CARDIO-ONCOLOGY: FROM BENCH TO BEDSIDE

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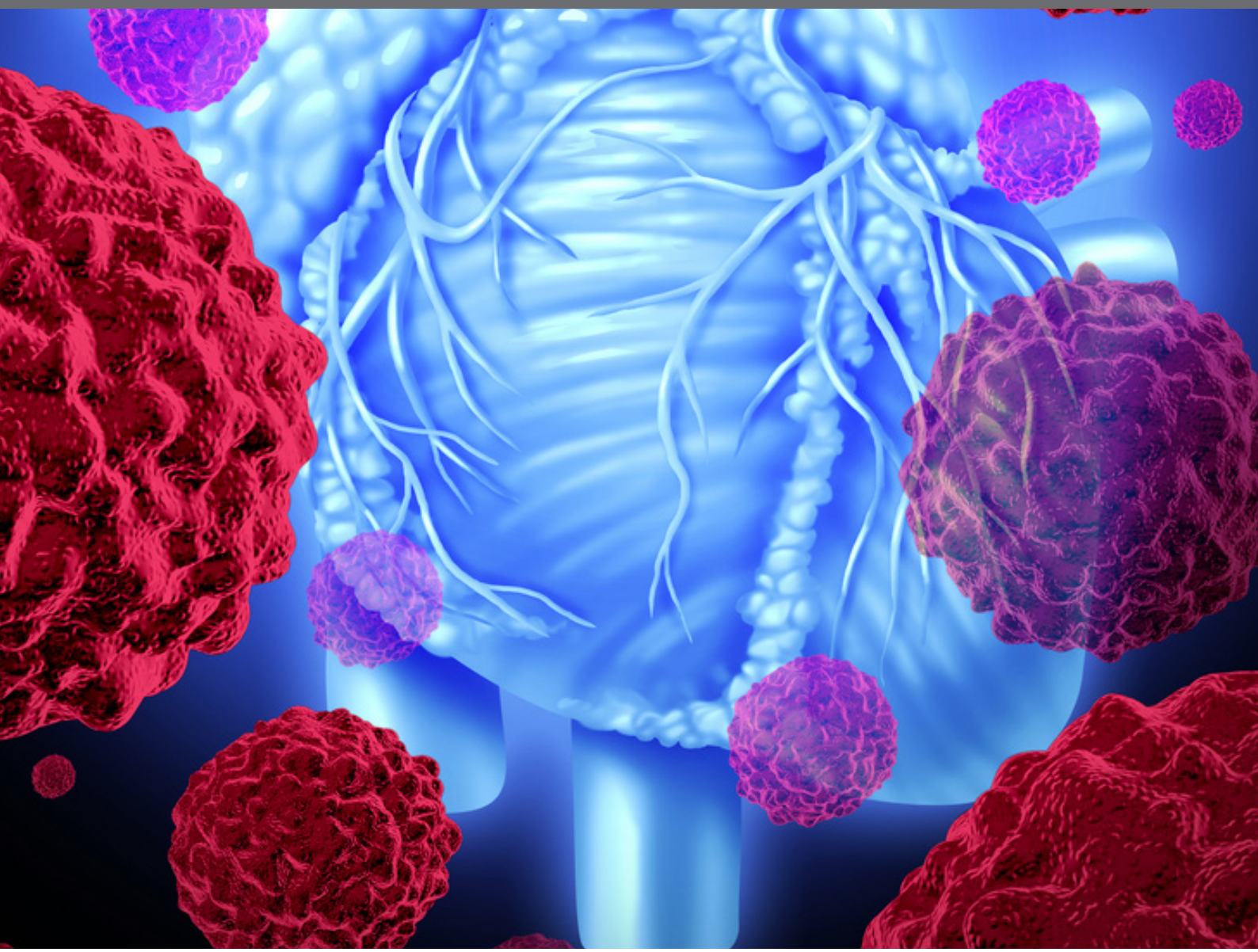

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CARDIO-ONCOLOGY: FROM BENCH TO BEDSIDE

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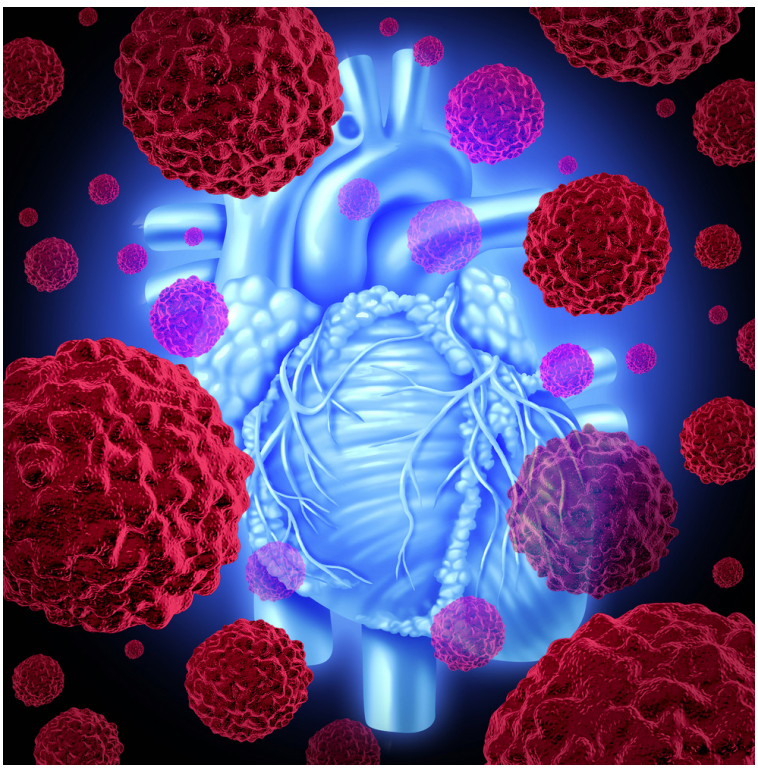

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Table of Contents

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EDITORIAL
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Editorial: Cardio-Oncology: From Bench to Bedside

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Keywords: cardio-oncology, onco-cardiology, cardio-toxicity, cardiovascular damages, anti cancer drugs

Editorial on the Research Topic

Cardio-Oncology: From Bench to Bedside

Although significant progress has been made by many investigators, arguably the field of cardio-oncology is still in early phases. We cannot create a field of cardio-oncology by just putting the cardiology and oncology in one box and mixing it. Only careful observations, patient care, thoughtful research, and enthusiasm can lead this field to the next step. Collaboration between oncologists and cardiologists is important, but the path to creating synergistic collaborations between oncologists and cardiologists has not been fully established. We believe that this is simply due to the lack of information, and additional platforms are needed to accelerate the communication between these disciplines and with other clinical and basic science groups. In this edition, we cover broad aspects related to the current practice and basic research of cardio-oncology.

Karlstaedt et al. proposed that by comparing metabolism in cancer cells and cardiomyocytes, we can obtain novel knowledge and therapeutic approaches for heart failure. Since it is thought that heart is the most "non-oncogenic" organ, this article reveals how important it is to learn cancer biology to fully understand cardiovascular disease.

The five review articles by clinician investigators provide an important message that clinical research based on diligent patient care and careful observations from the bed side form an essential part of cardio-oncology.

Fadol discusses the importance of the management of the cardiovascular complications of cancer therapy, which can significantly change the prognosis of chemotherapy-induced heart failure, and how important it is to generate evidence-based data to guide clinical decision making in the management of the cardiovascular complications of cancer therapy.

The remarkable progress of cancer therapy has led to a substantial increase in the number of childhood cancer survivors, many reaching childbearing age. This positive fact brings us a new and important question: can cardiac dysfunction be increased by pregnancy in women cancer survivors, especially among those treated by anthracyclines? Thompson's review provides an up-to date information about this important question and future goals to clarify this question.

It is well known that there is increased risk of venous and arterial thrombosis in cancer patients. Lee and Cameron discuss mechanism of thrombosis in cancer, and how to treat it. Liu et al. also describe the challenges for treating active coronary artery diseases in cancer patients, and provide strategy and guidelines for applying invasive cardiovascular procedures and intravascular imaging techniques to cancer patients. Lastly, Yusuf et al. have described the pathophysiology of radiation-induced cardiovascular disease. Although modern radiation therapy significantly minimizes the exposure of radiation to the heart, the dose of cardiac exposure to radiation remains high in many cancer patients.

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We include six articles from the basic science side. Additional animal models to study the effect of cancer treatments on the vasculature are needed. Ko et al. have established a reliable mouse model that recapitulates radiation-induced cardiovascular disease in cancer patients. Three articles offer up-to date information regarding modern radiation therapy-induced cardiovascular dysfunction and its possible mechanisms. First, Sylvester et al. summarize the types of radiation currently in clinical use, and current knowledge of the mechanisms by which they cause cardiovascular disease. Second, Menezes et al. have discussed the therapeutic dose of radiation used in modern radiation therapy and its relationship to the cardiovascular disease. Lastly, Vu et al. proposed the potential role of the p90RSK-ERK5 module in regulating radiation-induced endothelial cell apoptosis.

Paez-Mayorga et al. have reported an interesting role of ERK5 SUMOylation, which can explain ponatinib-induced endothelial damage. This is a good example to illustrate the importance of communication between basic science and clinical information to define a new mechanism of cancer therapy-induced cardiovascular disease.

In the review by Dong and Chen the importance to understand cancer treatment toxicity against not only heart but also vasculature is summarized. Because of this, we need to broaden our interests from heart alone to the entire circulatory system as potential targets of cancer therapy-induced cardiovascular disease.

We believe that the topic of cardio-oncology from bench to bedside offers the opportunity for readers to know the challenges and potential future directions of cardio-oncology today, and we hope that more researchers, nurses, paramedical aides, and clinicians will appreciate this important and interesting, but still developing field of cardio-oncology.

AUTHOR CONTRIBUTIONS

JA has prepared the editorial with the consent from JM and AS.

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REVIEW
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Actionable Metabolic Pathways in Heart Failure and Cancer—Lessons From Cancer Cell Metabolism

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Recent advances in cancer cell metabolism provide unprecedented opportunities for a new understanding of heart metabolism and may offer new approaches for the treatment of heart failure. Key questions driving the cancer field to understand how tumor cells reprogram metabolism and to benefit tumorigenesis are also applicable to the heart. Recent experimental and conceptual advances in cancer cell metabolism provide the cardiovascular field with the unique opportunity to target metabolism. This review compares cancer cell metabolism and cardiac metabolism with an emphasis on strategies of cellular adaptation, and how to exploit metabolic changes for therapeutic benefit.

Keywords: cardiac metabolism, cancer cell metabolism, heart failure, clinical trials as topic, targeted treatments, metabolic targets, systems biology, intermediary metabolism

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INTRODUCTION

The intermediary metabolism of substrates defines every living cell, including heart and cancer cells. Metabolism in mammalian cells has four specific functions: (a) to provide chemical energy from nutrients, (b) to convert exogenous nutrients into macromolecules or building blocks of cell components, (c) to assemble building blocks into proteins, nucleic acid, lipids, and other cell components, and (d) to synthesize or degrade biomolecules required in specialized functions of cells (1). Although intermediary metabolism involves a complex network of pathways, the function of metabolism is remarkably similar in living cells. Recent advances in mass-spectrometry-based proteomics, metabolomics and flux analysis facilitate a more precise dissection of the pathways involved in myocardial dysfunction on a molecular level (2–6). Eventually these tools may lead to actionable and individualized therapies for heart failure patients.

Cardiac metabolism is a dynamic process that adapts to stress by altering its activity to maintain cardiac contraction, thereby ensuring cell survival in the near term. Some metabolic responses shift cardiac metabolism toward an energetically unfavorable state, and turn an initial adaptation into maladaptation, which leads to further disease progression (7–9). Precisely how metabolism affects structural remodeling in the heart, how metabolic activities regulate this transformation, and how metabolic changes can be targeted for therapeutic strategies are key questions under investigation. This review compares cancer cell metabolism and cardiac metabolism with an emphasis on strategies of cellular adaptation, and how to exploit metabolic changes for therapeutic benefit. Here, we have only provided a brief overview on common concepts in the metabolism of heart disease and cancer due to the breadth of literature available in both fields. In cancer cells, the principle applies that alteration in metabolic activities supports the acquisition of biosynthetic material and maintenance of cell proliferation. In other words: reprogrammed metabolism is a hallmark of cancer (10–12). Similarly, alterations in cardiac metabolism contribute to disease progression and severity during cardiac hypertrophy, atrophy, and heart failure (8).

Metabolic adaptation in the heart supports contractile function by maintaining ATP provision. While cancer cells optimize metabolic flux to maximize cell proliferation and growth at pathological levels, the heart tries to optimize the use of energy-providing substrates to ensure cardiac contraction and cell survival. Both cancer cells and the heart show a high metabolic turnover to rapidly adapt themselves to changes in the chemical composition of their exogenous nutrients. The question arises: how do cancer cells successfully improve their cellular fitness by coopting metabolic machinery, while the heart fails to do so under long-term stress? Is it time to seriously rethink cardiac metabolism? To address this question, we will discuss modulation of energy substrate metabolism, biosynthesis of macromolecules, and redox balance in both cancer and the heart below. Regardless of whether specific metabolic activities provide benefits or liabilities to cancer cells or the heart, the rationale is that these activities may be exploited as therapeutic targets. For example, pharmacologic inhibition of fatty acid oxidation by agonists of the peroxisome proliferator-activated receptor (PPAR) ligand-activated nuclear hormone receptors decreases myocardial fatty acid oxidation; which, in turn, promotes increased glucose uptake and oxidation, and improves contractile function (13, 14). There is a strong precedent for using pharmacologic modification of metabolic pathways to improve our understanding and treatment heart diseases and cancer. We postulate that the analysis of metabolic patterns common to human cancers and the failing heart may also provide important insights into the relationships between energy substrates, and lead to metabolic targets in the heart.

COMPARING METABOLIC PATHWAYS IN CANCER AND THE HEART

We begin our comparison by considering how cancer cells and cardiomyocytes employ pathways that catalyze the degradation of nutrients and the recovery of part of their chemical energy as ATP. The metabolic control of enzyme-catalyzed reactions is tightly regulated in eukaryotic cells through spatial localization, cooperativity, allosteric interactions, substrate availability, expression, and post-translational modification of enzymes. In certain tumors, impairment of mitochondrial function by somatic mutations of Krebs cycle enzymes (e.g., succinate dehydrogenase and fumarate hydratase) leads to activation of glycolysis even in the presence of oxygen (15). Most of these tumors maintain their ability to provide ATP and thrive on glycolysis and glucose oxidation. This phenomenon has been first described by Otto Warburg, who discovered that ascites cancer cells from mice obtain approximately the same amount of ATP from fermentation as from respiration. In fact, limiting the complete oxidation of glucose by inhibiting pyruvate decarboxylation through modulation of pyruvate dehydrogenase activity in tumors fails to prevent tumorigenesis (16). Recent studies in cancer cell lines showed that a switch toward glycolysis is caused by impaired mitochondrial function and regulated by reductive carboxylation of glutamine (17–20). Upregulation of glycolysis allows cancer cells to satisfy their increased demand for biosynthetic intermediates that can be derived from glucose; hence increased glycolysis enables cell proliferation and growth. In the heart, plasma substrate composition and workload dictate nutrient utilization. Under normal physiologic conditions, the heart predominately oxidizes fatty acids (21, 22). However, this substrate preference can quickly shift toward carbohydrates or ketone bodies based on the availability of substrates, the workload, physical activity or periods of starvation. In fact, experimental studies of acutely stressed hearts (21, 23), ischemia and hypertrophy models of transverse aortic constriction (22, 24–26) reveal that glycolysis and glucose oxidation are preferred over fatty acid oxidation. However, this does not mean that the heart is not utilizing carbohydrates under normal physiologic conditions. Both *ex vivo* and *in silico* studies (21, 23, 27) showed that simultaneous oxidation of long-chain fatty acids and glucose allow most efficient ATP provision in the heart during physiologic workload. Degradation of glucose through glycolysis does not only ensure ATP provision, but also provides intermediates for other important pathways, in particular the pentose phosphate pathway and serine synthesis. In tumors and the heart, the glycolytic intermediate glyceraldehyde 3-phosphate is required for the generation of NADPH in the pentose phosphate pathway via glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and anabolic precursors for pentoses and nucleotide synthesis. Flux through the pentose phosphate pathway is regulated by GAPDH, and thus changes in GAPDH activity may impact redox regulation and synthesis of nucleic acids, as well as aromatic amino acids. Recent studies show that tumors exhibiting the Warburg effect are also characterized by increased GAPDH activity (e.g., non-small lung cancers, colorectal cancers) (28–31). Similarly, during myocardial infarction GAPDH activity increases in the heart and later decreases again during disease progression (32). This change in activity is potentially caused by post-ischemic myocardial reperfusion and may be linked to the production of reactive oxygen species, which have been shown to reduce GAPDH activity in the heart (28, 33). The central role of GAPDH in nucleotide synthesis and generation of reducing equivalents make it critical for survival of cells during stress, and therefore make it a potential target for pharmacologic strategies.

Fatty Acid Metabolism

Fatty acids are important metabolic building blocks for membranes to generate acetyl-CoA for post-translational protein modifications [e.g., histone acetylations; (34)], to provide reducing equivalents in the form of NADH and $FADH_2$, and to provide ATP through β-oxidation. *De novo* fatty acid synthesis includes several key regulatory enzymes: ATP citrate lyase (ACL) which generates acetyl-CoA from citrate; acetyl-CoA carboxylase which catalyzes the irreversible carboxylation of acetyl-CoA from malonyl-CoA, and fatty acid synthase (FASN) which catalyzes the sequential addition of carbon-units to assemble long-chain fatty acids. In most tissues, including the heart, FASN expression and *de novo* fatty acid synthesis is relatively low, indicating that most cells preferentially take up exogenous or dietary lipids from the blood to provide energy and macromolecule biosynthesis.

However, proliferating cells, like several human cancers, have been shown to up-regulate FASN expression (35) and take up free fatty acids to generate phospholipids (36–40). For example, KRAS-driven tumors (e.g., NSCLCs and ovarian cancer) increase fatty acid uptake and oxidation, thereby decreasing the need for *de novo* synthesis. Increased fatty acid oxidation may be driven by increased activation of AMP-activated protein kinase (AMPK) by reduced [ATP]:[AMP] ratio in RAS-mutant cells. Several studies in cancer cell lines and other mammalian tissues showed that ATP and AMP availability regulate the activation of AMPK and the mechanistic target of rapamycin (mTOR), which, in turn, regulate fatty acid metabolism on the molecular level [reviewed by Laplante et al. (41)]. These multiple levels of regulation enable tumors to optimize nutrient utilization and biomass synthesis.

Fatty acid oxidation is a major ATP source for the heart, and depends on cardiac energy demand, oxygen supply and free fatty acid supply from the blood. One of the hallmarks of metabolic perturbations during the development of cardiac hypertrophy and heart failure is decreased use of fatty acids. This metabolic pattern has been observed in both animal and human studies and has been compared to the metabolism in fetal hearts (42–45). Both the fetal and failing heart are characterized by a repression of various genes encoding rate-limiting enzymes of the fatty acid oxidation pathway [e.g., carnithine palmitoyl transferase 1 (CPT1), medium chain acyl-CoA dehydrogenase, and acetyl-CoA carboxylase] (43, 44) and their upstream regulators, including PPARα (46, 47). Downregulation of these genes is not fully understood. However, recent experimental evidence supports the hypothesis that fatty acid oxidation is less efficient (in terms of ATP per oxygen molecule consumed) during mitochondrial dysfunction and limited oxygen availability during ischemic heart disease (48). Therefore, in the short-term this metabolic reprogramming ensures energy provision and cardiac contractile function. In the long-term, reduction of fatty acid oxidation may cause an imbalance between the increased energy demand and simultaneously increased fatty acid availability during heart failure. Several studies have argued that a mismatch between fatty acid uptake and oxidation leads to an accumulation of acetyl-derivatives of CoA and acetyl-CoA as well as carnitine (49), which contributes to cell death and cardiac remodeling (50). In this way, lipotoxicity may contribute to cardiac dysfunction.

Ketone Bodies

In contrast to fatty acid and glucose metabolism, ketone body metabolism has been less investigated in both tumor metabolism and heart failure. Ketone bodies (e.g., acetate, acetoacetate, and beta-hydroxybutyrate) are released by the liver during a wide range of physiologic states; including fasting, starvation, low carbohydrate diets, the neonatal period, post-exercise, pregnancy and diabetes. In extrahepatic tissue (e.g., the heart, brain and skeletal muscle) ketone bodies play an important role for energy provision, post-translational modifications, as signaling mediators, and as modulators of inflammation and oxidative stress. Depending on the tumor type, ketone bodies can either support or diminish cancer cell progression. On the one hand, recent studies showed that ketone bodies support tumor progression and growth in breast cancer and glioblastoma by providing acetyl-CoA for *de novo* lipid synthesis, which is associated with shorter patient survival and increased metastasis of the tumor (51–53). On the other hand, ketogenic diets have been used in animal models and human studies, with potential benefits depending on the tumor location, type and time of diet initiation (54, 55). For example, ketone bodies inhibited growth, proliferation and glycolysis in pancreatic cancer and metastatic glioblastoma cell models and reduced *in vivo* tumor size and attenuated tumor-associated muscle loss (56). Similarly, recent studies indicate that altered cardiac ketone body metabolism contributes to the progression of heart failure (57, 58). These studies provide evidence that ketone body oxidation is increased in the failing heart. However, several questions remain unanswered, including which mechanisms are involved in upregulating ketone body utilization, whether increased use of ketone bodies is adaptive or maladaptive; whether normalization of ketone body oxidation is beneficial or detrimental for the non-failing and failing heart; and whether increased NAD+ levels during ketogenic diets may improve cardiovascular function. Additional work is needed to answer these questions, which will help understanding the role of ketone body metabolism in the pathogenesis of heart failure and evaluating potential risks during cancer treatments.

Amino Acid Metabolism

Compared to fatty acids and glucose, amino acids are not predominately used as substrates for energy provision. In general, amino acids are mostly used to provide substrates for protein synthesis or anaplerosis, and function as signaling molecules. For example, aspartate and leucine levels are sensed by mTOR complex 1 at the lysosomal membrane and promote activation of mTOR signaling (59–61). In cancer cells, the contribution of glutamine metabolism to energy provision and tumor progression has been widely studied (17, 20). Reductive carboxylation of glutamine is a common metabolic strategy, which enables cancer cells with somatic mutations of mitochondrial enzymes to maintain growth (e.g., *de novo* lipid synthesis). Furthermore, reductive glutamine carboxylation allows cancer cells to regenerate NADH and NADPH via malate dehydrogenase and/or isocitrate dehydrogenase. In the heart, amino acids predominately serve as fuels for protein synthesis and contribute only in a limited way to ATP provision. Under normal physiologic conditions, amidation of glutamate to glutamine occurs in the heart (62), but glutamine has only a marginal anaplerotic potential and may play a larger role in posttranslational modifications of proteins (e.g., β-linked N-acetylglucosamine) (63). During myocardial anoxia and ischemia, amino acids are used as anaplerotic substrates in the Krebs cycle (64, 65). The question remains whether glutamine plays a similar role in redox regulation in the heart as has been shown in cancer. Metabolomic analysis of failing mouse hearts and human plasma from heart failure patients showed that amino acid levels were increased (66–68). These changes suggest an association between amino acid levels and the progression of heart failure and have been attributed to increased protein breakdown in skeletal muscle. During heart failure, skeletal muscle serves as an additional amino acid source for the heart (69–71). Moreover, several

studies suggest that amino acid supplementation helps to increase cardiac function in heart failure. For example, branched chain alpha-keto acids are elevated in hearts of heart failure patients, indicating that breakdown of branched chain amino acids (BCAA) is impaired. However, pharmacologic activation of BCAA catabolism and BCAA supplementation increased BCAA oxidation and improved cardiac contractility in heart failure patients and animal models (67, 72, 73). Cachexia is an independent risk factor for mortality during heart failure (74). With this in mind, improving cardiac amino acid oxidation and protein synthesis in skeletal muscle may protect the heart.

BIOSYNTHESIS AND TURNOVER OF MACROMOLECULES

The biosynthesis of macromolecules is an essential aspect of metabolism in all living cells, as it ensures cellular homeostasis and is essential for cell proliferation and growth. Macromolecules are large molecules, commonly created by polymerization of a smaller subunit. In cells, these smaller subunits are nutrients (e.g., glucose, amino acids, fatty acids), which are converted into biosynthetic precursors through key pathways of intermediary metabolism, including glycolysis, the Krebs cycle, phospholipid pathways, and amino acid synthesis. These biosynthetic precursors then form the three most important biopolymers in the cell: proteins, lipids, and nucleic acids.

Protein biosynthesis is highly regulated in every living cell and requires sufficient supply of essential and non-essential amino acids. A complex network of growth factors, transporters, metabolic intermediates, and cofactors regulate activity of the mTOR signaling system, which is central for the activation of protein synthesis. Somatic mutations of either TSC1 or TSC2 genes causes the formation of hamartomas - a discovery that provided the first molecular link between mTOR and tumorigenesis (75). Phosphorylation and inhibition of TSC2 by AKT promotes activation of mTORC1, which is a common feature of oncogenic deregulation in cancer and may result from PTEN deletion, PIK3CA activating mutations or BCR-ABL translocation (76). Proliferating cancer cells further optimize uptake of amino acids and synthesis of non-essential amino acids through transamination of glutamate. For example, excess glutamine can be exported in exchange for leucine or other essential amino acids, which, in turn, ensures mTORC1 activation (77). At the same time, glutamate uptake and glutaminase activity are stimulated by mTORC1. This bidirectional regulation of mTORC1 activity and the glutamine pool further facilitates protein synthesis in cancer cells.

Protein synthesis and degradation in the heart are highly dynamic processes which are regulated by amino acid availability (78–81), regulation of specific mRNAs, oxygen supply and energy demand. Morgan et al. showed in perfused working hearts that the rate of protein synthesis could be increased by 40% when amino acids levels were increased by five-fold from normal plasma levels (80, 81). These early studies also showed that leucine primarily stimulates protein synthesis. However, overall, the net amount of protein synthesis in the adult heart is low compared to proliferating cells like tumors even when considering substantial increases in protein synthesis during physiologic as well as pathologic hypertrophy of the heart. Cardiac hypertrophy is mediated by protein phosphatases and kinases, such as MAPKs, Janus kinases (JAKs), and the PI3K/PDK/Akt pathway (82, 83). mTOR can be activated by the PI3K/PDK/Akt pathway. Chronic upregulation of mTORC1 is associated with increased cardiac hypertrophy (both physiologic and pathologic) (84, 85). Data from human subjects as well as animal studies show that decreased oxygen in the heart increases glutamine uptake and alanine release into the blood (86, 87). These effects are not seen until oxygen supply is decreased to less than 5% of normal oxygen concentrations, indicating that amino acids are necessary to provide protein precursors during stress (87). Cardiac remodeling is associated with increased glutamine deamination (glutaminolysis) (88). Thus, cardiac glutamine metabolism may enable mTORC1-mediated activation of protein synthesis in a way that mirrors cancer cells.

When nutrients are scarce, two main degradative pathways, autophagy and the ubiquitin proteasome system, enable cells to degrade macromolecules and replenish metabolic intermediates. Autophagy allows cells to maintain homeostasis by delivering protein aggregates and damaged organelles to the lysosome for degradation (89, 90). The formation of autophagosomes is controlled by specific yeast Atg-related proteins, which are tightly regulated by intracellular and extracellular nutrient availability and energy homeostasis of the cell (91–96). Autophagy functions as a cellular stress response that can increase the supply of amino acids by scavenging proteins. However, this contribution is unable to change net protein synthesis or increase nitrogen balance. Therefore, upregulation of glutamine uptake increases the cellular glutamate pool which is required for the synthesis of non-essential amino acids, and thus supports protein synthesis. Tumor cells use extracellular proteins as additional nitrogen source through micropinocytosis. Glutamine metabolism in cancer cells is highly dependent on the tumor type, oncogenic drivers, and the tumor microenvironment (97). Hypoxic tumor regions most distant from nutrient supply upregulate autophagy and sustain mitochondrial glutamine metabolism. Several studies have shown that tumor cells rely on extracellular amino acid supply to sustain cell growth (98). Therefore tumors may employ autophagy, or increase extracellular substrate uptake to buffet growth, while non-cancerous cells rely on autophagy alone during times of stress.

In the heart, autophagy recycles organelles and maintains supply of energy providing substrates during periods of reduced extracellular supply (e.g., starvation), oxygen deprivation (e.g., ischemia), or hemodynamic stress (e.g., valvular heart disease or systemic hypertension) (99–107). Tissue-specific deletion of *Atg5* in heart causes cardiac hypertrophy and contractile dysfunction, indicating that autophagy activation under physiologic conditions is required to maintain cardiomyocytes size and cardiac structure and function (108). Upregulation of autophagy in failing hearts is currently considered an adaptive response to protect cells from stress. However, the role of autophagy in regulating amino acid metabolism in the heart remains unknown, and it remains unclear whether prolonged

upregulation of autophagy is beneficial or detrimental in cardiovascular diseases.

De novo fatty acid synthesis is required in mammalian cells for membrane biosynthesis, lipidation reactions, signaling pathways and the formation of lipid rafts. Fatty acid synthesis depends on cytosolic acetyl-CoA levels and reducing equivalents in the form of cytosolic NADPH, which is provided through glycolysis via the pentose phosphate pathway. This link between carbohydrate, redox and fatty acid metabolism has been widely studied. Tumor cells in culture use glucose as a source for acetyl-CoA and fatty acid synthesis (19, 109). Most tumor cells also use glutamine, acetate or leucine degradation to enable lipogenesis when glucose availability is reduced, during hypoxia and when mitochondrial function is impaired (17, 34, 110–112). In contrast, the contribution of *de novo* fatty acid synthesis to the lipid pool in the heart is thought to be minimal under physiologic conditions and mostly limited to the synthesis of acetyl-CoA (113–115). The heart is capable taking up complex lipids through lipoprotein particles delivered by the liver (116, 117). Furthermore, glucose is used for glycerol synthesis and storage of fatty acids in the form of triacylglycerides. On the molecular level, Li et al. (118) showed that mTOR complex 1 directly affects *de novo* lipid synthesis through insulin-dependent activation and phosphorylation of S6 kinase (S6K), which then upregulates the sterol regulatory element binding protein 1 (SREBP1) [reviewed by Laplante et al. (41)]. Several questions remain unanswered regarding the metabolic regulation of (i) phospholipid synthesis/turnover, (ii) storage lipids accumulation/utilization, and (iii) cholesterol homeostasis. Recent advances in lipidomics and nutrient flux analysis will help further our understanding of these interconnected processes.

In addition to proteins and lipids, all living cells also rely on the synthesis of nucleic acids (e.g., RNA and DNA) from purines and pyrimidines. Therefore, it is not surprising that nucleotide analogs and antifolates targeting nucleotide biosynthesis have formed an integral part of cancer chemotherapeutic regimens. *De novo* synthesis of purines and pyrimidines requires non-essential amino acids and methyl groups donated from the one-carbon/folate pool. Aspartate and glutamine are required to synthesize the pyrimidine ring. Precursors for nucleotide synthesis are provided by central metabolic pathways including glycolysis, PPP, the serine-glycine pathways, the Krebs cycle and glutamine amidotransferase reactions. The metabolic energy required to enable nucleotide synthesis is substantial and proliferating cells have developed strategies to optimize flux into pathways providing precursors for nucleotides [reviewed by Lane et al. (119)]. The non-essential amino acid, aspartate, is a critical precursor, and most cells generate aspartate through deamination from glutamine rather than through uptake from the blood (120, 121). For many tumors, the rates of aspartate and folate synthesis limit proliferation and growth (122–124). In the heart, aspartate and other amino acids are preferentially used as anaplerotic substrates to provide ATP during ischemia reperfusion injury and cardiac hypertrophy (86, 87, 125). The purine nucleotide cycle in the heart provides fumarate from aspartate to replenish Krebs cycle intermediates (126). Recent reports indicate that purine and pyrimidine metabolism is potentially regulated by signaling pathways, including mTORC1 pathway (127). Depletion of purines, but not pyrimidines, is associated with mTORC1 inhibition, suggesting that in addition to leucine and aspartate, purines may play a role in regulating mTORC1 activity. Additional work is needed to determine how aspartate metabolism, the purine nucleotide cycle and other aspects of *de novo* nucleotide synthesis are regulated in the diseased heart to support protein synthesis as well as energy provision. In all, nucleic acid synthesis represents a rate-limiting step for the growth of tumors, which divert metabolic substrates to maintain cellular proliferation. Conversely, nucleotide metabolism in the heart helps to fuel the Krebs cycle, as there is currently no experimental evidence that its growth depends on nucleotide availability. However, more research may uncover a connection between growth and nucleotide synthesis in the heart.

TARGETING METABOLISM IN THE FAILING HEART

Despite striking similarities in the metabolism of the failing heart and cancer cells, there are fundamental differences that need to be considered for the development of pharmacologic treatments. Cancer treatments targeting metabolism have the goal to limit or prevent tumor growth and induce cell death. Interventions targeting cardiac metabolism during heart failure aim to reverse structural remodeling and improve cardiac function. Are there therapies that may target metabolism to reverse heart failure? Are there strategies that both protect the heart and target the cancer? Common pharmacologic strategies for both heart failure and cancer are summarized in **Table 1**.

