouridsen H, Crowther D, Verweij J, Buesa J, et al. High-dose epirubicin is not an alternative to standard-dose doxorubicin in the treatment of advanced soft tissue sarcomas. A study of the EORTC soft tissue and bone sarcoma group. *Br J Cancer*. (1998) 78:1634–9. doi: 10.1038/bjc.1998.735
38. Srokowski TP, Liebmann JE, Modiano MR, Cohen GI, Pro B, Romaguera JE, et al. Pixantrone dimaleate in combination with fludarabine, dexamethasone, and rituximab in patients with relapsed or refractory indolent non-Hodgkin lymphoma. *Cancer*. (2011) 117:5067–73. doi: 10.1002/cncr.26121
39. Rivankar S. An overview of doxorubicin formulations in cancer therapy. *J Cancer Res Ther.* (2014) 10:853–8. doi: 10.4103/0973-1482.139267
40. Xue K, Gu JJ, Zhang Q, Liu X, Wang J, Li XQ, et al. Cardiotoxicity as indicated by LVEF and troponin T sensitivity following two anthracycline-based regimens in lymphoma: Results from a randomized prospective clinical trial. *Oncotarget*. (2016) 7:32519–31. doi: 10.18632/oncotarget.8685
41. Hara T, Yoshikawa T, Goto H, Sawada M, Yamada T, Fukuno K, et al. R-THP-COP versus R-CHOP in patients younger than 70 years with untreated diffuse large B cell lymphoma: a randomized, open-label, noninferiority phase 3 trial. *Hematol Oncol.* (2018) 36:638–44. doi: 10.1002/hon.2524
42. Xu PP, Fu D, Li J-Y, Hu J-D, Wang X, Zhou J-F, et al. Anthracycline dose optimisation in patients with diffuse large B-cell lymphoma: a multicentre, phase 3, randomised, controlled trial. *Lancet Haematol.* (2019) 6:e328–37. doi: 10.1016/S2352-3026(19)30051-1
43. Kang YK, Ryoo BY, Kim TY, Im YH, Kim BS, Park YH, et al. A phase II trial of DA-125, a novel anthracycline, in advanced non-small-cell lung cancer. *Cancer Chemother Pharmacol.* (1999) 44:518–21. doi: 10.1007/s002800051127
44. Obasaju C, Manola J, Hudes GR, Khandekar JD, Citrin DL, Carbone P, et al. Phase II evaluation of menogaril in advanced prostate cancer: Eastern Cooperative Oncology Group EST P-A885. *Am J Clin Oncol.* (2001) 24:150–4. doi: 10.1097/00000421-200104000-00011
45. Hofheinz RD, Porta C, Hartung G, Santoro A, Hanauske AR, Kutz K, et al. BBR 3438, a novel 9-aza-anthrapyrazole, in patients with advanced gastric cancer: A phase II study group trial of the central European Society of Anticancer-Drug Research (CESAR). *Invest New Drugs*. (2005) 23:363–8. doi: 10.1007/s10637-005-1445-z
46. Tardi PG, Boman NL, Cullis PR. Liposomal doxorubicin. *J Drug Target*. (1996) 4:129–40. doi: 10.3109/10611869609015970
47. Reynolds JG, Geretti E, Hendriks BS, Lee H, Leonard SC, Klinz SG, et al. HER2-targeted liposomal doxorubicin displays enhanced anti-tumorigenic effects without associated cardiotoxicity. *Toxicol Appl Pharmacol.* (2012) 262:1–10. doi: 10.1016/j.taap.2012.04.008
48. Giotta F, Lorusso V, Maiello E, Filippelli G, Valerio MR, Caruso M, et al. Liposomal-encapsulated doxorubicin plus cyclophosphamide as first-line therapy in metastatic breast cancer: a phase II multicentric study. *Ann Oncol.* (2007) 18(Suppl. 6):vi66–9. doi: 10.1093/annonc/mdm228
49. Xing M, Yan F, Yu S, Shen P. Efficacy and cardiotoxicity of liposomal doxorubicin-based chemotherapy in advanced breast cancer: a meta-analysis of ten randomized controlled trials. *PLoS ONE*. (2015) 10:e0133569. doi: 10.1371/journal.pone.0133569
50. Fridrik MA, Jaeger U, Petzer A, Willenbacher W, Keil F, Lang A, et al. Cardiotoxicity with rituximab, cyclophosphamide, non-pegylated liposomal doxorubicin, vincristine and prednisolone compared to rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone in frontline treatment of patients with diffuse large B-. *Eur J Cancer*. (2016) 58:112–21. doi: 10.1016/J.EJCA.2016.02.004
51. Chan S, Davidson N, Juozaityte E, Erdkamp F, Pluzanska A, Azarnia N, et al. Phase III trial of liposomal doxorubicin and cyclophosphamide compared with epirubicin and cyclophosphamide as first-line therapy for metastatic breast cancer. *Ann Oncol.* (2004) 15:1527–34. doi: 10.1093/annonc/mdh393
52. Gabizon A, Shmeeda H, Barenholz Y. Pharmacokinetics of pegylated liposomal Doxorubicin: review of animal and human studies. *Clin Pharmacokinet.* (2003) 42:419–36. doi: 10.2165/00003088-200342050-00002
53. Lyass O, Uziely B, Ben-Yosef R, Tzemach D, Heshing NI, Lotem M, et al. Correlation of toxicity with pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in metastatic breast carcinoma. *Cancer*. (2000) 89:1037–47. doi: 10.1002/1097-0142(20000901)89:5<1037::aid-cncr13>3.0.co;2-z
54. O'Brien MER. Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYXTM/Doxil") versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Ann Oncol.* (2004) 15:440–9. doi: 10.1093/annonc/mdh097
55. Gil-Gil MJ, Bellet M, Morales S, Ojeda B, Manso L, Mesia C, et al. Pegylated liposomal doxorubicin plus cyclophosphamide followed by paclitaxel as primary chemotherapy in elderly or cardiotoxicity-prone patients with high-risk breast cancer: results of the phase II CAPRICE study. *Breast Cancer Res Treat.* (2015) 151:597–606. doi: 10.1007/s10549-015-3415-2
56. Ansari L, Shiehzadeh F, Taherzadeh Z, Nikoofal-Sahlabadi S, Momtazi-borojeni AA, Sahebkar A, et al. The most prevalent side effects of pegylated liposomal doxorubicin monotherapy in women with metastatic breast cancer: a systematic review of clinical trials. *Cancer Gene Ther.* (2017) 24:189–93. doi: 10.1038/cgt.2017.9
57. Miller K, Cortes J, Hurvitz SA, Krop IE, Tripathy D, Verma S, et al. HERMIONE: a randomized Phase 2 trial of MM-302 plus trastuzumab versus chemotherapy of physician's choice plus trastuzumab in patients with previously treated, anthracycline-naïve, HER2-positive, locally advanced/metastatic breast cancer. *BMC Cancer*. (2016) 16:352. doi: 10.1186/s12885-016-2385-z
58. Karimi M, Zangabad PS, Mehdizadeh F, Malekzad H, Ghasemi A, Bahrami S, et al. Nanocaged platforms: modification, drug delivery and nanotoxicity. Opening synthetic cages to release the tiger. *Nanoscale*. (2017) 9:1356–92. doi: 10.1039/c6nr07315h
59. Zhen Z, Tang W, Chen H, Lin X, Todd T, Wang G, et al. RGD modified apoferritin nanoparticles for efficient drug delivery to tumors NIH Public Access. *ACS Nano*. (2013) 7:4830–7. doi: 10.1021/nn305791q
60. Du B, Jia S, Wang Q, Ding X, Liu Y, Yao H, et al. A self-targeting, Dual ROS/pH-responsive apoferritin nanocage for spatiotemporally controlled drug delivery to breast cancer. *Biomacromolecules*. (2018) 19:1026–36. doi: 10.1021/acs.biomac.8b00012
61. Han X, Jiang Y, Li S, Zhang Y, Ma X, Wu Z, et al. Multivalent aptamer-modified tetrahedral DNA nanocage demonstrates high selectivity and safety for anti-tumor therapy. *Nanoscale*. (2018) 11:339–47. doi: 10.1039/c8nr05546g
62. Ji M, Qiu X, Hou L, Huang S, Li Y, Liu Y, et al. Construction and application of a liver cancer-targeting drug delivery system based on core-shell gold nanocages. *Int J Nanomed*. (2018) 13:1773–89. doi: 10.2147/IJN.S151043
63. Xu Q, Wan J, Bie N, Song X, Yang X, Yong T, et al. A biomimetic gold nanocages-based nanoplatform for efficient tumor ablation and reduced inflammation. *Theranostics*. (2018) 8:5362–78. doi: 10.7150/thno.27631
64. Schöffski P, Delord JPP, Brain E, Robert J, Dumez H, Gasmi J, et al. First-in-man phase I study assessing the safety and pharmacokinetics of a 1-hour intravenous infusion of the doxorubicin prodrug DTS-201 every 3 weeks in

patients with advanced or metastatic solid tumours. *Eur J Cancer*. (2017) 86:240–7. doi: 10.1016/j.ejca.2017.09.009
65. Kuruvilla SP, Tiruchinapally G, Crouch AC, ElSayed MEH, Greve JM. Dendrimer-doxorubicin conjugates exhibit improved anticancer activity and reduce doxorubicin-induced cardiotoxicity in a murine hepatocellular carcinoma model. *PLoS ONE*. (2017) 12:1–24. doi: 10.1371/journal.pone.0181944
66. Georgakopoulos P, Roussou P, Matsakas E, Karavidas A, Anagnostopoulos N, Marinakis T, et al. Cardioprotective effect of metoprolol and enalapril in doxorubicin-treated lymphoma patients: a prospective, parallel-group, randomized, controlled study with 36-month follow-up. *Am J Hematol*. (2010) 85:894–6. doi: 10.1002/ajh.21840
67. Zhang H, Shen WS, Gao CH, Deng LC, Shen D. Protective effects of salidroside on epirubicin-induced early left ventricular regional systolic dysfunction in patients with breast cancer. *Drugs R D*. (2012) 12:101–6. doi: 10.2165/11632530-000000000-00000
68. Seicean S, Seicean A, Plana JC, Budd GT, Marwick TH. Effect of statin therapy on the risk for incident heart failure in patients with breast cancer receiving anthracycline chemotherapy: an observational clinical cohort study. *J Am Coll Cardiol*. (2012) 60:2384–90. doi: 10.1016/j.jacc.2012.07.067
69. Chotenimitkhun R, D'Agostino R, Lawrence JA, Hamilton CA, Jordan JH, Vasu S, et al. Chronic statin administration may attenuate early anthracycline-associated declines in left ventricular ejection function. *Can J Cardiol*. (2015) 31:302–7. doi: 10.1016/j.cjca.2014.11.020
70. Tashakori Beheshti A, Mostafavi Toroghi H, Hosseini G, Zarifian A, Homaei Shandiz F, Fazlinezhad A. Carvedilol administration can prevent doxorubicin-induced cardiotoxicity: a double-blind randomized trial. *Cardiology*. (2016) 134:47–53. doi: 10.1159/000442722
71. Hao W, Liu S, Qin Y, Sun C, Chen L, Wu C, et al. Cardioprotective effect of *Platycodon grandiflorum* in patients with early breast cancer receiving anthracycline-based chemotherapy: study protocol for a randomized controlled trial. *Trials*. (2017) 18:386. doi: 10.1186/s13063-017-2140-z
72. Janbabai G, Nabati M, Faghihinia M, Azizi S, Borhani S, Yazdani J. Effect of enalapril on preventing anthracycline-induced cardiomyopathy. *Cardiovasc Toxicol*. (2017) 17:130–9. doi: 10.1007/s12012-016-9365-z
73. Nabati M, Janbabai G, Baghyari S, Esmaili K, Yazdani J. Cardioprotective effects of carvedilol in inhibiting doxorubicin-induced cardiotoxicity. *J Cardiovasc Pharmacol*. (2017) 69:279–85. doi: 10.1097/FJC.0000000000000470
74. Skrypnyk I, Maslova G, Lymanets T, Gusachenko I. L-arginine is an effective medication for prevention of endothelial dysfunction, a predictor of anthracycline cardiotoxicity in patients with acute leukemia. *Exp. Oncol*. (2017) 39:308–11.
75. Abuosa AM, Elshiekh AH, Qureshi K, Abrar MB, Kholeif MA, Kinsara AJ, et al. Prophylactic use of carvedilol to prevent ventricular dysfunction in patients with cancer treated with doxorubicin. *Indian Heart J*. (2018) 70:S96–S100. doi: 10.1016/j.ihj.2018.06.011
76. Cardinale D, Ciceri F, Latini R, Franzosi MG, Sandri MT, Civelli M, et al. Anthracycline-induced cardiotoxicity: a multicenter randomised trial comparing two strategies for guiding prevention with enalapril: The International CardioOncology Society-one trial. *Eur J Cancer*. (2018) 94:126–37. doi: 10.1016/j.ejca.2018.02.005
77. Gupta V, Kumar Singh S, Agrawal V, Bali Singh T. Role of ACE inhibitors in anthracycline-induced cardiotoxicity: A randomized, double-blind, placebo-controlled trial. *Pediatr Blood Cancer*. (2018) 65:e27308. doi: 10.1002/pbc.27308
78. Chung R, Maulik A, Hamarneh A, Hochhauser D, Hausenloy DJ, Walker JM, et al. Effect of remote ischaemic conditioning in oncology patients undergoing chemotherapy: rationale and design of the ERIC-ONC Study - a single-center, blinded, randomized controlled trial. *Clin Cardiol*. (2016) 39:72–82. doi: 10.1002/clc.22507
79. Kirkham AA, Paterson DI, Prado CM, Mackey JM, Courneya KS, Pituskin E, et al. Rationale and design of the Caloric Restriction and Exercise protection from Anthracycline Toxic Effects (CREATE) study: A 3-arm parallel group phase II randomized controlled trial in early breast cancer. *BMC Cancer*. (2018) 18:864. doi: 10.1186/s12885-018-4778-7
80. Milei J, Marantz A, Alé J, Vazquez A, Buceta JE. Prevention of adriamycin-induced cardiotoxicity by prenylamine: a pilot double blind study. *Cancer Drug Deliv*. (1987) 4:129–36. doi: 10.1089/cdd.1987.4.129
81. Shah RR, Stonier PD. Withdrawal of prenylamine: perspectives on pharmacological, clinical and regulatory outcomes following the first QT-related casualty. *Ther Adv Drug Saf*. (2018) 9:475–93. doi: 10.1177/2042098618780854
82. Nobili S, Landini I, Giglioni B, Mini E. Pharmacological strategies for overcoming multidrug resistance. *Curr Drug Targets*. (2006) 7:861–79. doi: 10.2174/138945006777709593
83. Stove C, Bracke M. Roles for neuregulins in human cancer. *Clin Exp Metastasis*. (2005) 21:665–84. doi: 10.1007/s10585-004-6917-6
84. Gao R, Zhang J, Cheng L, Wu X, Dong W, Yang X, et al. A phase II, randomized, double-blind, multicenter, based on standard therapy, placebo-controlled study of the efficacy and safety of recombinant human neuregulin-1 in patients with chronic heart failure. *J Am Coll Cardiol*. (2010) 55:1907–14. doi: 10.1016/j.jacc.2009.12.044
85. Galindo CL, Ryzhov S, Sawyer DB. Neuregulin as a heart failure therapy and mediator of reverse remodeling. *Curr Heart Fail Rep*. (2014) 11:40–9. doi: 10.1007/s11897-013-0176-2
86. Lenihan DJ, Koren M, Eisen A, Caggiano A. Sustained improvement of left ventricular ejection fraction with intravenous cimaglermin in patients with symptomatic systolic dysfunction. *J Am Coll Cardiol*. (2018) 71:A667. doi: 10.1016/s0735-1097(18)31208-7
87. Jay SM, Murthy AC, Hawkins JF, Wortzel JR, Steinhauser ML, Alvarez LM, et al. An engineered bivalent neuregulin protects against doxorubicin-induced cardiotoxicity with reduced proneoplastic potential. *Circulation*. (2013) 128:152–61. doi: 10.1161/CIRCULATIONAHA.113.002203
88. Zhang J, Cui X, Yan Y, Li M, Yang Y, Wang J, et al. Research progress of cardioprotective agents for prevention of anthracycline cardiotoxicity. *Am J Transl Res*. (2016) 8:2862–75.
89. Lipshultz SE, Rifai N, Dalton VM, Levy DE, Silverman LB, Lipsitz SR, et al. The effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. *N Engl J Med*. (2004) 351:145–53. doi: 10.1056/NEJMoa035153
90. Asselin BL, Devidas M, Chen L, Franco VI, Pullen J, Borowitz MJ, et al. Cardioprotection and safety of dexrazoxane in patients treated for newly diagnosed t-cell acute lymphoblastic leukemia or advanced-stage lymphoblastic non-hodgkin lymphoma: a Report of the Children's Oncology Group Randomized Trial Pediatric Oncology Group 9404. *J Clin Oncol*. (2016) 34:854–62. doi: 10.1200/JCO.2015.60.8851
91. Lemez P, Maresová J. Efficacy of dexrazoxane as a cardioprotective agent in patients receiving mitoxantrone- and daunorubicin-based chemotherapy. *Semin Oncol*. (1998) 25:61–5.
92. Swain SM, Whaley FS, Gerber MC, Weisberg S, York M, Spicer D, et al. Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. *J Clin Oncol*. (1997) 15:1318–32. doi: 10.1200/JCO.1997.15.4.1318
93. Marty M, Espié M, Llombart A, Monnier A, Rapoport BL, Stahalova V. Multicenter randomized phase III study of the cardioprotective effect of dexrazoxane (Cardioxane®) in advanced/metastatic breast cancer patients treated with anthracycline-based chemotherapy. *Ann Oncol*. (2006) 17:614–22. doi: 10.1093/annonc/mdj134
94. Elbl L, Hrstkova H, Tomaskova I, Michalek J. Late anthracycline cardiotoxicity protection by dexrazoxane (ICRF-187) in pediatric patients: echocardiographic follow-up. *Support Care Cancer*. (2006) 14:128–36. doi: 10.1007/s00520-005-0858-8
95. Cui N, Wu F, Lu WJ, Bai R, Ke B, Liu T, et al. Doxorubicin-induced cardiotoxicity is maturation dependent due to the shift from topoisomerase IIα to IIβ in human stem cell derived cardiomyocytes. *J Cell Mol Med*. (2019) 23:4627–39. doi: 10.1111/jcmm.14346
96. Deng S, Yan T, Jendrny C, Nemecek A, Vincetic M, Gödtel-Armbrust U, et al. Dexrazoxane may prevent doxorubicin-induced DNA damage via depleting both Topoisomerase II isoforms. *BMC Cancer*. (2014) 14:1–11. doi: 10.1186/1471-2407-14-842
97. Force T, Krause DS, Van Etten RA. Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition. *Nat Rev Cancer*. (2007) 7:332–44. doi: 10.1038/nrc2106