Glucose metabolism is an attractive target for the treatment of cancer and heart failure, because many solid tumors, as well as the failing heart, upregulate glucose utilization (167, 168). Glucose transporter 1 (GLUT1), a uniporter protein that facilitates glucose transport across the plasma membrane in mammalian cells, is a target for treatment of both cancer and heart failure (**Figure 1**). Genetic or pharmacologic inhibition of GLUT1 in lung and breast cancer diminishes tumor growth without systemic toxicity (**Table 1**). In the heart, the opposite approach has been studied. Cardiac-specific overexpression of GLUT1 has been shown to prevent cardiac hypertrophy in a transgenic mouse model subjected to pressure overload by transaortic constriction (169). Intriguingly, inhibition of Sodium glucose co-transporter 2 (SGLT2) in the kidney has proven to be an effective strategy in the treatment of type 2 diabetes with beneficial effects on the heart and at the same time implicated as a potential target in pancreatic and prostate cancers. Thus, depending on the disease progression and tumor type, inhibition of glucose transport may prevent cardiac hypertrophy and reduce cancer growth.

Another potential therapeutic target is Hexokinase II (HK-II; **Figure 1** and **Table 1**), which catalyzes the phosphorylation of glucose to glucose 6-phosphate as the first rate limiting of glycolysis. HK-II binds and inactivates mTORC1 during glucose deprivation, which in turn activates autophagy. Under normal physiologic conditions, mammalian cells predominately

TABLE 1 | Strategies to target metabolic enzymes for treatment of heart failure and cancer.

Pathway	Effect	Compound(s)	Target(s)	Rationale	Cancer field	Cardiovascular field
GLUCOSE OXIDATION						
	Inhibition	WZB117	GLUT1	Inhibition of glucose uptake; limiting nutrient supply	Preclinical data only (128, 129)	
	Inhibition	MK-2206	AKT	Inhibition of the PI3K/Akt signaling pathway and cell proliferation; induction of cell apoptosis	Phase II clinical trials (130, 131)	
	Inhibition	Empagliflozin, Canagliflozin	SGLT2	Inhibition of glucose reabsorption by the kidney; limiting nutrient supply	Preclinical data only (132)	In clinical trials (133–135)
	Inhibition	3-Bromopyruvate, 2-deoxyglucose methyl jasmonate dichloroacetate, clotrimazole and bifonazole, and some traditional Chinese medicinal plants	HK-II	Inhibition of glycolysis to decrease cell growth and survival	Clinical and preclinical data with unacceptable toxicity observed (136)	
	Inhibition	AR-C155858, AZD3965	MCT1, 2 or 4	Inhibition of lactate release, thus promoting increased mitochondrial metabolism; limiting cell growth and survival in cells with upregulated glycolysis and limited mitochondrial metabolism	Clinical and preclinical data (137–140)	
	Activation		GLUT1			Preclinical data only
	Activation	Dichloroacetate	PDH	used for treating lactic acidosis; in clinical trials for the treatment of pulmonary arterial hypertension, metastatic solid tumors and malignant gliomas	Clinical and preclinical data (141–144)	Clinical and preclinical data (45, 142, 145)
	Activation	GLP-1	Glucagon analog	Activation of glucose metabolism	Approved (146–148)	
	Activation		HX-II	Activation of glycolysis to increase glucose metabolism		Preclinical data only (149–151)
FATTY ACID OXIDATION/ LIPID SYNTHESIS						
	Inhibition	Trimetazidine, Ranolazine	3-KAT	Activation of glucose metabolism through inhibition of fatty acid metabolism	Approved in Europe and Asia (152, 153)	
	Inhibition	Etomoxir, Oxfenicine, Perhexiline	CPT1-inhibitor	Activation of glucose metabolism through inhibition of fatty acid transport	In clinical trials (Perhexiline); retired due to hepatotoxicity (etomoxir)	Tested in clinical trials; retired due to hepatotoxicity (Etomoxir); limited clinical trials (Oxfenicine, Perhexiline) (154, 155)
	Inhibition	TVB-2640	FASN	FASN is a rate limiting enzyme in *de novo* lipogenesis;	Clinical and preclinical data (137)	Preclinical data only (156, 157)
	Inhibition		GRK2	GRK2 enhances the ERK cascade and promotes partial inactivation of PPARG and FASN inhibition		Preclinical data only (158)
	Inhibition	ETC-1002; BMS303141; SB 204990	ACL	ACL catalyzes the conversion of citrate to acetyl-CoA, and is important for *de novo* lipogenesis	Preclinical data only (159)	
	Activation	ND-630; ND-646; MK-4074	ACC	ACC catalyzes the irreversible carboxylation of acetyl-CoA to malonyl-CoA; ACC inhibition stimulates FAO	Preclinical data only (160)	Preclinical data only (161, 162)

(Continued)

TABLE 1 | Continued

Pathway	Effect	Compound(s)	Target(s)	Rationale	Cancer field	Cardiovascular field
	Activation	Fenofibrate	PPARα	PPARα agonist with antihyperlipidemic activity by activation of lipoprotein lipase and reduction of the production of apoprotein C-III	Approved (163, 164)	
	Activation	Metformin	ETC complex I	Reduction of plasma levels for insulin and IGF-1; Activation of AMPK and inhibition of mTORC1	Approved in T2DM (165, 166)	
NUCLEIC ACID SYNTHESIS						
	Inhibition	Methotrexate; pemetrexed	DHFR	Inhibition of DHFR resulting in inhibition of purine nucleotide and thymidylate synthesis; immunosuppressant activities	Approved in various cancer (CVD side effects)	
	Inhibition	5-Fluorouracil	TYMS	Converted to active F-UMP; replacing uracil and inhibits RNA processing	Approved in various cancer (CVD side effects)	
	Inhibition	Hydroxyurea	RNR	RNR required to convert ribonucleoside diphosphate into deoxyribonucleoside diphosphates	Approved in leukemia (CVD side effects)	
	Inhibition	Gemcitabine; Fludarabine	RNR; DNA polymerase	Deoxycytidine analogs are onverted to dFdCDP and dFDCTP which compete with dCTP; prevents nucleotide incorporation	Approved in various cancer (CVD side effects)	
	Inhibition		TKTL1; GAPDH	TKTL1 allows non-oxidative ribose synthesis; GAPDH required for oxidative riobose synthesis and NADPH provision	Preclinical data only	Preclinical data only
AMINO ACID METABOLISM						
	Inhibition	Asparaginase	Asparagine availability	Asparaginase hydrolyzes L-aspargine, resulting in inhibition of protein synthesis, cell cyle arrest and apoptosis	Approved in leukemia (CVD side effects)	
	Inhibition	BPTES;CD-839	Glutamine availability	GLS1 inhibition; induces apoptosis, growth arrest and/or autophagy	Preclinical data only	Preclinical data only

ACC, acetyl-CoA carboxylase; ACL, AKT, protein kinase B; ATP citrate lyase; AMPK, AMP-activated protein kinase; CPT1, carnitine palmitoyltransferase 1; DHFR, dihydrofolate reductase; ERK, extracellular signal-regulated kinase; ETC, Electron transport chain, FASN, fatty acid synthase; GAPDH, glucose-6-phosphate dehydrogenase; GLS, glutaminase 1; GLUT1, glucose transporter 1; GRK2, G protein-coupled receptor kinase 2; HK-II, hexokinase 2; IGF-1, Insulin-like growth factor 1; MCT, monocarboxylate transporter; mTOR, mechanistic target of rapamycin; PDH, pyruvate carboxylase complex; PPAR, peroxisome proliferator-activated receptor; RNR, ribonucleotide reductase; SGLT2, Sodium-glucose co-transporter 2; TKTL1, transketolase-like protein 1; TYMS, thymidylate synthase.

express HK-I. Many tumors, including gliomas and NSCLCs, overexpress HK-II making it an attractive metabolic target for pharmacologic inhibitors that disrupt the binding between HK-II and mitochondria. However, HK-II inhibitors showed unacceptable systemic toxicity, e.g., development of cardiac cell death, in clinical and preclinical trials when used at high dosage (170, 171). These observations are supported by heterozygotic HK-II-knockout mouse models, which display increased cardiac susceptibility to ischemia and reperfusion injury, and increased hypertrophy and fibrosis in response to pressure overload (149). In contrast, overexpression of HK II in the heart attenuates cardiac hypertrophy by increasing flux through glycolysis and pentose phosphate pathway (150, 151, 172).

Another potential metabolic target is the PDH complex, which catalyzes the decarboxylation of the glycolytic product pyruvate to acetyl-CoA (**Figure 1**). The transcription factors c-MYC and HIF induce HK II and pyruvate dehydrogenase kinase (PDK) in a subset of lymphoma, which, in turn, decreases PDH activity. Pharmacologic activation of PDC by the PDK inhibitor dichloroacetate is currently in clinical trials for the treatment of pulmonary hypertension, as well as solid metastatic tumors, and gliomas. The rationale behind this strategy is to promote a tighter coupling between glucose uptake and oxidation. In the heart the premise is to increase ATP provision by increasing complete oxidation of glucose; while in cancer cells that are relying on glycolysis due to mitochondrial dysfunction, dichloroacetate potentially decreases tumor growth, and progression.

Fatty acid and mitochondrial metabolism have also emerged as targets for treatment of heart failure and cancer (173). The rationale in cancer treatment is to limit tumor proliferation by inhibiting *de novo* lipogenesis or stimulating fatty acid oxidation. Intriguingly, pharmacologic strategies when targeting fatty acid metabolism have been similar in heart disease and cancer. Modulation of fatty oxidation by selective inhibition of

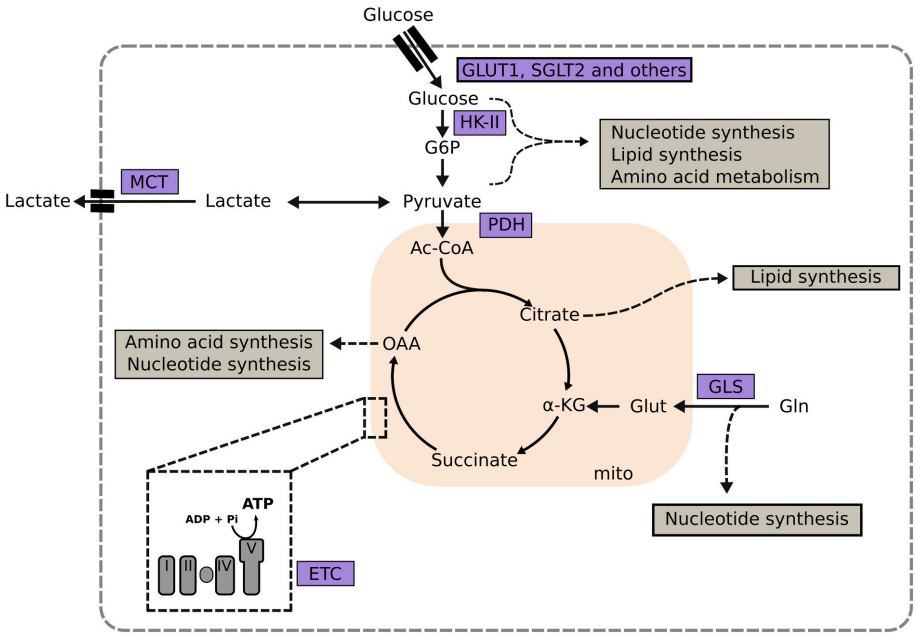

FIGURE 1 | Targeting metabolic enzymes as a strategy in heart failure and cancer. Central metabolic pathways and the involvement of key metabolic enzymes in the synthesis of macromolecules are depicted (shown in gray boxes). α-KG, α-ketoglutarate; G6P, glucose-6-phosphate; GLS, glutaminase; GLUT1, glucose transporter type 1; Glut, glutamate; Gln, glutamine; HK-II, hexokinase II; I, complex I; III, complex III; IV, complex IV; MCT, monocarboxylate transporter; OAA, oxaloacetate; PC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; Succ, succinate V, complex V.

3-ketoacyl coenzyme-A thiolase (3-KAT) and CPT1 have been either approved (e.g., 3-KAT inhibitors like trimetazidin) or tested in clinical trials for both heart failure and cancer (**Table 1**). However, application of CPT-1 inhibitors is limited due to hepatotoxicity and other severe side effects. Other approaches focus on limiting *de novo* lipid synthesis by inhibiting FASN or ATP citrate lyase (ACL). FASN is the rate limiting enzyme for *de novo* lipogenesis, while ACL catalyzes the conversion of glucose-derived citrate to acetyl-CoA and regulates cytosolic acetyl-CoA levels. Similarly, pharmacologic inhibition of GRK2 partially inactivates PPARγ and inhibits FASN through mitogen-activated protein kinases (MAPK) (158). Ongoing clinical trials indicate the efficacy for FASN inhibition in cancer. Similar trials in heart failure have not been successful. Another common metabolic target that may be employed is the electron transport chain (ETC), and specifically metformin, which has been increasingly used as an anti-cancer agent (174–177). By inhibiting ETC complex I (**Figure 1**), metformin decreases mitochondrial ATP provision (120, 178). In cancer cells, this inhibition increases the reliance on glycolysis for ATP provision, and makes cancer cells vulnerable when glucose availability is limited. Additionally, metformin reduces plasma levels of insulin and insulin-like growth factor 1 (IGF-1), which further constricts glucose availability to glycolysis-dependent cancer cells.

A further potential treatment strategy is targeting nucleic acid synthesis and amino acid metabolism. Among the various pharmacologic agents targeting nucleic acid synthesis that are available for cancer therapy, almost all have been reported to have cardiovascular side effects. Glutaminase inhibitors offer a potential way to inhibit mitochondrial amino acid metabolism (**Figure 1**), to induce apoptosis, growth arrest and autophagy. Certain tumors (e.g., NSCLCs and pancreatic tumors) show increased uptake and utilization of glutamine to support macromolecule synthesis and ATP provision (18, 179–181). Prolonged activation of autophagy may be involved with disease progression and decreased cardiac contractility (182, 183); thus, glutaminase inhibitors can reduce tumor burden and potentially improve cardiac function during advanced stages of heart failure.

OUTLOOK AND CONCLUSIONS

We presented several common metabolic strategies that both cancer cells and cardiomyocytes employ to optimize nutrient flux and cell growth. Metabolic reprogramming is a hallmark of both heart failure and malignant cells, which provides them with the ability to survive and sustain stress. Recent progress in molecular techniques (e.g., CRISPR/Cas9) and metabolic flux analysis using stable isotope labeling improved our understanding of mechanisms, biological consequences, and vulnerabilities associated with metabolic reprogramming in heart disease and cancer. Somatic mutations in metabolic reprogramming predominately stems from redirections of metabolic intermediates and increased ATP demand in the context of decreased cardiac contractility. Intermediary metabolites serve as signals that activate signaling pathways, modulate posttranslational modifications of proteins and alter gene expression. Examination of these relationships has inspired pharmacologic strategies that aim to either

correct or enhance metabolic vulnerabilities in cancers and the failing heart. In cancer, potential pharmacologic targets manifest in pathways that regulate energy homeostasis and macromolecule biosynthesis. In the heart, similar strategies are often accompanied by severe side effects and increased cell death. Developing rational therapeutic strategies for both cancer and cardiovascular diseases will be aided by integrating findings on a systems level from pre-clinical and clinical studies. Little is known about the metabolic interaction between tumors and the heart. However, recent studies show that oncometabolic dysregulation can promote cardiac dysfunction (27). Despite the vast metabolic differences and functions of cancer cells and the heart, their common metabolic requirements present opportunities to find intersections for new therapies. Recent experimental and conceptual advances in cancer cell metabolism [reviewed by Vander Heiden et al. (184)] provide the cardiovascular field with the unique opportunity to target metabolism. This strategy holds the potential for new therapies to combat heart failure, as well as chemotherapies that may protect the heart as much as they subvert cancer.

AUTHOR CONTRIBUTIONS

AK drafted and wrote the manuscript. WS edited the manuscript, critically evaluated and searched the literature. HT provided guidance and edited the manuscript.

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PERSPECTIVE
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Management of Chemotherapy-Induced Left Ventricular Dysfunction and Heart Failure in Patients With Cancer While Undergoing Cancer Treatment: The MD Anderson Practice

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Chemotherapy-induced cardiotoxicity resulting in heart failure (HF) is one of the most dreaded complications of cancer therapy that can significantly impact morbidity and mortality. With a high prevalence of cardiovascular disease in cancer patients, the risk of developing HF is significantly increased. A new discipline of Onco-Cardiology has evolved to address the cardiovascular needs of patients with cancer, however, there is limited evidence-based data to guide clinical decision-making in the management of the cardiovascular complications of cancer therapy. The department of cardiology at MD Anderson Cancer Center initiated the MD Anderson Practice (MAP) project and developed algorithms to guide the management of the cardiovascular complications of cancer therapy. For chemotherapy-induced HF, we initiated the Heart Success Program (HSP), a patient-centered program that promotes interdisciplinary collaboration for the management of concurrent HF resulting from chemotherapy-induced cardiotoxicity. After one year of HSP implementation, compliance with the Center for Medicare and Medicaid Services HF core measures has significantly improved. The measurement of LVEF and initiation of recommended pharmacologic therapy for HF (angiotensin converting enzyme inhibitor [ACE-I] or angiotensin receptor blocker for ACE-I intolerant patients) has improved to 100%; provision of discharge instruction has improved from 50 to 94%; and the 30-day hospital readmission rate decreased from 40 to 27%. This article will describe the MD Anderson Practice in the management of chemotherapy-induced cardiomyopathy and HF in cancer patients through the HSP. The novelty of the HSP has raised clinician's awareness of the magnitude of the clinical problem of HF in cancer and the

Keywords: chemotherapy-induced left ventricular dysfunction, heart failure, cancer, patient-centered care, interdisciplinary

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INTRODUCTION

Cardiotoxicity resulting in heart failure (HF) is one of the most dreaded complications of cancer therapy that can significantly impact patient care and clinical outcomes. HF can occur as a consequence of cancer treatment–related cardiotoxicity secondary to chemotherapy (1, 2), radiation therapy (3) and biotherapy (4). A majority of cancer patients are usually older at the time of their cancer diagnosis

and with multiple co-morbidities associated with aging which can increase the complexity of care. Additionally, cancer patients have a high prevalence of baseline cardiovascular risk factors and cardiovascular disease (CVD) prior to initiation of cancer therapy, making them more vulnerable to cardiovascular injuries, which may increase their risk of developing cardiomyopathy leading to HF and death. Management of patients with multiple comorbidities requires multiple providers and specialists which can predispose to the risk of fragmented and inefficient care (5). Over the course of cancer therapy, cancer patients with multiple comorbidities are managed by multidisciplinary teams with a concentrated effort on treating cancer, and the focus on the cardiotoxic effects of cancer treatments may be minimalized which can result in adverse cardiac outcomes and unplanned hospitalizations. A new discipline of Onco-Cardiology or Cardio-Oncology has evolved to address the cardiovascular needs of patients with cancer and optimize their care in a multidisciplinary approach. However, there is limited evidence-based data to guide clinical decision-making in many areas of Onco-Cardiology, although a number of documents attempting to define best practices from national societies is increasing (6–9).Because cancer patients are often excluded from cardiology trials, purely evidence-based data in the management of the cardiac complications of cancer therapy is almost impossible. At MD Anderson, we initiated the MD Anderson Practice (MAP) project to summarize our practice patterns into algorithms based on our cumulative experience from our everyday practice in the management of cancer patients. This article will describe the MD Anderson Practice (MAP) (**Figure 1**) in the management of chemotherapy-induced cardiomyopathy and HF in cancer patients through the Heart Success Program.

THE HEART SUCCESS PROGRAM

The high level of complexity in the care of patients with cancer and concurrent HF requires a comprehensive interdisciplinary approach to provide appropriate clinical management so that patients can continue to receive cancer treatment to improve survival and patient's quality of life. To promote collaboration among cardiologists, oncologists, nurses and other members of the health care team, we developed the Heart Success Program (HSP) (**Figure 2**), a patient-centered, interdisciplinary program to coordinate the management of concurrent cardiomyopathy (CMP) and HF while the patient is receiving cancer treatment. The HSP is based on the principles of disease management to bridge the gap between existing therapeutic options for cardiotoxicity and the realities of clinical practice of the new specialty of Onco-Cardiology. Evidence from meta-analyses of disease management programs have shown that such interventions has a significant impact on survival and hospitalization rates (11–15). The goals of the HSP are to: (1) identify cancer patients whose clinical history and oncologic treatment put them at higher risk for developing cardiomyopathy and HF while receiving cancer treatment; (2) provide timely initiation of recommended pharmacologic therapy for patients who developed chemotherapy-induced HF based on available research data; and (3) actively involve patients in the management of their illness through comprehensive patient education. This interdisciplinary approach fosters open communication and collaborative decision- making to develop the best plan of care for the patient. The HSP exemplifies the Institute of Medicine (IOM) recommendation of "Delivering High-Quality Cancer Care," to address the complex care needs of persons with multiple coexisting diseases, increased side effects from treatment, and greater need for social support (16).

Identification and Monitoring of Patients at Risk for Chemotherapy-Induced cardiomyopathy/Heart Failure

According to the American College of Cardiology and American Heart Association Heart Failure guidelines (17), patients receiving chemotherapy may be considered a stage A heart failure with an increased risk of developing left ventricular dysfunction (LVD). Identification of cancer patients at high risk for chemotherapy-induced cardiotoxicity is one key strategy to reduce morbidity and mortality from cardiovascular toxicity related to cancer therapy. A pre-existing cardiovascular risk factor is itself a strong predictor for the development of cardiovascular injury after chemotherapy, making

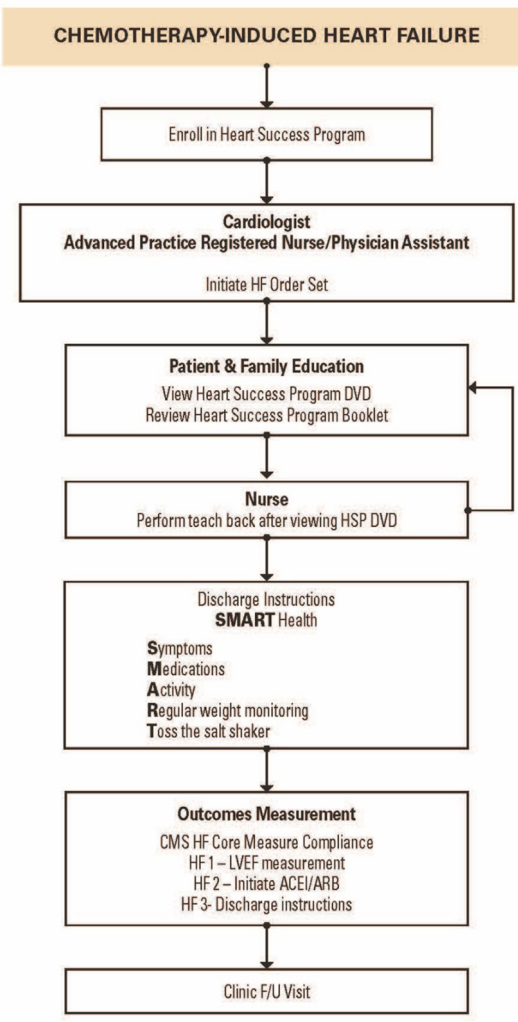

FIGURE 1 | The MD Anderson Practice (MAP) for Management of Chemotherapy-Induced Heart Failure (10).

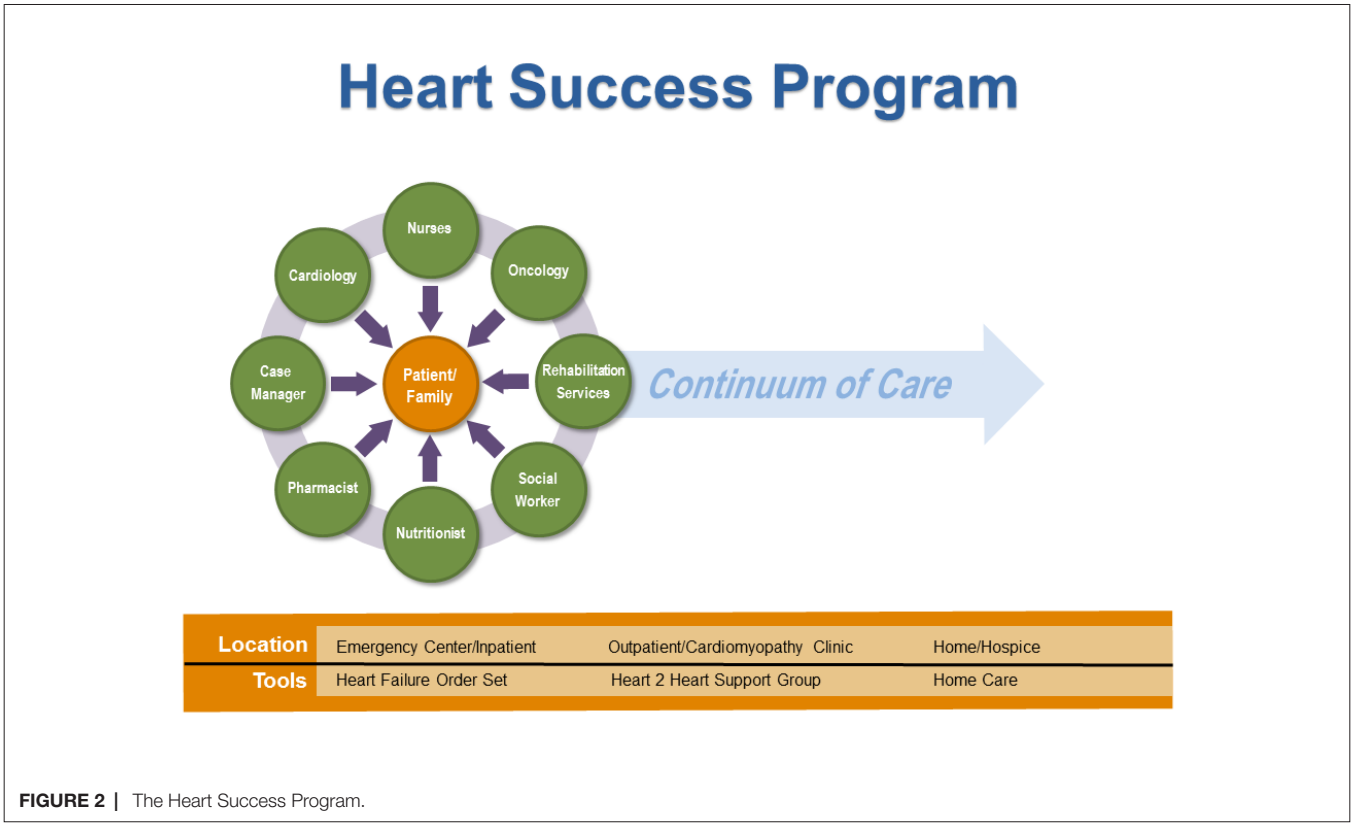

FIGURE 2 | The Heart Success Program.

the likely risk for cardiovascular disease much greater. However, clinical guidelines for screening and monitoring of cardiotoxicity during and after cancer therapies in adults are lacking. Certain professional organizations such as the European Society of Cardiology (ESC) (8) and the American Society of Clinical Oncology (ASCO) (18) have suggested recommendations for prevention and monitoring of cardiac dysfunction in patients with cancer and survivors of adult-onset cancers.

In the HSP, the interdisciplinary team are actively involved with screening patients for cardiac risk factors before receiving cancer treatments, particularly chemotherapeutic agents with potential cardiotoxicity. Patients with cardiac risk factors (e.g., diabetes mellitus, dyslipidemia, hypertension, smoking history, and obesity [BMI >30], etc.), or with history of cardiovascular disease (coronary artery disease (CAD), valvular disease, myocardial infarction, cardiomyopathy) are more vulnerable to cardiovascular injuries and increase their risk of premature cardiovascular death. During the oncologic-active treatment phase, every effort is made to continue and optimize the therapy of underlying cardiovascular diseases, as well as to correct pre-existing and newly acquired cardiovascular risk factors.

The most classic and frequent clinical manifestation of cardiotoxicity is the development of left ventricular dysfunction (LVD). All high risk patients are screened for LVD with an echocardiography with global longitudinal strain measurement. The development of LVD, particularly systolic dysfunction (LVEF <50%) even when asymptomatic, not only negatively affects patients' cardiologic outcome, but it also seriously limits their therapeutic opportunities when adjunctive chemotherapy is required. Moreover, the presence of impaired cardiac function restricts the choice of possible oncologic treatments to those considered less aggressive and, consequently, less effective. If the LVEF is ≤50%, the oncologist formulate the cancer therapeutic options in collaboration with the cardiologist, taking into consideration the patient's cardiac function. Nurses routinely screen patients and ensure that the Onco –Cardiology team are informed of patients who developed chemotherapy-induced CMP/HF, and patients with co-morbidities or CVD risk factors that are not optimally controlled.

Timely Initiation of Pharmacologic Therapy for Heart Failure

Interventions in HF management have become increasingly complicated for patients, in part due to multiple-drug therapy and complex regimens. Guidelines from both European and US cardiology societies do not provide specific recommendations for cancer patients who develop HF after cancer treatment (19, 20). However, small studies have shown that patients with decreased LVEF (≤50%) who are started on standard HF pharmacotherapy, including angiotensin-converting enzyme (ACE) inhibitors and beta blockers have shown improvement in cardiac function (21–24). A prospective study on cancer patients with anthracycline-induced cardiomyopathy demonstrated that the time elapsed from the end of chemotherapy to the start of HF therapy with ACE inhibitors and, when tolerated, with beta-blockers, was a crucial variable for recovery of cardiac dysfunction (23, 25). This finding emphasizes the crucial importance of the early detection of cardiotoxicity and timely

initiation of HF pharmacotherapy, and therefore should always be considered and attempted in all cases of anthracycline –induced cardiomyopathy. The clinical pharmacist work collaboratively with other members of the healthcare team particularly with the initiation and titration of drug regimens based on specific patient characteristics. In addition, the clinical pharmacist is accountable for determining the presence of specific drug-related problems (e.g., drug and/or allergy interactions, medical conditions without drug therapy, drugs without indications, therapeutic duplication, the presence or potential for drug-drug, drug-lab, or drug-food interactions, and the presence or potential for adverse reactions) in all HF patients. Moreover, the clinical pharmacist provides education to other healthcare professionals.

Patient/Family Education

After the patient is confirmed with a HF diagnosis, the inpatient HF order set is activated. The nurses initiate patient and family education and encourage patients to view a 15 min video presentation entitled, "Heart Success for Cancer Patients" (26). This video was developed at MD Anderson specifically for patients with cancer and HF. The video shows important information regarding HF, chemotherapeutic agents with potential cardiotoxicity, HF medications and adverse effects, signs and symptoms of HF exacerbation, and when to seek medical care. Individualized patient and family education are essential components of the Heart Success Program that enables patients to become active "co-managers" of their disease. Patient teaching is reinforced with the "Teach-Back" method, also referred to as "closing the loop", which allows patients to articulate, in their own words, their understanding of what they were taught by providers (27). The "teach back" process enables nurses to identify areas of HF management that patients are deficient and need further explanation. A copy of the patient education booklet "Heart Success: A Resource Guide for Individuals Living with Cancer and Heart Failure" (28) is provided to the patient and family when HF education is initiated. The patient education booklet highlights the essential learning points in the videotape and can be used by patients for reference at home after hospital discharge. Empowering patients requires increasing their comprehension of the disease process and managing their common symptoms such as decreasing salt intake to prevent lower extremity edema and shortness of breath, which can prevent unplanned hospital readmission.

Dietary issues of patients with cancer and HF is a major challenge. The oncology patient often have gastrointestinal symptoms related to cancer and adverse effects of cancer treatment which can have a major impact on the patient's nutritional status. The nutritionist has a major role in assisting patients with food choices to meet their nutritional needs, without compromising taste particularly with low sodium diet for patients with HF.

Prior to discharge, the nurses review with patients the essential information to guide them with their care at home using the "Heart SMART" guide. SMART is an acronym developed to help patients easily recall essential information related to symptoms, medications, activity, regular weight monitoring, and toss the salt shaker (for low sodium diet). Patients are also provided with instructions on what to do if symptoms worsen (20). Comprehensive discharge planning and post-discharge support for patients with heart failure has been shown to significantly reduce hospital readmission rates (12).

Successful transition to home after hospital discharge is critical to prevent unplanned hospital readmission. The case manager and social worker work collaboratively to facilitate the process, which includes assessment, planning and facilitating options and services to meet the individual's cardiac and oncology needs. Timely planning and intervention regarding available resources prevents unnecessary confusion and decreases stress for patients and families. The case manager also acts as a link between the hospital and the agencies when referrals are initiated. Participation in weekly HSP rounds allows discussions between the multidisciplinary team regarding interventions, and therefore promotes continuity of care.

Outcomes Measurement

Although specialty hospitals such as MD Anderson are currently exempt from public reporting of HF core measures as required by the Center for Medicare and Medicaid Services (CMS), we monitored the core measures with the HSP implementation which includes: (1) measurement of left ventricular ejection fraction (LVEF); (2) initiation of ACE-I or an angiotensin-receptor blocker for patients with LVD; and (3) discharge instructions. After one year of implementing the HSP in a medical telemetry unit, we reviewed the database to evaluate our compliance with the CMS core measures for HF. Our compliance with measurement of LVEF and initiation of ACE-I/ARB was 100%. The provision of discharge instruction has improved from 50 to 94% since the implementation of the HSP. The 30 day hospital readmission rate has also decreased from 40 to 27% since the implementation of the HSP in 2012. Using continuous improvement methodology, the team refined the tools based on the experience and lessons learned and disseminate the HSP to the other areas of the hospital, one clinical area at a time. Currently, the HSP is disseminated throughout MD Anderson. The HSP is a quality improvement (QI) initiative developed to promote higher standards in the management of cancer patients.

CONCLUSION

The growing awareness about cardiovascular side effects of anticancer drugs, and the increasing number of cancer survivors entails a host of novel challenges. Cardiovascular safety represents an emerging problem for patients with cancer and cancer survivors. The prevalence of cancer treatment–related cardiovascular disease is increasing, and its management demands a multidisciplinary approach from cardiologists, oncologists and the interdisciplinary team involved in the management of these patients. It is no longer sufficient to focus exclusively on the cancer diagnosis and associated cancer treatments. Providers must enlarge their focus to include pre-existing chronic illnesses as well as cancer treatment–related illness and disability. The novelty of the Heart Success Program has raised clinician's awareness of the magnitude of the clinical problem of heart failure in cancer and the importance of interdisciplinary

collaboration to improve clinical outcomes. The HSP provides a model for engaging patients and family members as partners with a shared goal of reducing the burden of HF in people with cancer.

AUTHOR CONTRIBUTIONS

AF is responsible for the conception of the project, drafting, revising and final approval of the manuscript prior to submission.

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MINI REVIEW
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Pregnancy and Cardiomyopathy After Anthracyclines in Childhood

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With advances in cancer therapy, there has been a remarkable increase in survival in children diagnosed with malignancies. Many of these children are treated with anthracyclines which are well known to cause cardiotoxicity. As more childhood cancer survivors reach childbearing age, many will choose to become pregnant. At this time, the factors associated with development of cardiomyopathy after anthracycline treatment are not clearly identified. It is possible that cardiac stress could predispose to cardiac deterioration in a patient with reduced functional reserve from prior anthracycline exposure. Pregnancy is one form of cardiovascular stress. The cardiac outcomes of pregnancy in childhood cancer survivors must be considered. In view of limited data, guidelines for pregnancy planning, management, and monitoring after cardiotoxic cancer therapy have not been established. This review summarizes the limited data available on the topic of pregnancy after anthracyclines in childhood.

Keywords: cardiotoxicity, pregnancy, anthracyclines, childhood, survivor

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INTRODUCTION

Anthracyclines are effective antineoplastic agents that have contributed to the remarkable improvement in survival in children diagnosed with malignancy. Approximately 60% of childhood cancers are treated with anthracyclines. Unfortunately, these drugs are also well known to cause cardiotoxicity (1). The risk of cardiotoxicity is known to increase with higher cumulative doses (2). No dose is without cardiac risk (1). Yet, some patients can tolerate high cumulative doses (in excess of 1,000 mg/m^2) without cardiotoxicity (3). Recently, topoisomerase (Top) 2B inhibition by anthracyclines has been identified as an important mediator of anthracycline-induced cardiotoxicity (4). Deletion of cardiomyocyte-specific Top2B has been shown to protect mice from anthracycline-induced cardiotoxicity, and patients with low levels of peripheral blood leukocyte Top2B have been shown to have higher resistance to anthracycline-induced cardiotoxicity. However, further study is needed before this information can be applied to clinical practice (5). As these drugs have high antineoplastic efficacy, and as it is not yet possible to identify patients with higher sensitivity, continued use of anthracyclines is expected despite the risk of cardiomyopathy with treatment. Thus, there will be an increase in the number of female childhood cancer survivors who reach childbearing age.

Cardiac dysfunction after treatment with anthracyclines is often subclinical. It has been reported that within 20 years, 25 to 50% of asymptomatic childhood cancer survivors have abnormal cardiac function found by echocardiographic evidence (1). The incidence increases with the length of time since treatment (6). Female sex and younger age at the time of cancer diagnosis are associated with increased risk of cardiac dysfunction (1). In young children, doxorubicin impairs myocardial growth resulting in a disproportionately small increase in left ventricular wall thickness in proportion to bodily growth. This excess afterload could contribute to the late development

of myocardial deterioration (7). There is some evidence that anthracycline exposure results in reduced functional reserve such that additional cardiac stress could lead to acute deterioration (3). Ali et al. described 5 patients who developed a sudden decrease in fractional shortening in the setting of a history of a cumulative doxorubicin dose greater than 300 mg/m^2 and development of viral infection (8). Pregnancy is another source of excess cardiac stress. The risk of development of cardiotoxicity with pregnancy in patients exposed to anthracyclines in childhood is not clearly known, but some limited data is available.

REVIEW OF "PREGNANCY AFTER ANTHRACYCLINE LITERATURE"

In 1988, Davis and Brown published a case report of peripartum heart failure in a patient previously treated with doxorubicin. The patient was treated with 525 mg/m^2 of doxorubicin at age six for osteosarcoma. At age 13, she developed acute systolic heart failure with an ejection fraction of 35% sixty-seven hours after delivering a full-term infant. She had an echocardiogram 2 years prior that showed normal systolic function. She underwent endomyocardial biopsy that showed cardiac fibrosis due to doxorubicin. She was thought to have subclinical doxorubicin fibrosis that lead to acute cardiac decompensation in the postpartum period (9).

In 1990, Goorin et al. reported 4 cases of initial congestive heart failure six to ten years after doxorubicin chemotherapy in childhood. One of the patients had development of symptoms 2 months after delivery of her first child and died 21 months later. This patient was noted to have reduced thickness of her left ventricular wall and increased afterload suggesting her cardiac development did not match her somatic growth (10).

In 1995, Lipshultz reported that female sex and higher rate of drug administration were independent risk factors for cardiac abnormalities after doxorubicin. Of the 12 patients with late congestive heart failure (mean of 10.2 years after doxorubicin treatment), 2 were women in the peripartum period (11).

In 1997, Katz et al. reported a case of a 28 year old primigravida patient who was diagnosed with acute heart failure 3 months postpartum. She had a history of large B cell lymphoma treated with 270 mg/m^2 of doxorubicin ten years prior. Her ejection fraction 2 months after completion of therapy was 58% by radionuclide scintigraphy. Her ejection fraction 3 months postpartum was 20%. One year later, her ejection fraction remained severely decreased at 25% despite medical therapy (12).

In 2002, Pan and Moore reported the anesthetic management of 3 deliveries in 2 patients with doxorubicin-induced cardiomyopathy. The first patient was a 35 year old with a history of osteosarcoma and doxorubicin 20 years prior. She was active and asymptomatic through 28 weeks gestation and then developed shortness of breath and was found to have an ejection fraction of 30%. She further deteriorated and her ejection fraction decreased to 10%. She underwent an urgent cesarean section with combined spinal-epidural technique. The other patient had a history of doxorubicin treatment for Ewing's sarcoma at age 16. She was active and had her first child at age 24 and developed heart failure with an ejection fraction of 35% five days after delivery. With medical therapy, her ejection fraction improved to 40–45%. She had a second pregnancy with a decrease in ejection fraction to 35%; vaginal delivery was successful with an epidural anesthetic. She had a third pregnancy and an ejection fraction of 25% at delivery. She had a combined spinal-epidural anesthetic and another successful vaginal delivery (13).

These case reports highlight the topic of doxorubicin associated cardiomyopathy with pregnancy. Asymptomatic female survivors of childhood cancer developed acute heart failure with pregnancy suggesting limited cardiac reserve after doxorubicin that was unmasked by the cardiovascular stress of pregnancy. Since these case reports, there have been 1 prospective and 3 retrospective reviews of this topic published, summarized in (**Table 1**).

Bar et al. prospectively reviewed 37 women over 17 years who were followed in the same center for surveillance after childhood cancer and through pregnancy. These women were all treated with <500 mg/m^2 of doxorubicin; only 4 received >450 mg/m^2. Cardiac function was assessed by echocardiograms using measurement with fractional shortening (FS). FS <30% defined cardiac dysfunction. 29 women had FS ≥30% at baseline and had no change in cardiac function during pregnancy. 8 had FS <30% with a mean decrease of FS by 19% after pregnancy. This finding was not statistically significant, possibly due to the small number of patients, but suggested patients

TABLE 1 | Comparison of studies of pregnancy in childhood cancer survivors (14–17).

	Bar	Van Dalen	Hines	Thompson
Type of study	Prospective	Retrospective	Retrospective	Retrospective
# of patients	37	53	847	58
Mean f/u time	17 years	20.3 years	26.5 years	20 years
Treatment/dose	<500 mg/m^2 of anthracycline	267 mg/m^2 of anthracycline	57% received anthracycline, 200 mg/m^2	97% received anthracycline, 272 mg/m^2
Definition of cardiotoxicity	FS <30% by echo	Signs and symptoms	Questionaires and echoes, SF <28%, EF <50%	EF <50% by echo
Conclusions	Pregnancy did not cause heart failure in those with normal baseline function; pregnancy did not increase risk of cardiotoxicity.	No heart failure occurred. Study was not powered to assess risk.	Pregnancy associated CMP in CCS was low but not insignificant, 1:500 vs. 1:3,000–4,000 in general population.	Subgroups identified with increased risk: 1. younger age at time of cancer diagnosis 2. Longer time from treatment to pregnancy 3. Higher anthracycline dose Pregnancy was an independent risk factor.

f/u, follow up; FS, fractional shortening; SF, shortening fraction; EF, ejection fraction; CMP, cardiomyopathy; CCS, childhood cancer survivor.

with baseline cardiac dysfunction might need special care. This study also suggested that pregnancy did not contribute to worsening cardiac function in those with normal baseline cardiac function and did not increase the risk of cardiotoxicity from prior anthracyclines (18).

Van Dalen et al. reviewed 53 childhood cancer survivors who delivered one or more children. Two of these patients had a history of acute congestive heart failure at the end of anthracycline therapy. In this study, the patients did not undergo routine evaluation with echocardiograms. Heart failure was defined based on clinical signs and symptoms. They received a mean cumulative dose of 267 mg/m^2 of anthracycline therapy. None of the patients developed heart failure during pregnancy. Thus, it was not possible to evaluate risk factors for the development of heart failure, and the study was not powered to assess cardiac risk.

The largest review was published by Hines et al. in 2015 and included 847 female cancer survivors with 1,554 live births. Patients were identified by self-report from questionnaires sent to survivors treated at St. Jude Children's Research Hospital between 1963 and 2006. Records were obtained to verify reports, and pregnancy-associated cardiomyopathy was defined as shortening fraction <28%, ejection fraction <50%, or treatment for cardiomyopathy during or up to 5 months after completion of pregnancy. Of the 847 patients, 484 (57%) received anthracyclines and 363 (43%) did not. The mean dose for those treated with anthracyclines was 200 mg/m^2. Of the 847 survivors, 3 developed pregnancy-associated cardiomyopathy, 14 developed cardiomyopathy >5 months post-partum, and 26 were diagnosed with cardiomyopathy prior to pregnancy. This study suggests that the development of pregnancy-associated cardiomyopathy in childhood cancer survivors is low but not insignificant, with 3 in 1,514 births which is approximately 1 in 500. In comparison, peripartum cardiomyopathy occurs in the general population less commonly, in 1 in 3,000 to 4,000 live births. The risk for deterioration of cardiac function with pregnancy was worse in those with a known prior decrease in cardiac function; 8 of 26 patients or 30% decompensated in that group. This study was limited by possible underreporting as patients were identified by questionnaires, and it was not possible to calculate risk factors for development of pregnancy-associated cardiomyopathy due to the low numbers of patients with the outcome (14).

At MD Anderson, we evaluated the cardiac outcomes of childhood cancer survivors who had pregnancies and were previously exposed to anthracyclines and/or chest radiation. We identified 58 patients from the Children's Cancer Hospital Longitudinal Database and the cardiology echocardiogram database who had pregnancies. We compared these women with pregnancies to a control group of 80 women from this same population with similar anthracycline dose and follow-up time who did not have pregnancies. 56 of the 58 women with pregnancies had received anthracyclines, and the mean dose was 272 mg/m^2. Median follow up time was 20 years. Adverse cardiac events were defined as the presence or worsening of cardiomyopathy based on at least two echocardiograms showing an ejection fraction (EF) <50% or coronary artery disease (CAD). Peripartum was defined as during pregnancy or within 1 year after delivery. Of the 58 women who had pregnancies, 17 (29%) had an adverse cardiac event, 3 prior to pregnancy, 9 in the peripartum time frame, and 5 after pregnancy. 16 had decreased EF and 1 had CAD. Of the 17 patients with adverse cardiac events, 2 died (1 with CAD), 8 had recovered EF at last follow up, and 7 had decreased EF at last follow up. In comparison, of the 80 women in the control group without pregnancies, 12 (15%) had an adverse cardiac event. We identified subgroups with increased risk of adverse cardiac outcomes with pregnancy. Younger age at time of cancer diagnosis, longer time from cancer treatment to first pregnancy, and higher total anthracycline dose were associated with increased risk. Pregnancy was also identified as an independent risk factor with a 2.35 fold increase in cardiac risk (15).

The MD Anderson findings suggest more concern regarding cardiac outcomes in cancer survivors with pregnancies than the

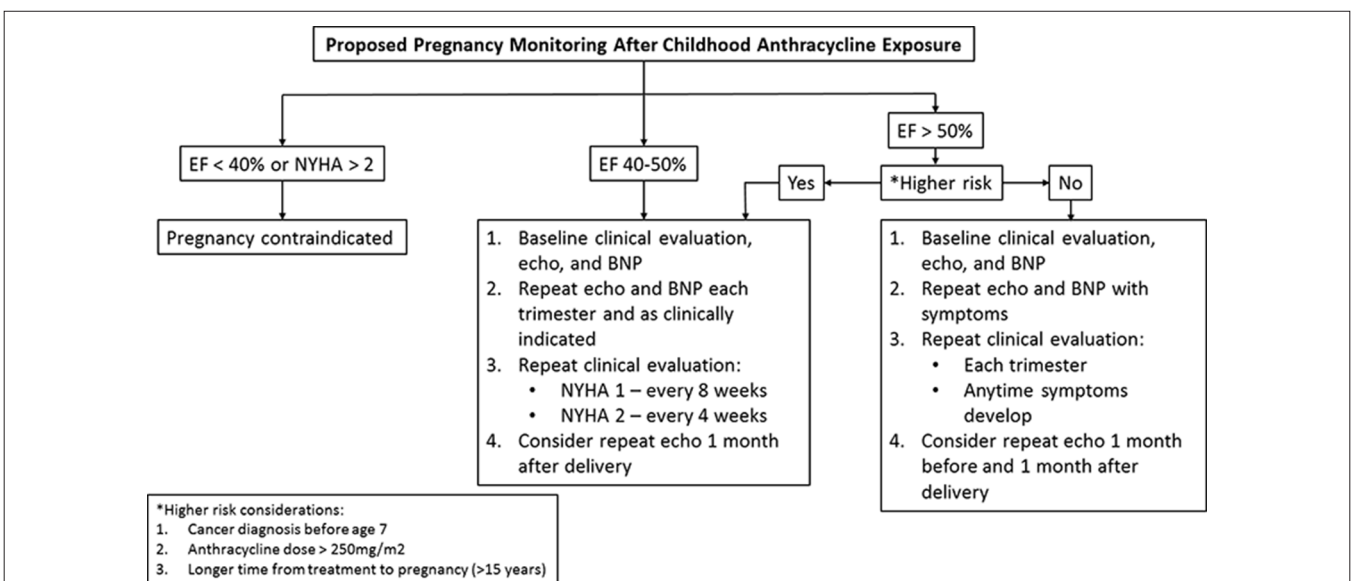

FIGURE 1 | Proposed Monitoring Algorithm.

other studies. Bar et al. reported successful outcomes of pregnancy in women without significant LV dysfunction prior to pregnancy (18). In the MD Anderson data, follow up time was longer, and some patients who tolerated pregnancy developed cardiac dysfunction later. The two deaths in our group occurred 1 and 5 years after tolerating pregnancy. It is difficult to compare the MD Anderson study to the study by van Dalen et al. as the definition of cardiac dysfunction was based on echocardiogram findings rather than symptoms alone (16). In the study by Hines et al., only 0.3% of patients developed pregnancy-associated cardiomyopathy compared to 16% in the MD Anderson study. The Hines study was based on self-report rather than on echocardiographic findings. Thus, subclinical cardiomyopathy was likely under-reported (14). The MD Anderson study group was also a higher risk group; fifteen percent of survivors in the group who did not have a pregnancy had a cardiac event (15). Compared to the Hines group, the MD Anderson patients received a higher median dose of anthracycline. We had a higher proportion of patients who were treated for sarcoma with high doses of doxorubicin and fewer with acute lymphocytic leukemia who are usually treated with lower doses of anthracyclines. And, our overall population had a high mean cardiac risk score by the method of Chow (19).

DISCUSSION

Pregnancy is associated with substantial changes in cardiac physiology and volume overloading. Yet, in normal hearts, chronic volume overloading with multiple pregnancies does not compromise left ventricular function (20). In patients with subclinical cardiac disease, the cardiac problem will often become manifest for the first time in pregnancy (21). In patients with a history of peri-partum cardiomyopathy, future pregnancies are generally not recommended, even if left ventricular (LV) function recovers as there can be an associated decrease in LV function and/or possible death. The risk seems higher in those who have a subsequent pregnancy with persistent LV dysfunction (22). Similarly, Siu et al. studied the outcomes of pregnancy in women with heart disease and identified four predictors of maternal cardiac complications and developed a risk index on the basis of these predictors. A prior cardiac event such as heart failure was one predictor which would be associated with a 27% estimated risk of a cardiac event with pregnancy according to this index (23). In patients with EF <40% or NYHA >2, pregnancy is not recommended (24). In patients with mild LV dysfunction or subclinical LV dysfunction, recommendations are less clear. For childhood cancer survivors, there are limited pregnancy guidelines based on minimal data. International guidelines suggest that surveillance for cardiotoxicity is reasonable before pregnancy or in the first trimester for all women previously treated with anthracyclines and/or chest radiation (25). There are no recommendations regarding ongoing surveillance in pregnant cancer survivors who have normal left ventricular systolic function prior to pregnancy or in the first trimester (2). Dutch guidelines recommend that all pregnant cancer survivors treated with any cardiotoxic treatment should have an echocardiogram in the third trimester of pregnancy (26). With the increasing number of childhood cancer survivors reaching child-bearing age, it would be ideal to develop pregnancy monitoring guidelines for this group of patients. For the present time, it is important for patients and doctors to recognize that there is increased cardiac risk with pregnancy in patients previously exposed to anthracyclines. Higher risk factors to consider based on MD Anderson data include younger age at time of cancer diagnosis, higher anthracycline dose, and longer time from cancer treatment to pregnancy. In addition, pregnancy is possibly an increased risk factor for development of cardiomyopathy in patients previously exposed to treatment with anthracyclines and/or radiation (15). Until registries are established to collect data and develop formal guidelines, I propose the following monitoring algorithm (**Figure 1**).

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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MINI REVIEW
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Cancer and Thrombotic Risk: The Platelet Paradigm

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Hematologic malignancies and solid tumors increase the risk of venous and arterial thrombosis and contribute greatly to patient morbidity and mortality. Thrombosis occurs when the intricate balance of circulating antithrombotic and prothrombotic blood elements are disrupted. In recent years, the interplay between paraneoplastic cells and platelets has become apparent, with a change in platelet phenotype causing dysregulated platelet activity. This review discusses mechanism of thrombosis in cancer, evidence for using drug therapy, and exciting research efforts to understand and hopefully control aberrant thrombotic events in patients with cancer.

Keywords: thrombosis, platelet activation, platelet dysfunction, cancer progression, dysregulated platelets, antiocoagulation

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INTRODUCTION

Patients afflicted with malignancies have a known propensity for thrombosis, with twenty to thirty percent of all first venous thromboembolisms (VTEs) associated with cancer (1). This thrombophilia may be a consequence of alterations in coagulation factor quantity and activity due to the underlying disease itself, the treatment for the disease, and alterations in platelet function (2–4). In addition, new evidence suggests that interactions between platelets and malignant cells lead to platelet activation and increased incidence of thrombosis (5, 6). The specific malignancy plays a role in the propensity for thrombosis, with higher rates associated with solid tumors of the pancreas, ovary, and brain in addition to hematologic malignancies, particularly, Hodgkin lymphoma (7, 8). While VTE is a well-documented risk in patient with malignancies, thrombosis in the arterial tree such as that observed in acute coronary syndrome (ACS) is also more common in patients with malignancy (9). Through understanding the pathophysiology of thrombosis in malignancies at the cellular level, we can investigate therapies and postulate future research avenues.

THE ROLE OF PLATELETS IN CANCER

There is an emerging body of evidence addressing the interactions between platelets and cancers. Not only do cells of paraneoplastic origin activate platelets, but platelets themselves appear to play a role in cancer propagation and metastatic spread in a process sometimes described as "tumor education." Direct interaction with tumor cells induces platelet aggregation in experimental pancreatic, colorectal, and renal cell lines (6). Additionally, cancer cells directly excrete thrombin and other mediators, which interact with platelet surface receptors *via* PAR-1 and PAR-4 receptors (thrombin is the agonist), $P2Y_{12}$ receptor (ADP is the agonist), and the thromboxane receptor (thromboxane A2 is the agonist). Tumors also secrete matrix metalloproteinases (MMPs) and IL-6, which have been shown to activate platelets directly (6, 10, 11). Specific study of human small cell and non-small cell lung cancer cell lines revealed *in vitro* induction of platelet aggregation both through direct cellular interactions observed under electron microscopy in SCLC and indirect cellular interactions *via* secreted thrombin and ADP mediators in NSCLC (12). Similarly, in a mouse xenograph of four different human pancreatic cell lines, two of the lines were found to express TF and release

TF-positive microparticles—both known thrombogenic entities (5). As may be expected, only the TF-positive cell lines activated coagulation pathways in mice. In breast cancer cell lines, secreted MMPs led to platelet activation *via* cellular actions, which were independent of TF concentration: the platelets changed shape to form pseudopodia and demonstrated an increased concentration of activated GPIIb/IIIa surface receptors, which are then able to bind with fibrinogen and form stable platelet aggregates (13). Von-Willebrand Factor (VWF) also plays a role in platelet aggregation and recruitment of platelets to the vascular endothelium as demonstrated in patients with melanoma and mouse models of melanoma. This phenomenon may be related to tumor-derived vascular endothelial growth factor (VEGF) secretion, which mediates endothelial cell activation and, therefore, promotes VWF expression in the tumor vessel lumen—a biological process, which promotes platelet recruitment and atheroembolism (14).

The important interactions between platelets and malignant cells are increasingly clear as there is a growing body of evidence for the platelet's role in metastatic spread of a variety of tumors. Early studies of renal sarcoma noted a correlation between the tumor's ability to enhance platelet aggregation and the tumor's metastatic potential (15). Platelet count itself also changes a patient's metastatic risk. Patients with renal cell carcinoma and thrombocytosis had worse prognosis than those with normal platelet counts, again suggesting that platelets play a role in disease progression (16). Further investigation with *in vitro* and *in vivo* models has elucidated an intricate interplay between malignant cells and platelets, which propagates metastatic spread. When a tumor cell infiltrates the vasculature, it activates platelets and induces platelet aggregation around the tumor cell. This shields the tumor cell from the host immune system, allowing the tumor to evade the immune system and promoting survival. Additionally, the platelet releases microparticles, which promote blood vessel permeability and extravasation, allowing transport of the tumor cell to a new location. Finally, VEGF released by platelets promotes angiogensis both locally within the tumor and systemically throughout the vasculature (17, 18). Tumor angiogenesis promotes tumor growth (19). There may be additional mechanisms of tumor-platelet interactions yet elucidated, as recent study of a mouse melanoma model showed that platelets inhibit T-cell function, allowing the tumor to evade the immune system and metastasize (20). In contrast, at the bone marrow level, there is evidence that megakaryocytes are protective against bone metastasis of prostate cancer and breast cancer cells (21, 22). Additional study is needed on the specific interactions between platelet progenitors and cancer.

The role of platelets in metastatic spread leads to the hypothesis that antiplatelet agents will decrease tumor progression (23, 24). Although there is no guideline or recommendation for antiplatelet agents as a treatment of known malignancy, there is evidence that ticagrelor, a $P2Y_{12}$ inhibitor, reduces metastases in murine models of melanoma and breast cancer (25). Rothwell et al. pooled a group of patients enrolled in randomized controlled trials of aspirin in vascular disease and performed a secondary analysis examining the incidence of distant metastases in patients who developed cancer both on and off aspirin (26). There was a significant decrease in distant metastases and death in patients taking aspirin who developed adenocarcinomas as opposed to those who were not taking aspirin. The use of aspirin did not change the risk of other fatal cancers (26). However, the role of antiplatelet agents in slowing malignant progression remains unclear. In a large population-based cohort study of patients who began low-dose aspirin therapy after diagnosis with colorectal cancer, there was no association with a reduction in colorectal cancer specific mortality (27). A meta-analysis of multiple cohort and one case-control study reached a similar conclusion that aspirin use after colorectal cancer diagnosis did not improve patient survival (28). In prostate cancer, aspirin use after diagnosis may only improve prostate cancer mortality in patients with high-risk cancer (29, 30). Overall, the evidence is not robust enough to advise routine use of antiplatelet agents as a component of the treatment armamentarium for cancer, though the use of antiplatelet agents in a personalized manner in select cases of cancer treatment should be investigated.

There are more robust data to support the prophylactic effect of aspirin in colorectal cancer. In fact, the language of the USPSTF recommendation for aspirin use combines primary prevention of coronary artery disease and colorectal cancer into the same statement and grade (31). Additionally, the use of aspirin in cancer prevention may extend to other solid tumors. An analysis of data from the Nurses' Health Study and the Health Professionals Follow-up Study revealed a reduced incidence of overall cancer in subjects regularly taking aspirin (32). The largest effect was seen in gastrointestinal tract cancers, particularly colorectal cancer. There is evidence that low-dose aspirin reduces the risk of developing epithelial ovarian cancer; however, prior aspirin may not improve survival in patients once they receive a diagnosis of ovarian cancer (33, 34). Overall, additional studies are needed to better elucidate which specific malignancies may respond to aspirin either as a prophylactic measure or as part of a post-diagnosis therapy regimen.

CANCER AND CARDIOVASCULAR RISK

The relationship between malignancy and platelet activation may be an underlying mechanism for increased thrombotic events seen in patients with cancer. Additionally, platelets are significantly involved in the pathophysiology of ACS and thus the hypothesis that patients with cancer are at higher risk for coronary events is compelling. There are data that patients with occult cancer have a higher risk of coronary events even 2 years prior to cancer diagnosis, compared to control patients. This risk was found to be highest in patients with colorectal cancer (9). After cancer diagnosis, the risk of subsequent coronary disease events was highest in the first 6 months but persisted to 10 years after initial diagnosis. This risk was further increased by the presence of metastases (35). Additionally, there is a significantly increased risk of bare metal coronary stent thrombosis in patients with solid tumors (36). It is unclear how to further treat these patients beyond recommended dual-antiplatelet therapy since, at this time, evidence for using Factor Xa inhibitors of vitamin K antagonists is lacking and conceivably would increase the risk of bleeding.

Cancer treatment regimens themselves may also increase the risk of cardiovascular events. In a meta-analysis of seven randomized controlled trials examining the risk of thrombotic and cardiovascular events in women with breast cancer treated with tamoxifen or aromatase inhibitors, Cuppone et al. reported a slight increase in the pooled outcome of cardiovascular adverse events (37). The authors postulate this difference is related to the cholesterol raising effect of aromatase inhibitors. However, the specific risk of myocardial infarction related to aromatase inhibitor or tamoxifen therapy is unclear (38). Mediastinal therapeutic radiation is also an important risk factor for future cardiovascular disease. The European Society of Cardiology recommends preventative therapy with an antiplatelet agent as well as regular screening for cardiac disease beginning 10–15 years after initial radiation treatment (39).

The question remains of how to manage patients with cancer and myocardial infarction. In the short term, practitioners may be reticent to treat patients with ACS with antiplatelet agents, particularly, if the patient has thrombocytopenia as a pathophysiological consequence of malignancy or secondary to chemotherapy agents. However, aspirin has been shown to decrease all-cause mortality in patients with an acute myocardial infarction, even those with severe thrombocytopenia (40). This observation raises the concern that a low platelet count in the absence of bleeding should not be a reason to discontinue antiplatelet therapy in a patient with active coronary disease since the platelet population may be "dysregulated" with enhanced thrombotic potential. An additional study showed that both beta blockers and aspirin improve mortality in patients with known cancer and an acute myocardial infarction, whereas revascularization did not have a significant impact on mortality in this patient cohort (41). Thus, it remains important to adhere to guideline-directed medical therapies of ACS even in patients with malignancy.

CANCER AND THROMBOEMBOLISM

The association between cancer and VTE is well-established and VTE is a dangerous complication of malignancy and chemotherapy treatments. Cancer is a hypercoagulable condition due to disease-related aberrations in the coagulation cascade. As discussed above, both platelets and tumor cells release tissue factor, which is one of the major factors in the extrinsic coagulation pathway. TF joins with factor VIIa to form a complex, which activates both factor IX to IXa and X to Xa, leading to thrombin formation and clots (42). Additionally, cancer procoagulant is a protease, which activates factor X independently of TF and has been detected in malignancies such as ovarian, colon, kidney, breast, prostate, and small cell lung cancer (43). Third, heparanase is an enzyme found in platelets, which enhances TF activity and is upregulated in some cancers. This leads to a positive feedback cycle of increased TF activation, which in turn activates platelets and promotes the release of additional heparanase (44). It is through these mechanisms that malignancies effect the coagulation cascade and promote thrombosis.

Additionally, the endothelium itself, endothelial modulators such as VEGF, and circulating blood cells play an important role in the thrombotic process. First, as a solid tumor grows, the hypoxic microenvironment of the tumor promotes reactive oxygen species generation by the mitochondria leading to release of angiogenic proteins such as VEGF and induction of transcription factors as well as increased endothelial permeability due to endothelial intracellular actin remodeling. The increased endothelial permeability exposes native and tumor extravascular TF expressing cells to circulating factor VIIa, initiating the coagulation cascade (45). Additionally, endothelial permeability allows for tumor cell metastasis, increased exposure of blood products to cancer-produced thrombin, and adhesion of pro-inflammatory cells to the vascular endothelium (45, 46). The malignancy's direct action on platelet activation and aggregation then couples with the increase in pro-inflammatory leukocytes in the endothelium (5, 13, 47). Activated platelets and leukocytes interact to form microthrombi within the vasculature and these microthrombi adhere to the endothelium creating a nidus for larger thrombi to form (6). These processes of localized thrombosis are hypothesized as the underlying etiology of the histologic findings of vascular pseudopalisades around a necrotic core in glioblastoma multiforme (48). An area of additional interest regarding the role of platelets in thrombosis could examine abnormal megakaryoblasts in acute megakaryocytic leukemia, a rare AML subtype in adults (1% of AML cases), which can present with thrombocytopenia but has limited data on prognosis and treatment (49). Mechanisms for enhanced thrombosis in cancer are summarized in **Table 1**.

The microscopic process of localized tumor thrombosis, neovascularization, and disruption of the coagulation cascade becomes important in clinical practice, as epidemiologic studies have found that 20–30% of all first VTE events are cancer-associated, with a cumulative incidence of VTE in cancer patients of 1–8% (1, 50). Diagnosis of thrombosis is a poor prognostic sign for patients with cancer. In a prospective study of patients receiving cancer chemotherapy, progression of disease was the leading cause of death, closely followed by thrombosis and infection (51). Thus, it is an issue of great importance with research focused both on the prevention of VTE and on appropriate treatment.

The risk of developing VTE depends on the time from initial cancer diagnosis, tumor origin, treatment modalities, and laboratory values. Pulmonary embolism (PE) may precede a diagnosis of lung cancer and also carries a sixfold higher risk than controls in the year following diagnosis (52). This risk is further elevated by receiving chemotherapy, with registry data indicating an eightfold increase in the risk of developing a PE compared to age-matched controls. Patients with pancreatic cancer and brain cancer have a higher risk of VTE and patients with prostate cancer and breast cancer have a lower risk. Additionally, surgical instrumentation and chemotherapy treatment increase a patient's VTE risk (50). Further independent risk factors for VTE include high platelet count and higher leukocyte count (53).

Clinically, a patient's VTE risk can be quantified using the Khorana Score. This is a comprehensive risk model validated in multiple cancer types, which uses parameters such as site of cancer, presence of anemia, platelet count, leukocyte count, and BMI to calculate an ambulatory patient's risk of VTE and guide the decision for prophylaxis (54, 55). The model is recommended by the American Society of Clinical Oncology (56). Data suggest that inhibiting plasma Factor Xa with low molecular weight heparin

TABLE 1 | Summary of pro-thrombotic elements observed in cancer.

Mediator	Origin	Mechanism
Tissue factor (TF)	○ Platelets ○ Malignant cell expressed ○ Malignant cell secreted microparticles	○ Activates platelets ○ Activates extrinsic clotting cascade ○ Binds with Factor VII/VIIa and this complex activates Factor IX to IXa and Factor X to Xa ○ Ultimately increases activation of prothrombin to thrombin
Matrix metaloprotease	○ Native cell secreted ○ Malignant cell secreted	○ Activates platelets independent of TF ○ Increases platelet binding to fibrinogen increases platelet aggregation
Vascular endothelial growth factor (VEGF)	○ Native cell secreted ○ Malignant cell secreted	○ Increases VonWillebrand Factor expression on endothelial cells, promoting platelet aggregation ○ Increases endothelial permeability, exposing cells and clotting factors to extravascular TF ○ Draws pro-inflammatory leukocytes, which complex with platelets and form microthrombi
Cancer Procoagulant	○ Malignant cells only	○ Activates FX to FXa independent of TF
Heparanase	○ Platelets ○ Neutrophils ○ Monocytes ○ Malignant cell cytoplasm malignant cell secreted	○ Enhances TF activity ○ Activates VEGF, increases neoangiogenesis

is the prophylactic agent of choice to reduce occurrence of VTE; however, its use does not impact survival (50, 57). Because of the known role of platelets in thrombus formation for patients with cancer, it is reasonable to hypothesize that aspirin would have a protective effect on VTE risk. In patients without malignancy, aspirin is useful for both prophylaxis and prevention of VTE recurrence (58, 59). However, in patients with malignancy, there is a trend to suggest that aspirin is effective in VTE prophylaxis, but the evidence is not strong enough to support routine use (60). If a patient with cancer does develop VTE, the treatment of choice remains low molecular weight heparin rather than vitamin K antagonists based upon open-label randomized controlled trials and a meta-analysis showing a decrease in recurrent VTE in cancer patients treated with LMWH compared to warfarin (50, 61).