98. Doherty KR, Wappel RL, Talbert DR, Trusk PB, Moran DM, Kramer JW, et al. Multi-parameter in vitro toxicity testing of crizotinib, sunitinib, erlotinib, and nilotinib in human cardiomyocytes. *Toxicol Appl Pharmacol.* (2013) 272:245–55. doi: 10.1016/j.taap.2013.04.027
99. Sharma A, Burridge PW, McKeithan WL, Serrano R, Shukla P, Sayed N, et al. High-throughput screening of tyrosine kinase inhibitor cardiotoxicity with human induced pluripotent stem cells. *Sci Transl Med.* (2017) 9:1–13. doi: 10.1126/scitranslmed.aaf2584
100. Li C, Zou R, Zhang H, Wang Y, Qiu B, Qiu S, et al. Upregulation of phosphoinositide 3-kinase prevents sunitinib-induced cardiotoxicity *in vitro* and *in vivo*. *Arch Toxicol.* (2019) 93:1697–712. doi: 10.1007/s00204-019-02448-z
101. Wang H, Sheehan RP, Palmer AC, Everley RA, Boswell SA, Ron-Harel N, et al. Adaptation of human iPSC-derived cardiomyocytes to tyrosine kinase inhibitors reduces acute cardiotoxicity via metabolic reprogramming. *Cell Syst.* (2019) 8:412–26.e7. doi: 10.1016/j.cels.2019.03.009
102. Kitani T, Ong SG, Lam CK, Rhee JW, Zhang JZ, Oikonomopoulos A, et al. Human-induced pluripotent stem cell model of trastuzumab-induced cardiac dysfunction in patients with breast cancer. *Circulation*. (2019) 139:2451–65. doi: 10.1161/CIRCULATIONAHA.118.037357
103. Eldridge S, Guo L, Mussio J, Furniss M, Hamre J, Davis M. Examining the protective role of ErbB2 modulation in human-induced pluripotent stem cell-derived cardiomyocytes. *Toxicol Sci.* (2014) 141:547–59. doi: 10.1093/toxsci/kfu150
104. Kurokawa YK, Shang MR, Yin RT, George SC. Modeling trastuzumab-related cardiotoxicity *in vitro* using human stem cell-derived cardiomyocytes. *Toxicol Lett.* (2018) 285:74–80. doi: 10.1016/j.toxlet.2018.01.001
105. Veerman CC, Kosmidis G, Mummery CL, Casini S, Verkerk AO, Bellin M. Immaturity of human stem-cell-derived cardiomyocytes in culture: fatal flaw or soluble problem? *Stem Cells Dev.* (2015) 24:1035–52. doi: 10.1089/scd.2014.0533
106. Denning C, Borgdorff V, Crutchley J, Firth KSA, George V, Kalra S, et al. Cardiomyocytes from human pluripotent stem cells: From laboratory curiosity to industrial biomedical platform. *Biochim Biophys Acta.* (2016) 1863:1728–48. doi: 10.1016/j.bbamcr.2015.10.014
107. Schwach V, Passier R. Native cardiac environment and its impact on engineering cardiac tissue. *Biomater Sci.* (2019) 7:3566–80. doi: 10.1039/c8bm01348a
108. Hansen A, Eder A, Bönstrup M, Flato M, Mewe M, Schaaf S, et al. Development of a drug screening platform based on engineered heart tissue. *Circ Res.* (2010) 107:35–44. doi: 10.1161/CIRCRESAHA.109.211458
109. Hirt MN, Boeddinghaus J, Mitchell A, Schaaf S, Börnchen C, Müller C, et al. Functional improvement and maturation of rat and human engineered heart tissue by chronic electrical stimulation. *J Mol Cell Cardiol.* (2014) 74:151–61. doi: 10.1016/j.yjmcc.2014.05.009
110. Wijnker PJM, Friedrich FW, Dutsch A, Reischmann S, Eder A, Mannhardt I, et al. Comparison of the effects of a truncating and a missense MYBPC3 mutation on contractile parameters of engineered heart tissue. *J Mol Cell Cardiol.* (2016) 97:82–92. doi: 10.1016/j.yjmcc.2016.03.003
111. Truitt R, Mu A, Corbin EA, Vite A, Brandimarto J, Ky B, et al. Increased Afterload Augments Sunitinib-Induced Cardiotoxicity in an engineered cardiac microtissue model. *JACC Basic to Transl Sci.* (2018) 3:265–76. doi: 10.1016/j.jacbts.2017.12.007
112. Kilickap S, Barista I, Akgul E, Aytemir K, Aksoy S, Tekuzman G. Early and late arrhythmogenic effects of doxorubicin. *South Med J.* (2007) 100:262–5. doi: 10.1097/01.smj.0000257382.89910.fe
113. Buza V, Rajagopalan B, Curtis AB. Cancer treatment-induced arrhythmias: focus on chemotherapy and targeted therapies. *Circ Arrhythmia Electrophysiol.* (2017) 10:1–12. doi: 10.1161/CIRCEP.117.005443
114. Chen S. Pharmacogenetics and drug discovery for anthracycline-induced cardiotoxicity enabled by sinoatrial node-like cells derived from human pluripotent stem cells. *Circ Res.* (2019) doi: 10.1161/res.125.suppl_1.476. [Epub ahead of print].
115. Birket MJ, Ribeiro MC, Verkerk AO, Ward D, Leitoguinho AR, Den Hartogh SC, et al. Expansion and patterning of cardiovascular progenitors derived from human pluripotent stem cells. *Nat Biotechnol.* (2015) 33:970–9. doi: 10.1038/nbt.3271
116. Devalla HD, Schwach V, Ford JW, Milnes JT, El-Haou S, Jackson C, et al. Atrial-like cardiomyocytes from human pluripotent stem cells are a robust preclinical model for assessing atrial-selective pharmacology. *EMBO Mol Med.* (2015) 7:394–410. doi: 10.15252/emmm.201404757
117. Protze SI, Liu J, Nussinovitch U, Ohana L, Backx PH, Gepstein L, et al. Sinoatrial node cardiomyocytes derived from human pluripotent cells function as a biological pacemaker. *Nat Biotechnol.* (2016) 351:35–56. doi: 10.1038/nbt.3745
118. Schwach V, Passier R. Generation and purification of human stem cell-derived cardiomyocytes. *Differentiation*. (2016) 91:126–38. doi: 10.1016/j.diff.2016.01.001
119. Strijdom H, Lochner A. Cardiac endothelium: more than just a barrier. *SA Hear.* (2017) 2:174–85. doi: 10.24170/6-3-1987
120. Leucker TM, Bienengraeber M, Muravyeva M, Baotic I, Weihrauch D, Brzezinska AK, et al. Endothelial-cardiomyocyte crosstalk enhances pharmacological cardioprotection. *J Mol Cell Cardiol.* (2011) 51:803–11. doi: 10.1016/j.yjmcc.2011.06.026
121. Talman V, Kivelä R. Cardiomyocyte—endothelial cell interactions in cardiac remodeling and regeneration. *Front Cardiovasc Med.* (2018) 5:101. doi: 10.3389/fcvm.2018.00101
122. Colliva A, Braga L, Giacca M, Zacchigna S. Endothelial cell-cardiomyocyte crosstalk in heart development and disease. *J Physiol.* (2019). doi: 10.1113/JP276758. [Epub ahead of print].
123. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. *Science*. (2010) 328:1662–8. doi: 10.1126/science.1188302
124. Passier R, Orlova V, Mummery C. Complex tissue and disease modeling using hiPSCs. *Cell Stem Cell*. (2016) 18:309–21. doi: 10.1016/j.stem.2016.02.011
125. Van Den Berg A, Mummery CL, Passier R, Van der Meer AD. Personalised organs-on-chips: functional testing for precision medicine. *Lab Chip*. (2019) 19:198–205. doi: 10.1039/c8lc00827b
126. Den Hartogh SC, Passier R. Concise review: fluorescent reporters in human pluripotent *stem cells*: contributions to cardiac differentiation and their applications in Cardiac Disease and Toxicity. *Stem Cells*. (2016) 34:13–26. doi: 10.1002/stem.2196
127. Weng C, Kurokawa YK, Hajek BS, Paladin JA, Shirure VS, George SC. Human iPS-cardiac-endothelial-tumor-on-a-chip to assess anti-cancer efficacy and cardiotoxicity. *Mary Ann Liebert*. (2019) 26:1–40. doi: 10.1089/ten.TEC.2019.0248
128. Skardal A, Shupe T, Atala A. Organoid-on-a-chip and body-on-a-chip systems for drug screening and disease modeling. *Drug Discov Today*. (2016) 21:1399–411. doi: 10.1016/j.drudis.2016.07.003
129. Prantil-Baun R, Novak R, Das D, Somayaji MR, Przekwas A, Ingber DE. Physiologically based pharmacokinetic and pharmacodynamic analysis enabled by microfluidically linked organs-on-chips. *Annu Rev Pharmacol Toxicol.* (2018) 58:37–64. doi: 10.1146/annurev-pharmtox-010716-104748
130. Li RA, Keung W, Cashman TJ, Backeris PC, Johnson BV, Bardot ES, et al. Bioengineering an electro-mechanically functional miniature ventricular heart chamber from human pluripotent stem cells. *Biomaterials*. (2018) 163:116–27. doi: 10.1016/j.biomaterials.2018.02.024
131. MacQueen LA, Sheehy SP, Chantre CO, Zimmerman JF, Pasqualini FS, Liu X, et al. A tissue-engineered scale model of the heart ventricle. *Nat Biomed Eng.* (2018) 2:930–41. doi: 10.1038/s41551-018-0271-5
132. Knowles DA, Burrows CK, Blischak JD, Patterson KM, Serie DJ, Norton N, et al. Determining the genetic basis of anthracycline-cardiotoxicity by molecular response QTL mapping in induced cardiomyocytes. *Elife*. (2018) 7:1–25. doi: 10.7554/eLife.33480.001

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REVIEW
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High Density Lipoprotein and Its Precursor Protein Apolipoprotein A1 as Potential Therapeutics to Prevent Anthracycline Associated Cardiotoxicity

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Cardiovascular disease and cancer are the leading causes of death in developed societies. Despite their effectiveness, many cancer therapies exhibit deleterious cardiovascular side effects such as cardiotoxicity and heart failure. The cardiotoxic effects of anthracyclines such as doxorubicin are the most well-characterized of cardiotoxic anti-cancer therapies. While other anti-neoplastic drugs also induce cardiotoxicity, often leading to heart failure, they are beyond the scope of this review. This review first summarizes the mechanisms of doxorubicin-induced cardiotoxicity. It then reviews emerging preclinical evidence that high density lipoprotein and its precursor protein apolipoprotein A1, which are known for their protective effects against ischemic cardiovascular disease, may also protect against doxorubicin-induced cardiotoxicity both directly and indirectly, when used therapeutically.

Keywords: HDL, ApoA1, anthracyclin, chemotherapy, cardiotoxicity, cardioprotective, doxorubicin

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INTRODUCTION

Advances in cancer treatment over the past few decades have led to substantial increases in cancer survivorship (1, 2). As cancer-related survival has improved, an unexpected increase in premature cardiovascular events, including myocardial ischemia, myocardial infarction, congestive heart failure (HF), QT interval prolongation, hypertension, and stroke has occurred (3, 4). A major contributing factor to cardiovascular outcomes is cardiotoxicity related to antitumor drugs, which may become apparent acutely during treatment, or often, well after treatment has ended (4, 5). In general terms, cancer therapy related cardiotoxicity ultimately leads to pathological alterations in the cardiac muscle tissue (5). Many different classes of chemotherapeutic agents have cardiotoxic effects. Anthracyclines such as doxorubicin (DOX) are, perhaps, the most well-studied cardiotoxic chemotherapeutic agents. They have a number of direct cardiotoxic effects, including DNA damage, interfering with mitochondrial function, induction of reactive oxygen species (ROS), alterations in autophagy and induction in apoptosis. Mechanisms of anthracycline induced cardiotoxicity will be discussed in more detail below. Other cardiotoxic chemotherapeutic agents/treatments include fluoropyrimidines, such as 5-fluorouracil, biologicals, such as trastuzumab and radiation therapy, all of which impact cardiomyocyte survival by triggering apoptosis through differing, but overlapping pathways

(summarized in **Figure 1**). For example, fluoropyrimidines, such as 5-fluorouracil (5-FU) and capecitabine, are used to treat different types of tumors, especially those that appear in the head, neck and breast (10, 11). In the case of these agents, cardiotoxicity appears to be due to both direct toxic effects of these drugs on cardiomyocytes, through the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to both oxidative and nitrosative stress, and activation of apoptosis and autophagy (12). Furthermore, these agents also appear to have indirect cardiotoxic effects through interaction with the coagulation system and autoimmune responses (8). Trastuzumab, an antibody targeting the Human epidermal growth factor 2 receptor (HER/ErbB2), and used as first choice therapy against breast cancer (13, 14), also inhibits this receptor in cardiomyocytes, affecting myocardial structure and survival pathways, triggering cardiomyocyte apoptosis and leading to asymptomatic decreased left ventricular ejection fraction, and eventually heart failure, particularly when it is used in combination with other agents, such as anthracyclines (15, 16). Radiation therapy also triggers cardiotoxicity through induction of DNA damage as well as ROS in cardiomyocytes, again affecting cell survival pathways and triggering apoptosis (17). Radiation therapy induced cardiovascular effects may manifest as pericarditis, coronary artery disease, myocardial infarction, valve heart disease, changes in rhythm, silent myocardial ischemia and damage to the conduction system (18). The risk of heart disease is mainly related to the total radiation dose and the volume of the heart receiving radiation (19).

As a result of these cardiotoxic effects, the mode of administration, time and dose of these treatments and the presence of pre-existing comorbidities such as cardiovascular and liver disease, diabetes mellitus and hypertension are

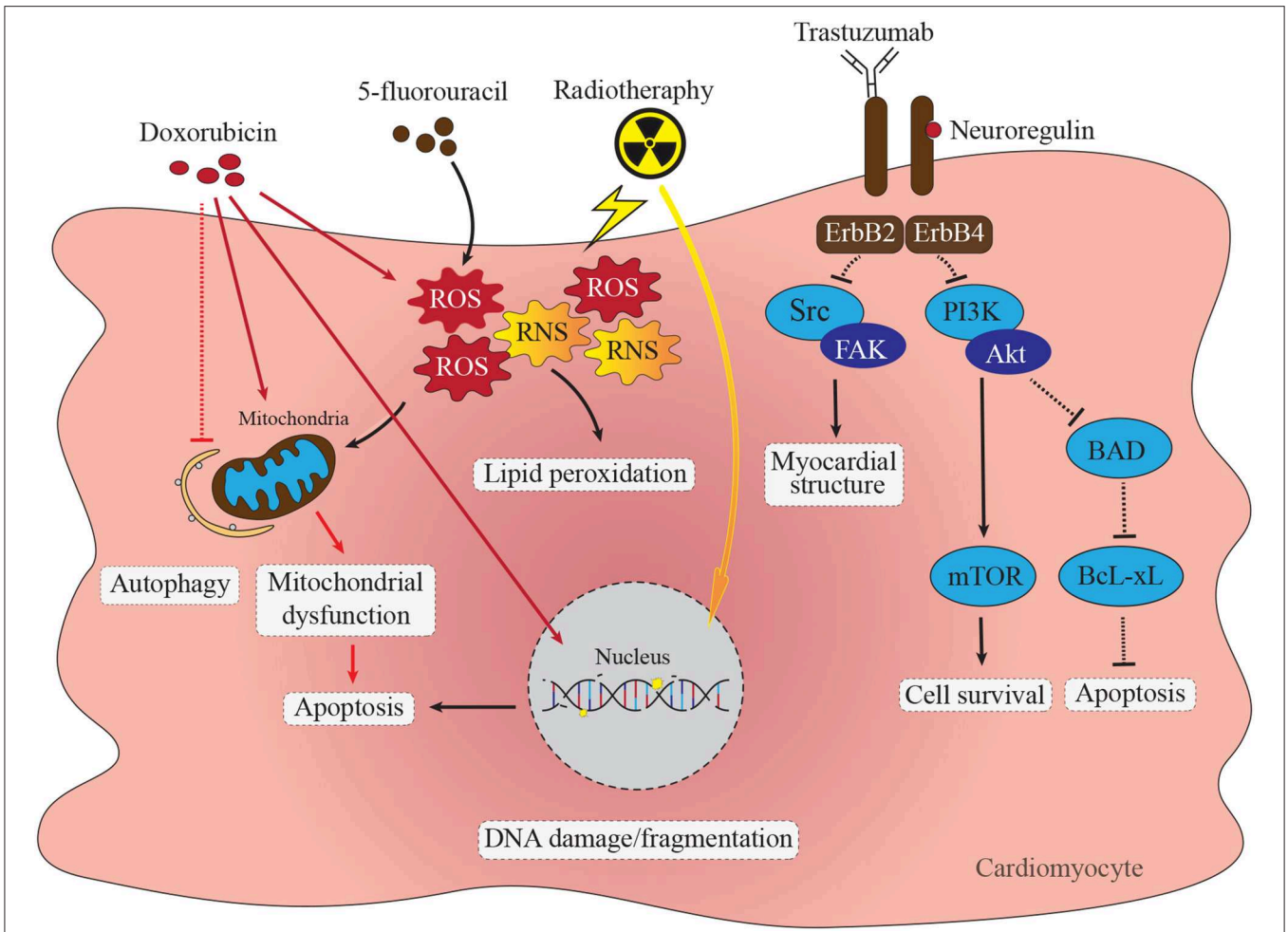

FIGURE 1 | Direct cardiotoxic effects of anti-cancer therapies in cardiomyocytes. Doxorubicin cardiomyocyte cytotoxicity through diverse pathways including DNA damage, accumulation of ROS and RNS, mitochondrial dysfunction, apoptosis and impaired autophagy. Inhibition of ErbB2/4 receptor complex with trastuzumab impacts several signaling pathways resulting in impaired protein synthesis and myocardial structure via the Src/Fak pathway (6). Trastuzumab mediated supression of ErbB2/4 stimulated PI3K-Akt signaling impacts cell survival via mTOR pathway as well as increases apoptosis via BAD/BcL-xL pathway (7). Chemotherapy with fluoropyrimidines, such as 5-fluorouracil (5-FU) cause increase in formation of ROS and RNS leading to mitochondrial dysfunction and activation of caspase-3 and apoptosis (8). Radiotherapy leads to ROS and RNS formation, mitochondrial dysfunction and single-stranded DNA breaks leading to apoptosis activation (9).

important factors to be taken into consideration upon treatment (20). Furthermore, long term follow-up for cardiovascular complications is important, particularly in those patients with pre-existing cardiovascular risk factors (21–23). Understanding the mechanisms by which chemotherapeutic agents cause cardiotoxicity, as well as the identification of pathways that can be targeted in the cardiomyocyte to selectively mitigate chemotherapy induced cardiotoxicity is of fundamental importance in decreasing the undesirable impact on normal tissues and improving cancer treatment outcomes.

High density lipoproteins (HDL) have long been associated with cardioprotection, with a major focus being on arteriosclerosis-associated ischemic heart disease and stroke (24–28). This has largely been associated with its role in the transport of cholesterol from the artery wall to the liver for excretion or recycling, a process called reverse cholesterol transport (RCT) (29). However, recent advances in our understanding of HDL properties and biological functions have revealed HDL associated functions extending beyond cholesterol transport, including direct cytoprotective effects on a number of cell types including cardiomyocytes (30, 31). These advances provide key insights into the potential of exploiting these cytoprotective properties for therapeutic approaches to mitigate chemotherapy associated cardiotoxicity.

Here-in, we will first review the mechanisms by which anthracyclines, the most well-characterized of the cardiotoxic chemotherapeutic agents, induce cardiotoxicity, focusing on DOX as a well-studied anthracycline. While other anti-neoplastic drugs also induce cardiotoxicity, often leading to heart failure, they are beyond the scope of this review. We will then review recent advances in our understanding of HDL functions and properties that can be exploited to mitigate DOX-associated cardiotoxicity. These include understanding the mechanisms by which HDL induces cytoprotective responses in cells including cardiomyocytes, the ability of HDL and synthetic particles based on it to encapsulate DOX and serve as delivery vehicles, and findings that therapeutic treatment with HDL's major structural protein, apolipoprotein A1 (ApoA1) appears to have direct antineoplastic effects in preclinical tumor models (32–34).

MECHANISMS OF ANTHRACYCLINE INDUCED CARDIOTOXICITY

Anthracyclines are a class of chemotherapeutics commonly prescribed to both adult and pediatric populations (35) and can be used alone or in combination with other cancer treatments. Since its discovery in the late 1960's, DOX (also called adriamycin, the prototypical anthracycline) has become widely prescribed due to its efficacy in treating cancers of both hematologic and solid origin (36, 37). Despite their widespread use, anthracyclines such as DOX are not specific in their cell target and exhibit cytotoxic effects in cardiomyocytes thereby limiting their long-term use due to dose-dependent cardiotoxicity (38). Immediate cardiac side effects of DOX infusion are detectable in the form of arrhythmias (39), and cardiotoxic outcomes can be measured following termination of treatment in both early (weeks to months) and late phases (years). These outcomes range from asymptomatic left ventricular dysfunction, to problematic arrhythmias and severe symptomatic congestive heart failure (37, 38). In a retrospective analysis of three trials of DOX therapy for breast or small cell lung cancers, the estimated cumulative percentage of patients with congestive heart failure was 5% in patients receiving a cumulative DOX dose of 400 mg/m^2, which increased to 26% at a dose of 550 mg/m^2, and 48% at 700 mg/m^2 (40). The incidence of cardiotoxicity is highest within the first year following the termination of chemotherapy in adults, although in cases of childhood cancer, onset of cardiotoxicity has been observed to be delayed in survivors by 4–20 years (40–43).

A number of pathways have been implicated in DOX-mediated cardiomyocyte cytotoxicity. These include the direct and indirect induction of oxidative stress, DNA damage, and mitochondrial dysfunction. These pathways, along with alterations in homeostatic processes such as autophagy directly and indirectly lead to induction of cell death pathways, including apoptosis and necrosis (44–46) (**Figure 2**). These pathways have been the subject of recent comprehensive reviews (51–55) and will be summarized in the following sections.

Oxidative Stress

Oxidative stress is the accumulation of oxygen and nitrogen free radicals resulting when their production exceeds the capacity of anti-oxidant enzymes to detoxify them (56). Oxidative stress has been proposed to be a major contributor to cardiomyocyte death and dysfunction following DOX treatment (56) (**Figure 2**). DOX can directly induce oxidative stress. The quinone moiety of DOX can act as an electron acceptor which can be reduced by a variety of enzymes to a semi-quinone, with the generation of oxygen free radicals. These can react with proteins, lipids and DNA, resulting in protein dysfunction, lipid peroxidation and DNA damage (50). DOX can also trigger reductions in the activities and expression of antioxidant enzymes, including matrix manganese superoxide dismutase (MnSOD) and glutathione (GSH) peroxidase (57), thereby reducing the antioxidant capacity of the cardiomyocyte. DOX can also interact with nitric oxide (NO) to generate reactive nitrogen species, contributing to nitrosative stress (56). DOX can also contribute to free radical formation and oxidative stress in cardiomyocytes through complex formation with iron (54). DOX treatment also increases accumulation of mitochondrial iron, once again manifesting in increased reactive oxygen and nitrogen species production (50). Dexrazoxane (DRZ) is an iron chelator which has seen limited clinical use to mitigate DOX-associated cardiotoxicity. On the other hand, pre-clinical studies of anti-oxidants have shown limited effectiveness at reducing DOX-associated cardiotoxicity, leading to doubt regarding the role of DOX-induced ROS formation in DOX associated cardiotoxicity (53).