CONCLUSION/FURTHER STUDY

In vivo and *in vitro* studies in human and animal models have described the interactions between platelets and cancer cells. With this background descriptive knowledge, it is a compelling hypothesis that antiplatelet agents are useful in decreasing malignant transformation as well as the occurrence of thrombosis. There is evidence for aspirin as a cancer preventative agent, particularly in colon cancer as reflected in the USPSTF guidelines. Unfortunately, the data for antiplatelet agents as an adjunctive treatment to chemotherapy in order to decrease the spread of malignancy is not as strong. Similarly, the use of antiplatelet agents for prophylaxis of VTE and other thrombotic events such as myocardial infarction has not been widely adopted. However, available data suggest that patients with cancer who develop ACS should be treated with guideline-based secondary prevention therapies, including aspirin even in the setting of thrombocytopenia (40). Areas of potential research include how to treat patients with malignancy and in-stent thrombosis despite dual antiplatelet therapy. Perhaps these patients would benefit from low molecular weight heparin treatment, though this would have to be carefully weighed against the risk of bleeding. Ongoing areas of interest include prophylaxis, treatment, and secondary prevention of thrombosis and VTE in cancer patients. Additionally, while the Khorana Score focuses on the risk of VTE, it may be helpful to incorporate malignancy into other risk scores such as the CHAD2S-VASC score for atrial fibrillation and embolic stroke risk. These are potential avenues of collaboration between the cardiovascular and oncology societies to formalize recommendations for this unique population of patients.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to this work.

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REVIEW
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Interventional Cardio-Oncology: Adding a New Dimension to the Cardio-Oncology Field

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The management of cardiovascular disease in patients with active cancer presents a unique challenge in interventional cardiology. Cancer patients often suffer from significant comorbidities such as thrombocytopenia and coagulopathic and/or hypercoagulable states, which complicates invasive evaluation and can specifically be associated with an increased risk for vascular access complications. Furthermore, anticancer therapies cause injury to the vascular endothelium as well as the myocardium. Meanwhile, improvements in diagnosis and treatment of various cancers have contributed to an increase in overall survival rates in cancer patients. Proper management of this patient population is unclear, as cancer patients are largely excluded from randomized clinical trials on percutaneous coronary intervention (PCI) and national PCI registries. In this review, we will discuss the role of different safety measures that can be applied prior to and during these invasive cardiovascular procedures as well as the role of intravascular imaging techniques in managing these high risk patients.

Keywords: interventional cardio-oncology, optical coherence tomography, intravascular imaging, thrombocytopenia, fractional flow reserve, instantaneous free-wave ratio, transcatheter aortic valve replacement, takotsubo cardiomyopathy

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INTERVENTIONAL CARDIO-ONCOLOGY

There are approximately 14.5 million cancer survivors in the United States alone, a number that is expected to reach 20 million within the next ten years. It is estimated that in 2016, over 1.6 million new cases of cancer were diagnosed in the United States, and approximately 600,000 people died from the disease (1). However, the death rate from cancer in the United States continues to decline. With improvements in early diagnosis, monitoring and treatment of various malignancies, cancer patients often are at higher risk of mortality from cardiovascular disease rather than recurrence of their cancer.

Patients with malignancies present challenges to the management of cardiovascular disease. They are often frail, particularly those with advanced age and those who sustain off-target effects from cancer treatments. Comorbidities must be appropriately evaluated and addressed for optimal management of cardiovascular health. Furthermore, cancer patients often have significant time constraints placed upon them, and care must be coordinated around various diagnostic and/or therapeutic oncologic procedures. Outside of the acute setting, cancer survivors experience increased cardiovascular

morbidity and mortality across all age groups (2, 3). Cancer is intrinsically linked to heart disease via multiple mechanisms: common risk factors, chronic inflammatory states brought on by malignancies, and cardiac and vascular toxicities of cancer therapy.

Coronary Artery Disease (CAD)

Cardiovascular disease and cancer share similar risk factors: increasing age, cigarette smoking, obesity, diabetes, hypertension, hyperlipidemia, and physical inactivity (4). These risk factors increase the short- and long-term cardiac mortality in cancer patients (5). At the time of cancer diagnosis there is a high prevalence of cardiac disease, which is often exacerbated by necessary cancer therapeutic agents (6). The pathogenesis and progression of cancer and cardiovascular disease also share an increased state of inflammation. Inflammation and endothelial damage are key in the development of atherosclerosis and plaque thrombosis (7, 8). Meanwhile, cancerous cells produce pro-inflammatory cytokines and chemokines that damage the endothelium and increase permeability of the microvasculature (9). This allows the formation of plaques as low-density lipoprotein cholesterol particles invade damaged vascular intima, creating a pro-atherosclerotic effect and contributing to increased risk of CAD in cancer patients.

Cancer therapeutic agents frequently cause cardiovascular complications, due to direct toxicity to the vascular endothelium and myocardium. Complications may include anginal chest pain, hypertension, acute coronary syndromes (ACS), stroke, arterial thrombosis leading to limb ischemia, venous thrombosis, arrhythmias, and heart failure. Development of cardiotoxic side effects from chemotherapy depends on several factors including the choice of drug, dose of drug administered, interval of administration, cumulative dose, route of administration, and association with radiotherapy (10). Those at the extremes of age are at increased risk of cardio-toxicity from chemotherapeutic agents, as are those with preexisting cardiovascular risk factors/disease and those with a history of radiation therapy to the chest (11).

Several forms of antineoplastic therapy including chemotherapy, hormonal therapy, and radiotherapy have been associated with a higher risk of atherosclerosis, CAD and cardiac ischemia (12). The presumed mechanism of cancer therapy causing CAD is highly variable and often unknown. Coronary vasospasm is one of the most frequently noted adverse effects of cancer therapy that may lead to myocardial ischemia or infarction, as seen in agents such as sorafenib, 5-fluorouracil and capecitabine (13–15). Apoptosis of endothelial cells, resulting in vasospastic angina and myocardial infarction, is an adverse effect related to antineoplastic agents such as etoposide, bleomycin, bevacizumab and vinblastine (16). Bevacizumab specifically, by inhibiting vascular endothelial growth factor (VEGF), increases expression of proinflammatory genes and decreases endothelial cell production of the vasodilator nitric oxide, causing increased platelet activity (17, 18). One study found that addition of bevacizumab to chemotherapy resulted in increased incidence of arterial thromboembolic events, from 3.1 events in those with standard chemotherapy compared to 5.5 events per 100 person-years in those additionally treated with bevacizumab (19). A recent meta-analysis found that bevacizumab increased the risk of arterial adverse events, including cardiac and cerebral ischemia, venous thromboses/pulmonary emboli, bleeding, and hypertension with higher risks in patients taking higher doses (18). Lenalidomide is an immunomodulatory agent used in the management of multiple myeloma that similarly increases the risk for both arterial and venous thrombo-embolic events, including myocardial infarction and cerebrovascular accidents (20).

Accelerated progression of atherosclerosis and elevated risk of ischemic events has similarly been reported in cancer survivors on long-term hormone deprivation therapies such as gonadotropin-releasing hormone agonists (21–24) or aromatase inhibitors (anastrozole, letrozole, exemestane) (25). Tamoxifen is a selective estrogen receptor modulator that does not seem to have increased cardiovascular risk based on randomized, placebo-controlled trials (26), but does increase thromboembolic risk (25).

Accelerated atherosclerosis has been well documented in cancer patients with exposure to radiotherapy. These survivors experience higher risk of ischemic heart disease beginning as soon as 5 years following exposure and continuing for life. The risk of major adverse events is dependent on the radiation dose received, with an increased risk of 7.4% per gray of radiation. There is not a safe threshold of exposure below which there is zero risk (27). Radiation to the heart causes these effects through direct damage to the endothelium resulting in inflammatory changes such as increased monocyte attachment which, in combination with elevated cholesterol, initiates the formation of fatty streaks and ultimately atherosclerosis (28–30). Damage also occurs to the microvasculature, causing inflammation and thrombus formation which obstructs the microvasculature ultimately leading to ischemia, fibrosis, and death of myocardial cells (31, 32). Thus, patients with history of exposure to chemotherapy or cardiac radiation may be considered for screening every 5 years with an ankle-brachial index, carotid ultrasound, stress test, and/or coronary CT angiography for evidence of advancing coronary and peripheral atherosclerosis (33). Table 1 contains a list of the cardiovascular effects associated with several anticancer therapies.

Recently published data on 279,719 pairs of patients with a new primary diagnosis of cancer and matched control patients found a 6 month cumulative incidence of arterial thromboembolism in 4.7% of cancer patients compared with 2.2% in control patients (34). Furthermore, risk for thromboembolism generally resolved by a year following cancer diagnosis and correlated with cancer stage. Since cancer patients are at higher risk for thrombotic events immediately following diagnosis, an additional concern for the cardio-oncologist is to achieve the proper balance of antiplatelet, antithrombotic, and statin medications in patients with potential bleeding diathesis.

Thrombocytopenia

Thrombocytopenia is frequent in cancer patients, occurring in anywhere from 10 to 25% of solid tumor patients treated with intensive chemotherapy as well as most acute leukemia, lymphoma, myelodysplastic syndrome and multiple myeloma patients (35). Complicating their management, 15 to 25% of thrombocytopenic patients experience thrombocytopenia refractory to platelet transfusion, which is defined as failure to increase platelet counts by 10,000/μl or more after transfusion of an appropriate dose

of platelets or >3,000/μl increase per unit (36). Clinical studies suggest that platelet function is more important than platelet count (37). Prophylactic platelet transfusion is not recommended in the general adult inpatient population for platelet counts over 10,000/μl (38). For patients receiving therapy for urologic, gynecologic, colorectal tumors or melanoma, or in extreme cases of known necrotic tumors, transfusion can be considered when the platelet count dips below 20,000/μl (39). Following transfusion, platelet count must be remeasured to ensure the desired level has been reached. In patients undergoing invasive procedures, platelets should be available on short notice in case a bleeding event occurs. Many cancer patients have a long history of receiving transfusions; for alloimmunized patients, histocompatible platelets must be available.

When cancer patients with thrombocytopenia require lifesaving interventions, thromboelastography (TEG) may be utilized in some centers to assess their ability to undergo certain procedures. TEG is a viscoelastic method of blood clotting assessment used at the bedside to analyze the entire process of clotting, including both platelet and coagulation function (40). TEG may determine whether pericardiocentesis would be safe in patients with platelet counts below 30,000 (36), but the current data is limited. Abnormal TEG results require correction prior to the procedure with a platelet transfusion or the necessary blood products. Unfortunately, data is lacking to support the use of TEG in PCI management, presenting a challenge in patients requiring PCI in this setting. As TEG is available at few centers, this limited experience is largely drawn from the anesthesia, cardiovascular and liver transplant surgical literature.

Thrombocytopenia is traditionally considered a relative contraindication to pericardiocentesis for patients with pericardial effusions (41). However, a recent study showed that pericardiocentesis was safe and effective in the setting of malignancy and thrombocytopenia (36, 42). Pericardiocentesis on thrombocytopenic patients may be performed under echocardiographic and/or fluoroscopic guidance. In appropriate patients, an intercostal approach is preferred to avoid possible trauma to the liver from a subxiphoid approach. Care must be taken to ensure that the needle is placed appropriately above the specific rib margin to avoid damage to the intercostal vessels and nerves (42, 43). Micro-puncture and small sheath size are recommended to minimize procedural risks (44).

Due to lower risk of bleeding complications such as retroperitoneal hemorrhage, pseudoaneurysm, arterio-venous fistula and excessive bleeding, radial artery access is preferred for invasive diagnosis and management of CAD in patients with thrombocytopenia (45). These patients still require anticoagulation administration while undergoing transradial diagnostic catheterizations; unfractionated heparin can be given intra-arterially or intravenously at decreased doses of 50 U/kg or 3,000 units in cancer patients platelet counts below 50,000/μl undergoing cardiac catheterization via radial access. Even in thrombocytopenic patients receiving anticoagulation and antiplatelet therapy, reductions in bleeding complications can be achieved. Meanwhile, radial access site catheterization allows for early ambulation, decreasing risk of complications from venous thrombosis (46, 47)

Evidence is lacking on dual antiplatelet therapy (DAPT) for stents placed in the setting of thrombocytopenia. However, given the hypercoagulable state that cancer presents, thrombocytopenic patients who undergo stent placement should receive DAPT. For the general populace, a recent focused update of the ACC/AHA guidelines on duration of DAPT in CAD patients offered a class IIb recommendation that ACS patients treated with DAPT following DES implantation with a high risk of bleeding or severe bleeding complications can be reasonably discontinued after 6 months of P2Y12 therapy (48).

TAKOTSUBO CARDIOMYOPATHY

Stress-induced cardiomyopathy (SC), also known as Takotsubo cardiomyopathy, mimics the clinical presentation of acute myocardial infarction with symptoms such as chest pain, dyspnea, hypotension, and electrocardiographic changes mimicking STEMI or NSTEMI (49). In patients undergoing cardiac catheterization for ACS there is an approximately 1% incidence of this disorder; the incidence in the cancer population appears to be significantly higher at 10–20% (50). While in the general population SC is appropriately categorized and discussed under the subject of "cardiomyopathy," this surprising insight suggests that the interventional cardio-oncologist is more likely to encounter SC and should be aware of the high incidence of SC in the cancer population as compared to the general population. In patients with SC, the circulating epinephrine and norepinephrine levels released from chromaffin cells as well as norepinephrine from sympathetic nerve terminals are elevated during the acute course of clinical presentation, suggesting that this cardiomyopathy is driven by excess adrenergic stimulation of cardiomyocytes (51). However, there is no clear explanation for the pathogenesis of this cardiomyopathy, and the mechanisms involved are likely to be heterogeneous and multifactorial. Possible mechanisms include emotional stress when receiving a frightening cancer diagnosis, catecholamine-induced microvascular vasospasm, inadequate increase in cardiac sympathetic nervous activity, modification of cardiomyocyte adrenergic receptors by the underlying malignancy, and reduction in estrogen (52). Antineoplastic agents such as 5-FU, Sunitinib, and Cytarabine may cause SC as an adverse effect (53, 54). Prognosis in patients with SC is generally good in the absence of significant underlying comorbidities. Anticancer therapy can be resumed within 2 to 4 weeks, and beta-blockers should be utilized indefinitely to reduce sympathetic stimulation of the heart.

INTRACORONARY IMAGING (OPTICAL COHERENCE TOMOGRAPHY)

Optical Coherence Tomography (OCT) is an important intravascular imaging modality in cardio-oncology. OCT is used for risk stratification of plaques, as plaque architecture affects risk of thrombosis. Those with a thin fibrous cap covering large thrombogenic cores, known as thin-cap fibroatheromas, are more susceptible to rupture and atherothrombosis (55).

A non-cardiac surgical procedure is unpredictably required within 12 months following stent implantation in approximately 5%

of patients with drug-eluting stents (DES) (56). Recent guidelines recommend DAPT for only six months with newer generation DES outside a setting of ACS (48). Moreover, a recent data analysis reported that discontinuation of DAPT 3 to 6 months following (predominantly new-generation) DES placement was not associated with an early increase in major adverse cardiac and cerebrovascular events (57). In fact, greater than 12 months of DAPT therapy was associated with an early increase in such events. In patients with newly diagnosed or existing cancer, DAPT may need to be prematurely discontinued for diagnostic biopsies, surgery or initiation of cancer therapy. Furthermore, the risk of thrombosis is increased due to the prothrombotic state of cancer patients (34). The optimal duration of DAPT therapy in cancer patients receiving coronary stents during the periprocedural period is unclear. The management of cancer patients with recent stent placement requiring urgent DAPT discontinuation remains largely empirical.

OCT can be useful in identifying whether a coronary stent has sufficiently healed and whether discontinuation of DAPT may be appropriate for the clinical scenario. Intravascular imaging such as intravenous ultrasound (IVUS) or OCT after stent placement ensures optimal stent expansion and apposition and absence of complications, given the potential for early DAPT interruption. A recently published single-center prospective study in cancer patients with DES within the past 12 months requiring premature DAPT discontinuation outlined a comprehensive strategy for determining the proper method of discontinuing DAPT in cancer patients (58). Patients classified as low risk were considered to have appropriate stent strut coverage, expansion, apposition, and the absence of in-stent restenosis or intraluminal masses. Low risk patients were allowed to temporarily discontinue DAPT and proceed with cancer related procedures. The incidence of adverse cardiovascular events was assessed after the procedure and at 12 months. Of 40 patients in the study, 27 low-risk by OCT criteria temporarily discontinued DAPT. The remaining 13 patients with one or more OCT findings were considered high risk and underwent bridging with low-molecular weight heparin and the appropriate further endovascular treatment. No cardiovascular events occurred in the low risk group, and one myocardial infarction occurred in the high-risk group. There were no cardiovascular deaths, but a total of 14 non-cardiac deaths occurred before 12 months due to cancer progression or cancer therapy. The median time between stent placement and follow-up OCT was 5.2 months (1.1–11.6 months), with 40% of patients having follow-up within 3 months of stenting. The median time interval over which DAPT was discontinued was 6 days (5–36 days), with 38.5% of patients discontinuing DAPT for over 7 days. It has been suggested that the aforementioned vascular toxicity of various antineoplastic agents can cause delayed stent endotheliazation in cancer patients, demonstrating the utility of real-time imaging of coronary stents to determine if stents are appropriately positioned for suspension of DAPT instead of applying broad, potentially inaccurate timelines for DAPT administration and discontinuation.

While further evidence regarding early discontinuation of DAPT is required to establish its safety and efficacy, an OCT-guided strategy is promising to identify cancer patients who have received DES who may need discontinuation of DAPT to proceed with cancer-related surgery and procedures.

Fractional Flow Reserve (FFR)-Guided PCI

FFR is a well established method of quantifying the functional severity of coronary artery stenosis during coronary angiography. Maximal hyperemia is induced with intravenous adenosine, allowing for correlation between blood flow and the blood pressure within a coronary artery (59). Comparison of distal coronary pressure to mean aortic pressure provides a functional evaluation of several hemodynamic parameters such as the mass of myocardium supplied by a specific coronary vessel, collateral blood flow and myocardial viability. This ratio provided by FFR adds a functional component to the anatomic assessment of lesion severity already provided by conventional coronary angiography.

The DEFER (Percutaneous Coronary Intervention of Functionally Nonsignificant Stenosis) trial showed that PCI can be safely deferred in patients with FFR above 0.75, with similar event-free survival and symptom recurrence between patients who deferred PCI and those who received PCI (60). group which deferred PCI had decreased rates of myocardial infarction at 15 year follow-up versus the group which received an intervention (61). The FAME study showed that routine measurement of FFR during PCI reduced death, myocardial infarction, and repeat revascularization at 1 year. By using a FFR-guided strategy, there was reduced usage of stents without decrease in functional status, quality of life, or procedure length (62). Several additional studies in recent years have confirmed the safety and reliability of FFR guided decision-making regarding PCI.

The American College of Cardiology guidelines on coronary revascularization state that FFR is reasonable to assess intermediate coronary lesions in the 50 to 70% diameter stenosis range, and can be useful for guiding revascularization decisions in CAD patients (Class IIa, Level A) (63). The most recent version of the appropriate use criteria on coronary revascularization endorses FFR for functional lesion assessment in CAD, as well as, the expanded use of intracoronary physiological testing (64).

Instantaneous wave-free ratio (iFR) is a more recent physiological method that assesses the functional severity of coronary stenoses without the need of hyperemic agents, based on the concept that a translesional gradient should be detectable at rest to have a significant effect on the delivery of blood to the myocardium. iFR is measured during the wave-free period of diastole, a portion of the cardiac cycle suitable for a pressure measurement of the hemodynamic impact of coronary stenosis (65). iFR can serve as a method to spare 60 to 70% of patients from administration of adenosine, which can cause dangerous side effects such as bradycardia and atrioventricular block (66), and multiple trials have shown that iFR is non-inferior to FFR in guiding PCI in the absence of ACS with respect to 1 year risk of all-cause mortality, nonfatal MI or unplanned revascularization (67). Additional benefits of iFR over FFR include shorter procedure length, lower incidence of patient-related discomfort, and the ability to assess serial lesions (68).

Data regarding use of FFR- and iFR-guided PCI in cancer patients is lacking. However, given its reliability in assessing the functional importance of coronary artery stenoses, we believe these are essential tools in the evaluation of patients with active malignancies undergoing cardiac evaluation. High-risk cancer patients with

TABLE 1 | Anticancer Therapies Associated With Vascular Side Effects.

Chemotherapy Agents	Adverse Cardiovascular Effects	Possible Mechanism
Antimetabolites		
5-Fluorouracil	Angina, vasospasm, MI, SC	Vasospasm
Capecitabine	Angina, vasospasm, MI, SC	Vasospasm
Gemcitabine	Angina, vasospasm, MI	Vasospasm
Antimicrotubule agents		
Paclitaxel	Angina, vasospasm, MI	Vasospasm
Vinblastine (16, 79)	Angina, MI	Endothelial injury
Monoclonal antibody-based tyrosine kinase inhibitor		
Bevacizumab	Angina, MI, SC	Endothelial injury
Small molecule tyrosine kinase inhibitors		
Sorafenib	Angina, vasospasm, MI	Vasospasm
Sunitinib	Angina, MI, SC	Unknown
BCR-ABL targeted tyrosine-kinase inhibitors		
Nilotinib	Angina, MI, progression of CAD, PAD	Unknown
Ponatinib	Angina, MI, progression of CAD	Unknown
Hormone therapy		
Aromatase inhibitors (anastrozole, letrozole, exemestane)	Angina, MI	Unknown
Gonadotropin-releasing hormone agonists (goserelin)	Angina, MI	Unknown
Radiotherapy	Angina, MI, progression of CAD, PAD	Endothelial injury

MI indicates myocardial infarction; SC, stress-induced cardiomyopathy; CAD, coronary artery disease; PAD, peripheral artery disease.

multiple comorbidities but nonsignificant stenosis measured by coronary physiology could avoid further invasive diagnostic or unnecessary therapeutic cardiovascular procedures. Given the risks that antiplatelet therapies present in cancer patients and the complexities associated with cancer care, deferral or avoidance of unnecessary stent placements that are associated with their own risks of thrombosis and would require that patients transiently be placed on DAPT is another major benefit, decreasing the risk of perioperative or chemotherapy-related bleeding complications.

Transcatheter Aortic Valve Replacement (TAVR)

TAVR was initially utilized as a treatment for patients with severe aortic stenosis (AS) and prohibitively high surgical risk (69). Recently, TAVR proved to be a safe and effective alternative to surgical aortic valve replacement (SAVR) in patients at intermediate surgical risk (70, 71). While several newer trials are still underway, there is no data on TAVR in cancer patients and cancer patients have been excluded from most TAVR studies (72). Meanwhile, the presence of cancer remains a common reason for declining surgical intervention in patients with severe aortic stenosis (73). Cancer survivors are at higher risk for SAVR due to prohibitive anatomy (e.g., mediastinal fibrosis, severe lung disease, porcelain aorta, and prior thoracic surgeries or chest radiation). This presents a problem, as cancer patients with severe AS who do receive AVR have improved survival, regardless of their cancer status (74). One case series involving six cancer patients demonstrated that balloon aortic valvuloplasty, which can be used as a bridge to SAVR, TAVR or non-cardiac surgery, is a viable option in cancer patients with severe AS (75). Recent expert consensus suggests that balloon aortic valvuloplasty and TAVR can be used as a palliative measure for symptomatic AS in cancer patients (39). One concern regarding TAVR is the increased rates of subclinical leaflet thrombosis and reduced leaflet motion seen in bioprosthetic aortic valves when compared to SAVR (76–78), especially in cancer patients who may already be hypercoagulable. Increased rates of transient ischemic attacks were associated with subclinical leaflet thrombosis, and therapeutic anticoagulation was found to resolve the condition (76). While the clinical significance of this finding is unclear, TAVR is associated with excellent outcomes which may be further improved with thorough investigation of this complication.

CONCLUSION

Interventional cardio-oncology is a new field in search of a path, seeking to match traditional cardiovascular research values such as randomization and large population-based data samples with the individualized, targeted and patient-specific treatments and science in oncology. Future directions for the field will be carried partially by the application of broader interventional cardiology trends in cancer patients. Third-generation DES feature safer stent designs which could improve outcomes in patients with challenging coronary anatomies as well as biodegradable polymers. The advent of bioabsorbable vascular scaffold is a highly impressive innovation with unclear clinical value to date but promising applications in the population of cancer survivors. As a field addressing the intricate intersection between the top two leading causes of death in the United States, the challenge in interventional cardio-oncology is real but the potential for growth and expansion is massive.

AUTHOR CONTRIBUTIONS

Each of the authors contributed to the writing and editing of this manuscript. The first two authors contributed equally to this manuscript and are co-first authors.

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REVIEW
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Radiation-Induced Cardiovascular Disease: A Clinical Perspective

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Cancer survival has improved dramatically, and this has led to the manifestation of late side effects of multimodality therapy. Radiation (RT) to the thoracic malignancies results in unintentional irradiation of the cardiac chambers. RT-induced microvascular ischemia leads to disruption of capillary endothelial framework, and injury to differentiated myocytes results in deposition of collagen and fibrosis. Coexistence of risk factors of metabolic syndrome and preexisting atherosclerosis in addition to RT exposure results in accelerated occurrence of major coronary events. Hence, it becomes pertinent to understand the underlying pathophysiology and clinical manifestations of RT-induced cardiovascular disease to devise optimal preventive and surveillance strategies.

Keywords: cardiovascular disease, cancer, radiation, ischemic heart disease, biomarkers

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INTRODUCTION

Cardiovascular diseases are the number one cause of mortality worldwide with an estimated 17.7 million people dying of it. Cancer is the second most common cause of death worldwide in 2015 with an estimated 8.8 million cases (1). The heavy burden of CVS disease and cancer portends a scenario where the number of patients with coexisting cardiac disease and cancer is going to increase exponentially. Cancer management has undergone significant changes in the past century. Better understanding of tumor biology and advent of modern therapeutic arsenal has led to improved survival outcomes. As survival improves, there is superimposition of age-related chronic diseases with the chronic side effects of multimodality cancer therapy. It is estimated that over 50% of all patients diagnosed with cancer undergo RT either as a curative and/or supportive therapy (2, 3). Cancer survivors tend to have comorbidities such as diabetes, hypertension (HT), hyperlipidemia, chronic renal disease, and vascular diseases, which might be additive to RT in causing accelerated atherosclerosis or *vice versa* (4). Incidental RT dose to heart or vascular structure has been documented to produce long-term cardiovascular side effects. The risk of development of CV disease increases with increasing radiation (RT) dose to the heart and can cause structural and functional abnormalities of coronary vessels, valves, pericardium, and myocardium (5). Breast, lung, esophageal cancer, thymoma, and mediastinal lymphoma are the most commonly treated malignancies with RT that are in close proximity to heart and those that have high probability of being enclosed in the RT portal. Early-stage breast cancer and lymphoma have most of the best survival rates and hence have higher possibility of manifesting the late RT-induced cardiac side effects. Population-based cohort studies have shown that in long-term survivors of Hiroshima bombing, the incidence of vascular diseases such as coronary events and stroke are increased with linear increase in RT dose. In addition, chronic renal failure and liver cirrhosis are also increased, which may potentially influence the CVS outcomes (6). The literature on cardiac events in cancer patients are predominantly based on those treated between

1950 and 1990s and in an era of orthovoltage machines, primitive dosimetry techniques, extended RT fields, and high RT doses (7). This may not be truly reflective of the CVS risk current RT protocols imparts on cancer patients. Yet, even in the current era of advanced RT techniques such as image-guided radiotherapy, three dimensional dosimetry, and strict adherence of RT protocols dose effect association between RT and cardiac effect seems to exist (8). Analysis of time trends in clinical and experimental studies reporting RT-induced CV disease confirms the increasing interest in understanding the causes, effects, and prevalence of this phenomenon (**Figure 1**). As cancer survivorship and quality of life come into focus it is of paramount importance to understand the basic underlying mechanism of RT-induced cardiac dysfunction. In this review, we briefly outline the pathophysiology of RT-induced CV disease genesis and elaborate on clinical manifestations of CV disease in cancer patients who received RT, our current understanding of factors contributing to this, and propose strategies that can be undertaken to minimize this risk.

Brief Overview of Pathophysiological Manifestations of RT-Induced Cardiovascular Injury

From an RT oncologist's perspective, the heart is a serial organ as well as a parallel organ with the myocardium functioning as individual functional units whereas the vessels function as a single functional unit. Prominent pathophysiological changes noted in individual components of the cardiovascular system are briefly highlighted here.

Pericardial changes following RT are characterized by disruption of microvascular endothelial cells of the pericardium with repeated episodes of ischemia leading to fibrosis and formation of initial fibrinous exudates that are eventually replaced by fibroblasts and collagen (9). Endocardial changes following RT are most notable in the coronary vessels where ultrastructural changes in the capillary networks result in reduced capillary: myocyte ratio, damage to the epicardial vessels leading to upregulation of transforming growth factor-beta leading to a prothrombotic state, and activation of nuclear factor-kB leading to sustained inflammation (10–13). This may predispose to acceleration of atherosclerosis in view of increased recruitment of monocytes and macrophages to sites of active inflammation as well as vessel lumen occlusion secondary to prothrombotic milieu. The damage to the endothelium leads to migration of monocytes to the tunica intima and engulfment of lipoproteins with eventual formation of fatty streaks even in the absence of preexisting atherosclerosis (14). Risk factors such as hyperlipidemia appear to shorten the time to atherosclerosis development, as supplementing animals with fat diets increases the degree of atherosclerosis in rabbits undergoing RT, suggesting an additive effect of irradiation and other risk factors in producing RT-induced atherosclerosis (15). Japanese atomic bomb survivors had increased blood levels of pro-inflammatory cytokines like interleukin-6, C-reactive protein, and tumor necrosis factor-alpha suggesting an indirect association between chronic inflammation and RT-induced vascular damage (16). In experimental mice models with established atherosclerosis, RT tends to cause ultrastructural alterations in plaques leading to intraplaque hemorrhage, infiltration of macrophages leading to an unstable plaque that is vulnerable to thrombosis (17). The RT-induced inflammatory changes and the alterations in the endothelium act in cohesion to modulate the process of atherosclerosis in the coronary vessels. Hence, RT alone can initiate atherosclerosis and in addition can act to accelerate already established atherosclerosis. Myocardial changes following RT include myocardial fibrosis as a consequence of endothelial cell degeneration of myocardial capillaries. Direct myocardial injury is compounded by endothelial injury resulting in collagen deposition in the lumen of capillaries, stenosis of these vessels, and worsening myocardial blood supply creating a vicious cycle of reduced blood supply and continual fibrotic remodeling of the myocardium (18).

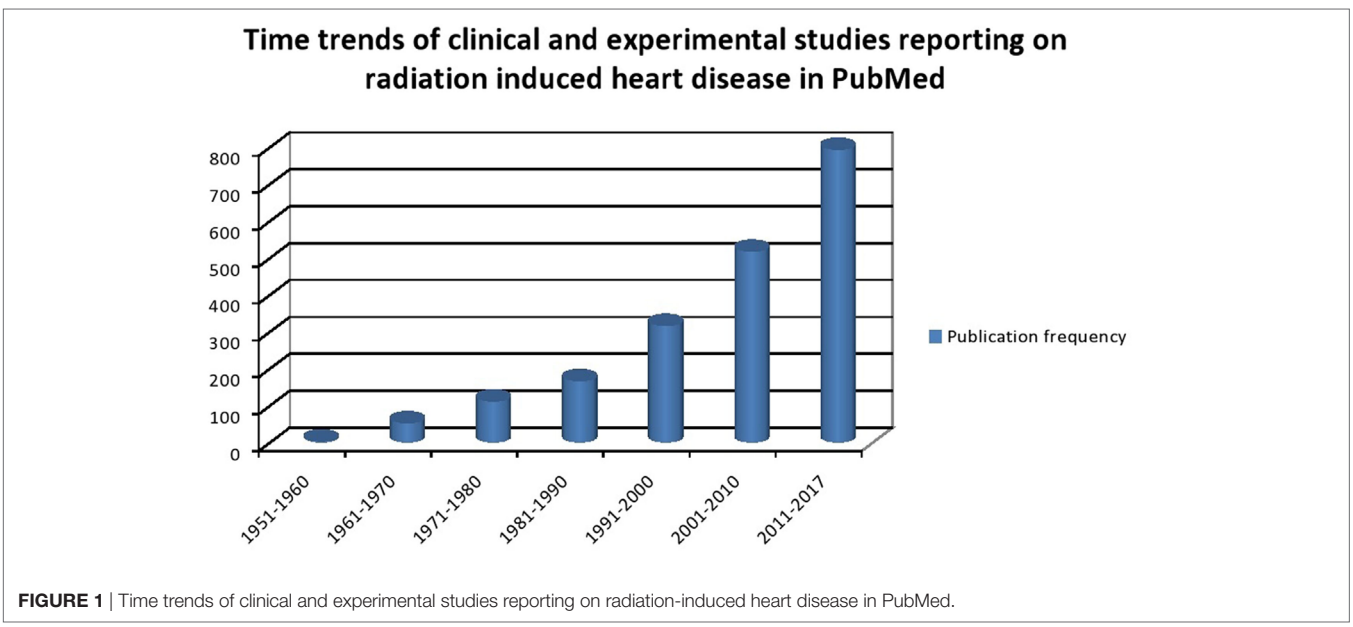

FIGURE 1 | Time trends of clinical and experimental studies reporting on radiation-induced heart disease in PubMed.

In addition to pericardial, myocardial, and endocardial injury, the cardiac valves and conduction system suffer from RT-induced injury as well. This may manifest as regurgitant valvular disease initially due to physical retraction of valves and stenotic disease later on due to fibrotic thickening, calcification, and valve retraction. Left-sided valves are found to be more frequently affected with one autopsy study showing diffuse valve fibrosis in 79% of mitral or aortic valves (19–21). RT-induced changes affect the vagus nerve/carotid sinus altering the baroreceptor reflex leading to elevated baseline heart rate (HR) and abnormal heart rate recovery (HRR) (22). Alternatively, a compensatory increase in the concentration of beta adrenergic receptors with increased stimulation of the sympathetic nerves resulting in autonomic dysfunction may occur following RT-induced myocardial injury (23). Diffuse fibrosis post-RT might lead to alteration of conduction pathways with associated fibrosis of sinoatrial node leading to rhythm changes and eventual complete heart block (CHB) (24).

Clinical Manifestations of RT-Related Heart Disease

Radiation can cause pericardial disease, ischemic heart disease (IHD), valvular disease, conduction system disease, autonomic changes, and cardiomyopathy (25, 26).

Pericardial Disease

Pericardial changes are the most frequent RT-induced CV disorder.

Acute Pericarditis

Acute pericarditis is a rare manifestation that may occur during or immediately after RT. Such is the rarity that even in high volume centers only eight RT-induced inflammatory pericarditis were reported over a period of 30 years (7). It presents with chest pain in the vicinity of RT therapy, in association with a rise in inflammatory markers such as neutrophil count and erythrocyte sedimentation rate. The electrocardiogram (ECG) may or may not show classic findings of pericarditis. Treatment is with non-steroidal anti-inflammatory drugs (NSAIDs) and colchicine. Steroid should only be used in resistant or cases unresponsive to NSAID, as its use is associated with high relapse of pericarditis (27). It is often benign and if it resolves with NSAIDs, RT need not be stopped. Patients who develop acute pericarditis should be followed up closely, since they have high risk of developing chronic pericarditis (28).

Delayed Pericarditis, with or without Tamponade

Chronic pericardial disease can develop months to years after completion of RT therapy and may present as large pericardial effusion. Accumulation of protein rich exudate in the pericardial sac may lead to pericardial effusion and the rapidity of accumulation may result in cardiac tamponade. Dyspnea, orthopnea, chest pain with clinical signs of distant heart sounds, hypotension, and distended jugular veins might serve as pointers for diagnosis. Echocardiography remains the gold standard for definitive diagnosis and to rule out a tamponade. Fukada et al. reported that mediastinal RT field width of >8 cm for esophageal irradiation was associated with increased incidence of pericardial effusion (29). Wei et al. in a series of inoperable carcinoma esophagus treated with chemo radiotherapy reported that the median time for onset of pericardial effusion was 5.3 months (range, 1.0–16.7 months). The dose volume parameter V30 (volume of pericardium receiving 30 Gy) more than 46% was associated with 73% pericardial effusion rate at 18 months post-therapy compared with 13% when V30 was less than 46% ($p = 0.001$) (30). RT-induced pericardial effusion should always be a diagnosis of exclusion with due emphasis on other causative factors. Most effusions are self-limiting, and cardiac tamponade requires emergency pericardiocentesis. Recurrent effusions might require a pericardiotomy.

Constrictive Pericarditis (CP)

Constrictive pericarditis is a long-term sequelae of any cardiac inflammatory pathology. Long-term survivors of pediatric Hodgkin's lymphoma were found to have an incidence of 7% CP among 86 patients (10). Patients may present with intractable heart failure. Surgical removal of parietal pericardium is the treatment of choice for CP. Avgerinos et al. reported that over a 15-year period of 36 patients undergoing pericardiotomy for CP post-RT related were 8.3% ($n = 3$) (31). Bertog et al. reported of 163 patients who underwent pericardiectomy for CP and found that post-RT CP had the worst 7-year survival rate of 27% (95% CI 9–58) (32).

Coronary Artery Disease (CAD)

Researchers from Sweden examined the breast cancer and CAD registry and tried to correlate the RT portals and stenosis of coronary vessels. Left-sided breast cancer patients tended to have stenosis in the distal left anterior descending (LAD) artery and distal diagonal artery. In the analyses of women with breast cancer and RT hotspot areas [>15 mm volume outside the planning target volume (PTV) which receives dose larger than 100% of the specified PTV dose], the severity of coronary stenosis increased with the increase in the hotspot areas. Correa et al. assessed in 961 patients the pattern of CAD for early-stage breast cancer who received RT and subsequently underwent cardiac stress testing and/or catheterization for cardiovascular symptoms. At a median time of 12 years post-RT (range, 2–24 years), higher rate of stress test anomalies was found in the left (59%) versus right-side irradiated patients (8%) ($p = 0.001$). 70% of coronary changes were in the LAD artery (33). In a further analysis, the authors reported that mortality from any cardiac cause was 3.5% in left-sided vs. 2% in right-sided breast cancer patients. In the second decade post-RT, cumulative risk of cardiac deaths was 6.4% (95% CI, 3.5–11.5) for left-side vs. 3.6% (95% CI, 1.8–7.2) for right-sided patients. Chest pain, CAD and myocardial infarction were higher in left-sided radiated patients ($p < 0.002$). HT was associated with higher risk of CAD (34). Pooled analysis of dose-escalation studies in 127 patients in stage III non-small cell lung cancer showed that the incidence of 2- and 4-year rates of symptomatic cardiac events were 10 and 18%. The cohort who had CVS events had higher heart doses than patients without events (heart mean dose, 20 vs. 10 Gy; V5Gy, 56% vs. 34%; V30Gy, 29% vs. 12%, respectively). Baseline CAD was also higher in the cohort with major events (35 vs. 8%), which suggests that RT may contribute to acceleration of atherosclerosis (35).

Darby et al. reported from a population based surveillance study for major coronary events in 2,168 women post-RT and

found that major coronary events had a linear dose response relationship with mean cardiac dose. Major coronary events in the first decade were 44% and 33% between 10 and 19 years and 23% greater than 20 years. Per gray increase in mean heart dose resulted in 7.4% (95% CI, 2.9–14.5; $p < 0.001$) linear increase in the incidence of major coronary events. Baseline existence of cardiac risk factors contributed to the magnified risk from irradiation (5). Left-sided irradiation resulted in higher coronary events compared with right-side irradiation. Women with preexisting IHD, diabetes, respiratory diseases, smokers, and circulatory diseases had higher risk of coronary events. Mean RT dose to the heart to ≥10 Gy resulted in 116% (95% CI, 59–195) increase in occurrence of major coronary events. The mean RT dose to heart was a better predictor of major coronary events compared with mean doses to LAD coronary artery (5). Early Breast Cancer Trialists' Collaborative Group reported that the vascular mortality was significantly increased with RT (death rate ratio 1.30; SE 0.09) (36). Caution should be emphasized in interpretation of above mentioned data, since most of these patients had received RT with older modalities, with extended internal mammary fields, lack of standardized RT protocols and lack of advanced volume based dosimetry tools.

Clinical presentation for post-RT-induced CAD is variable and includes chest pain, dyspnea, heart failure, syncope, or even sudden death. Post-surgery somatic phantom pain, RT-induced skin fibrosis, post herpetic neuralgia, costochondritis, RT-induced fatigue, and reflux disease in post-RT esophagus patients are potential confounders that may mimic angina chest pain. Treatment principles of RT-induced CAD are similar to general population. However, if coronary artery bypass graft is required, bypass using internal mammary graft may not be possible in all cases, as this vessel may also be affected by RT (37). There is also a higher rate of restenosis in stented and bypassed vessels (38). Patients may have asymptomatic IHD, and this is frequently identified on stress testing (39).

Valvular Heart Disease (VHD)

Valvular heart disease usually develops many years after completion of RT therapy. Patients irradiated >20 years before had increased incidence of aortic regurgitation (60 vs. 4%, $p < 0.0001$), tricuspid regurgitation (4 vs. 0%, $p = 0.06$), and aortic stenosis (16 vs. 0%, $p = 0.0008$) than patients within 10 years. Left ventricular fractional shortening and age- and gender-adjusted left ventricular mass was lower in irradiated patients (40). The risk of developing VHD in patient exposed to RT increases linearly with the RT dose. VHD rate correlated with the dose to the affected valve ($p < 0.001$) than to the prescribed mediastinal dose ($p = 0.003$) (41). Although majority of the patient have mild to moderate VHD, a close follow-up is essential as some patients may develop significant valvular disease, needing surgical or percutaneous intervention. Few reports have stated that the mean duration for an asymptomatic VHD to transform to symptomatic VHD is 5 years (20).

Cardiomyopathy and Congestive Heart Failure (CHF)

Exposure to high dose of RT leads to myocardial fibrosis. Diffuse myocardial fibrosis prevents the myocardium from functioning in unison and leads to systolic heart failure. The failing myocardium activates the renin–angiotensin–aldosterone mechanism and sympathetic overactivation resulting in ventricular remodeling which further exacerbates heart failure symptoms. The presentation may be similar to that of CP with effusion, but the symptoms are not resolved with fluid drainage or pericardial stripping (20). Left ventricular ejection fraction changes to the tune of 7–15% are found in patient's treated with predominantly anterior weighted fields (42). Mulrooney et al. reported cardiac outcomes in a cohort of 14,358 5-year survivors as part of Childhood Cancer Survivor Study. They found that the children had a hazard ratio of 5.9 (95% CI 3.4–9.6; $p < 0.001$) for the incidence of CHF. RT exposure to heart of ≥15 Gy increased the hazard of CHF, MI, and VHD by two to six times in comparison with non-irradiated survivors. The risk of adverse cardiac outcomes persisted even up to 30 years of follow-up (43). Lind et al. reported that myocardial perfusion abnormalities were seen up to 6% in the LAD distribution compared with baseline. They found that percent irradiated left ventricle ($p < 0.001$), hormonal therapy ($p = 0.005$), and pre-RT hypercholesterolemia ($p = 0.006$) were factors associated with the perfusion defects (44). These subclinical perfusion defects may lead to microvascular ischemic changes leading areas of infarction leading eventually to fibrosis, and this is a slow process with latency and eventual cardiac function compromise.

Conduction System

In the acute phase, most patients have nonspecific ECG changes in relation to RT therapy. Gomez et al. reported that poor R wave progression and septal ST changes were the most common findings. RT-associated right bundle branch block is seen in patients who have undergone mediastinal irradiation due to close proximity of right bundle to the endocardium (45). Patients may rarely present with CHB years after completion of RT, and some of these patients may need a permanent pacemaker. Timeline for occurrence for CHB is variable ranging from 1–23 years post-RT (46).

Autonomic Dysfunction

Patients undergoing mediastinal RT are at risk of developing autonomic dysfunction. Groarke et al. tried to correlate the exercise capacity parameters in patients of Hodgkin's lymphoma who received RT as part of treatment. Autonomic dysfunction parameters such as elevated resting HR (≥80 beats/min) and abnormal HRR at 1 min (≤12 beats/min if active cool-down or ≤18 beats/min if passive recovery) were elevated in irradiated patients. Incidence of autonomic dysfunction increases with RT dose and time as a factor. Elevated resting HR and abnormal HRR were associated with inferior exercise capacity. Abnormal HRR had a higher hazard of all-cause mortality (hazard ratio 4.60; 95% CI: 1.62–13.02) (22). Coexistence of DM may contribute to autonomic dysfunction, and angina chest pain changes may go unnoticed.

Pulmonary Veno-occlusive Disease

Pulmonary veno-occlusive disease is a rare accompaniment of post-RT and has been reported only in a few anecdotal case reports. Thrombus formation, fibrosis, hyperplastic lymphatics, haemosiderosis leads to alteration in the arterial and venular

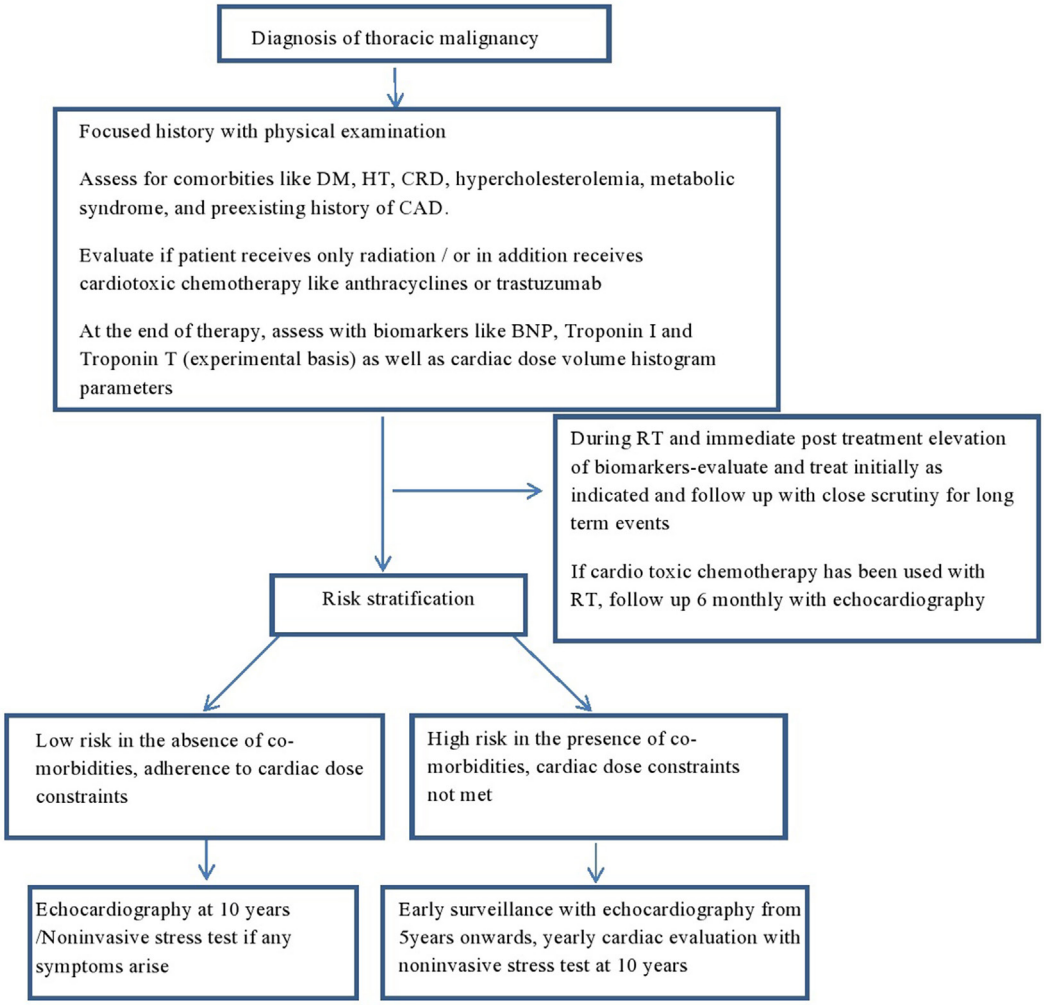

FIGURE 2 | Proposed algorithm for follow-up in irradiated patients with thoracic malignancies. DM, diabetes mellitus; HT, hypertension; CRD, chronic renal disease; CAD, coronary artery disease; BNP, brain natriuretic peptide; TnI, troponin I; TnT, troponin T; RT, radiation.

endothelium with arteriosclerosis and narrowing of vasculature may contribute to development of pulmonary HT (47).

Biomarkers of RT-Induced Cardiac Injury

Troponin T (TnT), troponin I (TnI), and brain natriuretic peptide (BNP) are potential biomarkers for cardiac injury. Gomez et al. did an exploratory study to assess TnI and BNP levels during RT for thoracic malignancies. The median BNP remained elevated for one patient post-RT ($p = 0.042$). BNP did not increase over time in the 18 patients who received only RT (45). Palumbo et al. tried to correlate BNP with cardiac dosimetry in left-sided breast cancer patients receiving adjuvant RT. BNP levels were elevated at ($p < 0.001$) 1 and 6 months after RT. Normalized BNP was significantly associated with V20, V25, V30, V45, mean heart dose, and maximum heart distance ($p < 0.05$). Four patients had coronary events and in the one patient with MI V20, V25, V30 and V45 were the highest, and BNP elevation was persistent from the 1–12 months of follow-up. BNP can serve as a surrogate marker for predicting post-RT cardiac events (48). TnT is an indicator of myocardial injury. Association of TnT levels with cardiac RT doses and echocardiography was assessed in a cohort of 58 breast cancer patients post-RT. TnT were elevated in 21% of patients during RT. Higher RT doses for the whole heart ($p = 0.02$), left ventricle ($p = 0.03$), volume of LAD artery receiving 15 Gy ($p = 0.03$), and 20 Gy ($p = 0.03$) were associated with elevation of TnT. Changes in the interventricular septum thickness and prolongation of the deceleration of ventricular contraction were also noted in irradiated patients (49). These studies only evaluated cardiac markers in the short term, whether it translates in predicting long-term outcomes remains to be elucidated.

SURVEILLANCE PRINCIPLES

Patients who are exposed to mediastinal RT or RT in the vicinity of the heart should be followed regularly and lifelong. A careful baseline history and physical examination is mandatory.

Any patient with murmur or clinical risk factor should have a baseline echocardiogram. In addition, all patients should have a baseline lipid and thyroid function tests. There is no large scale data to support any specific pattern of clinical or imaging follow-up, but it reasonable to do a yearly clinical follow-up, and in asymptomatic patients a follow-up echocardiogram at 5 years in high risk patients and at 10 years in rest of the patients with a functional non-invasive stress test at 5–10 years in the high risk group. Another approach would be to obtain an echocardiogram at 5 and 10 years only if heart-related symptoms or murmur and consideration of stress test or CT scan of coronary artery after 10 years following mediastinal RT. At all follow-up, a careful history and physical examination is crucial. As some patients may develop asymptomatic pericardial effusion, the chest X-ray and CT scans that are frequently and routinely done in these patients for follow-up and staging should be carefully reviewed on each visit. A proposed algorithm for follow-up is illustrated in **Figure 2**.

CONCLUSION

Cancer and cardiovascular disease are bound to present or develop as the life span of people keeps improving. Although most of the published studies on RT-associated cardiovascular events were from the older era of RT technology, some potential risk still persists as evidenced from the dose-escalation studies from lung cancer. Awareness of its underlying pathophysiology and the myriad of manifestations is required to detect and rehabilitate this cohort of patients. Risk prediction models and scrutiny of potential biomarkers may serve to predict the subset of patients who may develop cardiac events in the distant future. Heightened awareness of this complication following RT should result in adoption of careful surveillance programs during post-RT follow-up of patients. Increasingly, this will require close collaboration between RT oncologists, internists, radiologists, and cardiologists wherever possible. In an ideal program, a centralized registry of RT-induced CVS disease incidence and prevalence would be maintained at cancer centers in a manner similar to the tumor registry and would enlist active participation of oncologists, cardiologists, and radiologists.

AUTHOR CONTRIBUTIONS

Conception or design of the work, drafting the work, and final approval of the version to be published—SY, BV, LM, and SK. Agreement to be accountable to the accuracy or integrity of any part of the work are appropriately investigated and resolved—SY.

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Developing a Reliable Mouse Model for Cancer Therapy-Induced Cardiovascular Toxicity in Cancer Patients and Survivors

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Background: The high incidence of cardiovascular events in cancer survivors has long been noted, but the mechanistic insights of cardiovascular toxicity of cancer treatments, especially for vessel diseases, remain unclear. It is well known that atherosclerotic plaque formation begins in the area exposed to disturbed blood flow, but the relationship between cancer therapy and disturbed flow in regulating plaque formation has not been well studied. Therefore, we had two goals for this study; (1) Generate an affordable, reliable, and reproducible mouse model to recapitulate the cancer therapy-induced cardiovascular events in cancer survivors, and (2) Establish a mouse model to investigate the interplay between disturbed flow and various cancer therapies in the process of atherosclerotic plaque formation.

Methods and Results: We examined the effects of two cancer drugs and ionizing radiation (IR) on disturbed blood flow-induced plaque formation using a mouse carotid artery partial ligation (PCL) model of atherosclerosis. We found that doxorubicin and cisplatin, which are commonly used anti-cancer drugs, had no effect on plaque formation in partially ligated carotid arteries. Similarly, PCL-induced plaque formation was not affected in mice that received IR (2 Gy) and PCL surgery performed one week later. In contrast, when PCL surgery was performed 26 days after IR treatment, not only the atherosclerotic plaque formation but also the necrotic core formation was significantly enhanced. Lastly, we found a significant increase in p90RSK phosphorylation in the plaques from the IR-treated group compared to those from the non-IR treated group.

Conclusions: Our results demonstrate that IR not only increases atherosclerotic events but also vulnerable plaque formation. These increases were a somewhat delayed effect of IR as they were observed in mice with PCL surgery performed 26 days, but not 10 days, after IR exposure. A proper animal model must be developed to study how to minimize the cardiovascular toxicity due to cancer treatment.

Keywords: cancer treatment-related cardiovascular toxicity, atherosclerosis, disturbed blood flow, p90RSK, ionizing radiation

INTRODUCTION

Cancer therapy, including anthracyclines, cisplatin, tyrosine kinase inhibitors, hormone deprivation agents, and ionizing radiation (IR) can affect not only the heart but also the vasculature and its reactivity. For example, Chaosuwannakit et al. showed that proximal aortic wall stiffness increased 3 months after anti-cancer treatments after they had standardized other potential contributing factors such as age, sex, diabetes, and hypertension. The authors showed that the aortic stiffness increase occurred soon after the administration of cancer therapy and was equivalent to the stiffness increase due to 10 to 20 years of aging (1, 2). Of note, aortic stiffness increases with age (3), cardio-metabolic abnormalities (4, 5), and increased sodium intake (6), all of which are associated with left ventricular dysfunction (LVD) (7). The accelerated aortic stiffness in the patients with Hutchinson-Gilford progeria syndrome (premature aging) is well known (8). Importantly, the strong association between arterial stiffness and increased ventricular afterload, LVD, hypertrophy, and adverse vascular events including atherosclerosis and subsequent CV mortality has been well established (9, 10). In fact, higher incidence of CV events in cancer survivors has also been well established.

Recently, possible involvement of vascular dysfunction in the initiation of cancer therapy-related cardiac dysfunction (CTRCD) has been suggested. For example, Narayan et al. reported that ventricular-arterial coupling (effective arterial elastance [Ea]/end-systolic elastance [Ees_{sb}]) could be used to predict CTRCD (11). Importantly, they found that the association between Ea/Ees_{sb} and CTRCD was driven primarily by Ea. Therefore, they suggested that increased arterial elastance and alterations in arterial stiffness in ventricular-arterial coupling occur with and in advance of ejection fraction (EF) deterioration. Furthermore, Drafts et al. reported that thoracic aortic pulse wave velocity (PWV), as detected by phase-contrast cardiac magnetic resonance, increased within 6 months after a low to moderate dose of anthracycline-based cancer therapy, suggesting that cancer therapy is associated with an early and persistent increase in aortic PWV, which is a measure of arterial/aortic stiffness (12). Interestingly, Ohyama et al. recently reported a strong correlation between aortic arch stiffness and LV function (13). They found that a higher aortic arch PWV was associated with impaired LV systolic function. The association between PWV and a lower early diastolic strain rate, a marker of early diastolic dysfunction, was also demonstrated in this MRI study (13). Taken together, these data suggest that cancer therapy can affect not only cardiac function but also vascular function and subsequent development of atherosclerosis.

Radiation therapy (RT) is one of the crucial interventions to control primary thoracic malignancies, breast cancer, and lymphoma. It is now clear, however, that RT causes delayed negative effects on the CV system, leading to morbidity and mortality among cancer survivors. For example, among the survivors of Hodgkin's lymphoma, CV events after RT to the chest area are increased by up to 7-fold based on multivariate analyses controlling for other risk factors (14), and CV events including ischemic heart disease and heart failure are the leading causes of non-oncologic deaths (15). In addition, significantly increased mortality due to CV disease in breast cancer survivors after RT has also been reported(16). In patients with locally advanced non-small cell lung cancer, the 2 year incidence of grade ≥3 CV toxicity induced by RT was 11% in a combined analysis of 4 prospective studies (17). Lastly, it has been suggested that deaths linked to unavoidable IR exposure of the heart could offset any benefit of increasing the radiation dose to control the disease (18). Therefore, RT-induced CV toxicity is a significant factor when determining the survival of cancer patients who receive RT to the upper body. The incidence of numerous cardiovascular disorders is increased after RT, including coronary artery disease, congestive heart failure, and valvular disorders (19).

It is interesting to note that various forms of cancer treatments whose mechanisms of action are different cause similar cardiovascular pathological phenotypes. For example, Lipshultz et al. have reported that cancer survivors treated by so-called "non-cardiotoxic" drugs show pathological cardiovascular phenotypes. They have shown that not only the survivors treated with cardiotoxic drugs, but also those treated with so-called "non-cardiotoxic" drugs also exhibit decreased LV mass and cardiac dysfunction compared to healthy siblings. In addition, cancer survivors exposed to both cardiotoxic and so-called "non-cardiotoxic" drugs had a higher mean body mass index, higher fasting serum non-high-density-lipoprotein, higher insulin levels, and higher sensitivity C-reactive protein levels, suggesting that all cancer survivors exposed to any type of cancer therapy had a high risk of CTRCD (20). Lipshutz's group suggested that one of the common phenotypes observed in these patients is premature aging (20).

There are two types of blood flow in large arteries, namely, laminar flow (l-flow) and disturbed flow (d-flow). It has been well established that atherosclerotic plaques are rare in areas exposed to laminar flow, which induces anti-inflammatory signaling and anti-atherogenic gene expression in endothelial cells (ECs) (21, 22). In contrast, atherosclerotic plaques localize to areas of d-flow found in regions where vessels curve acutely, bifurcate, or branch. Previously, we have reported that d-flow induces p90RSK activation and expression (23), and up-regulates inflammation, apoptosis, and proliferation of ECs; and reduces vascular reactivity (24). Since both IR and d-flow can increase EC activation (25), it is important to study the possible interplay between d-flow and cancer treatments in regulating EC functions, but to our knowledge, there has been no study focused on the simultaneous assault of these two stimuli on ECs. Therefore, we decided to establish an animal model to investigate the effect of various cancer treatments including IR on d-flow-induced plaque formation in this study. The goals of this study are: (1) to generate an affordable, reliable, and reproducible mouse model that recapitulate the cancer therapy-induced cardiovascular events in cancer survivors and (2) to establish a mouse model to investigate the interplay between disturbed flow and various cancer therapies in the process of atherosclerotic plaque formation.

METHODS

Antibodies utilized for immunohistochemistry and immunofluorescence were against smooth muscle actin (SMA;#M0851, DAKO, CA), MAC3 (#550292, BD, NJ), pan p90RSK (cat #MAB2056, Novus Biologicals, LLC, CO), and phospho-p90RSK (Ser380, # 9341) from Cell Signaling Technology (Danvers, MA).

Doxorubicin and Cisplatin Treatment

Mice received doxorubicin (5 mg/kg of body weight; intraperitoneal injection; Cat# NDC 67457-436-50, Mylan Institutional LLC, IL) or saline on day 0, 7 and 14.

Mice received cisplatin (2.3 mg/kg of body weight; intraperitoneal injection; Cat# NDC 0703-5747-11, Teva Pharmaceuticals USA, Inc., PA) for 5 consecutive days, rested for the next 5 days, and then restarted the same daily injections for 5 more days. In the preliminary experiments, we found that mice showed severe weight loss after the cisplatin treatment. Therefore, we add 0.5 ml of phosphate-buffered solution (PBS; NaCl 137 mmol/L, KCl 2.7 mmol/L, Na_2HPO_4 10 mmpl/L, KH_2PO_4 1.8 mmol/L) subcutaneous infusion immediately after cisplatin treatment. The treatment regime we described in this study is within the range of human treatment regime variation (26–28). As we describe repeatedly the purposes of this study are (1) to generate tan affordable, reliable, and reproducible mouse model to recapitulate the cancer therapy-induced cardiovascular events in cancer survivors, and (2) to generate a mouse model to investigate the interplay between disturbed flow and various cancer therapy in the process of atherosclerotic plaque formation. And our purpose is not to find the regimen to cure cancer. Therefore, the rationales we used for these treatment regimens are: (1) the treatment regimen is within the range of human treatment and also used in the previous studies in mice and (2) the treatment regimens should not significantly affect the health of each mouse.

Housing and Husbandry

Mice were housed in pathogen-free conditions at the Texas A&M Institute of Biosciences and Technology and the University of Texas MD Anderson Cancer Center. The Program for Animal Resources is AAALAC certified and defined pathogen free facility for housing mice and rats. Cage level barrier system was used, diet was irradiated, ultra-filtered water, heat treated wood chip bedding. And enrichment material (nestlets) was provided. Cages and water were changed on a regular weekly basis. Animals were handled under hepa-filtered change station. Environmental parameters (room lighting, temperature and humidity) were computer monitored as follows, (1) Temperature: Set point 72F (high limit 74F, low limit 70F), (2) Humidity: Set point 45% (high limit 55%, low limit 40%), (3) Light cycle: 12 h light, 12 h dark, (4) Air changes 10–15 times per hour. The vivarium was staffed seven days a week by animal caretakers, including week-ends & holidays. Veterinary care and oversight was provided by contract veterinarian who visited facilities on a regular basis and was also available for consultation by phone/email.

Mice, Left Carotid Artery Partial Ligation, and Atherosclerosis

Ldlr$^{-/-}$ mice were obtained from The Jackson Laboratory, Bar Harbor, ME, USA. Eight- to twelve-week-old mice were fed an adjusted-calorie (high-fat) diet (HFD) consisting of 21% crude fat, 0.15% cholesterol, and 19.5% casein (cat. no. TD.88137; Envigo, NJ, USA) (24) as indicated. Since we did not find a significant difference in the response to cancer therapy, we used both males and females. The number of each sex in each experiment was added in each figure. The weight range of the mice was 15–25 g. Genotyping was performed based on the Jackson Lab protocol, and we confirmed that the mice were homozygous. No previous procedures were performed before the experiments in this study. To induce atherosclerosis by d-flow, we performed partial ligation of the left carotid artery (LCA) as we had described (23). We conducted a double-blind, randomized study and the persons who evaluated the size of the plaque were blinded until the data analysis was complete. Briefly, mice were anesthetized by 2.0% isoflurane, placed on a heated surgical pad, and subcutaneously given 5mg/kg Caprofen. Isoflurane was maintained at a level between 1.0 and 2.0%. Classic Vaporizer unit from Braintree Scientific (Cat. # EZ-150C) was used for the delivery and regulation of isoflurane during surgery. We followed the approved protocol by IAUCC and IBT committee for the choice of anesthetic drug, route of administration, and dose. After hair removal, a midline cervical incision was made and the internal and external carotid arteries were exposed and partially ligated with 6.0 silk suture, leaving the occipital artery patent (**Figure S1**). The skin was sutured with absorbable 6.0 silk suture in a running subcuticular pattern. Mice were allowed to recover in a clean cage on a heated pad. For the atherosclerosis study, mice were fed a HFD (TD88137, Envigo), at which time their carotid arteries were harvested. Each experiment was performed during 9 AM to 5 PM at central standard time in USA. Each experiment was performed in the vivarium designated for animal surgery at UT MD Anderson Cancer Center and at the Texas A&M Institute of Biosciences and Technology, Houston TX, 77030. Experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M Institute of Biosciences and Technology and also at the University of Texas MD Anderson Cancer Center.

Monitoring of Mice After Surgery

Mice were monitored until recovery in a chamber on a heating pad following surgery. We injected postoperative analgesia as needed for additional pain relief. We supported the post-operated mice by (1) Warmth: they were placed in a pre-warmed cage (cage was on a heating pad), (2) Fluids: pre-warmed normal saline (37°C, 5 ml per 100 g body weight) was given subcutaneously. We checked on mice every 10 min until mice were awake and moving. Daily for the first 3 days (date of surgery was day 1), then once per week afterwards till conclusion of study as described in our protocol approved by the IAUCC and IBT committee.

Sample Size Calculation

Initially we performed the sample size calculation based on our previous results (23). We expected that (1) the variation (SD) of lesion size in each group will be around 30% from the

mean value, (2) we can detect 100% increase in the lesion size by cancer therapy, and (3) the variation within the vehicle control and the cancer therapy will be similar. Therefore, the optimal number of animals needed to attain statistical significance of $p < 0.05$ with a 90% probability is 5 (based on the power calculator generated by the Laboratory Animal Services Centre at The Chinese University of Hong Kong: http://www.lasec.cuhk.edu.hk/sample-size-calculation.html).

Histology and Evaluation of Atherosclerotic Lesions After Partial Carotid Ligation

To determine disturbed flow-induced atherosclerotic lesions in histological sections, right (control) and left (surgery performed) carotid arteries were dissected out and all tissues were fixed in 10% neutralized buffered formalin. The fixed tissues were embedded in paraffin. Serial sections (5 μm) were made through the entire carotid arteries and stained with Masson's trichrome or H&E (**Figure S1**). To quantify atherosclerotic lesions, the intima area was calculated by subtracting the lumen area from the area circumscribed by the internal elastic lamina. The medial area was determined by the area between the internal and the external elastic laminae. These measurements were made by using ImageJ (http://imagej.nih.gov/ij/). The positions of the internal and the external elastic laminae were also confirmed by Masson's trichrome staining. The extent of atherosclerotic lesion was determined by detecting the intimal and media layers in both left and right carotid arteries.