DNA Damage

DOX also appears to act within the nucleus to trigger cytotoxic effects in cardiomyocytes (**Figure 2**). DOX can reportedly bind to the cytoplasmic proteasome, which assists in the translocation of DOX to the nucleus by an ATP-dependent nuclear pore-mediated

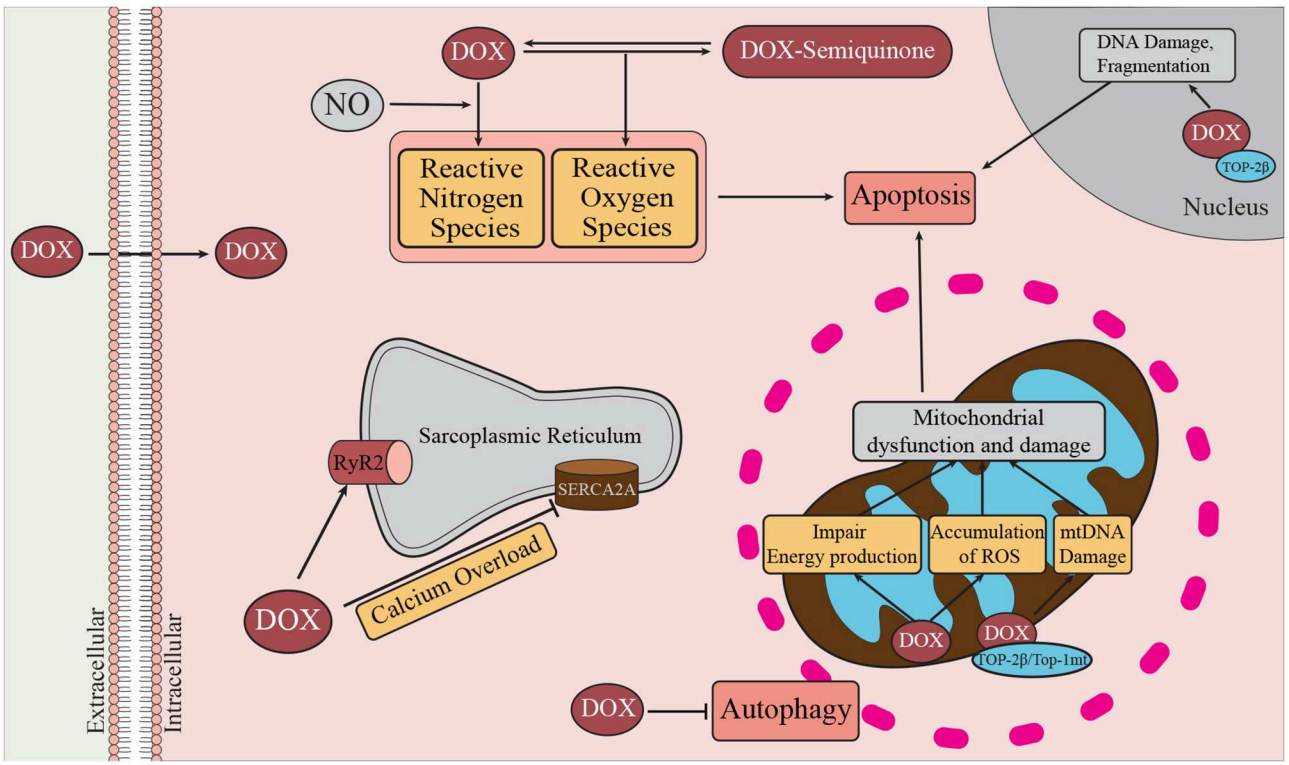

FIGURE 2 | Mechanisms of DOX-induced cardiotoxicity. DOX enters the cell by passive diffusion, and within the cytoplasm undergoes redox cycling resulting in the generation of ROS and RNS (47). Oxidative and nitrosative stress are known to contribute to activation of cell death pathways, such as autophagy, necrosis, and apoptosis. DOX can promote Ca^{2+} overload by inhibiting SERCA2a and transiently enhancing the activity of RyR2. DOX can bind cardiolipin, a major inner mitochondrial membrane lipid. At the mitochondria DOX promotes damage to mitochondrial DNA (mtDNA) through interactions with Top-2β, and Top-1mt. In the nucleus, DOX interacts with Top-2β and DNA to form the Top-2β–DOX—DNA cleavage complex (48). By binding to Top-2β, DOX disrupts the rejoining of DNA leading to the accumulation of double stranded DNA breaks thereby triggering apoptosis (49). Damage to mitochondrial DNA affects mitochondrial biogenesis and contributes to mitochondrial dysfunction and therefore reduced ATP production (50).

mechanism (58). Topoisomerase (Top)-2β was identified as a molecular mediator of DOX cardiotoxicity (48). Top-2β is located in both the nucleus and mitochondria, and is an important regulator of DNA topology by catalyzing the breaking and rejoining of DNA in order to allow for strands to pass by one another (59). DOX binding to Top-2β and DNA forms the Top-2β–DOX—DNA cleavage complex (48). By binding to Top-2β, DOX reportedly disrupts the rejoining of DNA leading to the accumulation of double stranded DNA breaks thereby triggering apoptosis (49). The importance of this pathway is highlighted by the finding that cardiomyocyte specific deletion of the gene encoding Top-2β protected cultured cardiomyocytes from DOX-induced cytotoxicity and protected mice from DOX-induced cardiotoxicity and heart failure (48). DOX has also been implicated in affecting mitochondrial DNA integrity via similar mechanism of ternary DOX—DNA—topoisomerase complex formation involving Top-2β residing in mitochondria as well as involving a mitochondrial specific topoisomerase, Top-1mt (60, 61).

Mitochondrial Dysfunction and Damage

Cardiomyocytes are highly dependent on mitochondrial function for energy production and proper contractile function (62–64). As outlined above, DOX can lead to the accumulation of iron within mitochondria and to the iron dependent and independent accumulation of reactive oxygen and nitrogen species which, themselves, can impair mitochondrial energy production (50, 65, 66). Furthermore, as mentioned above, through ternary complex formation between DOX, DNA and mitochondrial topoisomerases (Top-2β and Top-1mt), DOX can damage mitochondrial DNA by inducing double strand DNA breaks (48, 59). This can impair mitochondrial biogenesis as well as further impact mitochondrial function leading to insufficient energy production for the needs of the cardiomyocyte (60, 61).

Autophagy

Autophagy is a homeostatic mechanism whereby damaged or dysfunctional organelles are recycled to generate substrates for energy production or anabolic processes. Autophagy contributes to normal physiology of the heart and under conditions of acute cardiac stress can promote cardiac survival by releasing energy substrates and breaking down damaged organelles (67). Stimulation of autophagy results in the recruitment of autophagy-related proteins (ATGs) to a specific subcellular site and nucleation of an isolation membrane forming a structure called a phagophore and then an autophagosome, surrounding the damaged target organelle (68). Under conditions of cellular stress, such as nutrient deprivation, the serine/threonine kinase

master cell growth regulator, mTOR, is inhibited, resulting in autophagy (69, 70). ATGs that were phosphorylated by mTOR under normal conditions now become dephosphorylated and recruited for autophagosome formation (71). In addition, under conditions of prolonged stress increased autophagic activity can lead to atrophy (decreased size) of cardiac muscle and activation of cell death pathways (67). Tumor-bearing mice display signs of dysregulated cardiac autophagy (increased expression of autophagic markers) and atrophy (32–34), and several research groups have highlighted the importance of autophagy in cardiotoxicity resulting from treatment of mice with DOX (46, 51, 72).

Autophagy is orchestrated by a complex set of regulatory proteins which identify the target for autophagic disposal, form the limiting membrane and orchestrate fusion with lysosomes [reviewed in Li et al. and Koleini and Kardami (51, 52)]. Recent research has demonstrated DOX dysregulates autophagy in cardiomyocytes and that this appears to play an important role in DOX mediated cardiotoxicity. This has been the subject of a number of recent comprehensive reviews (51, 52). DOX appears to exert a dose-dependent disruption of the normal regulation of autophagy: low, clinically relevant doses, replicating chemotherapy, appear to suppress normal levels of basal autophagy, whereas high doses appear to induce autophagy above normal basal levels (51, 52). The impaired autophagy in cardiomyocytes, resulting from chronic low-dose treatment of mice with DOX, has been reported to involve DOX-mediated interference with lysosome mediated degradation of autophagosome contents (73). On the other hand, DOX, particularly at higher doses, appears to lead to an accumulation of PTEN-induced kinase (PINK) 1 and parkin (an E3 ubiquitin ligase) within mitochondria (74). PINK1 and parkin play a key role in regulating the balance between mitophagy and mitochondrial biogenesis; DOX appears to trigger a depolarization of the mitochondrial membrane potential resulting in an accumulation of PINK1 and parkin on mitochondria. PINK1 is a serine/threonine kinase which phosphorylates ubiquitin and allows parkin to ubiquitinate a number of mitochondrial outer membrane proteins, targeting the mitochondria for mitophagy (52, 74). This, possibly together with DOX-mediated interference with lysosome mediated degradation, results in an accumulation of autophagosomes containing mitochondria (52, 73, 74). An emerging consensus appears to be that DOX interferes with autophagic flux, as a result of simultaneous induction of early stages of mitophagy (triggering mitochondrial dysfunction/membrane depolarization, the marking of mitochondria for mitophagy and formation of autophagosomes surrounding dysfunctional mitochondria) and interference with later stages of autophagy (51, 52). As mentioned above, this may be through DOX-mediated interference with lysosome acidification, thereby preventing the lysosomal degradation of contents of autophagosomes (73). Alternatively, others have reported that DOX mediated mitochondrial damage leads to maladaptive activation of phosphatidylinositol 3-kinase (PI3K) γ signaling, which blocked autophagy, and that this was alleviated by cardiac specific PI3K γ inhibition in mice (75). The consequence of these effects appears to be a build-up of damaged/dysfunctional mitochondria and reactive oxygen species, exacerbating DOX-induced oxidative stress, and leading to activation of apoptotic pathways, triggering cell death (51).

Apoptosis

Apoptosis is a form of programmed cell death involving the induction of a caspase proteolytic cascade ultimately leading to nuclear condensation and fragmentation, phosphatidylserine exposure on the cell surface, formation of apoptotic bodies and their clearance by phagocytes by virtue of recognition of exposed phosphatidylserine. Apoptosis is characterized by the activation of caspase proteolytic cascades leading to the activation of effector caspases, such as caspases 3, 6, and 7 (76, 77). These activate nucleases and proteases which degrade DNA, nuclear, cytoplasmic, and cytoskeletal proteins (76, 77). These effector caspases are activated by two main pathways of apoptosis regulation, the extrinsic and intrinsic pathways. In the extrinsic pathway, ligation of "death receptors" such as the tumor necrosis factor (TNF) α receptor by their extrinsic ligands on the cell surface results in receptor dimerization, bringing together "death domains" leading to the formation of a death inducing signaling complex (DISC) that ultimately triggers the activation of caspase 8, which, in turn, goes on to activate the effector caspases 3, 6, and 7 (77).

On the other hand, mitochondria play central roles in the intrinsic apoptosis pathway, which is regulated by a series of soluble proteins that regulate pore formation in the mitochondrial membrane (76, 77). These factors belong to the Bcl-2 family of proteins and comprise 3 groups: the anti-apoptotic Bcl-2 family members, such as Bcl-2 itself, the pro-apoptotic effectors such as Bax and Bak, and the pro-apoptotic regulators such as Bim. Together these comprise the intrinsic apoptosis pathway. In this pathway, the anti-apoptotic family members bind to and sequester the pro-apoptotic effectors, preventing them from assembling as complexes on the mitochondrial membrane. The pro-apoptotic regulators, in turn, bind to the anti-apoptotic regulators, preventing them from sequestering the pro-apoptotic effectors and, additionally, assist the pro-apoptotic effectors in assembly on the mitochondria, where the pro-apoptotic effectors can form pores in the mitochondrial membrane. This leads to the leakage of mitochondrial cytochrome C and formation of a protein complex, called the apoptosome which serves as a platform for the activation of caspase 9, which can then activate effector caspases 3, 6, and 7 (76). In this intrinsic pathway, the relative amounts of pro-apoptotic effectors, regulators and anti-apoptotic factors determine whether apoptosis proceeds (76). Nuclear DNA damage and induction of oxidative stress and damage to mitochondria are all potent activators of the intrinsic apoptosis pathway (76, 77). In addition, DOX treatment of cardiomyocytes has been reported to result in increased transcription of the pro-apoptotic regulator Bim, thus enhancing the intrinsic apoptotic pathway (78). Additionally, recent work has identified increased expression of death receptors (TNF receptor 1, Fas, death receptor 4, death receptor 5) in human induced pluripotent stem cell derived cardiomyocytes following DOX treatment (44).

Atrophy

In addition to causing the death of cardiomyocytes, DOX also triggers cardiomyocyte atrophy, or a reduction in cardiomyocyte size. Ultrastructural changes in the myocardium, including myofibril structural disarray and atrophy due to DOX-cardiotoxicity appear well before clinical manifestations (79). The ubiquitin proteasome system regulates cardiomyocyte size by tagging proteins with polyubiquitin chains for subsequent degradation by the proteasome (80). The tagging of target proteins for proteolysis by the ubiquitin-proteasome system involves the assembly of polyubiquitin chains on target proteins mediated by E1 ubiquitin activating enzymes, E2 ubiquitin conjugating enzymes and E3 ubiquitin ligases. Atrogin-1 is a muscle specific E3 ubiquitin ligase that facilitates atrophic signaling in cardiomyocytes (80). It and other E3 ubiquitin ligases appear to be upregulated in cardiomyocytes by treatment with DOX and/or other anthracyclines (81, 82). This leads to the degradation of contractile proteins, and reduction in cardiomyocyte size (81). As cardiomyocyte size is related to overall contractile force generation, reduced cardiomyocyte size (atrophy) compounds reduced cardiomyocyte numbers in diseased states and eventually manifests as progressive reduction in cardiac function, as in the case of DOX-induced cardiotoxicity (83).

THERAPEUTIC STRATEGIES FOR PREVENTING OR TREATING ANTHRACYCLINE CARDIOTOXICITY

At present, monitoring and screening for cardiotoxicity following anthracycline therapy is imperative for timely treatment. In a recent prospective study of anthracycline-treated patients, of the 9% of patients who developed heart failure, 98% of cases occurred within a year following anthracycline treatment (40). Countless prophylactic therapeutics are currently under study in animal models but few have been assessed clinically. Furthermore, only a small number cardioprotective therapeutics that have been tested in humans reduce the cardiotoxic effects of anthracyclines and currently no clear guidelines or worldwide accepted therapies exist. β-blockers, angiotensin converting enzyme (ACE) inhibitors, statins, and dexrazoxane (DRZ) are drugs that have been assessed in small trials for protection against anthracycline cardiotoxicity. Results are promising, but data is limited by small study sizes and variability in study methods such as follow up time. Early identification of reduced cardiac function and immediate treatment with heart failure medication such as enalapril (an ACE inhibitor) alone or in combination with β-blockers (carvedilol, or bisoprolol) provided either full or partial improvement of cardiac function in 82% of patients (40). A small group of non-Hodgkins lymphoma patients receiving ramipril and/or bisoprolol as a prophylactic during anthracycline treatment also exhibited reductions in new symptoms of cardiotoxicity and projected prolonged survival (84).

Statins are commonly prescribed to reduce morbidity and mortality associated with atherosclerosis. Given the cardioprotective nature of statins, and the fact that cancer patients receiving chemotherapy may also be concurrently treated with statins for atherosclerosis, the prophylactic effect of statins on anthracycline cardiotoxicity has been examined in a number of small trials. Breast cancer patients on continuous statin therapy had reduced risk of heart failure following anthracycline treatment compared to those with non-continuous statin therapy (85), and similarly, anthracycline induced decline in left ventricular ejection fraction (LVEF) was reduced by statin treatment as compared to no statin treatment (86). In a small study of 40 patients, those receiving atorvastatin prior to chemotherapy infusion showed no significant change in LVEF at 6 months post therapy compared to a reduction in LVEF in those receiving anthracycline alone (87).

While statins and ACE inhibitors have shown promising results in mitigating resultant anthracycline cardiotoxicity or preventing cardiotoxicity when individuals happen to be taking them concurrently with chemotherapy for treatment of comorbidities, DRZ is currently the only United States Food and Drug Administration, and Health Canada approved prophylactic drug for use in combination with DOX to specifically limit cardiotoxicity in adults (88, 89). DRZ acts as an iron chelator, interferes with ROS production, can bind to Top-2β to inhibit complex formation with DOX, and also reduce Top-2β expression (90–92). Early multi-center randomized double blind trials of breast cancer patients receiving combination chemotherapy which included a cumulative DOX dose of 300 mg/m^2 reported a hazard ratio of cardiac events of 2.63 (placebo vs. DRZ); however, despite this promising effect of DRZ on heart function, patient survival was not improved (93). A systematic review also identified an association of DRZ with reduced risk of cardiovascular complications, but increased risk of secondary malignant neoplasms in children receiving chemotherapy (94). Given these results, Health Canada cautions against use of DRZ in children, as well as in elderly populations with reduced cardiac, hepatic, or renal function.

Identification of a treatment that protects against the cardiotoxic side effects without impacting the chemotherapeutic effects of DOX remains of utmost importance. Potential for development or further study of effective primary prevention therapies exists given the expansion of research uncovering the broad nature of mechanisms in the pathogenesis of anthracycline mediated heart failure.

HDL AND ROLE IN CANCER AND CANCER THERAPY

HDL has long been associated epidemiologically with reduced risk for cardiovascular disease. The main mechanistic explanation has traditionally been its apparent protection against atherosclerotic narrowing of arteries, thereby combating ischemic cardiovascular disease. However, recent research in pre-clinical models have increasingly suggested that HDL may exert direct cardioprotective effects on the heart itself (24). Furthermore, HDL based nanospheres have been developed as delivery vehicles for a variety of drugs including chemotherapeutic agents. Finally, pre-clinical studies have shown

that delivery of supra-physiological amounts of HDL's major apolipoprotein, ApoA1, may itself attenuate tumor growth. In the following sections, we provide an overview of HDL structure, composition, formation and function and then discuss recent findings demonstrating direct cardioprotective effects of HDL and/or its precursor, ApoA1 against DOX-induced cardiotoxicity, advances in the use of HDL based nanospheres for encapsulation of DOX for therapeutic delivery and direct anti-tumor effects of ApoA1.

HDL Structure and Composition

Lipoproteins are diverse biological particles that provide a means of transport for lipids between cells, tissues, and other lipoproteins, and can activate intracellular signaling pathways (95). Lipoproteins are separated into five classes (chylomicron, VLDL, IDL, LDL, and HDL) based on criteria including density, size, and relative content of lipids (cholesterol and triglyceride), and apolipoproteins (95). HDL represents one of the five major classes of lipoproteins, distinguishable from others based on their small particle size (5–11 nm), high density (1.063–1.21 g/ml), and unique apolipoprotein content (96). Unlike other lipoproteins, HDLs are unique in their cytoprotective actions and initiate anti-oxidative, anti-apoptotic, and anti-inflammatory effects. HDL can inhibit the oxidation of LDL and enhance endothelial function by inhibiting the expression of endothelial adhesion molecules (97). In addition, HDL can suppress atherosclerosis progression and inflammation by modulating production of monocytes and neutrophils (98).

HDL represents a class of particles of distinct protein and lipid composition. The proteome of HDL is diverse and can contain close to 100 proteins, more than can fit on a single particle, underscoring the notion that HDL represents a class of particles of distinct compositions. The HDL proteome includes apolipoproteins, diverse enzymes, lipid transfer proteins, acute phase proteins, and proteinase inhibitors, and other proteins of distinct functions (99). ApoA1 (243 amino acids, 28 kD) is the most abundant protein in HDL, comprising 70% of the protein carried by HDL (100). ApoA1 has over 90% amphipathic α-helical content, allowing for formation and stabilization of the HDL (101). ApoA1 is linked to several beneficial effects of HDL. Therefore, several research groups have reported that HDL quality is highly dependent on the abundance and function of ApoA1 (102).

HDL Formation and Function

ApoA1 is secreted by the liver (70%) and small intestine (30%) in a lipid-poor state, and is assembled into HDL by the addition of phospholipids and unesterified cholesterol aided by the ATP-binding cassette transporter A1 (ABCA1) at the cell surface, forming nascent HDL (an immature form of HDL). Next, the nascent HDL particle becomes mature HDL by activating lecithin: cholesterol acyltransferase (LCAT), which converts unesterified cholesterol to cholesteryl esters. The esterification of cholesterol increases its hydrophobicity, resulting in its movement into the core of the HDL particle and the particle itself adopting a spherical shape (103, 104).

HDL particles are continuously remodeled and catabolized by plasma and membrane proteins, thereby giving rise to dynamic subfractions. Membrane receptors including ATP-binding cassette transporter G1 (ABCG1) and the scavenger receptor class B type 1 (SR-B1) promote movement of lipids between cells and HDL, and plasma proteins such as cholesteryl ester transfer protein (CETP) assist in movement of lipids between lipoproteins. SR-B1 is a high affinity receptor for HDL, is highly expressed in liver and steroidogenic tissues, and plays a critical role in tissue uptake of HDL cholesterol, a key step in reverse cholesterol transport, which is the transport of cholesterol from peripheral tissues to the liver for repackaging into nascent lipoproteins or excretion (105). ApoA1 appears to play an important role in this process as adenoviral mediated or transgenic overexpression of human ApoA1 in mice leads to enhanced reverse cholesterol transport (106).

HDL Targeted Therapeutics

The Framingham Heart Study shows strong relationships between levels of HDL and the incidence of developing heart disease (25). Statins, inhibitors of hydroxymethylglutaryl coenzyme A (HMG-CoA), a key enzyme in the cholesterol biosynthetic pathway, have long been major therapeutic tools in the reduction of cardiovascular events. This is due to their ability to reduce production and increase clearance of VLDL and LDL, thereby reducing blood cholesterol levels. Many statins have also been shown to modestly raise HDL cholesterol levels by between 3 and 15% (107–109). The mechanisms and clinical benefit of statin triggered increases in HDL cholesterol are unclear and still the subject of debate (107–111). In part this may be due to the difficulty in evaluating the contribution of the relatively modest statin-induced increases in HDL-cholesterol levels in the context of dramatic reductions in LDL cholesterol.

The epidemiological association of increased HDL cholesterol with reduced risk for cardiovascular disease has, over the years, led to efforts to increase HDL levels pharmacologically. The focus, however, has been in increasing HDL-cholesterol as opposed to increasing HDL particles or, more subtly, to increasing functional HDL particles. This focus on raising HDL cholesterol levels has led to the development of CETP inhibitors which block the CETP mediated transfer of cholesterol from HDL to triglyceride rich lipoproteins such as VLDL. These drugs increase HDL cholesterol levels by more than 25% (up to 60%), but largely have not reduced cardiovascular events (107, 109, 112–118). More recent attempts at increasing HDL particle number and/or function have focused on infusion of reconstituted HDL, administration of ApoA1 mimetics, or upregulation of ApoA1 production by liver (119–124). CSL112 is a new reconstituted HDL (rHDL) made with human ApoA1. Clinical trials of CSL112 showed that it enhances cholesterol efflux capacity an important measure of HDL mediated reverse cholesterol transport mediated cardiovascular protection (124, 125). Also, the AEGIS-I trial suggests that CSL112 has advantages over other rHDL formulations (e.g., CSL-111, CER-001) or ApoA-I Milano because it is well-tolerated with no side effects in major organs (such as liver or kidney toxicity) or immunogenicity (126–128). However, the potential benefit of CSL112 in reducing

major adverse cardiovascular events in this group of high-risk patients still remains to be shown in the large phase III AEGIS-II study that is expected to be concluded in 2022 (124, 125).

Preclinical Studies of HDL Effects on DOX-Induced Cardiotoxicity

The protective effects of HDL against cardiovascular disease have long been the subject of intensive research. While most of the focus has been on the ability of HDL to protect against atherosclerosis and vascular dysfunction, and the impacts of that on ischemic cardiovascular disease, more recent focus has increasingly been placed on the direct cardioprotective effects of HDL, through its interactions with cardiomyocytes themselves (24). Epidemiological studies, as well as pre-clinical studies in animal models, have demonstrated that HDL can protect against cardiac disease independent of effects on coronary artery atherosclerosis, suggesting that it may also exert direct effects on the heart itself (129–132). For example, HDL treatment has been shown to protect hearts (*in vivo* and *ex vivo*) and isolated cardiomyocytes, from ischemia/reperfusion injury and infarction (130–132). In the context of DOX-induced cardiotoxicity, recent studies using isolated cardiomyocytes in culture (133–136) and in preclinical animal models (135, 137) demonstrate that HDL is able to protect against cardiomyocyte apoptosis and myocardial atrophy. For example pre-treatment of cultured cardiomyocytes with HDL prior to subsequent treatment with DOX, protects them against DOX induced apoptosis (137, 138). Similarly, HDL pretreatment of cultured cardiomyocytes protects them against other stresses leading to cytotoxicity, including necrosis resulting from oxygen and glucose deprivation (134), suggesting that HDL may protect cardiomyocytes against diverse forms of cell death. HDL mediated protection against DOX-induced cardiomyocyte apoptosis has been reported in different studies to involve the activation of AKT (134, 137) or the activation of the signal transducer and activator of transcription (STAT) 3 (138). Pharmacological or genetic inhibition of these signaling mediators has been shown to impair HDL mediated protection of cardiomyocytes against DOX-induced apoptosis (137, 138). The importance of the AKT pathway in cardioprotection against DOX has also been demonstrated by the finding that expression of constitutively active AKT1 in the myocardium inhibits DOX induced cardiotoxicity by preventing left ventricular dysfunction and cardiac atrophy (139). Furthermore, cardiac restricted overexpression of STAT3 in mice led to protection against DOX-induced atrophy and congestive heart failure, whereas cardiac specific knockout of STAT3 in mice was accompanied by increased cardiac fibrosis and age-dependent heart failure (140, 141). AKT and STAT3 form the respective cornerstones of the RISK (reperfusion injury salvage kinase) and SAFE (survivor activating factor enhancement) signaling pathways known to play important roles in cardioprotection, for example in the setting of ischemia/reperfusion injury (142, 143). These data also suggest that AKT and STAT3 are critical mediators of cardioprotection against DOX-induced cardiotoxicity (**Figure 3**). HDL dependent activation of AKT and STAT3 and other signaling pathways has been reported in different cell types to involve HDL mediated delivery of the bioactive lipid, sphingosine-1-phosphate (S1P), acting via the S1P receptors (136, 144–148) (**Figure 3**). In the case of cardiomyocytes, HDL and S1P mediated activation of STAT3 appears to be mediated by the S1P receptor 2 (S1PR2) (136). On the other hand, the involvement of S1P/S1P receptors in HDL mediated activation of AKT signaling in cardiomyocytes has not been demonstrated (134, 135, 137). However, HDL mediated activation of AKT signaling in cardiomyocytes and protection of cardiomyocytes against DOX-induced apoptosis appears to require the HDL receptor, SR-B1. SR-B1 is expressed by both mouse and human cardiomyocytes in culture and mouse cardiac tissue (137, 149). The ability of HDL to induce AKT phosphorylation and protection against DOX induced cytotoxicity in cultured mouse or human cardiomyocytes was lost when the gene for SR-B1 was either knocked out or knocked down (135, 137). SR-B1 mediates lipid transport between bound HDL particles and cells via a hydrophobic channel, suggesting that SR-B1 mediated transport of HDL bound, water insoluble S1P molecules from HDL, into the cell membrane, where they can access S1P receptors may be a potential mechanism for the involvement of SR-B1, and HDL associated S1P and S1P receptors in cardioprotection. This, however, remains to be demonstrated experimentally. The potential role for HDL associated S1P in cardioprotection against DOX-induced cardiotoxicity highlights the importance of understanding the role of HDL composition in evaluating HDL function and designing HDL based therapeutics such as reconstituted HDL-like particles (150).

These *in vitro* studies of HDL mediated protection against DOX-induced cardiotoxicity have recently been extended to *in vivo* models (135, 137) by examining the effects of increased circulating HDL levels on DOX-induced cardiotoxicity in mice. We first tested the effects of genetic overexpression of human ApoA1, on cardiotoxicity induced by repeated weekly DOX dosing in mice. Overexpression of transgenic human ApoA1 in mice has been shown to trigger dramatically increased circulating HDL levels by seeding the formation of new mature HDL particles (151). In one study, transgenic overexpression of human ApoA1 in mice virtually completely prevented chronic low dose DOX treatment from triggering myocardial apoptosis and atrophy, and protected mice from DOX-treatment induced reduction in left ventricular function (137). A drawback of this study was that although it represented a proof of concept, transgenic overexpression of ApoA1 led to levels of ApoA1 and HDL that were extremely high and therefore not likely to be therapeutically relevant (137). A more recent study, however, demonstrated that intraperitoneal injection of purified ApoA1 similarly prevented cardiotoxicity associated with chronic low dose DOX treatment in mice (135). Mice that were treated with five weekly injections of DOX alone exhibited substantial apoptosis in cardiomyocytes in hearts, and substantially reduced left ventricular function, whereas control mice that did not receive DOX displayed little myocardial apoptosis and normal left ventricular function (135). On the other hand mice that were treated with injection of ApoA1 alongside DOX were virtually completely protected against DOX-induced myocardial apoptosis and left ventricular dysfunction (135). Regardless of

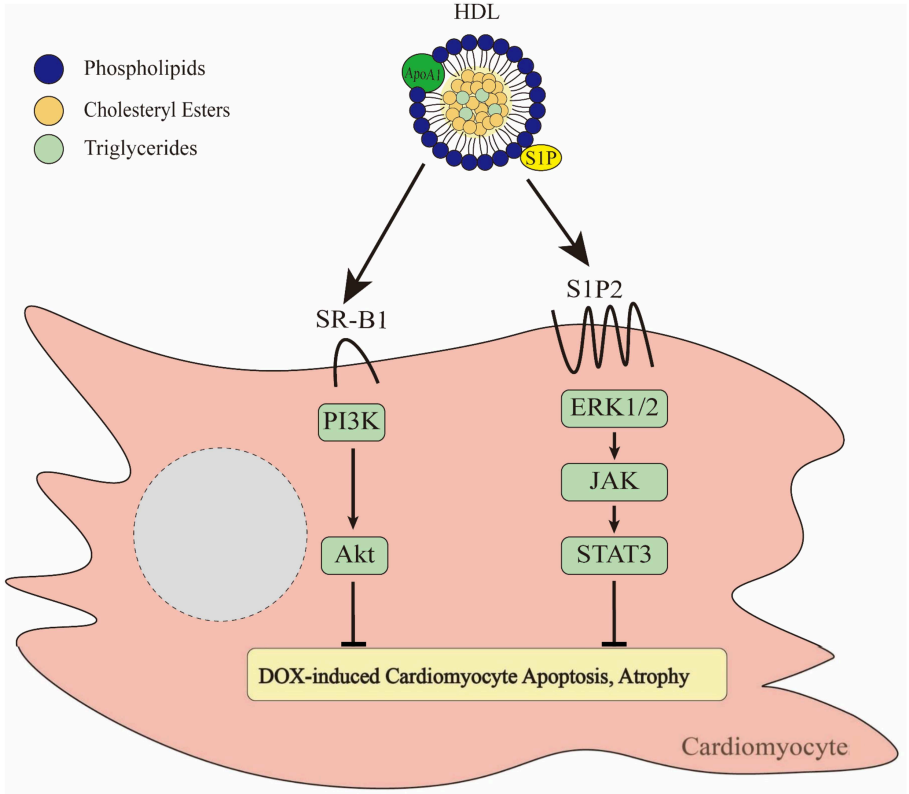

FIGURE 3 | Effects of HDL on DOX-induced cytotoxicity of cardiomyocytes. HDL binds to SR-B1 leading to activation of the PI3K/Akt pathway (137). In addition, HDL is able to activate ERK1/2-JAK-STAT3 signaling via a pathway involving HDL mediated delivery of the bioactive lipid, S1P acting via the S1P2 receptor (136). Together these pathways prevent DOX-induced apoptosis and atrophy.

means of HDL increase (ApoA1 transgenic expression or ApoA1 injection) cardioprotection was lost if mice lacked SR-B1 (135, 137). In fact, SR-B1 knockout mice were more susceptible to DOX induced cardiotoxicity than corresponding wild type mice. This effect of SR-B1 appeared to be associated with SR-B1 expression in cardiac tissue, consistent with observations that SR-B1 expression in cultured cardiomyocytes was required for HDL mediated protection against DOX-induced apoptosis (135, 137). These findings clearly demonstrate that in pre-clinical models, HDL-therapies such as injection of the HDL precursor ApoA1 have the potential to protect against DOX induced cardiotoxicity but are dependent on the expression of cardiomyocyte SR-B1 (**Figure 3**).

HDL Based Delivery of Chemotherapeutics

In addition to HDL's ability to protect cardiomyocytes against cytotoxicity induced by anti-cancer agents, reconstituted HDL (rHDL)-based nanoparticles have also been explored as drug delivery vehicles for chemotherapeutic agents such as DOX. The use of rHDL as a drug delivery system for DOX has been studied using both *in vitro* and *in vivo* methods. Yuan et al. showed that DOX encapsulated in HDL particles (rHDL-DOX) is more efficiently taken up by and more effective at inducing apoptosis in hepatocellular carcinoma cells, when compared to DOX alone or encapsulated in liposomes (45). Furthermore, in preclinical mouse tumor models, treatment with rHDL-DOX resulted in greater tumor regression than DOX alone (45). Wang et al. confirmed that incorporation of DOX into rHDL-based particles enhanced the cytotoxic effects of DOX on tumors *in vivo* and cancer cells *in vitro* (152). Furthermore, they demonstrated that the HDL receptor SR-B1 was required in tumor cells for rHDL mediated delivery of the encapsulated DOX (152). Interestingly, the authors measured DOX tissue distribution after treating mice with rHDL-DOX and showed that DOX uptake by the heart was low (152). Others have tested the effects of using rHDL to deliver paclitaxel (PTX) either alone or in combination with DOX. Co-delivery of PTX and DOX encapsulated in rHDL was shown to improve their anti-cancer effects over co-administration of non-encapsulated PTX and DOX (153). When used to treat preclinical models of liver cancer, the majority of PTX and DOX delivered via rHDL was found in the liver tumors (attributed to uptake via SR-B1) with little accumulation in the heart and very little cardiac damage (153). These findings suggest that, at least for liver cancer rHDL encapsulation can provide a means for targeted delivery of anti-cancer agents to tumor cells, sparing cardiac tissues. Whether the reduced cardiac damage was solely due to targeted delivery of the anti-cancer agents to the hepatic tumor over the heart or whether it also involved induction of survival signaling

at the heart (PI3K/AKT and STAT3 signaling as described above) remains to be determined. It also remains to be determined whether rHDL-mediated chemotherapeutic delivery is effective against other types of cancer or against tumor cells which do not express high levels of SR-B1. Nevertheless, these studies suggest the potential for rHDL based drug delivery systems to confer tissue selective delivery to at least some types of tumors, sparing the heart from cardiotoxic damage. More research is required to determine the full potential of this.

HDL and Cancer

In addition to research showing that HDL can protect cardiomyocytes from chemotherapy-induced cytotoxicity both directly by inducing survival signaling in the cardiomyocytes, and indirectly by acting as a targeted delivery system for anti-cancer agents, sparing the heart, other research has suggested that HDL and its precursor ApoA1 may also have direct anti-tumor effects themselves.

Endogenous HDL and Cancer Risk

Results of epidemiological studies of endogenous HDL cholesterol levels and the incidence of cancer are mixed with some studies reporting an inverse correlation between HDL cholesterol and cancer risk and/or mortality, while other studies report minimal association, particularly when corrected for confounding factors (152, 154–159). Contributing to this is uncertainty over the cause-vs.-effect relationship between low HDL-cholesterol and cancer, with some studies suggesting that tumor cells may drive the lowering of HDL-cholesterol levels by utilizing HDL-cholesterol to support tumor growth (159). Complicating matters further are reports that HDL prepared from cancer patients or from patients with other co-morbidities, such as type 2 diabetes or obesity exhibit altered functions as compared to HDL from unaffected individuals, for example, promoting rather than inhibiting migration and invasion of tumor cells in *in vitro* assays (160–166). Therefore, it is presently unclear what, if any, effects levels of endogenous HDL or variations in those levels have on cancer development.

Anti-cancer Therapeutic Potential of the HDL Precursor ApoA1

On the other hand, preclinical studies in mouse models have suggested that supra-physiological levels of ApoA1 may have therapeutic potential against tumor growth and metastasis. For example, Zamanian-Daryoush et al. reported that transgenic overexpression of human ApoA1 reduced, while complete knockout of endogenous ApoA1 increased tumor growth and metastasis in mice compared to control mice with normal levels of endogenous ApoA1 (167). They also demonstrated that pharmacological treatment with purified ApoA1 similarly attenuated both primary tumor development and metastasis in mouse models (167). They provided evidence that ApoA1 reduced tumor angiogenesis and recruited tumor cell targeting macrophages and $CD8^+$ cytotoxic T cells, thereby altering the tumor microenvironment to one less permissive for tumor development (167, 168). By using different tumor cell lines, including a human melanoma cell line (A375), they demonstrated that supra-physiological levels of ApoA1 may have general anti-neoplastic effects including toward human tumors (167, 168). Others have reported that synthetic ApoA1 mimetic peptides, which replicate the amphipathic properties of ApoA1, also exhibit anti-tumor properties, when used at pharmacological concentrations. For example, the ApoA1 mimetic peptide, L-5F was reported to prevent angiogenesis, suggesting that it may have therapeutic potential against angiogenesis associated diseases such as cancer (169). In preclinical studies, the ApoA1 mimetic peptide 4F has been reported to suppress ovarian tumorigenesis (170). Similarly, preclinical studies demonstrated that the recombinant ApoA1 mimetic peptide 6F reduced tumor burden in mouse models of metastatic lung cancer (171). However, ApoA1 mimetic peptides may exert anti-tumor effects via mechanisms distinct from ApoA1. For example ApoA1 mimetic peptides are thought to strongly bind and neutralize lysophosphatidic acid (LPA), which is known to stimulate cell proliferation, oncogenesis, and metastasis (172). On the other hand, neither transgenic overexpression of human ApoA1 nor ApoA1 knockout affected LPA levels in tumor-bearing mice (167). Whether ApoA1 (injected or overexpressed) or ApoA1 mimetic peptides exert anti-tumor effects by driving the increased formation of HDL-like particles or whether their anti-neoplastic effects are independent of HDL particle formation has not been examined. Other pre-clinical studies have reported that in certain cases, HDL may drive the development of breast cancer, particularly in circumstances when breast tumor cells overexpress the HDL receptor, SR-B1, since this receptor can mediate both survival signaling and uptake of cholesterol fueling tumor growth (173–177). Therefore, direct anti-tumor effects of ApoA1 or ApoA1-mimetic peptides may be restricted to tumors that do not overexpress SR-B1; although SR-B1 overexpression in tumors could be exploited by strategies that encapsulate

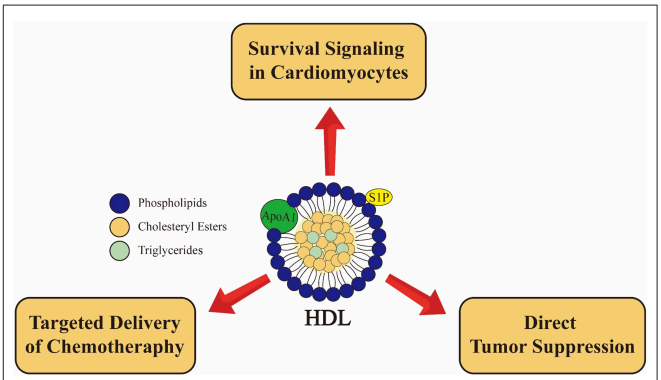

FIGURE 4 | Emerging roles of high density lipoprotein (HDL) in cancer and cancer therapy associated cardiotoxicity from preclinical studies. HDL induces cardioprotective effects in cardiomyocytes via SR-B1 receptor and activation of PI3K-Akt pathway as well as via S1P2 receptor leading activation of STAT3-JAK-ERK1/2 pathway (136, 137). rHDL used as a drug delivery system may allow for targeted delivery to at least some types of tumors, sparing cardiomyocytes (45, 152). Furthermore, the major apoliprotein of HDL (ApoA1) directly attenuates tumor growth and metastasis in preclinical models (167). These pathways are not necessarily muatually exclusive.

chemotherapeutic agents like DOX in HDL based nanoparticles (see previous section), which may be readily and preferentially taken up by tumor cells overexpressing SR-B1.

CONCLUSIONS

Preclinical studies suggest that HDL targeted therapies involving pharmacological treatment with supra-physiological levels of ApoA1, peptides based on ApoA1 (ApoA1 mimetic peptides) or rHDL like particles may show promise in the protection against chemotherapy related cardiotoxicity via a number of mechanisms (**Figure 4**). These include (1) direct HDL mediated survival signaling in cardiomyocytes leading to protection against cytotoxicity, as exemplified by studies using DOX as a cardiotoxic agent; (2) indirect protection afforded to the heart by utilizing rHDL-based nanoparticles as targeted delivery vehicles for chemotherapeutic agents which spare the heart and have the potential to target tumor cells which may overexpress SR-B1; and (3) indirect protection resulting from direct ApoA1 mediated tumor suppression (**Figure 4**). It remains to be demonstrated experimentally whether these mechanisms broadly impact diverse malignancies and chemotherapeutic agents or are specific for those that have been tested to date. Importantly, these mechanisms may not necessarily be mutually exclusive. For example, in the case of treatment with ApoA1 along with chemotherapeutic agents such as DOX, the ApoA1 may be acting by seeding the formation of new HDL particles which may incorporate the chemotherapeutic agent, act as targeted delivery systems for certain types of tumors, directly attenuate tumor growth, and directly induce survival signaling in cardiomyocytes, thus inducing both direct and indirect mechanisms of cardioprotection simultaneously. However, studies need to be designed to test whether these mechanisms do occur simultaneously, in the same preclinical models. Whether or not they do occur simultaneously, much more work remains to be done do determine the full potential for HDL targeted therapies as therapeutic approaches to prevent chemotherapy induced cardiotoxicity in human disease.

AUTHOR CONTRIBUTIONS

All authors listed above made substantial, direct and intellectual contributions to the work, and have approved the final version.