Immuno-Histochemistry

To identify macrophages and smooth muscle cells (SMCs) within the plaque area, immunohistochemistry (IHC) was performed. Epitope retrieval (HIER) was performed by heating de-paraffinized slides in the HIER buffer containing 10 mM sodium citrate and 0.05% Tween 20 (pH 6) at 100°C for 10–15 min. After cooling the slides down to room temperature (RT), slides were treated with 3% hydrogen peroxide and then were blocked with 5% normal goat serum (Vector laboratories) for 30 min at RT. Primary antibodies were against Mac-3 (1:100, Rat, BD550292) for macrophages and α-smooth muscle actin (SMA) (1:500, Mouse, Ab7817) for SMCs. Secondary antibodies (goat anti-mouse or anti-rat-Biotinylated) were used at 1:1,000 dilutions. Sections were developed by DAB substrate (ImmPACT DAB, SK-4105) and counterstained with hematoxylin.

Grading of Necrotic Core Formation

To quantify necrotic core formation, cross-sectioned carotid arteries were stained by hematoxylin and eosin, and the necrotic core formation was quantified by % of non-cellular area/total lesion area by using ImageJ (http://imagej.nih.gov/ij/). We graded each necrotic core as no necrotic core = 0, ≤5% = 1 or >5% =2 and scored at seven different levels within each carotid artery after partial carotid ligation as shown in **Figure S1**. For each mouse, the sum of the total grades was calculated.

Immunofluorescence Staining

Immunofluorescence staining was performed on paraffin slides as described previously (29). Briefly, the tissue sections were de-paraffinized and incubated with 10% normal goat serum for 30 min. Epitope retrieval (HIER) was performed by boiling de-paraffinized slides in the HIER buffer containing 10 mM sodium citrate and 0.05% Tween 20 (pH 6) at 100°C for 20 min. The slides were then incubated with primary antibodies at 4°C overnight, followed by incubation with Alexa Fluor 647-conjugated secondary antibodies for 60 min at RT. Expression of total p90RSK and phospho-p90RSK (S380) were imaged on an Olympus FX1200 confocal laser scanning microscope.

Irradiation

Mice were irradiated on a Cesium-137 Research-Irradiator "Mark-I Model M68A" (J. L. Shepherd & Associates, San Fernando, CA 91340). The unit is comprised of a sealed pencil-like (37 cm long) radioactive Cesium-137 source producing 662 keV gamma (γ) radiation. *Ldlr*$^{-/-}$ mice were placed in a cylindrical container (inner diameter 20.2 cm, height 13.4 cm). Thin black plastic strips were arranged to create wedge-shaped partitions for 8 mice per cylinder. A round top-cover of clear plastic with a wedge-shaped cut-out was used to conveniently place/retrieve mice one at a time. The mice-loaded container was placed on an acrylic stand 30.1 cm above the axis of the Cesium source. A dose of 2 Gy was delivered in this geometry. Dose uniformity was within 5%. The in-air dose-rate at the irradiation height was measured with an ion-chamber employing the Task-Group Report 61 of the AAPM (American Association of Physicists in Medicine). The chamber had been calibrated by M. D. Anderson's ADCL (Accredited Dosimetry Calibration Laboratory). The chamber calibration is traceable to NIST (National Institute of Standards and Technology).

Serum Lipid Profile Analysis

Mice fasted overnight were euthanized with CO_2, and whole blood was collected in a 1.5 ml tube. Whole blood was allowed to clot for 45 min at RT and centrifuged at 1,500 × g for 30 min at 4°C. The levels of cholesterol (high- and low-density lipoprotein, HDL and LDL, respectively) were determined using cholesterol assay kits (Cat#EHDL-100, Bioassay System, USA).

Quantification of Total and Phosphorylated p90RSK After PCL

For total p90RSK and phosphorylated p90RSK density analysis after PCL, nonsaturated images from the LCA of IR and non-IR treated groups were analyzed with ImageJ software to quantify integrated density of total and phosphorylated p90RSK staining per relevant area (intima vs. media) within each image.

Statistical Analysis

Data are presented as means ± SEM. Differences between two independent groups were determined using the Student *t*-test (two-tailed) and, when applicable, one-way ANOVA followed by Bonferroni post hoc testing for multiple group comparisons

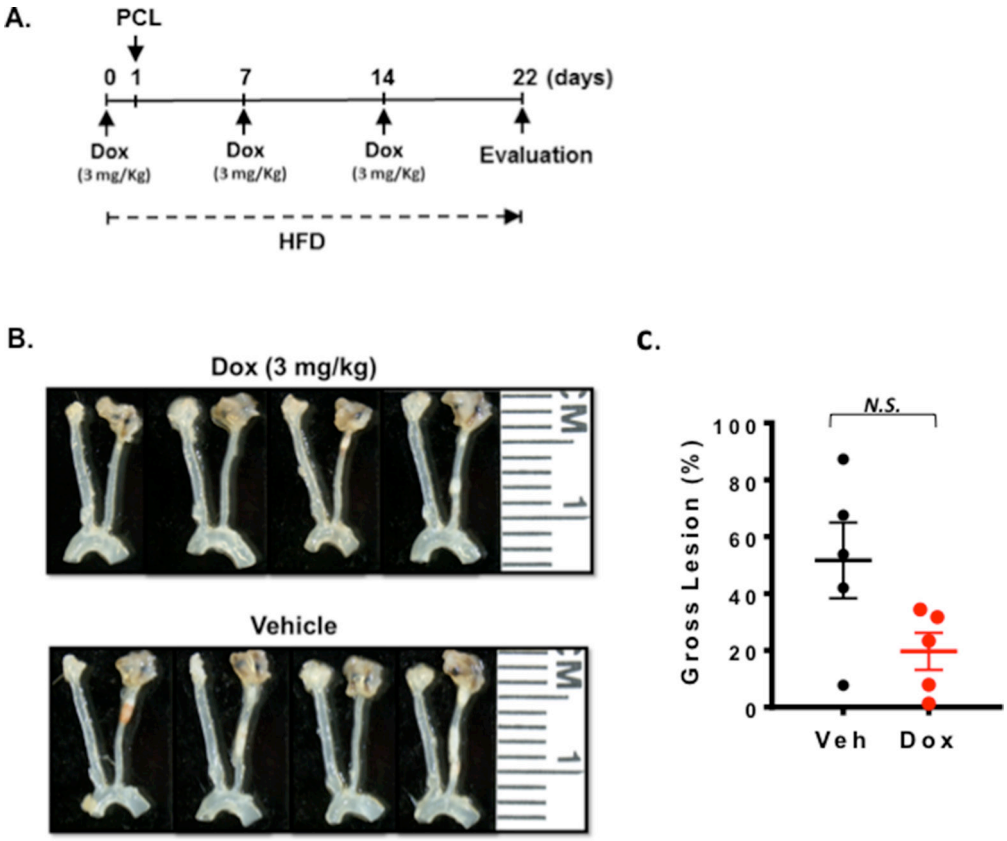

FIGURE 1 | Doxorubicin effect on plaque formation after PCL. All the animals did not show any clear health problem before each experiment. **(A)** The scheme of the study design of doxorubicin treatment and PCL. **(B)** Representative gross lesions from doxorubicin- or saline-treated groups after 3 weeks of PCL in *Ldlr*$^{-/-}$ mice. Left carotid artery is the right-side vessel (smaller of the two carotids) in each sample. **(C)** Gross lesion size after 3 weeks of PCL surgery (% lesion area of total LCA area). 5 males in doxorubicin-treated group and 3 males and 2 female in a vehicle group.

using the Prism software program (GraphPad Software, La Jolla, CA, USA). When groups exhibited unequal variances, Welch's ANOVA was applied to perform multiple group comparisons. *p* values less than 0.05 were considered statistically significant and indicated by an asterisk in the figures.

RESULTS

Effect of Doxorubicin (Dox) on D-Flow-Induced Plaque Formation After PCL

As we presented in the introduction, Dox may induce vascular injury (30, 31). To test this in mice, we injected 3 mg/kg of Dox before and after PCL and harvested carotids 3 weeks later (**Figure 1A**). As shown in **Figure 1B, C**, we found that Dox treatment did not accelerate d-flow-induced plaque formation compared to saline treated mice with this study design.

Cisplatin Injection Before 5 Weeks of PCL Did Not Accelerate Plaque Formation

The role of cisplatin in promoting vascular injury has been reported (32). We first treated mice with 2.3 mg/kg cisplatin via 2 cycles of daily intra-peritoneal injections for 5 days with 5 days of rest between the two cycles. We waited for 5 weeks until the mouse condition became stable after the chemotherapy, and then performed PCL (**Figure 2A**). We harvested carotids and studied plaque sizes. We found that cisplatin treatment showed no effect on body weight (**Figure 2B**) and plaque formation after PCL under this experimental condition (**Figure 2C,D**).

Plaque Formation Was Accelerated by IR After PCL

We could not detect accelerated plaque formation by doxorubicin and cisplatin in our PCL model under the conditions we studied. Mancuso et al. have reported that atherosclerotic lesion formation in irradiated athero-prone ApoE$^{-/-}$ mice was accelerated, especially in the d-flow area (33). Therefore, we applied 2 Gy of γ radiation and performed PCL surgery 1 week later. We harvested carotids 3 weeks after PCL (**Figure 3A**). As shown in **Figure 3B**, we found a significant decrease in body weight after IR compared to the non-IR group. We found no difference in d-flow-induced plaque formation between IR and control groups (**Figure 3C,D**). Another group of mice was allowed to regain body weight after IR, and PCL surgery was performed

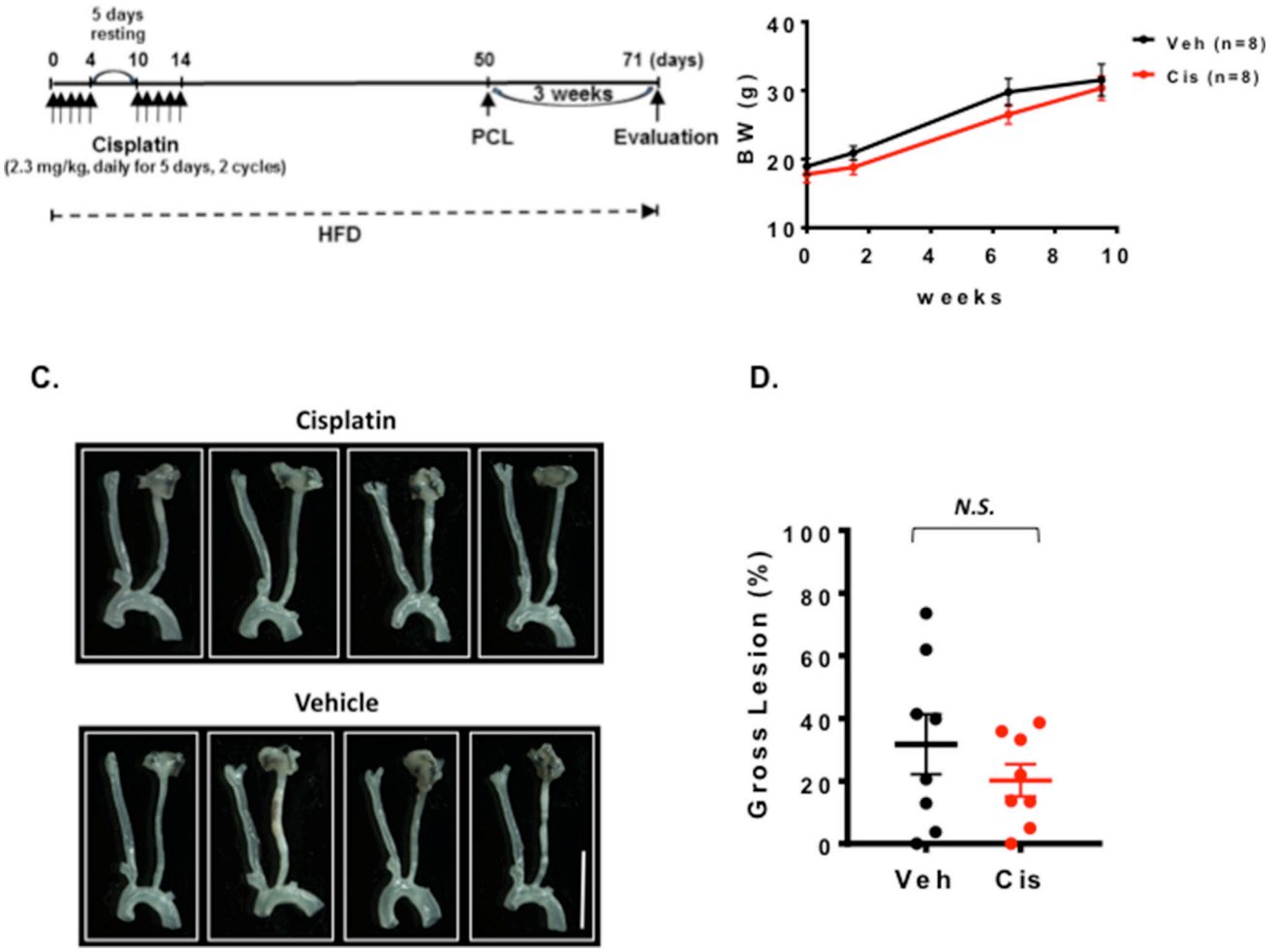

FIGURE 2 | Cisplatin treatment did not change the plaque size after PCL. All the animals did not show any clear health problem before each experiment. **(A)** The scheme of the study design of cisplatin treatment and PCL. (B) Changes in body weight after saline, cisplatin, and PCL surgery (mean ± SEM). **(C)** Representative gross lesions from cisplatin- and saline-treated groups after 3 weeks of PCL in *Ldlr*$^{-/-}$ mice. Scale bar, 0.5 cm. **(D)** Gross lesion size after 3 weeks of PCL surgery (% lesion area of total LCA area). 5 males and 3 females in both cisplatin and vehicle-treated groups.

subsequently (**Figure 4A**). In this regimen, mice were fed a high fat diet for 9 days, received 2 Gy of IR, and fed a normal chow diet for 14 days during which the body weight of the irradiated mice recovered. The normal diet was given during the recovery period in order to avoid the extra plaque formation induced by high fat diet in both IR and non-IR mice. High fat diet feeding was resumed when the body weight returned to the level of the non-irradiated, age matched normal mice, and PCL surgery was performed 12 days later. Carotids were harvested 3 weeks afterwards for atherosclerotic plaque formation (**Figure 4A,B**). With this study design, we were able to detect IR-induced enhancement of plaque formation after PCL surgery at the gross anatomical level (**Figure 4C,D**). We found significant increase of LDL levels in both non-IR and IRgroups after HFD and surgery, but we did not observe any differences in HDL and LDL levels between IR and non-IR groups (**Figure 4E,F**). These lipid data after surgery (non-IR and IR groups) were obtained at the time of sacrifice. The relatively large variation of HDL levels in the non-IR group may be due to the combination of the surgery and normal chow diet feeding for two weeks. IR may decrease this procedure-related variation.

Plaques formed under these conditions were characterized using routine histological methods. Robust plaques were seen in the irradiated and partially ligated carotid artery compared to the non-IR counterpart (**Figure 5A,B**). Interestingly, we found a clear necrotic core (non-cellular area) formation in the plaques of the IR-treated group, but less in the non-IR group (**Figure 5C,D**). In plaques of both groups, we detected a significant increase in macrophage infiltration into the plaque, which distinguishes these plaques from the restenotic lesions seen during vascular intimal formation after vascular injury (**Figure 6**). We did not observe a significant difference in the anti-α-SMA-positive cells in the intimal layer between control and IR groups (**Figure 6**). These data suggest that IR treatment

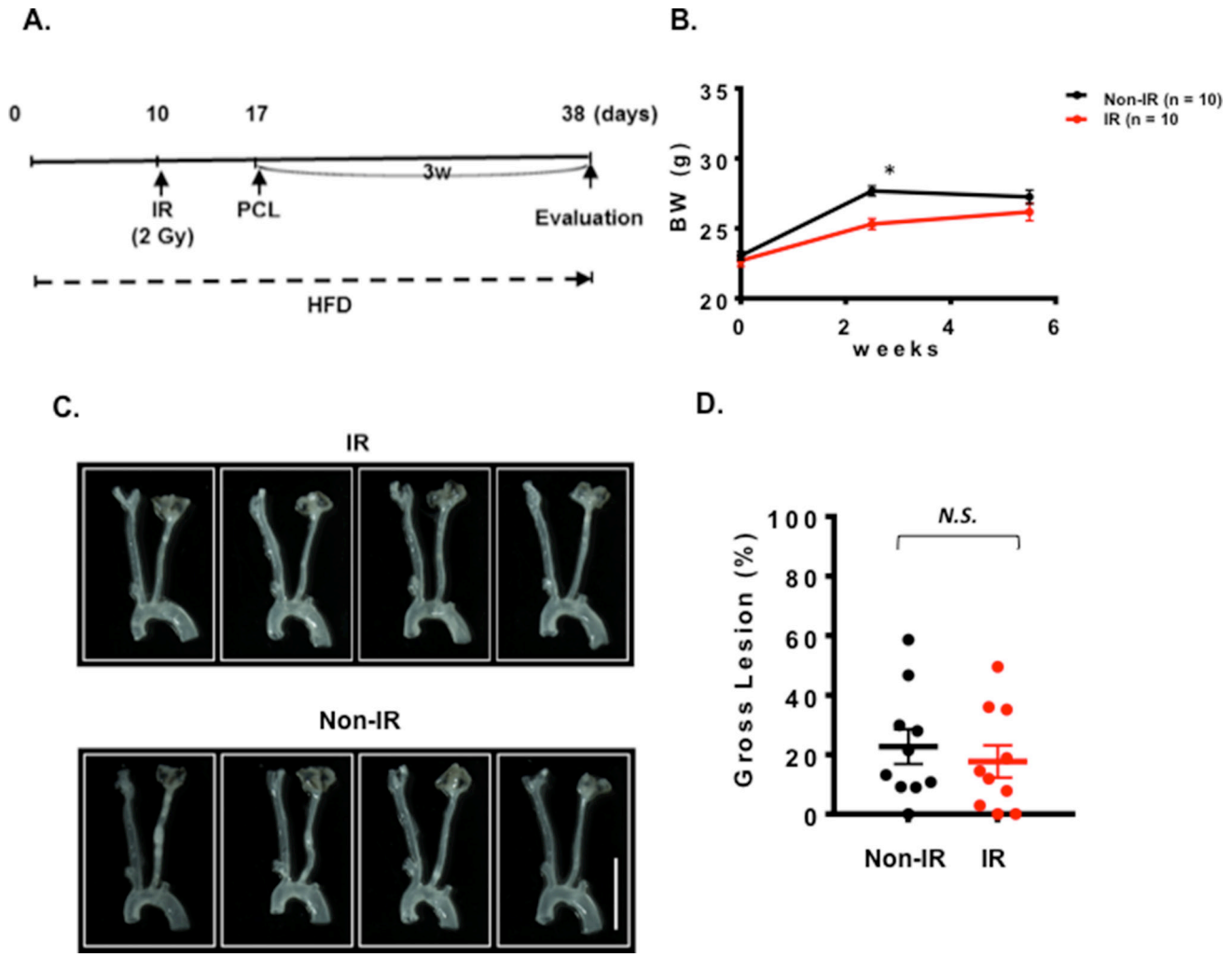

FIGURE 3 | One week interval between IR treatment and PCL did not change the plaque size after PCL. All the animals did not show any clear health problem before each experiment. **(A)** The scheme of the study design of IR treatment and PCL. **(B)** Changes in the body weight after IR, or non-IR treated group and PCL surgery. (mean ± SEM) **(C)** Representative gross lesions from IR- or non-IR-treated groups after 3 weeks of PCL in *Ldlr*$^{-/-}$ mice. Scale bar, 0.5 cm. **(D)** Gross lesion size after 3 weeks of PCL surgery (% lesion area of total LCA area). 10 males in IR-treated group and 10 males on control non-IR group.

induces not only larger plaques but also a more advanced unstable type of plaques characterized by the increased necrotic cores.

IR Increased p90RSK Activation in Vivo

Previously, we have reported the crucial role of p90RSK activation in atherosclerotic plaque formation (24). However, it remains unclear whether p90RSK is activated in the plaques after IR. As shown in **Figure 7**, we found a significant increase of phosphorylated p90RSK in the plaque intima after IR treatment compared to those from non-IR group. We did not find any difference in the total p90RSK expression between IR and non-IR groups. These data suggest the possible role of p90RSK activation in IR-mediated enhancement of plaque formation.

DISCUSSION

It is clear that cancer survivors suffer from higher incidence of cardiovascular events than individuals in the general population. Therefore, it is crucial to establish appropriate animal models that recapitulate this phenomenon for studying the molecular mechanisms and developing therapeutics for the cancer treatment-induced cardiovascular malady. It is well known that disturbed flow accelerates the process of EC inflammation and subsequent atherosclerosis (AS). However, the relationship between disturbed flow and cancer treatment in regulating the development of AS remains unclear. In this study we found that IR (2 Gy) treatment enhanced the size of atherosclerotic plaques after PCL. We also found that IR significantly accelerated formation of vulnerable plaques with the characteristic necrotic core formation. These data suggest that *Ldlr*$^{-/-}$ mouse treated by IR can be a good model for studying AS in cancer survivors.

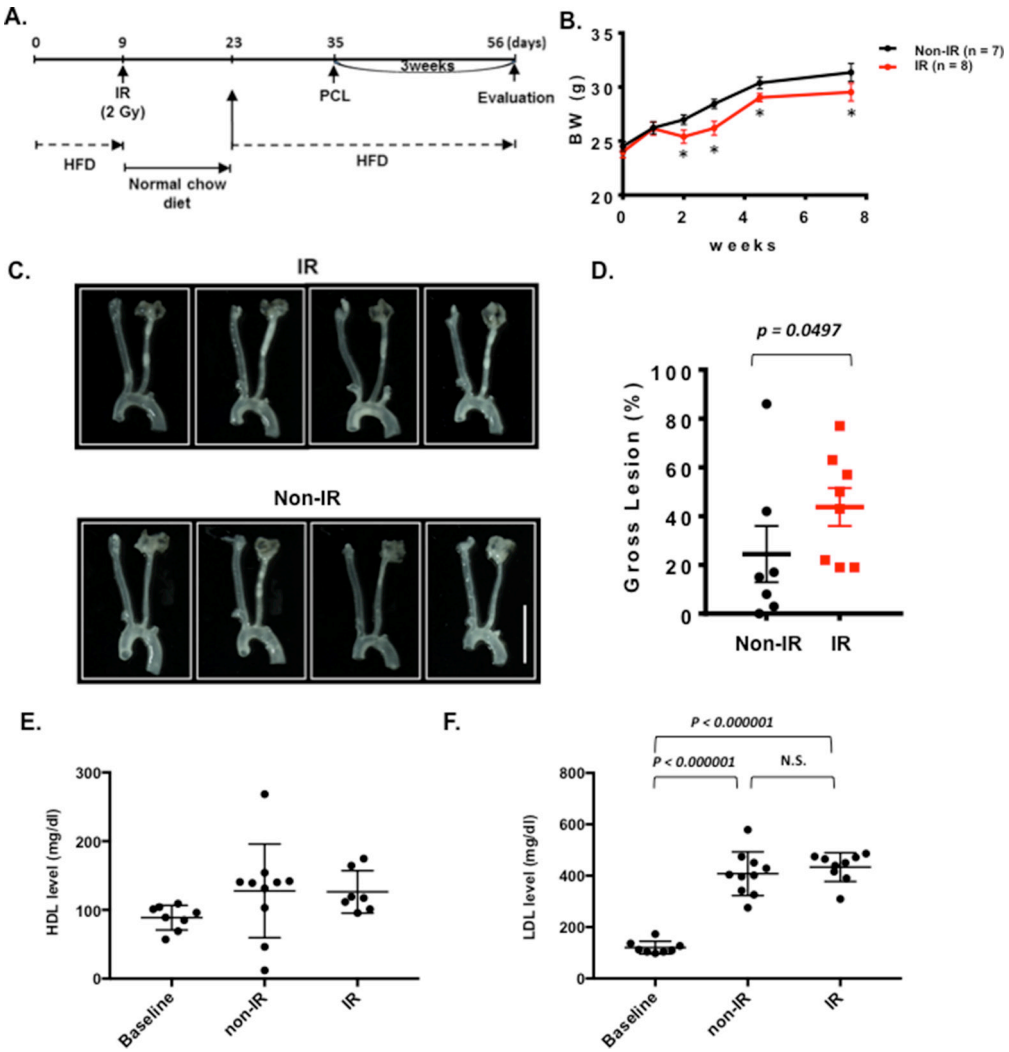

FIGURE 4 | Interval of 26 days between IR treatment and PCL accelerates plaque development after PCL. All the animals did not show any clear health problem before each experiment. **(A)** The scheme of the study design of IR treatment and PCL. **(B)** Changes in body weight in the IR and non-IR treatment groups. (mean ± SEM) **(C)** Representative gross lesions from the IR and non-IR treatment groups 3 weeks after PCL in *Ldlr*$^{-/-}$ mice. Scale bar, 0.5 cm. **(D)** Gross lesion size after 3 weeks of PCL surgery (% lesion area of total LCA area). **(E-F)** HDL and LDL levels in IR and non-IR treatment groups. 8 males in IR-treated group and 7 males in control group.

The IR-enhanced plaque formation in conventional ApoE$^{-/-}$ mice has already been reported (33, 34). But the study reported by Mancuso et al lasted almost 1 year to see enhanced atherosclerosis lesion formation by IR (33). This is not an easy and "affordable" method, and significantly limited the research in cardio-oncology. In addition, the other study did not show any lesion size difference with or without radiation (34). Therefore, previous reported were in disagreement in terms of the size of atherosclerotic plaques. In this report, we have described an affordable and reproducible experimental method to study the effect of cancer therapy on atherosclerotic lesion formation. In addition, atherosclerosis lesion formation at the aortic root and aortic arch areas may not be directly related to disturbed flow-induced atherogenesis, because it has been reported that fibronection expression is higher in these areas (35). By focusing on the lesions formed after PCL, we can evaluate the role of disturbed flow in cancer therapy-mediated vascular dysfunction.

We also tested Dox and cisplatin in our model, but we could not detect accelerated AS with the protocol tested in this study. The crucial role of macrophage proliferation during the process of plaque formation has been well established. Since we treated mice with Dox after PCL surgery, it is possible that Dox treatment inhibited macrophage proliferation in the plaque and inhibited its size. To detect the long term effects of Dox treatment on AS, we may need to add a certain length of waiting period between the end of Dox treatment and PCL surgery. As for the cisplatin treatment, we could not detect any enhancement effect on plaque formation after PCL, even after waiting for 5 weeks after cisplatin treatment to perform PCL surgery. It is well known that cisplatin can cause renal

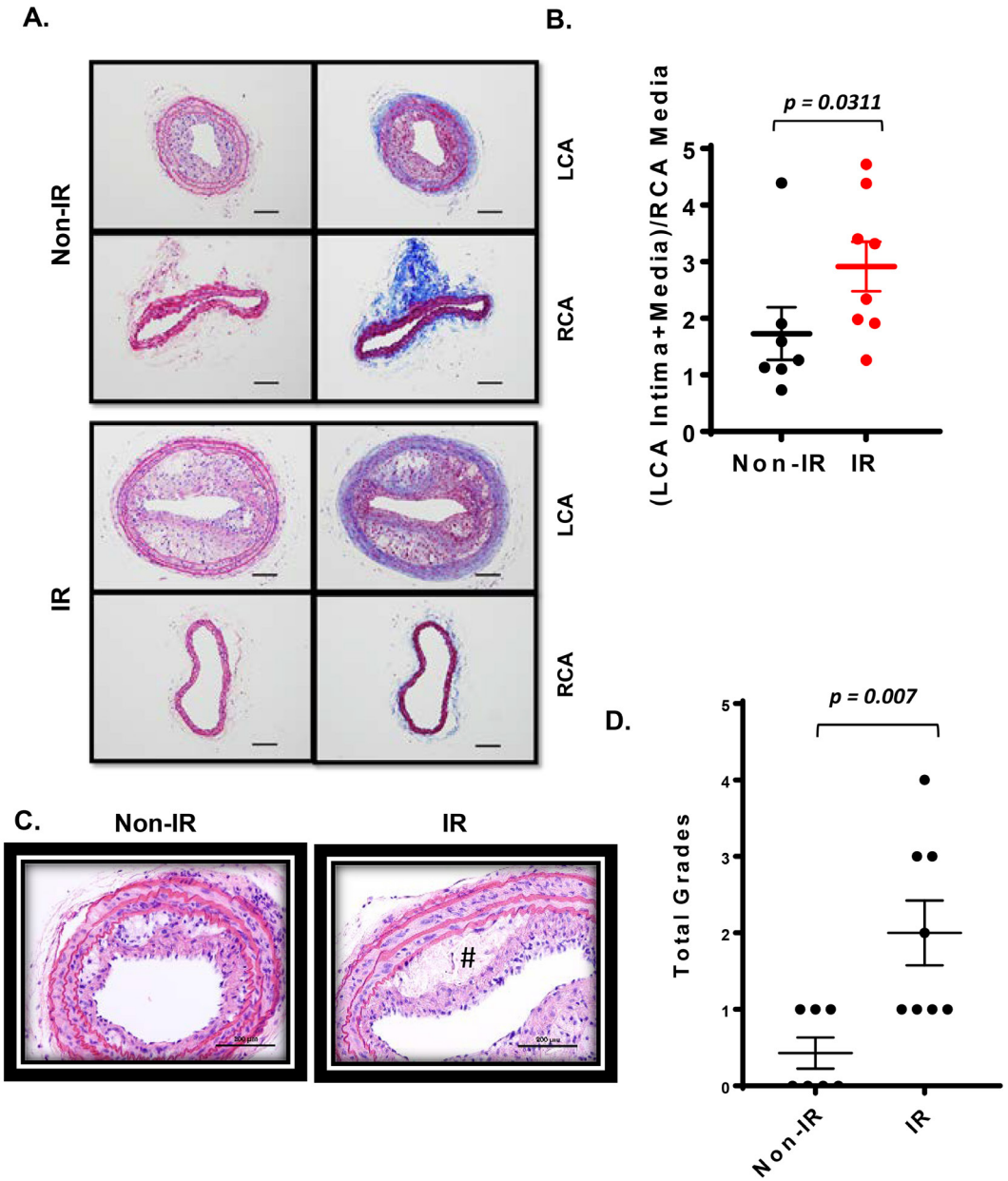

FIGURE 5 | Interval of 26 days between IR treatment and PCL enhanced the plaque size and vulnerable plaque formation detected in cross-sections. All the animals did not show any clear health problem before each experiment. **(A)** Atherosclerotic lesions 3 weeks after PCL in the left carotids isolated from the IR and non-IR treatment groups are shown. The unligated right carotid artery (RCA) from the same mouse is used as control (shown in lower panels of IR and control). Representative images are shown after H&E (left) and Masson trichrome (right) staining. Scale bars, 200 μm. **(B)** The intimal lesion area and the media area were determined in cross-sections of both ligated LCAs and control RCAs, and the ratio of (LCA intima + media)/RCA media was calculated. Data represent mean ± SEM. $^*p < 0.05$. **(C)** Representative images of the necrotic core from the IR and non-IR treatment groups are shown after H&E staining. # denotes necrotic core. **(D)** Necrotic core formation was quantified by a grading system as described in the methods. 8 males in IR-treated group and 7 males in control group.

dysfunction. Although we did not examine renal function in this study, it is possible that cisplatin-induced renal dysfunction affected the extent of plaque formation after PCL. These data provide a lesson that it is critical to control non-cardiovascular toxicity of drugs when investigating cardiovascular toxicity of cancer treatments. We could not observe statistically significant differences in the development of atherosclerosis between control and chemotherapy (Dox and cisplatin)-treated groups during our relatively short study periods. It is

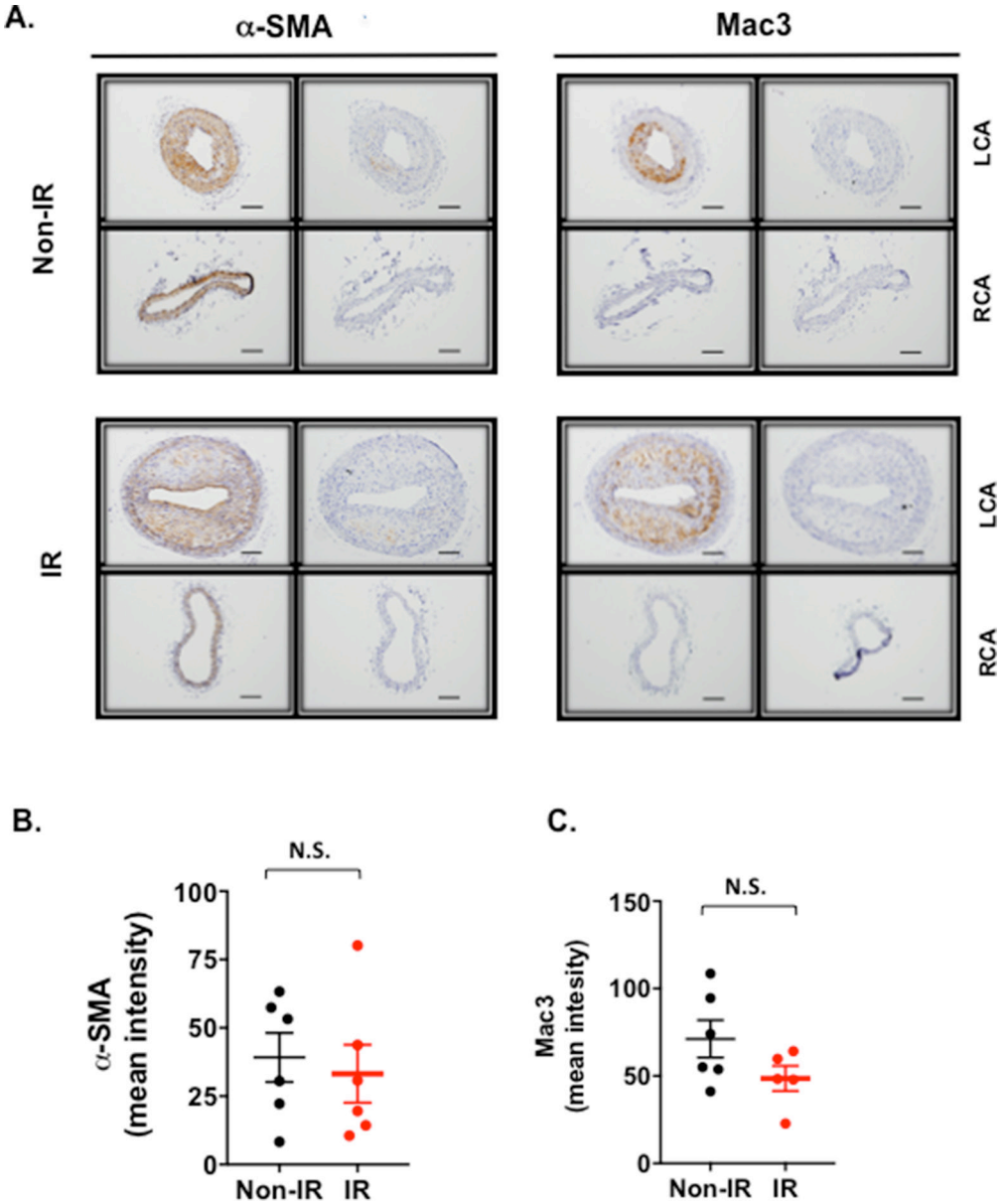

FIGURE 6 | Vascular smooth muscle cell and macrophage contents in lesions after partial carotid ligation. **(A)** LCA and RCA sections obtained from d-flow-induced atherosclerotic areas were immunostained with Mac3 (macrophages) or α-SMA (smooth muscle cells) antibodies. Representative immuno-histochemical images show macrophage infiltration into the intimal lesions in LCA but not in RCA. They also show that macrophage infiltration is accelerated in IR-treated mice. Scale bars, 200 μm. **(B, C)** The mean pixel intensities of α-SMA **(B)** and Mac3 **(C)** staining signals in the intima layer were measured as described in methods. Data represent mean ± SEM. 5 males in IR-treated group and 5 males in control group.

possible that we may need to extend our study to a much longer time course in order to detect increased atherosclerotic plaque formation by these anti-cancer drugs. To study the late effects of chemotherapy reagents, it will be crucial to minimize the non-cardiovascular acute side effects induced by the chemotherapy reagents and more careful optimization will be necessary.