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REFERENCES

1. de Moor JS, Mariotto AB, Parry C, Alfano CM, Padgett L, Kent EE, et al. Cancer survivors in the United States: prevalence across the survivorship trajectory and implications for care. *Cancer Epidemiol Biomarkers Prev.* (2013) 22:561–70. doi: 10.1158/1055-9965.EPI-12-1356
2. DeSantis CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, Kramer JL, et al. Cancer treatment and survivorship statistics, 2014. *CA Cancer J Clin.* (2014) 64:252–71. doi: 10.3322/caac.21235
3. Moslehi JJ. Cardiovascular toxic effects of targeted cancer therapies. *N Engl J Med.* (2016) 375:1457–67. doi: 10.1056/NEJMra1100265
4. Jain D, Russell RR, Schwartz RG, Panjrath GS, Aronow W. Cardiac complications of cancer therapy: pathophysiology, identification, prevention, treatment, and future directions. *Curr Cardiol Rep.* (2017) 19:36. doi: 10.1007/s11886-017-0846-x
5. Tocchetti CG, Cadeddu C, Di Lisi D, Femminò S, Madonna R, Mele D, et al. From molecular mechanisms to clinical management of antineoplastic drug-induced cardiovascular toxicity: a translational overview. *Antioxidants Redox Signal.* (2019) 30:2110–53. doi: 10.1089/ars.2016.6930
6. Kuramochi Y, Guo X, Sawyer DB. Neuregulin activates erbB2-dependent src/FAK signaling and cytoskeletal remodeling in isolated adult rat cardiac myocytes. *J Mol Cell Cardiol.* (2006) 41:228–35. doi: 10.1016/j.yjmcc.2006.04.007
7. Gassmann M, Casagranda F, Orloli D, Simon H, Lai C, Kleint R, et al. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature.* (1995) 378:390–4. doi: 10.1038/378390a0
8. Sara JD, Kaur J, Khodadadi R, Rehman M, Lobo R, Chakrabarti S, et al. 5-fluorouracil and cardiotoxicity: a review. *Ther Adv Med Oncol.* (2018) 10:1758835918780140. doi: 10.1177/1758835918780140
9. Sylvester CB, Abe J, Patel ZS, Grande-Allen KJ. Radiation-induced cardiovascular disease: mechanisms and importance of linear energy transfer. *Front Cardiovasc Med.* (2018) 5:5. doi: 10.3389/fcvm.2018.00005
10. Myers CE. The pharmacology of the fluoropyrimidines. *Pharmacol Rev.* (1981) 33:1–15.
11. Labianca R, Beretta G, Clerici M, Fraschini P, Luporini G. Cardiac toxicity of 5-fluorouracil: a study on 1083 patients. *Tumori.* (1982) 68:505–10. doi: 10.1177/030089168206800609
12. Lamberti M, Porto S, Marra M, Zappavigna S, Grimaldi A, Feola D, et al. 5-Fluorouracil induces apoptosis in rat cardiocytes through intracellular oxidative stress. *J Exp Clin Cancer Res.* (2012) 31:60. doi: 10.1186/1756-9966-31-60
13. Wahler J, Suh N. Targeting HER2 positive breast cancer with chemopreventive agents. *Curr Pharmacol Reports.* (2015) 1:324–35. doi: 10.1007/s40495-015-0040-z
14. Wang J, Xu B. Targeted therapeutic options and future perspectives for HER2-positive breast cancer. *Signal Transduct Target Ther.* (2019) 4:34. doi: 10.1038/s41392-019-0069-2
15. Ahmed S, Talat A, Iqbal N, Emara M, Le D, Sami A, Xiang J. Cardiac toxicity of HER2-directed therapy in women with breast cancer: epidemiology, etiology, risk factors, and management. In: Pham PV, editor. *Breast Cancer - From Biology to Medicine.* London: IntechOpen (2017). doi: 10.5772/66437
16. Guo S, Wong S. Cardiovascular toxicities from systemic breast cancer therapy. *Front Oncol.* (2014) 4:346. doi: 10.3389/fonc.2014.00346
17. Soucy KG, Lim HK, Kim JH, Oh Y, Attarzadeh DO, Sevinc B, et al. HZE ^{56} Fe-Ion irradiation induces endothelial dysfunction in rat aorta: role of xanthine oxidase. *Radiat Res.* (2011) 176:474–85. doi: 10.1667/RR2598.1
18. Rygiel K. Cardiotoxic effects of radiotherapy and strategies to reduce them in patients with breast cancer: An overview. *J Cancer Res Ther.* (2017) 13:186–92. doi: 10.4103/0973-1482.187303
19. Lewis GD, Farach A. Cardiovascular toxicities of radiation therapy. *Methodits Debakey Cardiovasc J.* (2019) 15:274–81. doi: 10.14797/mdcj-15-4-274
20. Hershman DL, McBride RB, Eisenberger A, Tsai WY, Grann VR, Jacobson JS. Doxorubicin, cardiac risk factors, and cardiac toxicity in elderly patients with diffuse B-cell Non-Hodgkin's Lymphoma. *J Clin Oncol.* (2008) 26:3159–65. doi: 10.1200/JCO.2007.14.1242
21. Einarson TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence

from across the world in 2007-2017. *Cardiovasc Diabetol.* (2018) 17:1–19. doi: 10.1186/s12933-018-0728-6
22. Lee SI, Patel M, Jones CM, Narendran P. Cardiovascular disease and type 1 diabetes: Prevalence, prediction and management in an ageing population. *Ther Adv Chronic Dis.* (2015) 6:347–74. doi: 10.1177/2040622315598502
23. Kalofoutis C, Piperi C, Kalofoutis A, Harris F, Phoenix D, Singh J. Type II diabetes mellitus and cardiovascular risk factors: current therapeutic approaches. *Exp Clin Cardiol.* (2007) 12:17−28.
24. Van Linthout S, Frias M, Singh N, De Geest B. Therapeutic potential of HDL in cardioprotection and tissue repair. In: *Handbook of Experimental Pharmacology*. Newyork, NY: Springer New York LLC. pp. 527–65.
25. Castelli WP. Cholesterol and lipids in the risk of coronary artery disease. The Framingham Heart Study. *Can J Cardiol.* (1988) 4:5A−10A.
26. He Y, Kothari V, Bornfeldt KE. High-density lipoprotein function in cardiovascular disease and diabetes mellitus. *Arterioscler Thromb Vasc Biol.* (2018) 38:e10–6. doi: 10.1161/ATVBAHA.117.310222
27. Rye KA, Barter PJ. Thematic review series: high density lipoprotein structure, function, and metabolism cardioprotective functions of HDLs 1. *J Lipid Res.* (2014) 55:168–79. doi: 10.1194/jlr.R039297
28. Annema W, von Eckardstein A, Kovanen PT. HDL and atherothrombotic vascular disease. In: von Eckardstein A, Kardassis D, editors. *High Density Lipoproteins. Handbook of Experimental Pharmacology*. Vol 224. Heidelberg: Springer (2015). p. 371–403. doi: 10.1007/978-3-319-09665-_11
29. Lewis GF, Rader DJ. New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circ Res.* (2005) 96:1221–32. doi: 10.1161/01.RES.0000170946.56981.5c
30. Mineo C, Shaul PW. Novel biological functions of high-density lipoprotein cholesterol. *Circ Res.* (2012) 111:1079–90. doi: 10.1161/CIRCRESAHA.111.258673
31. Kosmas CE, Martinez I, Sourlas A, Bouza K V., Campos FN, Torres V, et al. High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. *Drugs Context.* (2018) 7:212525. doi: 10.7573/dic.212525
32. Cosper PF, Leinwand LA. Cancer causes cardiac atrophy and autophagy in a sexually dimorphic manner. *Cancer Res.* (2011) 71:1710–20. doi: 10.1158/0008-5472.CAN-10-3145
33. Musolino V, Palus S, Tschirner A, Drescher C, Gliozzi M, Carresi C, et al. Megestrol acetate improves cardiac function in a model of cancer cachexia-induced cardiomyopathy by autophagic modulation. *J Cachexia Sarcopenia Muscle.* (2016) 7:555–66. doi: 10.1002/jcsm.12116
34. Manne NDPK, Lima M, Enos RT, Wehner P, Carson JA, Blough E. Altered cardiac muscle mTOR regulation during the progression of cancer cachexia in the ApcMin/+ mouse. *Int J Oncol.* (2013) 42:2134–40. doi: 10.3892/ijo.2013.1893
35. Volkova M, Russell R. Anthracycline cardiotoxicity: prevalence, pathogenesis and treatment. *Curr Cardiol Rev.* (2012) 7:214–20. doi: 10.2174/157340311799960645
36. Arcamone F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C, et al. Adriamycin, 14-hydroxydaimomycin, a new antitumor antibiotic from *S. Peucetius* var. caesius. *Biotechnol Bioeng.* (1969) 11:1101–10. doi: 10.1002/bit.260110607
37. Shakir D. Chemotherapy induced cardiomyopathy: pathogenesis, monitoring and management. *J Clin Med Res.* (2009) 1:8–12. doi: 10.4021/jocmr2009.02.1225
38. Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer.* (2003) 97:2869–79. doi: 10.1002/cncr.11407
39. Nousiainen T, Vanninen E, Rantala A, Jantunen E, Hartikainen J. QT dispersion and late potentials during doxorubicin therapy for non-Hodgkin's lymphoma. *J Intern Med.* (1999) 245:359–64. doi: 10.1046/j.1365-2796.1999.00480.x
40. Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, et al. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. *Circulation.* (2015) 131:1981–8. doi: 10.1161/CIRCULATIONAHA.114.013777
41. Lipshultz SE, Lipsitz SR, Sallan SE, Dalton VM, Mone SM, Gelber RD, et al. Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. *J Clin Oncol.* (2005) 23:2629–36. doi: 10.1200/JCO.2005.12.121
42. Feijen EAM, Leisenring WM, Stratton KL, Ness KK, Van Der Pal HJH, Caron HN, et al. Equivalence ratio for daunorubicin to doxorubicin in relation to late heart failure in survivors of childhood cancer. *J Clin Oncol.* (2015) 33:3774–80. doi: 10.1200/JCO.2015.61.5187
43. Hilfiker-Kleiner D, Ardehali H, Fischmeister R, Burridge P, Hirsch E, Lyon AR. Late onset heart failure after childhood chemotherapy. *Eur Heart J.* (2019) 40:798–800. doi: 10.1093/eurheartj/ehz046
44. Zhao L, Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. *Sci Rep.* (2017) 7:44735. doi: 10.1038/srep44735
45. Yuan Y, Wang W, Wang B, Zhu H, Zhang B, Feng M. Delivery of hydrophilic drug doxorubicin hydrochloride-targeted liver using apoAI as carrier. *J Drug Target.* (2013) 21:367–74. doi: 10.3109/1061186X.2012.757769
46. Zhang YW, Shi J, Li YJ, Wei L. Cardiomyocyte death in doxorubicin-induced cardiotoxicity. *Arch Immunol Ther Exp.* (2009) 57:435–45. doi: 10.1007/s00005-009-0051-8
47. Doroshow JH. Effect of anthracycline antibiotics on oxygen radical formation in rat heart. *Cancer Res.* (1983) 43:460–72.
48. Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med.* (2012) 18:1639–42. doi: 10.1038/nm.2919
49. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologie developments in antitumor activity and cardiotoxicity. *Pharmacol Rev.* (2004) 56:185–29. doi: 10.1124/pr.56.2.6
50. Ichikawa Y, Ghanefar M, Bayeva M, Wu R, Khechaduri A, Naga Prasad S V., et al. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *J Clin Invest.* (2014) 124:617–30. doi: 10.1172/JCI72931
51. Li M, Russo M, Pirozzi F, Tocchetti CG, Ghigo A. Autophagy and cancer therapy cardiotoxicity: from molecular mechanisms to therapeutic opportunities. *Biochim Biophys Acta Mol Cell Res.* (2020) 1867:118493. doi: 10.1016/j.bbamcr.2019.06.007
52. Koleini N, Kardami E. Autophagy and mitophagy in the context of doxorubicin-induced cardiotoxicity. *Oncotarget.* (2017) 8:46663–80. doi: 10.18632/oncotarget.16944
53. Kalyanaraman B. Teaching the basics of the mechanism of doxorubicin-induced cardiotoxicity: have we been barking up the wrong tree? *Redox Biol.* (2020) 29:101394. doi: 10.1016/j.redox.2019.101394
54. Damiani RM, Moura DJ, Viau CM, Caceres RA, Henriques JAP, Saffi J. Pathways of cardiac toxicity: comparison between chemotherapeutic drugs doxorubicin and mitoxantrone. *Arch Toxicol.* (2016) 90:2063–76. doi: 10.1007/s00204-016-1759-y
55. Hahn VS, Lenihan DJ, Ky B. Cancer therapy-induced cardiotoxicity: basic mechanisms and potential cardioprotective therapies. *J Am Heart Assoc.* (2014) 3:e000665. doi: 10.1161/JAHA.113.000665
56. Chen Y, Jungsuwadee P, Vore M, Butterfield DA, St. Clair DK. Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues. *Mol Interv.* (2007) 7:147–56. doi: 10.1124/mi.7.3.6
57. Li T, Singal PK. Adriamycin-induced early changes in myocardial antioxidant enzymes and their modulation by probucol. *Circulation.* (2000) 102:2105–10. doi: 10.1161/01.CIR.102.17.2105
58. Kiyomiya KI, Matsuo S, Kurebe M. Mechanism of specific nuclear transport of adriamycin: the mode of nuclear translocation of adriamycin-proteasome complex. *Cancer Res.* (2001) 61:2467–71.
59. Sobek S, Boege F. DNA topoisomerases in mtDNA maintenance and age. *Exp Gerontol.* (2014) 56:135–41. doi: 10.1016/j.exger.2014.01.009
60. Nitiss KC, Nitiss JL. Twisting and ironing: doxorubicin cardiotoxicity by mitochondrial DNA damage. *Clin Cancer Res.* (2014) 20:4737–9. doi: 10.1158/1078-0432.CCR-14-0821
61. Khiati S, Rosa ID, Sourbier C, Ma X, Rao VA, Neckers LM, et al. Mitochondrial topoisomerase i (Top1mt) is a novel limiting factor of doxorubicin cardiotoxicity. *Clin Cancer Res.* (2014) 20:4873–81. doi: 10.1158/1078-0432.CCR-13-3373
62. Piquereau J, Caffin F, Novotova M, Lemaire C, Veksler V, Garnier A, et al. Mitochondrial dynamics in the adult cardiomyocytes: which roles for a highly specialized cell? *Front Physiol.* (2013) 4:102. doi: 10.3389/fphys.2013.00102

63. Martín-Fernández B, Gredilla R. Mitochondria and oxidative stress in heart aging. *Age.* (2016) 38:225–38. doi: 10.1007/s11357-016-9933-y
64. Zhao Q, Sun Q, Zhou L, Liu K, Jiao K. Complex regulation of mitochondrial function during cardiac development. *J Am Heart Assoc.* (2019) 8:e012731. doi: 10.1161/JAHA.119.012731
65. Gorini S, Angelis A De, Berrino L, Malara N, Rosano G, Ferraro E. Chemotherapeutic drugs and mitochondrial dysfunction: focus on doxorubicin, trastuzumab, and sunitinib. *Oxid Med Cell Longev.* (2018) 2018:7582730. doi: 10.1155/2018/7582730
66. Guven C, Sevgiler Y, Taskin E. Mitochondrial dysfunction associated with doxorubicin. In: Taskin E, Guven C, Sevgiler Y, editors. *Mitochondrial Diseases.* London: IntechOpen (2018). doi: 10.5772/intechopen.80284
67. Lavandero S, Troncoso R, Rothermel BA, Martinet W, Sadoshima J, Hill JA. Cardiovascular autophagy. *Autophagy.* (2013) 9:1455–66. doi: 10.4161/auto.25969
68. Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. *J Pathol.* (2010) 221:3–12. doi: 10.1002/path.2697
69. González A, Hall MN. Nutrient sensing and TOR signaling in yeast and mammals. *EMBO J.* (2017) 36:397–408. doi: 10.15252/embj.201696010
70. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell.* (2017) 168:960–76. doi: 10.1016/j.cell.2017.02.004
71. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, et al. Nutrient-dependent mTORCl association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell.* (2009) 20:1981–91. doi: 10.1091/mbc.e08-12-1248
72. Lu L, Wu W, Yan J, Li X, Yu H, Yu X. Adriamycin-induced autophagic cardiomyocyte death plays a pathogenic role in a rat model of heart failure. *Int J Cardiol.* (2009) 134:82–90. doi: 10.1016/j.ijcard.2008.01.043
73. Li DL, Wang ZV, Ding G, Tan W, Luo X, Criollo A, et al. Doxorubicin blocks cardiomyocyte autophagic flux by inhibiting lysosome acidification. *Circulation.* (2016) 133:1668–87. doi: 10.1161/CIRCULATIONAHA.115.017443
74. Yin J, Guo J, Zhang Q, Cui L, Zhang L, Zhang T, et al. Doxorubicin-induced mitophagy and mitochondrial damage is associated with dysregulation of the PINK1/parkin pathway. *Toxicol Vitr.* (2018) 51:1–10. doi: 10.1016/j.tiv.2018.05.001
75. Li M, Sala V, De Santis MC, Cimino J, Cappello P, Pianca N, et al. Phosphoinositide 3-kinase gamma inhibition protects from anthracycline cardiotoxicity and reduces tumor growth. *Circulation.* (2018) 138:696–711. doi: 10.1161/CIRCULATIONAHA.117.030352
76. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. *Nat Rev Mol Cell Biol.* (2019) 20:175–93. doi: 10.1038/s41580-018-0089-8
77. Cavalcante GC, Schaan AP, Cabral GF, Santana-da-Silva MN, Pinto P, Vidal AF, et al. A cell's fate: an overview of the molecular biology and genetics of apoptosis. *Int J Mol Sci.* (2019) 20:4133. doi: 10.3390/ijms20174133
78. Xia P, Chen J, Liu Y, Fletcher M, Jensen BC, Cheng Z. Doxorubicin induces cardiomyocyte apoptosis and atrophy through cyclin-dependent kinase 2-mediated activation of forkhead box O1. *J Biol Chem.* (2020) 295:4265–76. doi: 10.1074/jbc.RA119.011571
79. Zhuo C, Wang Y, Wang X, Wang Y, Chen Y. Cardioprotection by ischemic postconditioning is abolished in depressed rats: role of Akt and signal transducer and activator of transcription-3. *Mol Cell Biochem.* (2011) 346:39–47. doi: 10.1007/s11010-010-0589-0
80. Willis MS, Bevilacqua A, Pulinilkunnil T, Kienesberger P, Tannu M, Patterson C. The role of ubiquitin ligases in cardiac disease. *J Mol Cell Cardiol.* (2014) 71:43–53. doi: 10.1016/j.yjmcc.2013.11.008
81. Yamamoto Y, Hoshino Y, Ito T, Nariai T, Mohri T, Obana M, et al. Atrogin-1 ubiquitin ligase is upregulated by doxorubicin via p38-MAP kinase in cardiac myocytes. *Cardiovasc Res.* (2008) 79:89–96. doi: 10.1093/cvr/cvn076
82. Sishi BJN, Bester DJ, Wergeland A, Loos B, Jonassen AK, van Rooyen J, et al. Daunorubicin therapy is associated with upregulation of E3 ubiquitin ligases in the heart. *Exp Biol Med.* (2012) 237:219–26. doi: 10.1258/ebm.2011.011106
83. Burchfield JS, Xie M, Hill JA. Pathological ventricular remodeling: mechanisms: part 1 of 2. *Circulation.* (2013) 128:388–400. doi: 10.1161/CIRCULATIONAHA.113.001878
84. Długosz-Danecka M, Gruszka AM, Szmit S, Olszanecka A, Ogórka T, Sobocinski M, et al. Primary cardioprotection reduces mortality in lymphoma patients with increased risk of anthracycline cardiotoxicity, treated by R-CHOP regimen. *Chemotherapy.* (2018) 63:238–45. doi: 10.1159/000492942
85. Seicean S, Seicean A, Plana JC, Budd GT, Marwick TH. Effect of statin therapy on the risk for incident heart failure in patients with breast cancer receiving anthracycline chemotherapy: an observational Clinical Cohort Study. *J Am Coll Cardiol.* (2012) 60:2384–90. doi: 10.1016/j.jacc.2012.07.067
86. Chotenimitkhun R, D'Agostino R, Lawrence JA, Hamilton CA, Jordan JH, Vasu S, et al. Chronic statin administration may attenuate early anthracycline-associated declines in left ventricular ejection function. *Can J Cardiol.* (2015) 31:302–7. doi: 10.1016/j.cjca.2014.11.020
87. Acar Z, Kale A, Turgut M, Demircan S, Durna K, Demir S, et al. Efficiency of atorvastatin in the protection of anthracycline-induced cardiomyopathy. *J Am Coll Cardiol.* (2011) 58:988–9. doi: 10.1016/j.jacc.2011.05.025
88. Kutateladze TG. Translation of the phosphoinositide code by PI effectors. *Nat Chem Biol.* (2010) 6:507–13. doi: 10.1038/nchembio.390
89. *Cancer Drug Manual*. Available online at: http://www.bccancer.bc.ca/health-professionals/clinical-resources/cancer-drug-manual (accessed December 3, 2019).
90. Seifert CF, Nesser ME, Thompson DF. Dexrazoxane in the prevention of doxorubicin-induced cardiotoxicity. *Ann Pharmacother.* (1994) 28:1063–72. doi: 10.1177/106002809402800912
91. Hasinoff BB, Kuschak TI, Yalowich JC, Creighton AM. A QSAR study comparing the cytotoxicity and DNA topoisomerase II inhibitory effects of bisdioxopiperazine analogs of ICRF-187 (dexrazoxane). *Biochem Pharmacol.* (1995) 50:953–8. doi: 10.1016/0006-2952(95)00218-O
92. Deng S, Yan T, Jendrny C, Nemecek A, Vincetic M, Gödtel-Armbrust U, et al. Dexrazoxane may prevent doxorubicin-induced DNA damage via depleting both Topoisomerase II isoforms. *BMC Cancer.* (2014) 14:842. doi: 10.1186/1471-2407-14-842
93. Swain SM, Whaley FS, Gerber MC, Weisberg S, York M, Spicer D, et al. Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. *J Clin Oncol.* (1997) 15:1318–32. doi: 10.1200/JCO.1997.15.4.1318
94. Shaikh F, Dupuis LL, Alexander S, Gupta A, Mertens L, Nathan PC. Cardioprotection and second malignant neoplasms associated with dexrazoxane in children receiving anthracycline chemotherapy: a systematic review and meta-analysis. *J Natl Cancer Inst.* (2016) 108:djv357. doi: 10.1093/jnci/djv357
95. Mahley RW, Innerarity TL, Rall SC, Weisgraber KH. Plasma lipoproteins: apolipoprotein structure and function. *J Lipid Res.* (1984) 25:1277–94.
96. Silverman DI, Ginsburg GS, Pasternak RC. High-density lipoprotein subfractions. *Am J Med.* (1993) 94:636–45. doi: 10.1016/0002-9343(93)90217-D
97. Tso C, Martinic G, Fan WH, Rogers C, Rye KA, Barter PJ. High-density lipoproteins enhance progenitor-mediated endothelium repair in mice. *Arterioscler Thromb Vasc Biol.* (2006) 26:1144–9. doi: 10.1161/01.ATV.0000216600.37436.cf
98. Seetharam D, Mineo C, Gormley AK, Gibson LL, Vongpatanasin W, Chambliss KL, et al. High-density lipoprotein promotes endothelial cell migration and reendothelialization via scavenger receptor-B Type I. *Circ Res.* (2006) 98:63–72. doi: 10.1161/01.RES.0000199272.59432.5b
99. Shah AS, Tan L, Long JL, Davidson WS. Proteomic diversity of high density lipoproteins: our emerging understanding of its importance in lipid transport and beyond. *J Lipid Res.* (2013) 54:2575–85. doi: 10.1194/jlr.R035725
100. Rader DJ. Molecular regulation of HDL metabolism and function: implications for novel therapies. *J Clin Invest.* (2006) 116:3090–100. doi: 10.1172/JCI30163
101. Mei X, Atkinson D. Lipid-free apolipoprotein A-I structure: insights into HDL formation and atherosclerosis development. *Arch Med Res.* (2015) 46:351–60. doi: 10.1016/j.arcmed.2015.05.012
102. Smith JD. Dysfunctional HDL as a diagnostic and therapeutic target. *Arterioscler Thromb Vasc Biol.* (2010) 30:151–5. doi: 10.1161/ATVBAHA.108.179226
103. Ahsan L, Ossoli AF, Freeman L, Vaisman B, Amar MJ, Shamburek RD, et al. Role of lecithin: cholesterol acyltransferase in HDL metabolism