If we increase the number of animals, we will most likely detect a difference between control and Dox treatment regarding lesion size. Gross lesion (%) SD was much bigger than cross-section size analysis, which we have performed before. Therefore, based on the current data the optimal number of animals needed to attain statistical significance of $p < 0.05$ with a 90% probability is 22 for each group. But this result will show that atherosclerosis will be down-regulated by Dox in this mouse model. We would like to emphasize here that the goals of this study are: (1) to generate an affordable, reliable, and reproducible mouse model that recapitulates the cancer therapy-induced cardiovascular events in cancer survivors and (2) to generate a mouse model to investigate the interplay between disturbed flow and various cancer therapy in the process of atherosclerotic plaque formation. Therefore, even if we increased the mouse number to 22

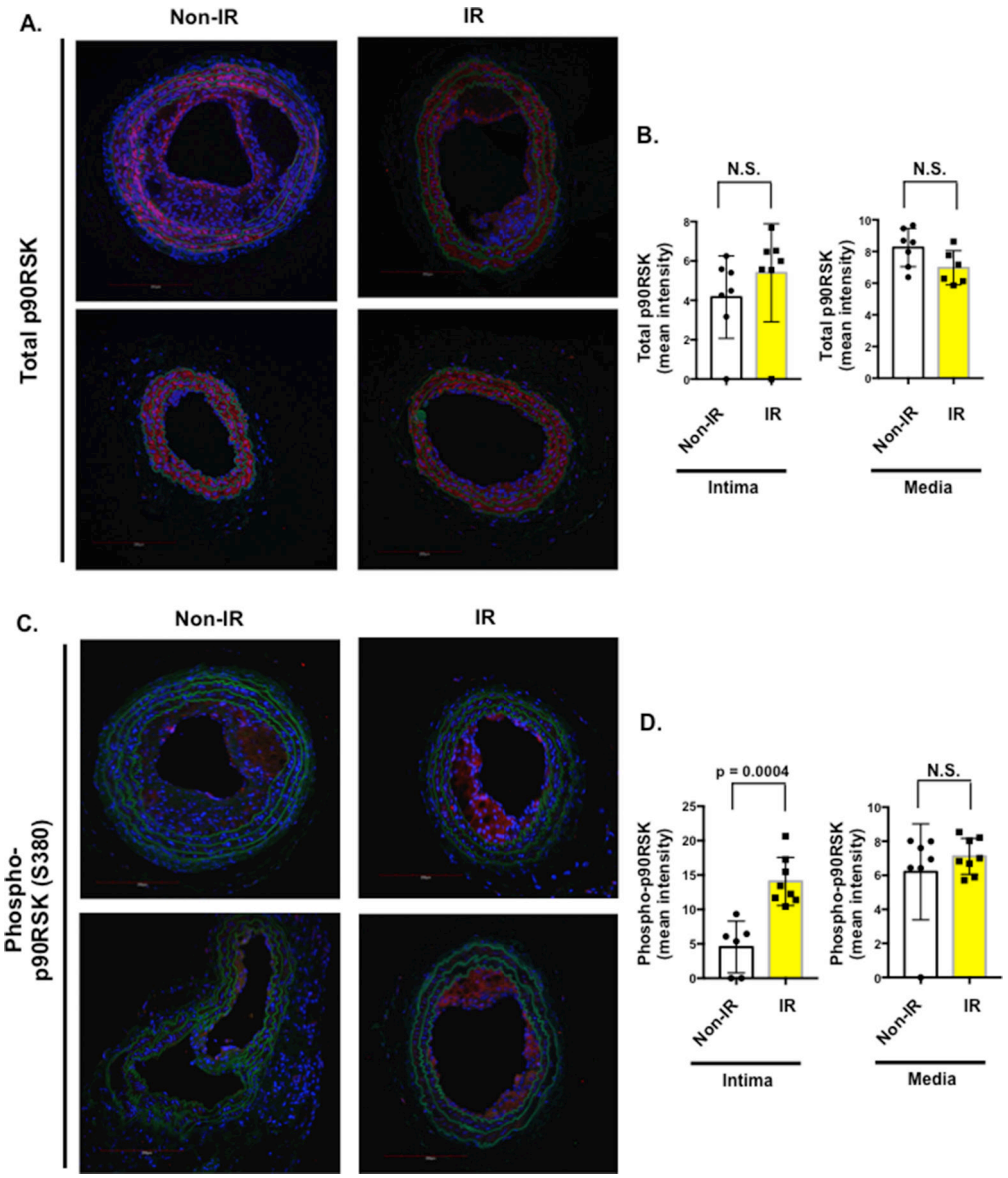

FIGURE 7 | Interval of 26 days between IR treatment and PCL accelerated both phosphorylation and expression of p90RSK after PCL. All the animals did not show any clear health problem before each experiment. **(A, C)** LCA sections after PCL were immuno-stained with anti-total-p90RSK **(A)** or anti-phopho-p90RSK antibody **(C)**. Two representative images for total-p90RSK **(A)** and anti-phopho-p90RSK **(C)** in atherosclerotic lesions are shown. Note that the level of phosphorylation but not expression of p90RSK was increased in IR-treated mice compared to non-IR group. Scale bars, 200 μm. **(B, D)** Quantification of the mean pixel intensity of total p90RSK **(B)** and phosphorylated p90RSK **(D)** expression in the intima and media after PCL in non-IR and IR groups. Mean ± SD, $^*p < 0.05$.

for each group, we would only confirm the data based on the $n = 5$ (for each group) studies and this would not lead us to generate a mouse model that recapitulate the cancer therapy-induced cardiovascular events in cancer survivors. Although it is unlikely, we might be able to show that Dox treatment could enhance lesions if we increased the number of animals. For such experiments, we would need more than 28 animals for each group to get the results counter to the data obtained from $n = 5$. This is against our goal to obtain "an affordable, reliable, and reproducible mouse model." Again, based on our results we could not conclude that Dox was unable to enhance atherosclerotic lesions compared to vehicle control in mice. However, it seems fair to state that increasing the animal number by more than 5 times does not satisfy our goal to create an affordable, reliable, and reproducible mouse model for studying mechanisms of cardio-oncology.

When we treated mice with IR and waited for a week to perform PCL surgery, we were unable to find any difference in the plaque size between non-IR and IR groups. As shown in **Figure 3B**, we saw a rapid decrease in body weight after IR. It is possible that this is due to lost appetite or reduced nutrient absorption in the intestine after IR, and this may affect the development of plaque formation. In a different group, we waited for 26 days for the body weight to recover and then performed PCL surgery. In such mice, we detected significantly enhanced IR-induced plaque formation. In order to develop a cancer therapy-induced AS model, it may be important

to maintain the body weight after cancer therapy interventions for enhancing the development of plaque formation.

In this study we also found increased phosphorylation of p90RSK in the plaque. Previously, we have reported a crucial role of p90RSK activation in plaque formation and that IR can increase p90RSK activation in both endothelial cells and macrophages (data not shown). Therefore, it is possible that the increase in p90RSK activation in the plaque after IR contributes to accelerated development of AS. Future study will be necessary to clarify this issue.

There are several limitations in this study. For example, the role of p90RSK activation in radiation-mediated plaque formation remains unclear. However, we have already reported that p90RSK can phosphorylates ERK5, which has a significant role in atherosclerotic plaque formation (24). Since we found in this study that p90RSK activation was significantly increased in the plaque, to determine the biological consequence of the increased p90RSK in this model, it will be necessary to generate macrophage- or vascular smooth muscle cell-specific triple p90RSK 1, 2, 3 knock out mice and to determine the total p90RSK null effect on the plaque formation. However, this will be beyond the scope of this study, and we will perform these experiments in our future studies. Since the purpose of this study is to develop and provide a mouse model to the research community for studying the cancer therapy-induced cardiovascular disease and its relationship to disturbed flow, we think that studying the role of p90RSK in this model is not critical at this time. Because, at present, there is no mouse model for studying cardio-oncology, we feel that there is a strong need to develop an animal model of the cancer therapy-induced cardiovascular toxicity.

In this paper, we included certain negative data. These data provide the research community what kind of issues one needs to be aware of for establishing an animal model for studying cardio-oncology. The negative data (not increased atherosclerosis formation after partial carotid ligation) by using Dox and cisplatin are not to conclude that they cannot increase plaque formation, but rather to state that under the conditions we tested, we could not detect increased plaque formation after the Dox and cisplatin treatment. It is still possible that under different experimental conditions, these drugs have athero-enhancing effects, and these issues can be investigated in future studies by us or other investigators. It is hoped that our negative data can help other investigators to save their time, and give some ideas how to optimize the conditions of cancer treatment regimens to establish animal models for studying cardio-oncology. These issues are also supported by the ARRIVE guideline (36), because ARRIVE guideline clearly states that animal models should have translational aspect to human biology, otherwise they strictly prohibit to do animal studies which do not recapitulate human illnesses. Animal studies are necessary which phenocopy human pathology and this cannot be done using non-animal experiments such as cell systems. We did not waste animals to perform experiments which are not relevant to human biology and cardio-oncology research. For example, we did not pursue the Dox effect on inhibiting plaque formation (which was opposite to the human pathology), in accordance of the policy of ARRIVE guide line (36). Lastly, showing negative data will provide the idea how to reduce the future use of animals.

In summary, we have developed a susceptible mouse model, using partial carotid ligation protocols, for the study of disturbed flow effects on atherosclerosis in cancer treatments. This protocol can be used to investigate the effects of IR on d-flow-induced atherosclerosis. In our future work, the contribution of endothelial, vascular smooth muscle cells and macrophage p90RSK activation on IR-induced enhancement of atherosclerotic lesion will be investigated with this model.

ETHICS STATEMENT

Experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M Institute of Biosciences and Technology at the University of Texas MD Anderson Cancer Center.

AUTHOR CONTRIBUTIONS

KK performed experiments, interpreted data and wrote the manuscript. YW, SKo, YF, HV, BV, TT, JM, and YG performed experiments and interpreted data. JG-A, ZP, SM, SKr, and MH interpreted data. KF and JA interpreted data and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fcvm.2018.00026/full#supplementary-material

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REVIEW
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Radiation-Induced Cardiovascular Disease: Mechanisms and Importance of Linear Energy Transfer

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Radiation therapy (RT) in the form of photons and protons is a well-established treatment for cancer. More recently, heavy charged particles have been used to treat radioresistant and high-risk cancers. Radiation treatment is known to cause cardiovascular disease (CVD) which can occur acutely during treatment or years afterward in the form of accelerated atherosclerosis. Radiation-induced cardiovascular disease (RICVD) can be a limiting factor in treatment as well as a cause of morbidity and mortality in successfully treated patients. Inflammation plays a key role in both acute and chronic RICVD, but the underling pathophysiology is complex, involving DNA damage, reactive oxygen species, and chronic inflammation. While understanding of the molecular mechanisms of RICVD has increased, the growing number of patients receiving RT warrants further research to identify individuals at risk, plans for prevention, and targets for the treatment of RICVD. Research on RICVD is also relevant to the National Aeronautics and Space Administration (NASA) due to the prevalent space radiation environment encountered by astronauts. NASA's current research on RICVD can both contribute to and benefit from concurrent work with cell and animal studies informing radiotoxicities resulting from cancer therapy. This review summarizes the types of radiation currently in clinical use, models of RICVD, current knowledge of the mechanisms by which they cause CVD, and how this knowledge might apply to those exposed to various types of radiation.

Keywords: cardiovascular disease, radiation, cancer, charged particle, linear energy transfer, chronic inflammation, space radiation

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INTRODUCTION

Radiation therapy (RT) has been used since the 1890s to treat cancer. RT can be used as a primary treatment or adjuvant to a combination of surgery, chemotherapy, targeted small molecules, or biologic drugs. Traditionally, low-linear energy transfer (LET) radiation such as photons (X-rays and γ-rays), have been the mainstay of RT, but since the 1950s, charged particle therapy (CPT) in the form of proton beams have been available and have showed superiority to photon therapies against some cancers (1). More recently, therapies using high-LET (densely ionizing) heavy charged particles such as carbon are being used because they can more precisely deliver higher intensity energy while decreasing the dose to healthy tissues in the path of radiation.

Use of high-LET therapies remain limited to small cohorts and most high-LET treatment centers are outside of the United States, primarily in Germany and Japan, but centers are now being built at the University of Texas Southwestern and University of California San Francisco medical centers in

the United States (2). The complications of low-LET RT exposure have been well-reviewed both in this journal (3) and elsewhere (4–6), but the effects of high-LET radiation from heavy charged ions are not well-characterized. Aside from its use as RT, the effects of high-LET radiation are relevant to the National Aeronautics and Space Administration (NASA) because of possible effects of space radiation on astronauts during extended missions.

Radiation-induced cardiovascular disease (RICVD) is one well-known complication of low-LET radiation exposure. RICVD can occur in individuals at otherwise low risk for cardiovascular disease (CVD) (7, 8), or it can exacerbate existing CVD (9, 10). The incidence of most cancers increases with age, as does the prevalence of traditional CVD. Thus, the population that most often needs treatment with radiation is the most at risk for complications of RICVD. RICVD can be a treatment-limiting factor in those who receive RT, especially to the thorax (9–11). Evidence suggesting that radiation independently causes CVD includes its development after radiation exposure in healthy or younger populations in whom the disease is almost uniformly absent (8, 12); the development of RICVD in areas directly exposed to radiation (7, 13); and accelerated progression of chronic CVD in at-risk or affected individuals (10). In addition to studies of patients exposed to therapeutic radiation, several groups exposed to nuclear radiation occupationally have had longitudinal follow-up (6). Study of these therapeutically and occupationally exposed groups has revealed a temporally bimodal distribution of RICVD. Short-term effects of RICVD such as acute pericarditis occur within weeks after doses >30 Gy (4). Long-term effects of RT such as atherosclerosis and coronary artery disease manifest more than a decade after exposure (4). High-LET radiation may affect the cardiovascular system in a different manner than that of traditional low-LET radiation (14, 15), and study of how high-LET radiation affects the cardiovascular system is underway. This review will focus on the available data on the effects of low- and high-LET radiation on the cardiovascular system, and how these results may impact those who will be exposed to high-LET radiation in the future.

COMPARISON OF LOW-LET AND HIGH-LET MODALITIES

Linear energy transfer refers to the amount of energy deposited into a material as an ionizing particle passes through it. Energy deposition, and thus ionization, of a beam increases with increasing LET. The exact LET depends on both the radiation type and the material traversed. Because of the heterogeneity of biological materials, LET must be considered in the context of both the tissue being irradiated and the type of radiation being transmitted, but in general LET increases with the mass of the particles used for irradiation.

External beam low-LET photon RT (X-rays or γ-rays) has been the primary modality of RT since its first clinical implementation and has remained the most commonly used modality of RT. Photons typically deliver the greatest dose of radiation at the first surface of tissue encountered, and the dose delivered decreases linearly as the beam pass through tissues (**Figure 1A**). The linear dose delivery of photon therapies means that healthy tissues in the path of the beam may also be damaged. Two techniques, dose fractionation and conformal radiation, have been used to reduce the amount of damage to off-target tissues (16, 17). In dose fractionation, the total dose to be delivered is divided over several treatments, allowing normal tissues to recover between doses. RT can be hypofractionated, with a larger dose delivered over fewer sessions, or hyperfractionated, with many smaller doses received as often as twice a day. Conformal radiation, which is often used in conjunction with dose fractionation, involves using multiple beams that converge on the target tissue to deliver a higher dose there while reducing the dose to collateral tissues which are only in a single beam's path.

In contrast to photon RT, CPT deposits a high dose of radiation as particles slow down within tissues, a phenomenon called the Bragg peak (**Figure 1B**). Because CPT releases more energy as ions slow down, the dose of radiation delivered to superficial tissues is much smaller than the dose delivered to deeper tissues. The depth at which the Bragg peak occurs can be varied by using particles of different energies. Since a single peak is often too small to irradiate the entire volume of a tumor, multiple beams of varying energies are used to overlap the Bragg peaks and distribute the dose to the entire tumor. The summation of these beams creates a spread-out Bragg peak (SOBP) (**Figure 1C**). By taking advantage of the SOPB, CPT delivers a high radiation dose to the targeted tumor tissue with minimal dose deposition to surrounding tissues. While the principals for delivering CPT is similar across types of ions used, there are differences in the dose deposition of different charged particles. For example, carbon-13 (^{13}C), the most common experimental high-LET RT source, has a higher ratio of dose delivered in the Bragg peak to dose delivered in the initial plateau region (**Figure 1B**) compared to protons. The increased Bragg peak-to-plateau energy deposition of carbon ions results in higher energy deposition in target tissues with less collateral tissue damage and is the basis for the increasing clinical use of carbon ions.

Regardless of the type of radiation used, the first events after exposure that lead to cytotoxicity in healthy and targeted tissues are the formation of DNA breaks (18) and reactive oxygen species (ROS) (19) in tissues in the radiation path. In the nucleus, ionization leads to DNA breaks which in turn leads to aberrant DNA base pairs (20) and epigenetic changes (21, 22). In response to radiation-induced DNA breaks, multiple repair mechanisms are activated, most notably the ataxia-telangiectasia mutated kinase (ATM) and ATM- and Rad3-related kinase (23). These cause a signaling cascade that induces cell cycle arrest and DNA repair proteins *via* p53 (24). The complexity of DNA damage determines whether the cell will survive or whether apoptosis is initiated.

Low-LET photon beams cause diffuse and homogenous ionization and cause ROS formation throughout cells (**Figure 2A**), which mainly causes single-stranded DNA breaks (SSB). The sparsely ionizing low-LET therapies are most effective in the G2/M checkpoint of the cell cycle (25). In contrast, the high-LET beams of heavier ions cause dense ionization (**Figure 2B**) especially at the Bragg peak, and ROS rapidly associate with surrounding structures (26, 27). High-LET radiation causes more double-stranded DNA breaks (DSB) than low-LET RT (28). The resulting DSB are more complex and more likely to lead to

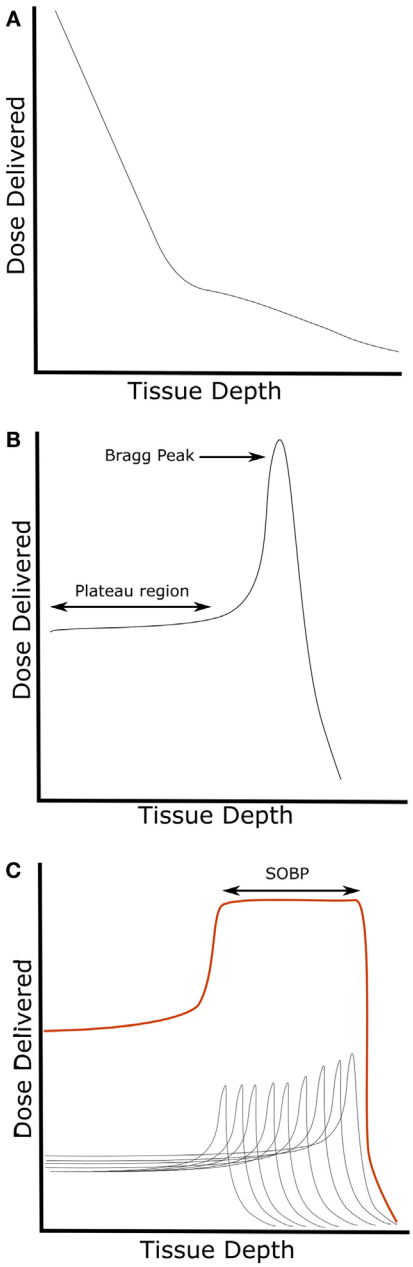

FIGURE 1 | Representative dose delivery patterns of **(A)** low-LET photons and **(B)** charged particles. **(B)** Charged particles demonstrate a low-LET plateau region before the high-LET Bragg peak. **(C)** Exposures with varying initial energies (gray lines) can be used to create a SOBP (red line) and cover the entire volume of targeted tissue with approximately the same dose of radiation. Abbreviations: LET, linear energy transfer; SOBP, spread-out Bragg peak.

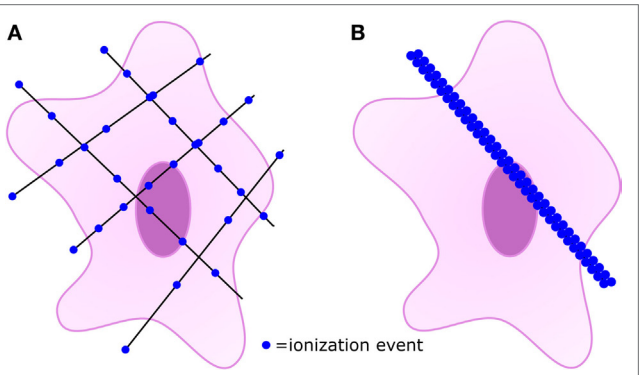

FIGURE 2 | The main factor differentiating low-linear energy transfer (LET) and high-LET therapies is the amount of ionization along the path of the beam. **(A)** Low-LET radiation is sparely ionizing, and multiple exposures may be used to adequately irradiation target tissues. **(B)** High-LET radiation causes dense ionization along the course of the beam.

cell death, whereas SSB are more easily repaired and are more likely to be sublethal (29). High-LET therapies are effective in all stages of the cell cycle, especially S-phase (27). Additionally, in experimental models, high-LET RT has shown the ability to overcome two major causes of tumor radioresistance: tumor stem cells (30) and hypoxia effect (31). Tumor stem cells are a subset of cancer cells believed to be a source of radioresistance because of increased antioxidant and DNA-repair capabilities (32). The tumor hypoxia effect refers to the decreased efficacy of radiation in hypoxic tumors. Although the exact mechanism by which tumor hypoxia leads to radioresistance is unknown, one hypothesis is that the presence of oxygen is necessary to create an organic peroxide intermediate with the broken strand of DNA induced by radiation. The peroxide then reacts with surrounding structures and fixes the break in place, contributing to signals favoring apoptosis. In hypoxic tissues, SSB are more likely to be reduced by surrounding sulfhydryl groups. The reduced carbon is more easily repaired and signals favoring repair and survival are induced. The DSB caused by high-LET radiation are not affected by oxygen concentration and mediate effective killing under hypoxic and normoxic conditions (2).

Most studies of radiation for oncological applications have focused on the effects of radiation in the nucleus, but more recently effects in the cytoplasm have become apparent and may be equally important to the study of RICVD. In the cytoplasm, ROS formation causes damage to the cell membrane, organelles (33), and the ligand-independent activation of multiple pathways, especially receptor tyrosine kinases (34). Not only does radiation directly produce ROS, it also causes ROS release from mitochondria (35). ROS disruption of normal cytoplasmic function and membrane structures can lead to cell death independently of or in conjunction with effects in the nucleus. However, in sublethally irradiated cells of collateral tissues, both nuclear and cytoplasmic damage results in signaling cascades that converge at signaling through nuclear factor kappa B (NF-κB) (36–38), which leads to multiple signaling cascades that suppress apoptosis, induce radioresistance, and induce inflammation (39).

MECHANISMS OF RICVD

Reactive oxygen species formation has been shown to be an important factor in the development of RICVD, and decreased ability to clear free radicals causes a worsening of cardiovascular effects (19). ROS formation in healthy endothelial cells and subsequent

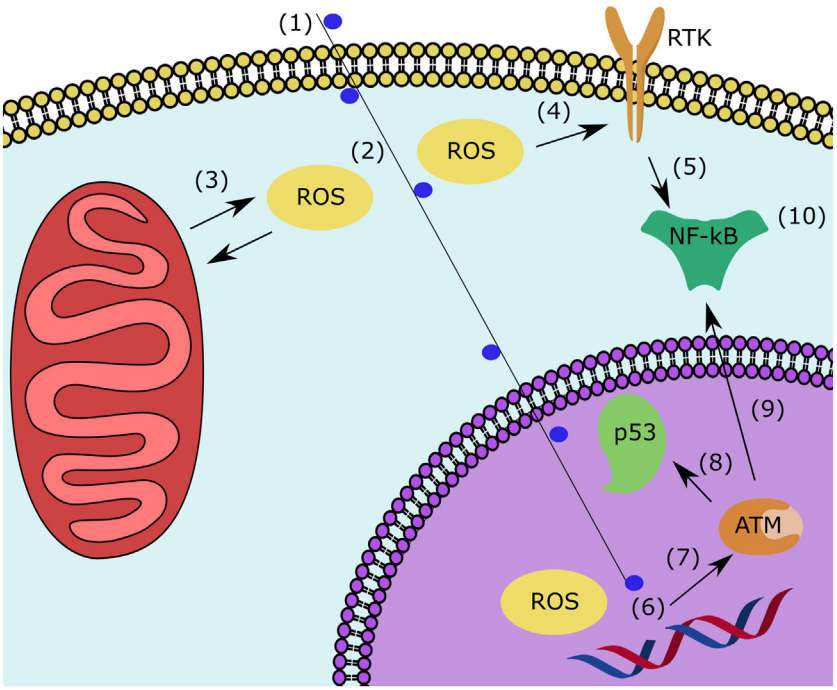

FIGURE 3 | After sublethal irradiation, multiple events in the nucleus and cytoplasm contribute to the inflammatory state responsible for RICVD. In the cytoplasm, (1) sublethal irradiation of an off-target cell leads to (2) ROS formation and (3) ROS-induced-ROS release from the mitochondria. (4) ROS lead to ligand-independent activation of RTK which leads to expression of many pathways that (5) induce NF-κB. In the nucleus, (1) sublethal ionizing radiation leads to (6) ROS formation and single-stranded DNA breaks. (7) In response to DNA damage, ATM and ATM and AT and Rad3-related kinase are activated. (8) They induce p53 activation leading to cell cycle arrest and DNA repair as well as (9) inducing NF-κB. (10) NF-κB activation from both pathways induces factors that lead to antiapoptotic, radiation resistance, and inflammatory signaling. The presented mechanism is generally expected to occur in all sublethally irradiated cell types, but the pathogenesis of RICVD is first seen in the endothelial cells. Abbreviations: RICVD, radiation-induced cardiovascular disease; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; NF-κB, nuclear factor kappa B; ATM, ataxia-telangiectasia mutated kinase.

signaling *via* NF-κB leads to an inflammatory state *via* expression of interleukin-1, interleukin-6, tumor necrosis factor-α (40), intercellular adhesion molecule-1 (41), and matrix metalloproteinases (42, 43). ROS levels remain elevated long after exposure to radiation. In animal models whose hearts were directly exposed to high-LET radiation, inflammation and apoptosis were shown to persist for at least 6 months (43, 44). This prolonged inflammation leads to a persistent but ineffective healing and remodeling response (45) marked by chronic inflammation of macrophages and mononuclear cells (46). The chronic inflammatory response is necessary for remodeling of damaged tissues, but the low levels of inflammation seen early in RICVD may be ineffective to fully restore tissue structure and function (47). Further, angiogenesis is disturbed after exposure to radiation due to the decrease in vascular endothelial growth factor secretion (48) and decreased tubule formation (14). The continuous attempts at repair induce the physiologic formation of more ROS (47), which contributes to a smoldering continuous inflammatory state. The vasculature's inability to appropriately remodel from the initial radiation injury is further worsened by a decrease of endothelium-dependent relaxation (49, 50) worsening the effect of turbulent blood flood, another important factor in atherosclerotic development (51). Later, intimal thickening and atherosclerosis occurs, especially at areas of disturbed flow (44). The atherosclerotic effects of radiation are seen in models of low-LET radiation (52–54) as well as high-LET radiation (44, 49, 50).

Additionally, high-LET radiation has been shown to upregulate connexin-43 in the cardiac myocytes of animal models (55–58). Connexin-43 is implicated in the development of atherosclerotic plaques (59), and downregulation of connexin-43 has been shown to reduce atherosclerosis formation in animal models (60, 61). The exact role of connexin-43 in the development of RICVD is still unclear, but it likely plays a role in communication between vascular cells and inflammatory cells (62).

Figure 3 illustrates the interrelation of proposed pathological mechanisms at the cellular level. In summary, sub-lethal DNA damage in the nucleus and ROS formation and release in the cytoplasm both activate NF-κB. NF-κB mediates a pro-survival and pro-inflammatory state in which ineffective remodeling leads to a vicious cycle of continuous ROS formation and persistent inflammation. The inflammatory state leads to impaired healing and endothelial dysfunction, making the vasculature more vulnerable to damage from non-laminar flow. Compensatory mechanisms manifest as intimal thickening and eventually atherosclerosis as inadequately healed endothelial injuries accumulate.

CASE REPORTS AND MODELS OF RICVD IN HIGH-LET THERAPIES

No large-scale clinical trial data is available for high-LET therapies. The first full trial with high-LET radiation began in 1994 (2), and most trials of high-LET therapy do not look at RICVD as an endpoint. Several small trials involving high-LET therapy near cardiovascular structures have been conducted, but none are large enough to determine the frequency or type of RICVD caused by these newer therapies. A case report showed the efficacy of carbon ion therapy against cardiac angiosarcoma, a tumor that is usually resistant to most forms of radiation and chemotherapy, without major off-target effects up to 1.5 years (63). In a case series published by Amino et al., eight patients treated for mediastinal cancer showed no cardiac toxicity up to five years post-^{12}C irradiation. It should be noted that six of the eight patients were deceased at 5-year follow up due to non-CV-related progression of their disease (64). In small trials, carbon-ion therapy has been shown to be effective in controlling hepatocellular carcinoma, even inoperable tumors near the porta hepatis, and these trials have shown no acute effects to the vasculature of the liver (65–67). These preliminary results of the effectiveness of high-LET RT show promise for the treatment of tumors that were previously thought of as radioresistant, and the works do not show any evidence of acute RICVD. Increased sample size and longitudinal follow-up will be required to determine the rate of chronic RICVD caused by high-LET therapies.

Because of the paucity of clinical information about RICVD from high-LET therapies, most knowledge of the cardiovascular effects of high-LET radiation comes from animal and *in vitro* experiments. Mice are most commonly used to model the effects of RICVD. However, due to their inherently low plasma low-density lipoprotein (LDL) and high plasma high-density lipoprotein (HDL) coupled with a short lifespan, many murine strains are resistant to atherosclerosis, a principle measurable endpoint in RICVD. Mutant mice with defects in lipid metabolism such as ApoE- and Ldlr-knockouts and ApoE Leiden- and ApoB-100-mutants acting in a dominant negative fashion are often used in the study of non-radiation-related atherosclerosis (68). These models are also used for the study of RICVD because they increase the sensitivity of the mice to the cardiovascular effects and reduce the time to observable effects of atherosclerosis (69). The choice of mouse line used to model RICVD is important because different effects will be seen in mice of different genetic makeups (70).

Animal models used to study RICVD show similarities between the CV effects of high- and low-LET radiation. Loss of vascular reactivity appears to be a sentinel event and can be seen as early as 5 weeks postradiation in animal models (49, 50). High-LET causes an upregulation of genes related to cell senescence and oxidative stress (71), which is similar to the response seen after low-LET radiation. Further, supporting the role of oxidative stress after high-LET radiation exposure, the level of serum antioxidants are reduced after exposure, and antioxidant-rich diets reduce this effect (72). Xanthine oxidase contributes to ROS production and nitric oxide reduction in whole-body irradiated mice (19). The above animal studies use whole-body radiation to assess various aspects of the pathophysiology of RICVD. This approach is useful for eliciting broad response, but takes away from the improved targeting capability of CPT, i.e., the ability to avoid irradiating non-target tissues. Yu et al. examined the effects of high-LET radiation on vasculature using a targeted approach. In their study, non-irradiated arteries from test mice as well as arteries from sham-irradiated mice were used as controls for exposure to ^{56}Fe radiation. They demonstrated that the high-LET ^{56}Fe ions accelerate atherosclerosis in target arteries but not controls. They also demonstrated that different arteries have different sensitivities to high-LET IR in a similar manner to low-LET modalities (44). Additionally, studies investigating difference sequences of exposure to low- and high-LET radiation have shown differential cellular responses (73, 74). While animal models are useful for examining the phenotypic characteristics of RICVD, they have several setbacks including the expense required to maintain the mouse lines and time required for animal testing. Murine atherosclerotic models also have key differences from human atherosclerosis, such as location plaques occur, stability of the plaques that form, and structure of HDLs expressed (75), which may make it difficult to tease apart what may be subtle differences in the effects of low- and high-LET RICVD.