and atherosclerosis. In: Komoda T, editor. *The HDL Handbook: Biological Functions and Clinical Implications.* 2nd ed. Elsevier Inc. (2013). p. 159–94.
104. Thacker SG, Rousset X, Esmail S, Zarzour A, Jin X, Collins HL, et al. Increased plasma cholesterol esterification by LCAT reduces diet-induced atherosclerosis in SR-BI knockout mice. *J Lipid Res.* (2015) 56:1282–95. doi: 10.1194/jlr.M048629
105. Rigotti A, Trigatti B, Babitt J, Penman M, Xu S, Krieger M. Scavenger receptor BI–a cell surface receptor for high density lipoprotein. *Curr Opin Lipidol.* (1997) 8:181–8. doi: 10.1097/00041433-199706000-00009
106. Zhang YZ, Zanotti I, Reilly MP, Glick JM, Rothblat GH, Rader DJ. Overexpression of apolipoprotein A-I promotes reverse transport of cholesterol from macrophages to feces *in vivo*. *Circulation.* (2003) 108:661–3. doi: 10.1161/01.CIR.0000086981.09834.E0
107. McTaggart F, Jones P. Effects of statins on high-density lipoproteins: a potential contribution to cardiovascular benefit. *Cardiovasc Drugs Ther.* (2008) 22:321–38. doi: 10.1007/s10557-008-6113-z
108. Chapman MJ. Are the effects of statins on HDL-cholesterol clinically relevant? *Eur Hear J.* (2004) 6 (Suppl.):58–63. doi: 10.1016/j.ehjsup.2004.04.002
109. Barter P. Options for therapeutic intervention: how effective are the different agents? *Eur Hear J.* (2006) 8 (Suppl.):47–53. doi: 10.1093/eurheartj/sul041
110. Sacks FM, Tonkin AM, Shepherd J, Braunwald E, Cobbe S, Hawkins CM, et al. Effect of pravastatin on coronary disease events in subgroups defined by coronary risk factors: the prospective pravastatin pooling project. *Circulation.* (2000) 102:1893–900. doi: 10.1161/01.CIR.102.16.1893
111. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet.* (1994) 344:1383–9. doi: 10.1016/S0140-6736(94)90566-5
112. Doggrell SA. No cardiovascular benefit with evacetrapib–is this the end of the road for the 'cetrapibs'? *Expert Opin Pharmacother.* (2017) 18:1439–42. doi: 10.1080/14656566.2017.1365838
113. Grabie M, Tai CH, Frishman WH. Is anacetrapib better than its CETP inhibitor counterparts? *Cardiol Rev.* (2019) 27:242–8. doi: 10.1097/CRD.0000000000000245
114. Garg A, Sharma A, Krishnamoorthy P, Garg J, Virmani D, Sharma T, et al. Role of niacin in current clinical practice: a systematic review. *Am J Med.* (2017) 130:173–87. doi: 10.1016/j.amjmed.2016.07.038
115. Batuca JR, Amaral MC, Favas C, Paula FS, Ames PRJ, Papoila AL, et al. Extended-release niacin increases anti-apolipoprotein A-I antibodies that block the antioxidant effect of high-density lipoprotein–cholesterol: the EXPLORE clinical trial. *Br J Clin Pharmacol.* (2017) 83:1002–10. doi: 10.1111/bcp.13198
116. Parhofer KG. Increasing HDL-cholesterol and prevention of atherosclerosis: a critical perspective. *Atheroscler Suppl.* (2015) 18:109–11. doi: 10.1016/j.atherosclerosissup.2015.02.020
117. McCarthy M. Niacin fails to reduce vascular events in large randomised trial. *BMJ.* (2014) 349:2–3. doi: 10.1136/bmj.g4774
118. AIM-HIGH Investigators, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med.* (2011) 365:2255–67. doi: 10.1056/NEJMoa1107579
119. Chyu KY, Shah PK. HDL/ApoA-1 infusion and ApoA-1 gene therapy in atherosclerosis. *Front Pharmacol.* (2015) 6:1–9. doi: 10.3389/fphar.2015.00187
120. Shah PK. Apolipoprotein A-I / HDL infusion therapy for plaque stabilization- regression : a novel therapeutic approach stimulation of reverse cholesterol transport. *Current.* (2007) 13:1031–8. doi: 10.2174/138161207780487520
121. Dunbar RL, Movva R, Bloedon LAT, Duffy D, Norris RB, Navab M, et al. Oral apolipoprotein A-I mimetic D-4F lowers HDL-inflammatory index in high-risk patients: a first-in-human multiple-dose, randomized controlled trial. *Clin Transl Sci.* (2017) 10:455–69. doi: 10.1111/cts.12487
122. Gilham D, Wasiak S, Tsujikawa LM, Halliday C, Norek K, Patel RG, et al. RVX-208, a BET-inhibitor for treating atherosclerotic cardiovascular disease, raises ApoA-I/HDL and represses pathways that contribute to cardiovascular disease. *Atherosclerosis.* (2016) 247:48–57. doi: 10.1016/j.atherosclerosis.2016.01.036
123. Bloedon LAT, Dunbar R, Duffy D, Pinell-Salles P, Norris R, DeGroot BJ, et al. Safety, pharmacokinetics, and pharmacodynamics of oral apoA-I mimetic peptide D-4F in high-risk cardiovascular patients. *J Lipid Res.* (2008) 49:1344–52. doi: 10.1194/jlr.P800003-JLR200
124. Valanti EK, Dalakoura-Karagkouni K, Sanoudou D. Current and emerging reconstituted hdl-apoa-i and hdl-apoe approaches to treat atherosclerosis. *J Pers Med.* (2018) 8:1–12. doi: 10.3390/jpm8040034
125. Gille A, D'Andrea D, Tortorici MA, Hartel G, Wright SD. CSL112 (Apolipoprotein A-I [Human]) enhances cholesterol efflux similarly in healthy individuals and stable atherosclerotic disease patients. *Arterioscler Thromb Vasc Biol.* (2018) 38:953–63. doi: 10.1161/ATVBAHA.118.310538
126. Chenevard R, Hürlimann D, Spieker L, Béchir M, Enseleit F, Hermann M, et al. Reconstituted HDL in acute coronary syndromes. *Cardiovasc Ther.* (2012) 30:e51–7. doi: 10.1111/j.1755-5922.2010.00221.x
127. Tardif J-C, Ballantyne CM, Barter P, Dasseux J-L, Fayad ZA, Guertin M-C, et al. Effects of the high-density lipoprotein mimetic agent CER-001 on coronary atherosclerosis in patients with acute coronary syndromes: a randomized trial. *Eur Heart J.* (2014) 35:3277–86. doi: 10.1093/eurheartj/ehu171
128. Kempen HJ, Gomaraschi M, Simonelli S, Calabresi L, Moerland M, Otvos J, et al. Persistent changes in lipoprotein lipids after a single infusion of ascending doses of MDCO-216 (apoA-IMilano/POPC) in healthy volunteers and stable coronary artery disease patients. *Atherosclerosis.* (2016) 255:17–24. doi: 10.1016/j.atherosclerosis.2016.10.042
129. Berge KG, Canner PL, Hainline A. High-density lipoprotein cholesterol and prognosis after myocardial infarction. *Circulation.* (1982) 66:1176–8. doi: 10.1161/01.CIR.66.6.1176
130. Calabresi L, Rossoni G, Gomaraschi M, Sisto F, Berti F, Franceschini G. High-density lipoproteins protect isolated rat hearts from ischemia-reperfusion injury by reducing cardiac tumor necrosis factor-α content and enhancing prostaglandin release. *Circ Res.* (2003) 92:330–37. doi: 10.1161/01.RES.0000054201.60308.1A
131. Frias MA, Pedretti S, Hacking D, Somers S, Lacerda L, Opie LH, et al. HDL protects against ischemia reperfusion injury by preserving mitochondrial integrity. *Atherosclerosis.* (2013) 228:110–6. doi: 10.1016/j.atherosclerosis.2013.02.003
132. Gordts SC, Muthuramu I, Nefyodova E, Jacobs F, Van Craeyveld E, De Geest B. Beneficial effects of selective HDL-raising gene transfer on survival, cardiac remodelling and cardiac function after myocardial infarction in mice. *Gene Ther.* (2013) 20:1053–61. doi: 10.1038/gt.2013.30
133. Brinck JW, Thomas A, Brulhart-Meynet MC, Lauer E, Frej C, Dahlbäck B, et al. High-density lipoprotein from end-stage renal disease patients exhibits superior cardioprotection and increase in sphingosine-1-phosphate. *Eur J Clin Invest.* (2018) 48:e12866. doi: 10.1111/eci.12866
134. Durham KK, Chathely KM, Trigatti BL. High-density lipoprotein protects cardiomyocytes against necrosis induced by oxygen and glucose deprivation through SR-B1, PI3K, and AKT1 and 2. *Biochem J.* (2018) 475:1253–65. doi: 10.1042/BCJ20170703
135. Durham KK, Kluck G, Mak KC, Deng YD, Trigatti BL. Treatment with apolipoprotein A1 protects mice against doxorubicin-induced cardiotoxicity in a scavenger receptor class B, type I-dependent manner. *Am J Physiol Circ Physiol.* (2019) 316:H1447–57. doi: 10.1152/ajpheart.00432.2018
136. Frias MA, James RW, Gerber-Wicht C, Lang U. Native and reconstituted HDL activate Stat3 in ventricular cardiomyocytes via ERK1/2: Role of sphingosine-1-phosphate. *Cardiovasc Res.* (2009) 82:313–23. doi: 10.1093/cvr/cvp024
137. Durham KK, Chathely KM, Mak KC, Momen A, Thomas CT, Zhao Y-Y, et al. HDL protects against doxorubicin-induced cardiotoxicity in a scavenger receptor class B type 1-, PI3K-, and Akt-dependent manner. *Am J Physiol Circ Physiol.* (2018) 314:H31–44. doi: 10.1152/ajpheart.00521.2016
138. Frias MA, Lang U, Gerber-Wicht C, James RW. Native and reconstituted HDL protect cardiomyocytes from doxorubicin-induced apoptosis. *Cardiovasc Res.* (2010) 85:118–26. doi: 10.1093/cvr/cvp289
139. Taniyama Y, Walsh K. Elevated myocardial Akt signaling ameliorates doxorubicin-induced congestive heart failure and promotes heart growth. *J Mol Cell Cardiol.* (2002) 34:1241–7. doi: 10.1006/jmcc.2002.2068
140. Jacoby JJ, Kalinowski A, Liu MG, Zhang SSM, Gao Q, Chai GX, et al. Cardiomyocyte-restricted knockout of STAT3 results in higher sensitivity to

inflammation, cardiac fibrosis, and heart failure with advanced age. *Proc Natl Acad Sci USA*. (2003) 100:12929–34. doi: 10.1073/pnas.2134694100
141. Kunisada K, Negoro S, Tone E, Funamoto M, Osugi T, Yamada S, et al. Signal transducer and activator of transcription 3 in the heart transduces not only a hypertrophic signal but a protective signal against doxorubicin-induced cardiomyopathy. *Proc Natl Acad Sci USA*. (2000) 97:315–9. doi: 10.1073/pnas.97.1.315
142. Lecour S. Activation of the protective Survivor Activating Factor Enhancement (SAFE) pathway against reperfusion injury: does it go beyond the RISK pathway? *J Mol Cell Cardiol.* (2009) 47:32–40. doi: 10.1016/j.yjmcc.2009.03.019
143. Sussman MA, Völkers M, Fischer K, Bailey B, Cottage CT, Din S, et al. Myocardial AKT: the omnipresent nexus. *Physiol Rev.* (2011) 91:1023–70. doi: 10.1152/physrev.00024.2010
144. Kimura T, Tomura H, Mogi C, Kuwabara A, Damirin A, Ishizuka T, et al. Role of scavenger receptor class B type I and sphingosine 1-phosphate receptors in high density lipoprotein-induced inhibition of adhesion molecule expression in endothelial cells. *J Biol Chem.* (2006) 281:37457–67. doi: 10.1074/jbc.M605823200
145. Ruiz M, Frej C, Holmér A, Guo LJ, Tran S, Dahlbäck B. High-density lipoprotein-associated apolipoprotein M limits endothelial inflammation by delivering sphingosine-1-phosphate to the sphingosine-1-phosphate receptor 1. *Arterioscler Thromb Vasc Biol.* (2017) 37:118–29. doi: 10.1161/ATVBAHA.116.308435
146. Gonzalez L, Qian AS, Tahir U, Yu P, Trigatti BL. Sphingosine-1-phosphate receptor 1, expressed in myeloid cells, slows diet-induced atherosclerosis and protects against macrophage apoptosis in ldlr KO mice. *Int J Mol Sci.* (2017) 18:2721. doi: 10.3390/ijms18122721
147. Al-Jarallah A, Chen X, González L, Trigatti BL. High density lipoprotein stimulated migration of macrophages depends on the scavenger receptor class B, type I, PDZK1 and Akt1 and is blocked by sphingosine 1 phosphate receptor antagonists. *PLoS ONE.* (2014) 9:e106487. doi: 10.1371/journal.pone.0106487
148. Galvani S, Sanson M, Blaho VA, Swendeman SL, Conger H, Dahlbäck B, et al. HDL-bound sphingosine 1-phosphate acts as a biased agonist for the endothelial cell receptor S1P1 to limit vascular inflammation. *Sci Signal.* (2015) 8:ra79. doi: 10.1126/scisignal.aaa2581
149. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* (1996) 271:518–20. doi: 10.1126/science.271.5248.518
150. Galvani S, Hla T. Quality versus quantity: making HDL great again. *Arterioscler Thromb Vasc Biol.* (2017) 37:1018–19. doi: 10.1161/ATVBAHA.117.309441
151. Rubin EM, Ishida BY, Clift SM, Krauss RM. Expression of human apolipoprotein A-I in transgenic mice results in reduced plasma levels of murine apolipoprotein A-I and the appearance of two new high density lipoprotein size subclasses. *Proc Natl Acad Sci USA*. (1991) 88:434–8. doi: 10.1073/pnas.88.2.434
152. Wang B, Yuan Y, Han L, Ye L, Shi X, Feng M. Recombinant lipoproteins reinforce cytotoxicity of doxorubicin to hepatocellular carcinoma. *J Drug Target.* (2014) 22:76–85. doi: 10.3109/1061186X.2013.839687
153. Rui M, Xin Y, Li R, Ge Y, Feng C, Xu X. Targeted biomimetic nanoparticles for synergistic combination chemotherapy of paclitaxel and doxorubicin. *Mol Pharm.* (2017) 14:107–23. doi: 10.1021/acs.molpharmaceut.6b00732
154. Kuai R, Li D, Chen YE, Moon JJ, Schwendeman A. High-density lipoproteins: nature's multifunctional nanoparticles. *ACS Nano.* (2016) 10:3015–41. doi: 10.1021/acsnano.5b07522
155. Ahn J, Lim U, Weinstein SJ, Schatzkin A, Hayes RB, Virtamo J AD. Prediagnostic total and high-density lipoprotein cholesterol and risk of cancer. *Cancer Epidemiol Biomarkers Prev.* (2009) 2814–21. doi: 10.1158/1055-9965.EPI-08-1248
156. Jafri H, Alsheikh-Ali AA, Karas RH. Baseline and on-treatment high-density lipoprotein cholesterol and the risk of cancer in randomized controlled trials of lipid-altering therapy. *J Am Coll Cardiol.* (2010) 55:2846–54. doi: 10.1016/j.jacc.2009.12.069
157. Penson P, Long DL, Howard G, Howard VJ, Jones SR, Martin SS, et al. Associations between cardiovascular disease, cancer, and very low high-density lipoprotein cholesterol in the REasons for Geographical and Racial Differences in Stroke (REGARDS) study. *Cardiovasc Res.* (2019) 115:204–12. doi: 10.1093/cvr/cvy198
158. Sirniö P, Väyrynen JP, Klintrup K, Mäkelä J, Mäkinen MJ, Karttunen TJ, et al. Decreased serum apolipoprotein A1 levels are associated with poor survival and systemic inflammatory response in colorectal cancer. *Sci Rep.* (2017) 7:1–8. doi: 10.1038/s41598-017-05415-9
159. Pirro M, Ricciuti B, Rader DJ, Catapano AL, Sahebkar A, Banach M. High density lipoprotein cholesterol and cancer: marker or causative? *Prog Lipid Res.* (2018) 71:54–69. doi: 10.1016/j.plipres.2018.06.001
160. Pan B, Ren H, Ma Y, Liu D, Yu B, Ji L, et al. High-density lipoprotein of patients with type 2 diabetes mellitus elevates the capability of promoting migration and invasion of breast cancer cells. *Int J Cancer.* (2012) 131:70–82. doi: 10.1002/ijc.26341
161. Furberg AS, Jasienska G, Bjurstam N, Torjesen PA, Emaus A, Lipson SF, et al. Metabolic and hormonal profiles: HDL cholesterol as a plausible biomarker of breast cancer risk. The Norwegian EBBA study. *Cancer Epidemiol Biomarkers Prev.* (2005) 14:33—40.
162. Pan B, Ren H, Lv X, Zhao Y, Yu B, He Y, et al. Hypochlorite-induced oxidative stress elevates the capability of HDL in promoting breast cancer metastasis. *J Transl Med.* (2012) 10:1–3. doi: 10.1186/1479-5876-10-65
163. Besler C, Heinrich K, Rohrer L, Doerries C, Riwanto M, Shih DM, et al. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. *J Clin Invest.* (2011) 121:2693–708. doi: 10.1172/JCI42946
164. Hansel B, Giral P, Nobecourt E, Chantepie S, Bruckert E, Chapman MJ, et al. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. *J Clin Endocrinol Metab.* (2004) 89:4963–71. doi: 10.1210/jc.2004-0305
165. Riwanto M, Rohrer L, Roschitzki B, Besler C, Mocharla P, Mueller M, et al. Altered activation of endothelial anti-and proapoptotic pathways by high-density lipoprotein from patients with coronary artery disease: role of high-density lipoprotein-proteome remodeling. *Circulation.* (2013) 127:891–904. doi: 10.1161/CIRCULATIONAHA.112.108753
166. Navab R, Strumpf D, Bandarchi B, Zhu CQ, Pintilie M, Ramnarine VR, et al. Prognostic gene-expression signature of carcinoma-associated fibroblasts in non-small cell lung cancer. *Proc Natl Acad Sci USA*. (2011) 108:7160–5. doi: 10.1073/pnas.1014506108
167. Zamanian-Daryoush M, Lindner D, Tallant TC, Wang Z, Buffa J, Klipfell E, et al. The cardioprotective protein apolipoprotein a1 promotes potent anti-tumorigenic effects. *J Biol Chem.* (2013) 288:21237–52. doi: 10.1074/jbc.M113.468967
168. Zamanian-Daryoush M, DiDonato JA. Apolipoprotein A-I and cancer. *Front Pharmacol.* (2015) 6:265. doi: 10.3389/fphar.2015.00265
169. Gao F, Vasquez SX, Su F, Roberts S, Shah N, Grijalva V, et al. L-5F, an apolipoprotein A-I mimetic, inhibits tumor angiogenesis by suppressing VEGF/basic FGF signaling pathways. *Integr Biol.* (2011) 3:479–89. doi: 10.1039/c0ib00147c
170. Marinho AT, Lu H, Pereira SA, Monteiro E, Gabra H, Recchi C. Anti-tumorigenic and platinum-sensitizing effects of apolipoprotein A1 and apolipoprotein A1 mimetic peptides in ovarian cancer. *Front Pharmacol.* (2019) 9:1–13. doi: 10.3389/fphar.2018.01524
171. Chattopadhyay A, Yang X, Mukherjee P, Sulaiman D, Fogelman HR, Grijalva V, et al. Treating the intestine with oral ApoA-I mimetic Tg6F reduces tumor burden in mouse models of metastatic lung cancer. *Sci Rep.* (2018) 8:1–12. doi: 10.1038/s41598-018-26755-0
172. Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. *Nat Rev Cancer.* (2003) 3:582–91. doi: 10.1038/nrc1143
173. Cao WM, Murao K, Imachi H, Yu X, Abe H, Yamauchi A, et al. A mutant high-density lipoprotein receptor inhibits proliferation of human breast cancer cells. *Cancer Res.* (2004) 64:1515–21. doi: 10.1158/0008-5472.CAN-03-0675
174. Pussinen PJ, Karten B, Wintersperger A, Reicher H, McLean M, Malle E, et al. The human breast carcinoma cell line HBL-100 acquires exogenous cholesterol from high-density lipoprotein via CLA-1 (CD-36 and LIMPII analogous 1)-mediated selective cholesteryl ester uptake. *Biochem J.* (2000) 349:559–66. doi: 10.1042/bj3490559

175. Danilo C, Gutierrez-Pajares JL, Mainieri MA, Mercier I, Lisanti MP, Frank PG. Scavenger receptor class B type I regulates cellular cholesterol metabolism and cell signaling associated with breast cancer development. *Breast Cancer Res.* (2013) 15:R87. doi: 10.1186/bcr3483
176. Yuan B, Wu C, Wang X, Wang D, Liu H, Guo L, et al. High scavenger receptor class B type I expression is related to tumor aggressiveness and poor prognosis in breast cancer. *Tumor Biol.* (2016) 37:3581–8. doi: 10.1007/s13277-015-4141-4
177. Cedó L, Reddy ST, Mato E, Blanco-Vaca F, Escolà-Gil JC. HDL and LDL: potential new players in breast cancer development. *J Clin Med.* (2019) 8:853. doi: 10.3390/jcm8060853

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REVIEW
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Review on the Role of Epigenetic Modifications in Doxorubicin-Induced Cardiotoxicity

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Use of anthracyclines such as doxorubicin (DOX), for the treatment of cancer, is known to induce cardiotoxicity, begetting numerous evaluations of this adverse effect. This review emphasizes the mechanism of how consideration of DOX-induced cardiotoxicity is important for the development of cardioprotective agents. As DOX is involved in mitochondrial dysfunction, enzymes involved in epigenetic modifications that use mitochondrial metabolite as substrate are most likely to be affected. Therefore, this review article focuses on the fact that epigenetic modifications, namely, DNA methylation, histone modifications, and noncoding RNA expression, contribute to DOX-associated cardiotoxicity. Early interventions needed for patients undergoing chemotherapy, to treat or prevent heart failure, would, overall, improve the survival, and quality of life of cancer patients. These epigenetic modifications can either be used as molecular markers for cancer prognosis or represent molecular targets to attenuate DOX-induced cardiotoxicity in cancer patients.

Keywords: cardiotoxicity, chemotherapy, epigenetics, cancer, doxorubicin

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INTRODUCTION

Cardiotoxicity, in simpler terms, is defined as "toxicity which damages the heart," often during or after chemotherapeutic treatment (1). Treatment options for cancer have been improving significantly in recent years, and the rates of survival in several human cancers have increased significantly with reduced recurrences (2). However, the applicability of these drugs is limited by the risk of cardiotoxicity (1). Doxorubicin (DOX)–induced cardiomyopathy can occur within a few days of its administration or delayed until decades after chemotherapy, thus affecting morbidity, mortality, and quality of life of cancer patients (3–6). However, the mechanism of DOX-induced cardiotoxicity is not fully understood.

Epigenetic modifications, including DNA methylation, histone modifications, and noncoding RNA (ncRNA) expression, play an important role in regulating gene expression and are considered as a hallmark of several human diseases, such as cardiovascular disease [review in Kimball and Vondriska (7)]. In this review, we discuss the mechanism of how aberrant epigenetic modifications contribute to DOX-induced cardiotoxicity and possible alternative therapeutic options that could forestall or prevent chemotherapy-induced cardiotoxicity (8).

CHEMOTHERAPEUTIC-ASSOCIATED CARDIOTOXICITY

In the 1960s, DOX (Adriamycin®), first isolated from *Streptomyces* actinobacteria, was found as one of the first anthracyclines (9), to be used for several cancer treatments, including breast carcinomas, sarcomas, leukemias, non-Hodgkin and Hodgkin lymphoma, and many other cancers (10, 11). At the molecular level, DOX acts to stabilize topoisomerase DNA isomers and therefore blocks DNA replication and transcription (12, 13). It has been reported in several studies over the last 15 years that despite the successful development of small molecules and targeted therapies, anthracycline-based chemotherapy still plays not only prominent anticancer but also overall detrimental roles in many types of cancer treatment (14). Concerning the latter, DOX causes a cumulative, irreversible, and dose-dependent cardiomyopathy that ultimately leads to congestive heart failure (15). Previous studies have demonstrated that cardiotoxicity is a repercussion of dose-dependent administration of DOX, with those exceeding 500 mg/m^2 greatly increasing the risk of congestive heart failure tremendously (16). Understanding the mechanism involved in DOX is important in developing novel preventive measures, and treatment strategies, against DOX-induced cardiotoxicity.

Cardiotoxicity is one of the major adverse effects of chemotherapy, and a leading cause of increased mortality and morbidity, in cancer patients (6, 17). Cardiotoxicity can occur in the early or late stages of the course of the disease and may vary from subclinical myocardial dysfunction to irreversible heart failure or death (18). Documented reports are limited to the mechanism of the appearance of cardiac dysfunction during chemotherapy and the susceptibility of patients to develop cardiotoxicity (1, 19). However, a proposed clinical study demonstrated that among all cancer patients, the overall occurrence of DOX-induced cardiotoxicity was ∼9%, and most cases occurred immediately during the first year after the completion of chemotherapy and have even been noticed after a follow-up of 4 years (20). Complications emerging from chemotherapy-induced cardiotoxicity are potentially life-threatening, further limiting the clinical use of various chemotherapeutic agents (particularly anthracyclines) (8), thus strongly supporting the need for improved cardioprotective agents.

MECHANISMS OF DOX-INDUCED CARDIOTOXICITY

One widely accepted mechanism for DOX-induced cardiotoxicity is the generation of reactive oxygen species (ROS) after DOX treatment in cardiac mitochondria; this occurrence marks as the primary initiating event in the cascade of intracellular modifications (21). In mitochondria, DOX is reduced by NADH dehydrogenase and undergoes redox cycling, generating ROS (22). Elevated levels of ROS result in cellular damage, also known as oxidative stress, which is initiated when the delicate balance between the ROS-generating system and antioxidant measures is disrupted (8). Cardiomyocytes are highly susceptible to oxidative stress, as treatment with DOX reduced the levels of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (23). Cancer patients receiving DOX treatment also undergo immediate systemic oxidative stress, which is due to a decrease in glutathione and total antioxidant capacity of plasma (24).

Production of ROS also affects the DNA, RNA, proteins, and lipids and can also act as secondary signaling molecules in various pathways that are involved in homeostasis, including cell proliferation and cell death (25, 26). Thus, maintenance of a proper level of ROS in the intracellular and extracellular environment is of vital importance. Hence, it could be inferred that oxidative stress could be a leading cause of cellular hypertrophy in the heart (27), due to gene expression alterations (28), cell death activation (29), extracellular matrix transformation (30), ventricular remodeling (29), and calcium transient perturbation (31), all of which could result in the pathophysiological changes that lead to cardiomyopathy and heart failure.

On the other hand, DOX can also disrupt cellular and mitochondrial metabolism, a phenomenon not fully explored. For example, DOX can reduce mitochondrial NADH accumulation and impair oxidative phosphorylation in heart tissues, events associated with reduced glucose uptake (32). Doxorubicin can also induce the opening of mitochondrial permeability transition pore, resulting in the loss of mitochondrial membrane potential, thus explaining DOX-mediated apoptosis in cardiomyocytes. Moreover, DOX can reduce both the protein level and AMPK phosphorylation, thus contributing to stress and metabolic dysfunction (33, 34). More recently, one study found that the noncanonical function of the tumor suppressor p53 is involved in DOX-mediated cardiotoxicity (35). Doxorubicin treatment of *TP53*-depleted mice resulted in left ventricular systolic dysfunction, in association with decreased oxidative metabolism, and reduced mitochondrial volume and DNA transcription. Taken together, induction of oxidative stress and disruption of metabolism in mitochondria are crucial to the development of cardiotoxicity by DOX.

ROLE OF EPIGENETIC ALTERATIONS IN DOX-INDUCED CARDIOTOXICITY

Mitochondrial metabolites constitute a large number of cofactors for several enzymes involved in human biochemical pathways, including epigenetic modifications (36). For example, S-adenosylmethionine (SAM) is the universal substrate for DNA and histone methylation. It is therefore believed that mitochondrial disruption may likely affect cardiomyocyte genomic chromatin (7). Indeed, DNA methylation and histone modifications, as well as non-coding RNA expression, have recently been found to play a role in DOX-induced cardiotoxicity. Furthermore, *in vivo* experiments also demonstrated that rat deficient in methyl donors developed cardiomyopathy

with disrupted mitochondrial alignment in the myocardium (37). This effect was due to the reduced activity of PGC-1α, the master regulator for mitochondrial biogenesis (38). Interestingly, such reduced PGC-1α activity was found to be due to increased acetylation and a decreased methylation of PGC-1α, through downregulation of the histone modifiers, SIRT1 deacetylase, and PRMT1 methyltransferase, thus further supporting the interplay between metabolism and epigenetic modifications (37). The role of DOX in the alteration of gene expression via epigenetic modifications is illustrated in **Figure 1**.

EPIGENETIC MODIFICATION: DNA METHYLATION

DNA methylation is often referred to as the "fifth" DNA based, because of its ubiquitousness in occurring at the 5′ position of cytosine in CpG dinucleotide (39). 5-Methylcytosine is established, maintained, and removed by several enzymes, including DNA methyltransferases (DNMTs) and Tet (the ten-eleven translocation hydroxylases) family protein. DNA methylation at the promoter region of a gene is associated with transcriptional repression by recruitment of transcriptional repressors and histone modifiers (such as histone deacetylases and histone methyltransferase), resulting in a repressive chromatin. The interplay between DNA methylation and histone modifications has been reviewed elsewhere (40, 41) and will not be discussed here.

DNA methyltransferases and Tet require SAM or α-ketoglutarate (α-KG) for the formation of 5-methylcytosine or 5-hydroxymethylcytosine (5hMC), in the process of DNA methylation and demethylation, respectively (42). In particular, the metabolic pathway from mitochondria generates SAM and α-KG; mitochondrial dysfunction associated with chronic DOX therapy may affect epigenetic machinery.

Indeed, in one of the studies, mouse cardiomyoblast H9c2 cells were used to analyze the effect of DOX (21). Together with a decrease in glycolytic activity and basal respiration in DOX-treated cells, dysregulation of mitochondrial DNA transcripts was observed. Importantly downregulation of DNMT1 (a maintenance methyltransferase), accompanied by a decrease in global DNA methylation, was also observed. This effect is in agreement with a previous animal study that global DNA hypomethylation, accompanied by a dysregulated expression of mitochondrial gene products encoded from both nuclear and mitochondrial genome, was observed in the hearts of rats treated with DOX (15). It is also interesting to point out that Ferreira et al. (21) found that pre-exposure of DOX can confer resistance to subsequent exposure of DOX in H9c2 cells, probably due to mitochondrial adaptation. As DNA methylation of the mitochondrial genome is maintained by DNMT1, the only DNMT member that can be translocated into the mitochondria (43, 44), downregulation of DNMT1 by oxidative stress may

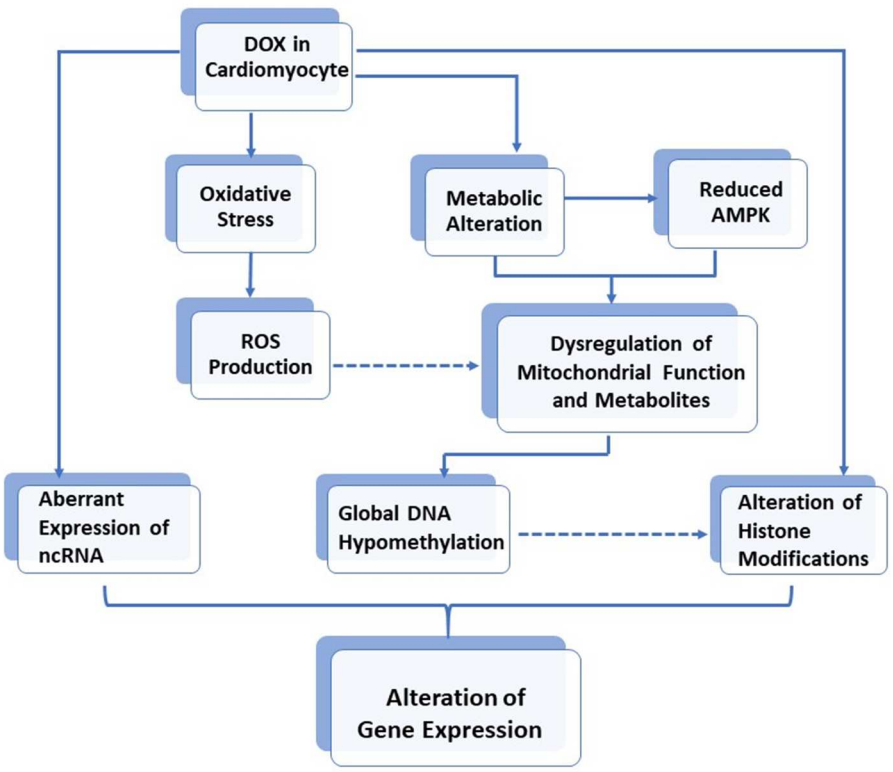

FIGURE 1 | The role of DOX in alteration of gene expressions via epigenetic modifications in cardiomyocytes.

eventually affect the methylation of mitochondrial genome. Taken together, these studies thus suggest that DOX may affect global DNA methylation via dysregulation of mitochondrial function and related metabolites.

Notably, a recent animal study demonstrated that the involvement of mitochondrial genome was not observed, as genes showing significant differential methylation in DOX-treated rats were all encoded from the nucleus. However, a global DNA hypomethylation in the DOX-treated group was still observed (45). This discrepancy may be due to the methods used in these studies. Study from Nordgren et al. (45) utilized a sequencing-based approach Reduced representation bisulfite sequencing (RRBS) that can only interrogate DNA methylation at the CpG rich region, whereas studies from Ferreira et al. used a candidate gene approach to analyze the change of the mitochondrial genome (21). In this regard, further unbiased experiments are required to analyze the role of DOX in the change of the methylome in mitochondria and nucleus.

HISTONE MODIFICATION

Besides DNA methylation, histone modifications are also involved in DOX-induced cardiotoxicity (**Table 1**). These modifications can give rise to synergistic or antagonistic interactions with chromatin-associated protein, resulting in dynamic switching between transcriptionally active (accessible euchromatin) and silent (condensed heterochromatin) states (50). For example, histone deacetylase, HDAC6, was found to be upregulated in DOX-treated primary rat cardiomyocytes, *in vitro*, and mice model, *in vivo*, resulting in deacetylation of α-tubulin (48). The upregulation of other HDACs (**Table 1**) has also been observed in the heart tissue of mice treated with DOX (46). In this regard, Song et al. (48) demonstrated that genetic or pharmacological inhibition of HDAC6 in mice showed a cardioprotective effect against DOX by restoring autophagic flux.

Furthermore, a recent study using H9c2 cardiac myoblast cells also demonstrated that expression of several histone modifiers was dysregulated in association with downregulation of global acetylation of histone H3 (**Table 1**). In this study, Hanf et al. (47) demonstrated that expression levels of histone deacetylases (SIRT1 and HDAC2) were affected upon DOX treatment. In particular, different isoforms of SIRT1 displayed a contradictory expression level. However, pterostilbene, a natural analog of resveratrol and antioxidant, has been found to alleviate DOX-induced cardiotoxicity both *in vitro* and *in vivo* (51). This effect is due to enhanced deacetylation activity of SIRT1, suggesting its cardioprotective effect against DOX. In the case of HDAC2, treatment with low-dose DOX resulted in decreased expression of HDAC2, but no significant changes in high-dose treatment, as compared to control. Consistently, HDAC2 downregulation was observed in the heart tissue of mice treated with DOX (46). As most of the HDACs were found to be upregulated in DOX-treated cardiomyocytes, it is reasonable to observe the cardioprotective effect of HDAC inhibitors on DOX (52). Intriguingly, studies found that trichostatin A, a pan-HDAC inhibitor, can enhance DOX-mediated hypertrophy and apoptosis in H9c2 rat cardiomyoblasts (53, 54). In one of the studies, Ma et al. (54) found that DOX-induced cardiotoxicity is mediated through Rac1, a GTP-binding protein, and subunit of NADPH oxidase, resulting in the suppression of HDAC activity and upregulation of p53. Importantly, this process is ROS-independent. In this regard, treatment of HDAC inhibitor further enhances the effect of DOX-mediated cardiotoxicity. The involvement of specific HDAC isoforms in this process, however, remains to be determined.

TABLE 1 | Changes of histone modifications and modifiers in DOX treated cardiomyocytes.

Modifiers or Modifications	Changes	References
HISTONE DEACETYLASES		
HDAC2[1,2]	Downregulated	(46, 47)
HDAC4[1]	Upregulated	(46)
HDAC5[1]	Upregulated	(46)
HDAC6[1,3]	Upregulated	(46, 48)
HDAC7[1]	Upregulated	(46)
HDAC10[1]	Upregulated	(46)
HDAC11[1]	Upregulated	(46)
SIRT1[2]	Contradictory	(47)
HISTONE LYSINE DEMETHYLASES		
KDM3A[2]	Upregulated	(47)
LSD1[2]	Downregulated[5]	(47)
HISTONE LYSINE METHYLTRANSFERASE		
SET7[2]	Upregulated[6]	(47)
SMYD1[2]	Upregulated[6]	(47)
HISTONE MODIFICATIONS		
H3Ac[2]	Downregulated	(47)
Histone[4]	Loss[7]	(49)
H3K4me3[4]	Downregulated[8]	(49)

Experimental model: *[1]Mice (C57BL/6); [2]H9c2 rat cardiomyocyte; [3]HDAC$^{-/-}$ mice and primary rat cardiomyocyte; [4]mice (unspecified); [5]long term (48 h treatment); [6]high dose and long term (48 h treatment); [7]histone eviction; [8]downregulation of H34me3 and a shift of peak toward the transcription start site.*

Moreover, the histone lysine demethylase, KDM3A, was significantly upregulated upon DOX treatment of H9C2 cells; however, long-term DOX treatment also significantly decreased the lysine-specific histone demethylase 1 (i.e., LSD1). In parallel, significant upregulation of the histone lysine methyltransferases, SET7 and SMYD1, was only observed in long-term and high-dose DOX treatment. Notably, a heart-specific transcriptional alteration was only observed in mice treated with DOX, but not etoposide, a nonanthracycline (49). This event was due to the inhibition of topoisomerase 2β (55), as "eviction" of specific histones from chromatin, resulting in a shift of histone modification (H3K4me3), and chromatin structure, around the promoter region of a gene.

NONCODING RNA EXPRESSION

Another recognized epigenetic modification is the regulation of ncRNAs, including long noncoding RNAs (lncRNAs) and microRNAs (miRNAs). Noncoding RNAs are involved in numerous human biological processes, as well as human

diseases (56). Up to 30% of gene expression in humans is regulated by ∼1,000 known miRNAs, ranging from 18 to 25 nucleotides. MicroRNAs may originate from either independent genes or introns of protein coding genes and are transcribed by RNA polymerase II. Subsequently, these "primary miRNAs" are processed into mature miRNAs and then assembled into argonaute family proteins containing ribonucleoprotein complexes called miRNA-induced silencing complexes. These complexes then bind to their mRNA target sequences in 3′ UTR (untranslated region) of mRNA transcripts, resulting in either translational blockage or mRNA degradation.

Aberrant expression of several miRNAs has been shown involved in DOX-mediated cardiotoxicity (**Table 2**). For example, upregulation of miR-15 was observed in DOX-induced apoptotic H9c2 cardiomyocytes (57). This effect was probably due to suppression of *Bmpr1a*, a target of miR-15 and BMP receptor, previously found to be involved in cardiac contractility (68). Activation of BMP signaling by Bmpr1a agonist is therefore able to rescue DOX-mediated cardiotoxicity in H9c2 cells (57). Similarly, upregulation of miR-23a (58), miR-34a (61, 62), miR-140 (63), miR-146a (64), and miR-532 (66) were observed either *in vitro* or *in vivo* models of DOX-induced cardiotoxicity. Interestingly, upregulation of miR-34a, a well-known tumor suppressive miRNA, could epigenetically suppress *SIRT1* (61, 62), thus partially explaining the downregulation of this HDAC, by DOX, in the aforementioned study (47).

Therapeutically, adenovirus-mediated overexpression of miR-212/132 cluster has been shown to prevent DOX-induced cardiotoxicity in a mouse model (65). This effect may be partially due to direct targeting of *Fitm2*, a transmembrane protein involved in fat storage, by miR-232/132. Moreover, downregulation of miR-29b (59) and miR-30 (60) was also observed in DOX-treated cardiomyocytes in an animal model, leading to de-repression of BAX, a proapoptotic protein, and β-adrenoceptor (β_1- and β_2AR), involved in myocyte contraction, respectively.

On the other hand, lncRNAs, which are more than 200 nucleotides long, regulate gene expression by diverse mechanisms (69). For example, lncRNAs can serve as a scaffold to recruit activators or repressors to regulate gene expression. The molecular function and clinical application of lncRNAs in cardiovascular disease have been recently reviewed (70, 71). Particularly, several studies have provided evidence to demonstrate that lncRNA can directly "sponge" or bind to miRNAs, thus regulating the activity of those miRNAs through a competing endogenous RNA (ceRNA) mechanism (71–74). For example, DOX can upregulate the lncRNA, LINC00339 (**Table 2**), resulting in the suppressing of miR-484 by ceRNA mechanism, in cardiomyocytes *in vitro* and in an animal model (67).

CONCLUSION

Although DOX is still the mainstay anthracyclines (9) for the treatment of several human cancers, a major concern

TABLE 2 | Expression changes of ncRNA in cardiomyocytes treated with DOX.

ncRNA	Changes	Targets	Experimental model	References
miR-15b	Upregulated	Bmpr1a, Gata4, Nkx2-5	H9c2 rat cardiomyocyte	(57)
miR-23a	Upregulated	PGC-1α	Rat (Sprague–Dawley); Primary rat cardiomyocyte	(58)
miR-29b	Downregulated	Bax	Rat (Wistar); Primary rat cardiomyocyte	(59)
miR-30	Downregulated	β_1AR, β_2AR, Giα-2, BNIP3L	Rat (Sprague–Dawley); primary rat cardiomyocyte; H9c2 rat cardiomyocyte	(60)
miR-34a	Upregulated	Bcl-2, SIRT1	Rat (Sprague–Dawley); H9c2 rat cardiomyocyte	(61, 62)
miR-140	Upregulated	Nrf2, SIRT2	Rat (Sprague–Dawley); mice (C57BL/6); H9c2 rat cardiomyocyte	(63)
miR-146a	Upregulated	ErBb4	Mice (C57BL/6); primary rat cardiomyocyte	(64)
miR-212/132	Overexpression[1]	Fitm2, Sgk3, Rbfox1	Mice (C57BL/6N); primary rat cardiomyocyte; human iPSC-derived cardiomyocyte	(65)
miR-532	Upregulated	ARC	Primary rat and mice cardiomyocyte	(66)
LINC00339	Upregulated	miR-484	Rat (Sprague–Dawley); primary rat cardiomyocyte; H9c2 rat cardiomyocyte	(67)

[1]*Adenovirus-mediated overexpression.*

is the side effect of cardiotoxicity. In this review, we have summarized recent findings that epigenetic modifications were observed in cardiomyocytes treated with DOX, both *in vitro* and *in vivo*. Although the causal relationship between cardiotoxicity and epigenetic modifications has not been fully explored, epigenetic modifications may contribute to either a cardiotoxic or cardioprotective process. Whether this process is contributed by DOX-mediated ROS or specific signaling pathways may require further investigation (54). Therapeutically, combinations of chemotherapeutic agents with epigenetic therapies, such as small molecule inhibitor of epigenetic writer/reader/eraser or miRNAs manipulations, may confer protection of patients from cardiotoxicity. However, whether such a potential cardioprotective agent will affect the efficacy of DOX or create other side effects requires further clinical investigation (i.e., the colloquial "double-edged sword"). For example, dexrazoxane, the only Food and Drug Administration–approved cardioprotective agent, has been shown to prevent DOX-mediated cardiotoxicity (75). However, the beneficial effect of dexrazoxane is still debated because of the risk for the development of acute myeloid leukemia and myelodysplastic syndrome in children (76, 77). In conclusion, epigenetic modifications may play a role in DOX-mediated apoptosis and atrophy in cardiomyocytes. Delineation of specific epigenetic therapies as detrimental vs. beneficial cardioprotective merits further investigation.

AUTHOR CONTRIBUTIONS

W-HH and MC performed literature search. HK and MC wrote the manuscript.

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REFERENCES

1. Brana I, Tabernero J. Cardiotoxicity. *Ann Oncol*. (2010) 7:vii173–9. doi: 10.1093/annonc/mdq295
2. Falzone L, Salomone S, Libra M. Evolution of cancer pharmacological treatments at the turn of the third millennium. *Front Pharmacol*. (2018) 9:1300. doi: 10.3389/fphar.2018.01300
3. Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. *J Mol Cell Cardiol*. (2012) 52:1213–25. doi: 10.1016/j.yjmcc.2012.03.006
4. Chatterjee K, Zhang J, Honbo N, Karliner JS. Doxorubicin cardiomyopathy. *Cardiology*. (2010) 115:155–62. doi: 10.1159/000265166
5. Pugazhendhi A, Edison T, Velmurugan BK, Jacob JA, Karuppusamy I. Toxicity of Doxorubicin (Dox) to different experimental organ systems. *Life Sci*. (2018) 200:26–30. doi: 10.1016/j.lfs.2018.03.023
6. Steinherz LJ, Steinherz PG, Tan CT, Heller G, Murphy ML. Cardiac toxicity 4 to 20 years after completing anthracycline therapy. *JAMA*. (1991) 266:1672–7. doi: 10.1001/jama.266.12.1672
7. Kimball TH, Vondriska T. Metabolism epigenetics, and causal inference in heart failure. *Trends Endocrinol Metab*. (2019) 3:181–91.
8. Angsutararux P, Luanpitpong S, Issaragrisil S. Chemotherapy-induced cardiotoxicity: overview of the roles of oxidative stress. *Oxid Med Cell Longev*. (2015) 2015:795602. doi: 10.1155/2015/795602
9. Arcamone F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C, et al. Peucetius var. caesius. Reprinted from Biotechnology and Bioengineering, Vol. XI, 1969, Issue 6, Pages 1101–1110. *Biotechnol Bioeng*. (2000) 67:704–13. doi: 10.1002/(SICI)1097-0290(20000320)67:6<704::AID-BIT8>3.0.CO;2-L
10. Simunek T, Sterba M, Popelova O, Adamcova M, Hrdina R, Gersl V. Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron. *Pharmacol Rep*. (2009) 61:154–71. doi: 10.1016/S1734-1140(09)70018-0
11. Sterba M, Popelova O, Vavrova A, Jirkovsky E, Kovarikova P, Gersl V, et al. Oxidative stress, redox signaling, and metal chelation in anthracycline cardiotoxicity and pharmacological cardioprotection. *Antioxid Redox Signal*. (2013) 18:899–929. doi: 10.1089/ars.2012.4795
12. Tewey KM, Rowe TC, Yang L, Halligan BD, Liu LF. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science*. (1984) 226:466–8. doi: 10.1126/science.6093249
13. Marinello J, Delcuratolo M, Capranico G. Anthracyclines as topoisomerase II poisons: from early studies to new perspectives. *Int J Mol Sci*. (2018) 19: doi: 10.3390/ijms19113480
14. McGowan JV, Chung R, Maulik A, Piotrowska I, Walker JM, Yellon DM. Anthracycline chemotherapy and cardiotoxicity. *Cardiovasc Drugs Ther*. (2017) 31:63–75. doi: 10.1007/s10557-016-6711-0
15. Ferreira A, Cunha-Oliveira T, Simoes RF, Carvalho FS, Burgeiro A, Nordgren K, et al. Altered mitochondrial epigenetics associated with subchronic doxorubicin cardiotoxicity. *Toxicology*. (2017) 390:63–73. doi: 10.1016/j.tox.2017.08.011
16. Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity. *Cancer*. (1973) 32:302–14. doi: 10.1002/1097-0142(197308)32:2<302::AID-CNCR2820320205>3.0.CO;2-2
17. Cardinale D, Colombo A, Lamantia G, Colombo N, Civelli M, De Giacomi G, et al. Anthracycline-induced cardiomyopathy: clinical relevance and response to pharmacologic therapy. *J Am Coll Cardiol*. (2010) 55:213–20. doi: 10.1016/j.jacc.2009.03.095
18. Mercuro G, Cadeddu C, Piras A, Dessi M, Madeddu C, Deidda M, et al. Early epirubicin-induced myocardial dysfunction revealed by serial tissue Doppler echocardiography: correlation with inflammatory and oxidative stress markers. *Oncologist*. (2007) 12:1124–33. doi: 10.1634/theoncologist.12-9-1124
19. Khakoo AY, Liu PP, Force T, Lopez-Berestein G, Jones LW, Schneider J, et al. Cardiotoxicity due to cancer therapy. *Tex Heart Inst J*. (2011) 38:253–6.
20. Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, et al. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. *Circulation*. (2015) 131:1981–8. doi: 10.1161/CIRCULATIONAHA.114.013777
21. Ferreira LL, Cunha-Oliveira T, Veloso CD, Costa CF, Wallace KB, Oliveira PJ. Single nanomolar doxorubicin exposure triggers compensatory mitochondrial responses in H9c2 cardiomyoblasts. *Food Chem Toxicol*. (2019) 124:450–61. doi: 10.1016/j.fct.2018.12.017
22. Doroshow JH, Davies KJ. Redox cycling of anthracyclines by cardiac mitochondria. II. Formation of superoxide anion, hydrogen peroxide, hydroxyl radical. *J Biol Chem*. (1986) 261:3068–74.
23. Babaei-Kouchaki S, Babapour V, Panahi N, Badalzadeh R. Effect of troxerutin on oxidative stress and expression of genes regulating mitochondrial biogenesis in doxorubicin-induced myocardial injury in rats. *Naunyn-Schmiedeb Arch Pharmacol*. (2020) doi: 10.1007/s00210-020-01818-0. [Epub ahead of print].
24. Panis C, Herrera AC, Victorino VJ, Campos FC, Freitas LF, De Rossi T, et al. Oxidative stress and hematological profiles of advanced breast cancer patients

subjected to paclitaxel or doxorubicin chemotherapy. *Breast Cancer Res Treat*. (2012) 133:89–97. doi: 10.1007/s10549-011-1693-x
25. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. *J Toxicol*. (2012) 2012:645460. doi: 10.1155/2012/645460
26. Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. *J Assoc Phys*. (2004) 52:794–804.
27. Sabri A, Hughie HH, Lucchesi PA. Regulation of hypertrophic and apoptotic signaling pathways by reactive oxygen species in cardiac myocytes. *Antioxid Redox Signal*. (2003) 5:731–40. doi: 10.1089/152308603770380034
28. Siwik DA, Tzortzis JD, Pimental DR, Chang DL, Pagano PJ, Singh K, et al. Inhibition of copper-zinc superoxide dismutase induces cell growth, hypertrophic phenotype, and apoptosis in neonatal rat cardiac myocytes *in vitro*. *Circ Res*. (1999) 85:147–53. doi: 10.1161/01.RES.85.2.147
29. Kwon SH, Pimentel DR, Remondino A, Sawyer DB, Colucci WS. H(2)O(2) regulates cardiac myocyte phenotype via concentration-dependent activation of distinct kinase pathways. *J Mol Cell Cardiol*. (2003) 35:615–21. doi: 10.1016/S0022-2828(03)00084-1
30. Siwik DA, Pagano PJ, Colucci WS. Oxidative stress regulates collagen synthesis and matrix metalloproteinase activity in cardiac fibroblasts. *Am J Physiol Cell Physiol*. (2001) 280:C53–60. doi: 10.1152/ajpcell.2001.280.1.C53
31. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertens*. (2000) 18:655–73. doi: 10.1097/00004872-200018060-00002
32. Montaigne D, Marechal X, Baccouch R, Modine T, Preau S, Zannis K, et al. Stabilization of mitochondrial membrane potential prevents doxorubicin-induced cardiotoxicity in isolated rat heart. *Toxicol Appl Pharmacol*. (2010) 244:300–7. doi: 10.1016/j.taap.2010.01.006
33. Gratia S, Kay L, Potenza L, Seffouh A, Novel-Chate V, Schnebelen C, et al. Inhibition of AMPK signalling by doxorubicin: at the crossroads of the cardiac responses to energetic, oxidative, genotoxic stress. *Cardiovasc Res*. (2012) 95:290–9. doi: 10.1093/cvr/cvs134
34. Tokarska-Schlattner M, Zaugg M, da Silva R, Lucchinetti E, Schaub MC, Wallimann T, et al. Acute toxicity of doxorubicin on isolated perfused heart: response of kinases regulating energy supply. *Am J Physiol Heart Circ Physiol*. (2005) 289:H37–47. doi: 10.1152/ajpheart.01057.2004
35. Li J, Wang PY, Long NA, Zhuang J, Springer DA, Zou J, et al. p53 prevents doxorubicin cardiotoxicity independently of its prototypical tumor suppressor activities. *Proc Natl Acad Sci USA*. (2019) 116:19626–34. doi: 10.1073/pnas.1904979116
36. Bahat A, Gross A. Mitochondrial plasticity in cell fate regulation. *J Biol Chem*. (2019) 294:13852–63. doi: 10.1074/jbc.REV118.000828
37. Garcia MM, Gueant-Rodriguez RM, Pooya S, Brachet P, Alberto JM, Jeannesson E, et al. Methyl donor deficiency induces cardiomyopathy through altered methylation/acetylation of PGC-1alpha by PRMT1 and SIRT1. *J Pathol*. (2011) 225:324–35. doi: 10.1002/path.2881
38. Handschin C, Spiegelman BM. Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. *Endocr Rev*. (2006) 27:728–35. doi: 10.1210/er.2006-0037
39. Ehrlich M, Lacey M. DNA methylation and differentiation: silencing, upregulation and modulation of gene expression. *Epigenomics*. (2013) 5:553–68. doi: 10.2217/epi.13.43
40. Flavahan WA, Gaskell E, Bernstein BE. Epigenetic plasticity and the hallmarks of cancer. *Science*. (2017) 357: doi: 10.1126/science.aal2380
41. Baylin SB, Jones PA. Epigenetic determinants of cancer. *Cold Spring Harb Perspect Biol*. (2016) 8: doi: 10.1101/cshperspect.a019505
42. Clare CE, Brassington AH, Kwong WY, Sinclair KD. One-Carbon metabolism: linking nutritional biochemistry to epigenetic programming of long-term development. *Ann Rev Anim Biosci*. (2019) 7:263–87. doi: 10.1146/annurev-animal-020518-115206
43. Shock LS, Thakkar PV, Peterson EJ, Moran RG, Taylor SM. DNA methyltransferase 1, cytosine methylation, and cytosine hydroxymethylation in mammalian mitochondria. *Proc Natl Acad Sci USA*. (2011) 108:3630–5. doi: 10.1073/pnas.1012311108
44. Sharma N, Pasala MS, Prakash A. Mitochondrial DNA: epigenetics and environment. *Environ Mol Mutag*. (2019) 60:668–82. doi: 10.1002/em.22319
45. Nordgren KKS, Hampton M, Wallace KB. Editor's highlight: the altered dna methylome of chronic doxorubicin exposure in sprague dawley rats. *Toxicol Sci*. (2017) 159:470–9. doi: 10.1093/toxsci/kfx150
46. Piotrowska I, Isalan M, Mielcarek M. Early transcriptional alteration of histone deacetylases in a murine model of doxorubicin-induced cardiomyopathy. *PLoS ONE*. (2017) 12:e0180571. doi: 10.1371/journal.pone.0180571
47. Hanf A, Oelze M, Manea A, Li H, Münzel T, Daiber A. The anti-cancer drug doxorubicin induces substantial epigenetic changes in cultured cardiomyocytes. *Chemico-Biol Inter*. (2019) 313:108834. doi: 10.1016/j.cbi.2019.108834
48. Song R, Yang Y, Lei H, Wang G, Huang Y, Xue W, et al. HDAC6 inhibition protects cardiomyocytes against doxorubicin-induced acute damage by improving alpha-tubulin acetylation. *J Mol Cell Cardiol*. (2018) 124:58–69. doi: 10.1016/j.yjmcc.2018.10.007
49. Pang B, Qiao X, Janssen L, Velds A, Groothuis T, Kerkhoven R, et al. Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin. *Nat Commun*. (2013) 4:1908. doi: 10.1038/ncomms2921
50. Al-Hasani K, Mathiyalagan P, El-Osta A. Epigenetics, cardiovascular disease, cellular reprogramming. *J Mol Cell Cardiol*. (2019) 128:129–33. doi: 10.1016/j.yjmcc.2019.01.019
51. Liu D, Ma Z, Xu L, Zhang X, Qiao S, Yuan J. PGC1alpha activation by pterostilbene ameliorates acute doxorubicin cardiotoxicity by reducing oxidative stress via enhancing AMPK and SIRT1 cascades. *Aging*. (2019) 11:10061–73. doi: 10.18632/aging.102418
52. Tarasenko N, Kessler-Icekson G, Boer P, Inbal A, Schlesinger H, Phillips DR, et al. The histone deacetylase inhibitor butyroyloxymethyl diethylphosphate (AN-7) protects normal cells against toxicity of anticancer agents while augmenting their anticancer activity. *Invest New Drugs*. (2012) 30:130–43. doi: 10.1007/s10637-010-9542-z
53. Karagiannis TC, Lin AJ, Ververis K, Chang L, Tang MM, Okabe J, et al. Trichostatin A accentuates doxorubicin-induced hypertrophy in cardiac myocytes. *Aging*. (2010) 2:659–68. doi: 10.18632/aging.100203
54. Ma J, Wang Y, Zheng D, Wei M, Xu H, Peng T. Rac1 signalling mediates doxorubicin-induced cardiotoxicity through both reactive oxygen species-dependent and -independent pathways. *Cardiovasc Res*. (2013) 97:77–87. doi: 10.1093/cvr/cvs309
55. Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med*. (2012) 18:1639–42. doi: 10.1038/nm.2919
56. Dykxhoorn DM, Novina CD, Sharp PA. Killing the messenger: short RNAs that silence gene expression. *Nat Rev Mol Cell Biol*. (2003) 4:457–67. doi: 10.1038/nrm1129
57. Wan GX, Cheng L, Qin HL, Zhang YZ, Wang LY, Zhang YG. MiR-15b-5p is involved in doxorubicin-induced cardiotoxicity via inhibiting Bmpr1a signal in H9c2 cardiomyocyte. *Cardiovasc Toxicol*. (2019) 19:264–75. doi: 10.1007/s12012-018-9495-6
58. Du J, Hang P, Pan Y, Feng B, Zheng Y, Chen T, et al. Inhibition of miR-23a attenuates doxorubicin-induced mitochondria-dependent cardiomyocyte apoptosis by targeting the PGC-1alpha/Drp1 pathway. *Toxicol Appl Pharmacol*. (2019) 369:73–81. doi: 10.1016/j.taap.2019.02.016
59. Jing X, Yang J, Jiang L, Chen J, Wang H. MicroRNA-29b regulates the mitochondria-dependent apoptotic pathway by targeting bax in doxorubicin cardiotoxicity. *Cell Physiol Biochem*. (2018) 48:692–704. doi: 10.1159/000491896
60. Roca-Alonso L, Castellano L, Mills A, Dabrowska AF, Sikkel MB, Pellegrino L, et al. Myocardial MiR-30 downregulation triggered by doxorubicin drives alterations in beta-adrenergic signaling and enhances apoptosis. *Cell Death Dis*. (2015) 6:e1754. doi: 10.1038/cddis.2015.89
61. Piegari E, Russo R, Cappetta D, Esposito G, Urbanek K, Dell'Aversana C, et al. De angelis, microRNA-34a regulates doxorubicin-induced cardiotoxicity in rat. *Oncotarget*. (2016) 7:62312–26. doi: 10.18632/oncotarget.11468
62. Zhu JN, Fu YH, Hu ZQ, Li WY, Tang CM, Fei HW, et al. Activation of miR-34a-5p/Sirt1/p66shc pathway contributes to doxorubicin-induced

cardiotoxicity. *Sci Rep*. (2017) 7:11879. doi: 10.1038/s41598-017-12192-y
63. Zhao L, Qi Y, Xu L, Tao X, Han X, Yin L, et al. MicroRNA-140-5p aggravates doxorubicin-induced cardiotoxicity by promoting myocardial oxidative stress via targeting Nrf2 and Sirt2. *Redox Biol*. (2018) 15:284–96. doi: 10.1016/j.redox.2017.12.013
64. Horie T, Ono K, Nishi H, Nagao K, Kinoshita M, Watanabe S, et al. Acute doxorubicin cardiotoxicity is associated with miR-146a-induced inhibition of the neuregulin-ErbB pathway. *Cardiovasc Res*. (2010) 87:656–64. doi: 10.1093/cvr/cvq148
65. Gupta SK, Garg A, Avramopoulos P, Engelhardt S, Streckfuss-Bomeke K, Batkai S, et al. miR-212/132 cluster modulation prevents doxorubicin-mediated atrophy and cardiotoxicity. *Mol Ther*. (2019) 27:17–28. doi: 10.1016/j.ymthe.2018.11.004
66. Wang JX, Zhang XJ, Feng C, Sun T, Wang K, Wang Y, et al. MicroRNA-532-3p regulates mitochondrial fission through targeting apoptosis repressor with caspase recruitment domain in doxorubicin cardiotoxicity. *Cell Death Dis*. (2015) 6:e1677. doi: 10.1038/cddis.2015.41
67. Li J, Li L, Li X, Wu S. Long noncoding RNA LINC00339 aggravates doxorubicin-induced cardiomyocyte apoptosis by targeting MiR-484. *Biochem Biophys Res Commun*. (2018) 503:3038–3043. doi: 10.1016/j.bbrc.2018.08.090
68. El-Bizri N, Wang L, Merklinger SL, Guignabert C, Desai T, Urashima T, et al. Smooth muscle protein 22alpha-mediated patchy deletion of Bmpr1a impairs cardiac contractility but protects against pulmonary vascular remodeling. *Circ Res*. (2008) 102:380–8. doi: 10.1161/CIRCRESAHA.107.161059
69. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. *Mol Cell*. (2011) 43:904–14. doi: 10.1016/j.molcel.2011.08.018
70. Hobuss L, Bar C, Thum T. Long Non-coding RNAs: At the Heart of Cardiac Dysfunction? *Front Physiol*. (2019) 10:30. doi: 10.3389/fphys.2019.00030
71. Zhou H, Wang B, Yang YX, Jia QJ, Zhang A, Qi ZW, et al. Long noncoding RNAs in pathological cardiac remodeling: a review of the update literature. *Biomed Res Int*. (2019) 2019:7159592. doi: 10.1155/2019/7159592
72. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell*. (2011) 146:353–8. doi: 10.1016/j.cell.2011.07.014
73. Yuan JH, Yang F, Wang F, Ma JZ, Guo YJ, Tao QF, et al. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. *Cancer Cell*. (2014) 25:666–81. doi: 10.1016/j.ccr.2014.03.010
74. Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. *Nature*. (2014) 505:344–52. doi: 10.1038/nature12986
75. Kalam K, Marwick TH. Role of cardioprotective therapy for prevention of cardiotoxicity with chemotherapy: a systematic review and meta-analysis. *Eur J Cancer*. (2013) 49:2900–9. doi: 10.1016/j.ejca.2013.04.030
76. Shaikh F, Dupuis LL, Alexander S, Gupta A, Mertens L, Nathan PC. Cardioprotection and second malignant neoplasms associated with dexrazoxane in children receiving anthracycline chemotherapy: a systematic review and meta-analysis. *J Natl Cancer Inst*. (2016) 108:djv357. doi: 10.1093/jnci/djv357
77. Reichardt P, Tabone MD, Mora J, Morland B, Jones RL. Risk-benefit of dexrazoxane for preventing anthracycline-related cardiotoxicity: re-evaluating the European labeling. *Future Oncol*. (2018) 14:2663–76. doi: 10.2217/fon-2018-0210

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