In vitro models are cheaper, faster, and offer more control than animal models. They allow for the isolation of parameters and simple measuring of outputs of cells *via* media and molecular techniques. Monocultures are most often used and may consist of cells derived from animal or human sources. Cell types commonly used to study CVD include human umbilical vein endothelial cells (76), cardiac myocytes, embryonic stem cells (77), and vascular support cells such as fibroblasts. High-LET ^{56}Fe radiation has been shown to cause more DSB in HUVECS (14). The DNA damage caused by high-LET charged ions also appears more durable than those of low-LET radiation (78–80). Additionally, high-LET radiation more effectively induces endothelial cell adhesiveness which would contribute to inflammatory cell adhesion (41, 81). Reproducing CV cells exposed to high-LET radiation show sustained genomic damage and decreased functionality (77). The angiogenic capabilities of endothelial cells are more effectively reduced by high-LET radiation. It appears that the angiogenic inhibition is due to a decrease in secreted VEGF (48) leading to tubule inhibition in multiple endothelial cell types (14, 48). High-LET radiation has also been shown to reduce endothelial cell adhesiveness in culture which could be an analog for increased vascular permeability (42), and decreases the mitochondrial membrane potential due to leaking of ROS from the mitochondria into the cytoplasm (76). While most of the above studies have compared the effects of low- and high-LET radiation, LET is not the sole determinant of the cellular effects of radiation, and different types of radiation have been shown to have different effects even at the same LET (78). The *in vitro* techniques described above have provided useful insights into the mechanisms of RICVD such as DNA damage, cytokine response, and effects on individual cells. However, atherosclerotic development in RICVD is a multifaceted process likely occurring both acutely and over many years and involving multiple cell types. Traditional cell culture techniques may be insufficiently complex to appropriately model some aspects of RICVD such as matrix remodeling, reaction to disturbed flow, and cellular migration.

To address gaps present in animal and 2D *in vitro* models, 3-dimensional (3D) cultures are being considered for use as a model in studying RICVD (14, 78, 82, 83). 3D matrices can be used to construct multilayer cocultures that have a greater fidelity to the physiological state of tissues being test compared to 2D *in vitro* cultures. 3D cultures also offer more control, homogeneity, and ease than animal models. Hydrogel models can be used to examine cytokine and morphological changes in response to stimuli. Constructs of vascular endothelial and interstitial cells can be used to replicate endothelial cell behavior (14) and model the damage caused after radiation (82). Flow cells can be used to recreate shear stress from blood flow (84) including pathological shear on vessel walls. 3D cultures have been especially useful in studying the effects of low- and high-LET radiation affects microvasculature. In a series of studies, Grabham et al. showed that the damage caused by high-LET ^{56}Fe ions on both mature and developing vessels compared to proton or photon IR which preferentially affected developing vessels (78). Additionally, they used 3D culture to show that ^{56}Fe radiation inhibits late stage angiogenesis, namely endothelial cell migration and tube formation, rather than early motile tip and intracellular adhesion, which is inhibited by low-LET radiation (14). The development and testing of more complex 3D models is underway and may provide new insights into the pathogenesis of RICVD.

IMPLICATIONS ON TREATMENT

The link between radiation and CVD is well established in human cohorts at doses greater than 0.5 Gy (85). Interestingly, low-dose high-LET radiation may have some anti-inflammatory effects in a dyslipidemic murine model (86), but the dose-rate and state of the disease affect the modification radiation has on atherosclerosis progression (87). The dose cancer patients receive varies widely for the disease being treated but are often well in excess of the doses known to cause RICVD (9, 88–90). The importance of cardiovascular health during and after treatment is well recognized, but there is still a lack of national guidelines (91).

The prevention and treatment of RICVD consists of optimizing traditional cardiovascular risk factors including hypertension, blood sugar, heart failure, coagulability, and blood lipids, as well as encouraging healthy diet, exercise, and medication adherence. RICVD may be resistant to some aspects of treatment (92, 93), making the optimization of all modifiable factors important in patients undergoing treatment. Additionally, patients often receive surgery and chemotherapy with radiation for the treatment cancer, and certain chemotherapies, such as anthracyclines and trastuzumab, are known to be directly toxic to the heart. The presence of CVD is not an absolute contraindication to the use of RT. Rather, clinicians administering cardiotoxic drugs with or without radiation should keep in mind patients' comorbidities and risk factors and weigh them against the therapeutic advantage granted in terms of tumor control (94).

RELEVANCE OF RICVD TO NASA

The effects of radiation are also of interest to NASA (95), as well as other space programs, as it poses a significant risk for manned spaceflight. RICVD is among the radiation-related health risks of concern. The types of radiation found in the space environment are significantly more damaging than those found on Earth and include galactic cosmic radiation (GCR), solar particle events, and trapped protons and electrons. GCR consists of high atomic number and high energy (HZE) nuclei, like carbon and iron, as well as high energy protons (96). There are similarities with charged ion RT which uses single ion beams of carbon or proton. Differences between the space radiation environment and clinical RT protocols includes dose levels, dose-rates, whole body vs. partial body irradiations, along with the mixed ion fields present in space versus single ion beams used for CPT.

National Aeronautics and Space Administration maintains a research portfolio to evaluate effects of high-LET radiation on CVD in order to characterize and mitigate radiation risks posed to astronauts on exploration missions (95, 97). Evidence comes from a body of cell and animal work as well as from terrestrial epidemiology analyses of atomic bomb survivors and nuclear workers showing a demonstrated risk for RICVD at doses greater than 0.5 Gy (6). However, at lower, space-relevant doses and radiation types, the association between exposure and cardiovascular pathology is more varied and unclear. Recent work has reiterated that, to date, there is no evidence in the astronaut cohort of increased risk of CVD (98, 99). This confirms the healthy worker effect expected in an astronaut population but also highlights the limitations of such a cohort, including small sample size and large confounding effects as well as the relatively low doses of radiation that astronauts have experienced to date. Exploration missions with longer durations and outside the LEO will result in larger radiation exposures to the astronauts, and a mission to Mars predicted to last several years (95, 100) will result in doses nearing the 0.5 Gy threshold for RICVD observed in terrestrial cohorts. Therefore, NASA requires risk characterization and mitigation strategies for the risk of RICVD for a Mars mission or other longer exploration missions. NASA relies on cellular models (both 2D and 3D), animal studies, and ongoing epidemiological analyses with both low- and high-LET exposures to inform its knowledge gaps. This research strategy is detailed within the NASA Human Research Roadmap (97), where current and planned work is described within the eight knowledge gaps for the "Risk of Cardiovascular Disease and Other Degenerative Tissue Effects From Radiation Exposure and Secondary Spaceflight Stressors." Advances within NASA's research program as well as within terrestrial work with CPT can inform both the risk of RICVD as well as mitigation strategies. Specifically, countermeasures already approved for use for CVD and evaluated in clinical radiotherapy cohorts will be a first priority for mitigation strategies in astronauts for RICVD.

CONCLUSION

The increasing number of patients being treated with radiation justifies further research into mechanisms and druggable targets for treatment and prevention of RICVD. High-LET CPT is an innovative form of RT that shows promise in the early phases of testing. There is not sufficient clinical data to draw conclusions about the efficacy of high-LET RT, and cost remains a practical

barrier to studying and implementing of CPT (101, 102). Still, its potential to overcome radioresistance in tumors and improve targeting around sensitive organs warrants further research.

In terms of RICVD, the complex pathophysiology remains to be fully elucidated. Population data does not yet show any increased risk of CVD in populations exposed to <0.5 Gy. *In vitro* models have shown that multiple variables beyond total dose contribute to differential responses. Factors shown to be important in cardiovascular cells' response to radiation include dose rate, LET, particle type regardless of LET, and genetic makeup of the model being used. Better models, such as 3D cocultures, which are more representative than standard 2D cell culture and faster, cheaper, and more tunable than animal models, are currently under development for use in the study of RICVD. They may offer even better insight into pathological progression after exposure to radiation. Finally, while most studies of RICVD revolve around cancer patients, this information is also relevant to NASA. Future space missions will be longer and outside of the earth's magnetic field, exposing astronauts to greater radiation doses. NASA's current research on RICVD, which relies on cellular and animal ground-based studies, can both contribute to and benefit from concurrent work informing radiotoxicities resulting from cancer therapy.

AUTHOR CONTRIBUTIONS

CS wrote the manuscript and prepared the figures. J-iA, ZP, and KG-A contributed to conception and editing of the manuscript.

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Radiation Matters of the Heart: A Mini Review

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Radiation Therapy (RT) has been critical in cancer treatment regimens to date. However, it has been shown that ionizing radiation is also associated with increased risk of damage to healthy tissues. At high radiation doses, varied effects including inactivation of cells in treated tissue and associated functional impairment are seen. These range from direct damage to the heart; particularly, diffuse fibrosis of the pericardium and myocardium, adhesion of the pericardium, injury to the blood vessels and stenosis. Cardiac damage is mostly a late responding end-point, occurring anywhere between 1 and 10 years after radiation procedures. Cardiovascular disease following radiotherapy was more common with radiation treatments used before the late 1980s. Modern RT regimens with more focused radiation beams, allow tumors to be targeted more precisely and shield the heart and other healthy tissues for minimizing the radiation damage to normal cells. In this review, we discuss radiation therapeutic doses used and post-radiation damage to the heart muscle from published studies. We also emphasize the need for early detection of cardiotoxicity and the need for more cardio-protection approaches where feasible.

Keywords: radiation therapy, proton therapy, heavy ion radiotherapy, ionizing radiation cardiotoxicity, charged particle therapy, cardiovascular disease, radiation damage to the heart

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INTRODUCTION

Cancer associated heart disease has become a prominent cause of mortality in the industrialized world (1). Modern treatment using radiotherapy has resulted in a dramatic improvement in the chances of cancer patient's survival. While the high energy ionized radiation treatment successfully kill cancer cells, they at the same time harm healthy cells, leading to several side effects including increased cardiovascular disease in cancer survivors (2).

It is well known that nuclear industry workers and survivors of nuclear catastrophes have a significantly higher incidence of cardiovascular diseases than the general population (3–5). For the last couple of decades, it had been found that radio therapy (RT) increases the risk of associated radiation related cardiac damage in cancer survivors (6). However, a significant increase of death rate in the follow up after 10 year was found in patients post radiation therapy (7). Later studies also revealed that radiotherapy increased the cardiovascular mortality in women treated for left breast compared to those who are treated only to the right breast from earlier studies during 1970s and 1980s (8). Several population studies show that RT induced heart disease develops very slowly and often seen around 15 years after the first exposure to radiation (9).

Subsequent studies have focused on the risk of radiation-induced heart mortality as a linear-quadratic function at moderate dose levels (10) and at high dose levels a more linear response (11–13). However, no threshold dose studies have been reported; we therefore suggest that the radiation dose exposed to the heart must be minimized and limited as there is no such thing as safe radiation dose to the heart.

Studies to-date show that radiation-associated cardiac disease emerged from studies of breast cancer (14) and Hodgkin's lymphoma (15, 16) There exists enough scientific evidence to now support radiation-related heart injury as a direct effect of RT to the chest (8) (Early Breast Cancer Trialists' Collaborative Group, EBCTCG-2000). At doses above 30 Gy, heart disease may occur within a year or two of radiation exposure with concomitant increase in the risk factors for cardiovascular disease with higher radiotherapy doses. At lower doses, the latency period is longer and can extend to more than a decade (17). Cardiovascular disease as a direct side effect of radiation was more common with radiation treatment regimens used before the late 1980s. Newer radiation protocols with lower radiation doses and more focused radiation beams allow tumors to be targeted more precisely and shield the heart and other healthy tissue from direct impact of radiation. In this review we discuss radiation induced damages to the heart tissue and effectiveness of current approaches to minimize the damage.

RADIATION INDUCED CARDIAC DAMAGE

A study of radiation doses used between the 1950s and the 1990s comparing whole heart doses for left vs. right-sided breast cancer indicate that heart doses for left-sided were higher than that for the right. The dose range was shown to be 13–17 Gy for the left breast and 2–10 Gy for the right (18). Breast radiotherapy practiced in the 1970s and 1980s resulted in more exposure to the myocardium of the heart and thereby damage, which was higher when left breast was treated (**Table 1**). Higher cardiovascular mortality following irradiation of the left breast as opposed to the right has been attributed to this difference (19). Swedish cancer registry documents increased mortality from myocardial infarction for patients treated for left compared with right sided tumors during 1970 and 1985 (20).

A correlation exists between RT to the thoracic region and ischemic cardiac disease with older clinical trials that are perhaps no longer standards for radiation treatment care (**Table 2**). It is also noted that RT for Hodgkin's lymphoma and breast cancer increases the risk factor for cardiovascular disease (26). A 3–5-fold greater incidence of cardiovascular disease has been observed in patients treated for Hodgkin's lymphoma and thereafter followed for a median of 18 years (27).

In contrast, most of these complications are reduced significantly with recent modern radiotherapeutic approaches that are designed to minimize direct cardiac dose such as three dimensional conformal radiotherapy (3DCRT) [(28–30)] and field-in-field techniques (31). Modern advances also contain better imaging technology approaches that help minimize the radiation doses to critical organs including the exposure to the heart. Among these, image guided radiotherapy (IGRT) (32), intensity modulated radiotherapy (IMRT) (33) and stereotactic body radiotherapy (SBRT) (34) provide more efficient conformation around the tumor volume, sparing organs at risk. IMRT (35–38) and accelerated partial breast irradiation (39, 40) along with practices such as deep inspiration breath hold (DIBH) vs. free breathing reduce the mean heart dose by about 50% with mean heart doses 2–3 Gy (41–46).

In a study investigating the linkage of radiotherapy to cardiovascular associated deaths, the absolute risk was seen to increase within the first 10 years for coronary disease and from the next 10 for mortality (47). To this effect, earlier measurement of cardiac damage becomes crucial to better clinical therapeutic intervention (**Figure 1**).

A suggested protocol to identify cardiac damage - Methods that would reliably predict the progression from radiotherapy to late, irreversible cardiac damage would facilitate the development of better therapeutic measures to cardiac safety. A way to identify patients at risk for cardiac failure would help generation of some early preventive measures, individualized toward the patient. Methods could be set in place to detect and/or measure early cardiac damage such as biochemical tests. Studies for improving prediction and preventing lesions to cardiac tissue surrounding tumors such as BACCARAT (BreAst Cancer and Cardiotoxicity induced by RAdioTherapy) could improve patient care and overall quality of life (49). Atrial natriuretic peptide (ANP) levels are seen as increased in patients irradiated for Hodgkin's disease and breast cancer. This alludes to the possibility that ANP plasma levels may be an identification marker for radiation induced cardiac dysfunction (50).

RADIATION INDUCED VASCULAR CHANGES

It is well documented that RT induces vascular endothelial dysfunction, which ultimately results in clinical cardiovascular events, manifesting many years after completion of therapy (51). Radiation induced heart conditions are described in selected studies (**Table 3**). The linkage of senescence of endothelial cells and atherosclerosis has been well established (68). In the preclinical setting, irradiation of the heart has been associated with endothelial cell dysfunction leading to accelerated atherosclerosis (69).

A more focused study with rodent models indicate that the radiation causes microvascular damage. Microvascular damage is manifested by a decrease in capillary density, resulting in chronic myocardial ischemia and fibrosis, whereas macrovascular disease is due to an accelerated onset of age-related atherosclerosis (70). Experimental data (53) lead to formulation of two possibilities for a mechanistic explanation of increased death from coronary artery dysfunction that follows exposure to radiation. The first, being radiation increases the frequency of myocardial dysfunction by affecting the biological pathway of age-related atherosclerosis. The second that radiation reduces the heart's tolerance to acute infarctions due to damage to the microvasculature, thereby increasing lethality. These two possible explanations may be contiguous and not necessarily exclusive acting together to produce heart disease.

TABLE 1 | Relative risk of Cardiac mortality after radiation for left vs. right breast cancer laterality at 95% Confidence Interval (CMR, Cardiac Mortality Ratio).

	CMR (Left vs. right tumor laterality)	CMR (Left vs. Right tumor laterality)	CMR (Left vs. Right tumor laterality)
Diagnosis	**<10 years**	**10–14 years**	**≥15 years**
1973–1982	1.2 (1.04–1.38)	1.42 (1.11–1.82)	1.58 (1.29–1.95)
1983–1992	1.04 (0.91–1.18)	1.27 (0.99–1.63)	NA
1993–2001	0.96 (0.82–1.12)	NA	NA

TABLE 2 | Selected studies with significance for heart condition post radiation treatment.

Author-year	Tissue/neoplasm	Average dose to heart (Gy) (mean, range)	Heart studies (endpoints)	Sample size
Cohn et al. (6)	Hodgkin's, breast, cervix, esophagus	1.5–9	Pericardial effusion/Cardiac Damage	21
Brosius et al. (21)	Hodgkin's	3–8.8	Thickened pericardia, interstitial myocardial fibrosis, fibrous thickening of mural and valvular endocardium	16
Applefeld and Wiernik (22)	Hodgkin's thorax	3–4	Constrictive or occult constrictive pericarditis, abnormal hemodynamic response, coronary artery disease, left ventricular dysfunction	48
Orzan et al. (23)	Hodgkin's, lymphoma,breast, seminoma	45–122	Aortic stenosis, regurgitation, pericardial effusion, constrictive pericarditis, mitral/tricuspid regurgitation, myocardial infarction, pericardial effusion	15
Veinot and Edwards (24)	Hodgkin's thorax	1.3–4	Pericardial fibrosis, constrictive pericarditis, endocardial fibrosis, and valvular dysfunction, non-ischemic myocardial fibrosis, obstructive coronary artery disease with myocardial ischemia, damage to the great vessels and conduction system dysfunction	27
Darby et al. (12)	Breast	4.9 (0.03–27.72)	Myocardial infarction, coronary revascularization, ischemic heart disease	2,168 (936 cases, 1,205 control)
Erven et al. (25)	Breast/chest wall	5	Decrease in cardiac strain and strain rate	75

CHARGED PARTICLE THERAPY AND HEART

Particle radiation therapy applied today uses more advanced techniques and safer approaches. About 137,518 (by 2014) patients worldwide were treated with particle therapy between 1954 and 2014, 86% of which were treated with protons and 14% with carbon ions and with other particles (71). Between 2014 and 2016, in just 2 years, the total number of patients treated with particle therapy increased by 27% or 36,994 new patients to a total of 174,512 (by 2016), about 27% increase. This includes a 37% increase in new carbon ion therapy patients from 15,736 (in 2014) to 21,580 (in 2016) by 5,844. On the other hand, proton therapy patients were increased by about 26% from 118,195 (in 2014) to 149,345 by about 31,150 patients worldwide. This is a significant increase in the total number of patients who are treated with more precise radiation treatment options. A study for the late effects of radiotoxicity to the heart from this new class of patient database after 5 and 10 years is of great importance for detailed studies and assessment. Such studies are anticipated and expected to dominate the published literature in the next few years. More details of the ion therapy data worldwide are shown in **Figure 3** for protons and carbon ions and in **Table 4** for all other particle therapy patients.

Adjuvant breast radiotherapy dramatically reduced radiation dose to the heart and substantially decreased the risk of death from cardiovascular heart disease (72, 73). More efficient planning with CT scanners and accurate delivery with IMRT could be ways to protect the heart and lungs from unintentional radiation (74).

Radiation treatment with x-rays and gamma particles, which emit high energy electromagnetic radiation is absorbed completely into the target tissue, resulting in an increase of radiation dose per tissue depth. Proton and heavy ions such as carbon ions which constitute charged particles, deposit minimal energy at the entrance of the body where their velocity is greater and deposit most of the energy at the end of its range (as planned and calculated for the Bragg peak) in the tumor. Charged particles therefore present a newer advancement to RT to achieve lower and more targeted dose to tumor and reduce organ at risk (OAR). Since cardiac damage is a late event, long term follow-up data to study its effects on the heart are limiting. Charged particles operate by delivering high energy more effectively than x-rays or gamma particles, therefore they have an advantage of

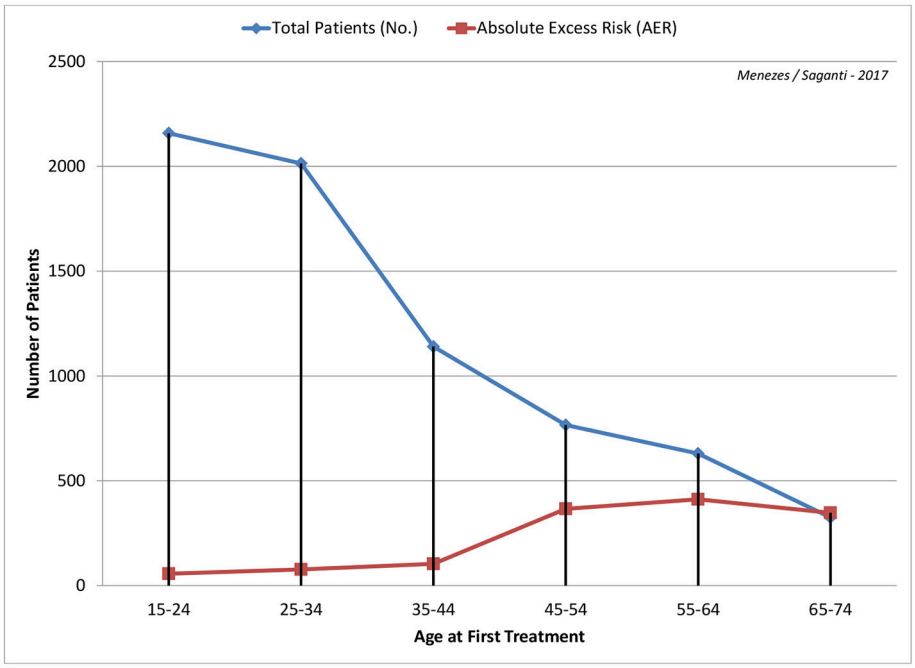

FIGURE 1 | Age at first radiation treatment from 15 years through 74 years are shown with calculated Absolute Excess Risk (AER) per 1,000 patients is depicted with data from Swerdlow et al. (48). Higher the age, the greater the risk with about 50% around age 45 years and almost 100% by age 65 years.

exhibiting a higher control of the tumor, lower probability of damage to healthy tissue, low risk of complications and a good prognosis for a rapid recovery after therapy (75); thus it is most promising for cardio-protection than conventional radio therapy.

Proton therapy may spare radiation exposure to the heart and reduce cardiotoxicity (18). The main benefit of proton therapy in breast cancer is to spare the heart from direct radiation exposure (76). The heart dose is dramatically reduced in proton therapy. A study on left breast cancer treatment using intensity radiotherapy and proton therapy using normal tissue probability showed that proton therapy has less radiation dose and damage to the heart (77). However, whether the cardiovascular disease is reduced in breast cancer survivors from proton therapy remains unclear. An undergoing study will reveal whether proton therapy decreases radiation induced cardiovascular disease in breast cancers.

In addition to the advantage of proton therapy, carbon therapy delivers higher linear energy transfer radiation (LET). High LET radiation increases radiation sensitivity to radioresistant cancer and overcomes the oxygen enhancement ratio (OER). Carbon ion therapy has also been used to stage I breast cancer without surgery at National Institute of Radiological Science (NIRS; Chiba, Japan) (78). Significant sparing of normal tissue has been demonstrated with IMRT (Intensity-modulated radiation therapy) proton treatment (79, 80), such that the dose delivered to 90% of the cochlea was reduced from 101.2% with conventional x-rays to 33.4% for IMRT beams and 2.4% for proton beams. Dose calculations to the heart recorded a reduction from 72.2% with conventional x-rays to 29.5% with IMRT and merely 0.5% with protons (**Figure 2**).

TABLE 3 | Radiation induced heart conditions for selected studies.

Radiation study	Observed condition	Description
Murros and Toole (52); Stewart et al. (53)	Arteriosclerosis	Thickening of heart wall and loss of elasticity
Gujral et al. (54)	Cardiac valve diseases	Heart Valve Abnormalities
Posner et al. (55)	Cardiac arrhythmias	Irregular Heart Rate
Stewart et al. (56); McChesney et al. (57)	Cardiomyopathy	Heart muscle becomes enlarged, thick or rigid
Wright and Bresnan (58); Ivanov et al. (59); Morris et al. (60); Smith et al. (61)	Cerebrovascular disease	Lack of oxygen to brain through blood
McReynolds et al. (62); Gyenes (63); Darby et al. (12)	Ischemic heart disease	Cholesterol plaque build-up in arteries, blocking flow of blood and oxygen
Morton et al. (64); Morton et al. (65); Brosius et al. (21); Posner et al. (55); Mill et al. (66); Stewart and Fajardo (67)	Pericarditis	Inflammation of the pericardium

NON RADIATION APPROACHES FOR PREVENTING DAMAGE TO THE HEART

Just as any other decease-prevention, mitigation, and treatment of radiation-induced cardiac injury also demands early detection. The sequences of events leading to cardiac damage that

TABLE 4 | Total number of patients who received treatment with protons, carbon, pion, helium, and other ions around the world through 2017.

Country	Protons	Carbon	Pion	Helium	Other	All
Belgium	21					21
Canada	196		367			563
China	1,239	563				1,802
Czech Rep.	1,538					1,538
England	3,020					3,020
France	13,903					13,903
Germany	8,556	2,870				11,426
Italy	846	816				1,662
Japan	23,842	17,331				41,173
Poland	167					167
Russia	7,061					7,061
South	2,799					2,799
Sweden	1,716					1,716
Switzerland	8,106		503			8,609
Taiwan	439					439
USA	75,896		230	2,054	433	78,613
Grand total	149,345	21,580	1,100	2,054	433	174,512

Data is adopted from PTCOG, Particle Therapy Co-Operative Group (https://www.ptcog.ch/).

result from radiation are of several facets. To identify an early detection marker to predict risk of radiation induced cardiovascular disease is a key to prevent the late effects. Ionizing radiation induce premature aging in cultured endothelial cells (ECs) can be seen as increased apoptosis and expression of inflammatory markers (81) which *in vivo* are associated with EC dysfunction and atherosclerotic plaque formation (82). It has been also reported that the biological effects of ionizing radiation exposure activate NF-κB, and reduces anti-inflammatory gene expression, which *in vivo* are pro-atherogenic conditions (83). Also, p90RSK is a unique serine/threonine kinase with two distinct functional kinase domains (84) that has been well characterized for its role in heart failure (85, 86). Perhaps, phenomena can be used as an early detection marker of radiation induced late cardiovascular diseases.

Pharmaceutical approach to prevent RT-related cardiac injury - since the endothelium of the vasculature is thought to be one target for injury induced by radiation, pharmaceutical interventions to maintain endothelial functions are one potential strategy to mitigate and treat radiation-induced cardiac damage. The pharmaceutical drug *Captopril*, which is currently used to treat hypertension and congestive heart failure because of its function as angiotensin-converting enzyme (ACE) inhibitor, has been known to be able to prevent structural changes to the heart, when administered after radiation exposure (20 Gy), but there is no evidence seen in its ability to prevent the decline in cardiac function (87). However, ACE inhibitors are not evaluated for cardio protective ability with lower doses of radiation (10 Gy or lower). Similarly, the drug *Simvastatin*, a lipid-lowering medication for lowering cholesterol has been observed to be capable of decreasing the radiation-associated injury to rats (88). However, critical data is lacking for understanding the ability of Simvastatin to mitigate cardiac damage following radiation (89). The plant polyphenol curcumin has been shown to have a potent anti-inflammatory and antioxidant properties (90).

Cardiac muscle toxicity can result in a concomitant loss of cardiac muscle and deterioration of the vasculature, ultimately resulting in cardiac failure. Current heart failure care can alleviate symptoms but cardiac myocytes that are killed during cancer therapies cannot be replaced or regenerated with current pharmaceuticals administered to-date. In light of the fact that most pharmaceutical interventions have not yet been demonstrated to be effective to repair cardiac damage, there arises a need for early detection of cardiac toxicity (91) and development of a new generation of therapeutics that are better able to more effectively prevent the cardiac injury caused by existing cancer therapeutics (92).

Cell based therapy to prevent RT-related cardiac injury—it has been investigated as a possible future treatment strategy for heart failure patients. Co-culturing stem cells with primary cells *in vitro* followed by injecting *in vivo* have demonstrated the ability of stem cells to engraft and differentiate into cells of cardiac nature. Myocytes isolated from cardiac tissue of rats have been shown as capable of inducing cardio- myogenic differentiation of endothelial progenitor cells (93, 94) and mesenchymal stem cells (95, 96). Mesenchymal stem cells injected into hearts of pig (97) or sheep (98) following myocardial infarction, have been shown to engraft long-term, express muscle-specific proteins as well as cells of vascular and smooth muscle origin (98). Despite the expression of cardiac proteins which are good indicators of cardiac differentiation, data is lacking for the stem cell's ability for differentiating into heart cells *in vivo*, alluding to the fact that merely injecting stem cells into heart may not be the best approach for cardiac muscle regeneration.

Activating stem cells residing within the heart may hold more promise as a therapeutic intervention strategy for heart regeneration. Scientific data exists for the ability of resident cardiac stem cells toward differentiating into the cardiac lineage. More specifically, the percentage of this population of dividing cardiac stem cells are shown to be increased in hearts undergoing acute infarction and those with end-stage cardiomyopathy when compared with normal cardiac tissue. Additionally, these cardiac stem cells display an increased commitment toward differentiation to the cardiac myocyte, smooth muscle and endothelial cell lineages within the infarcted and end-stage hearts as compared to hearts without abnormality or disease (99–101). Ongoing research is currently aimed at this differentiation process for understanding how to selectively increase the population of cells capable of regeneration which have highly sought after value for their functionality. Therefore, perhaps the best cell source for heart muscle regeneration is most likely the resident, cardiac stem cells if the proportion that becomes a thriving functioning heart cells could be enhanced. Further studies are needed to develop the cell based therapy specially targeted RT-induced cardiac injury.

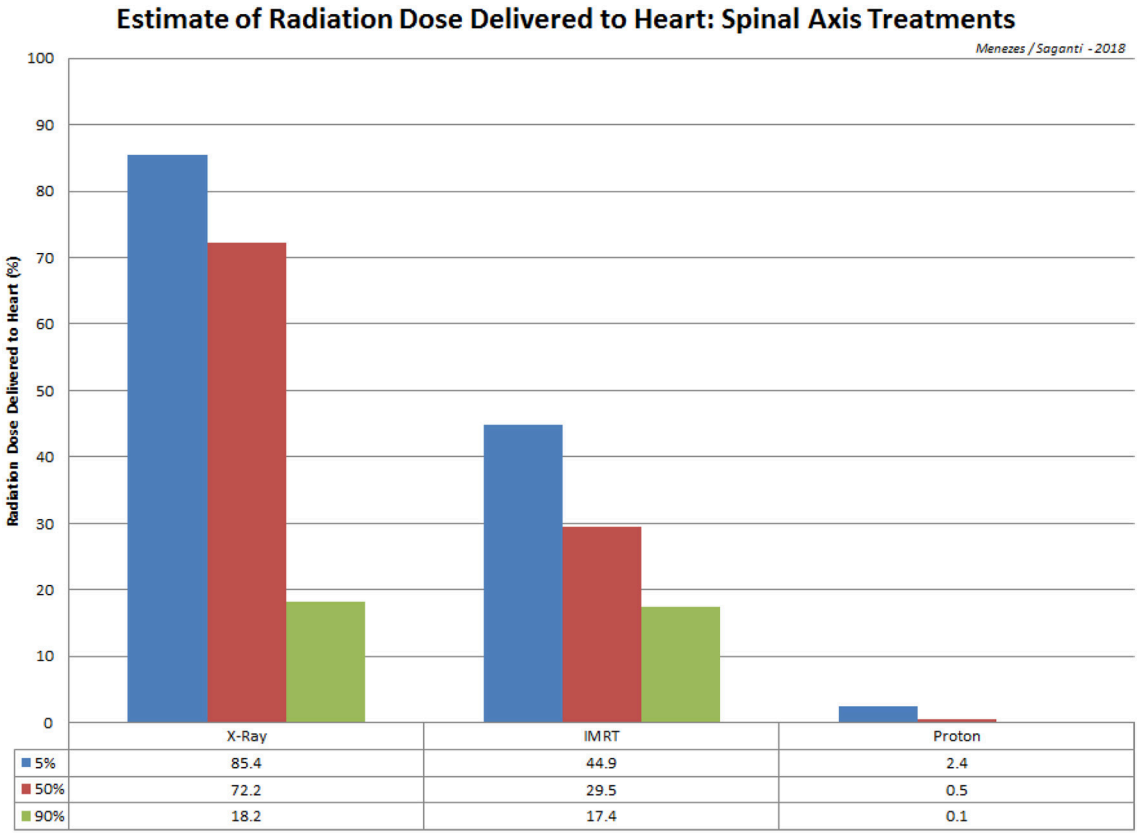

FIGURE 2 | A comparison of radiation treatment via spinal axis and the estimated dose received at the heart for X-Ray, IMRT, and Proton procedures. Data is adopted from St Clair et al. (80).

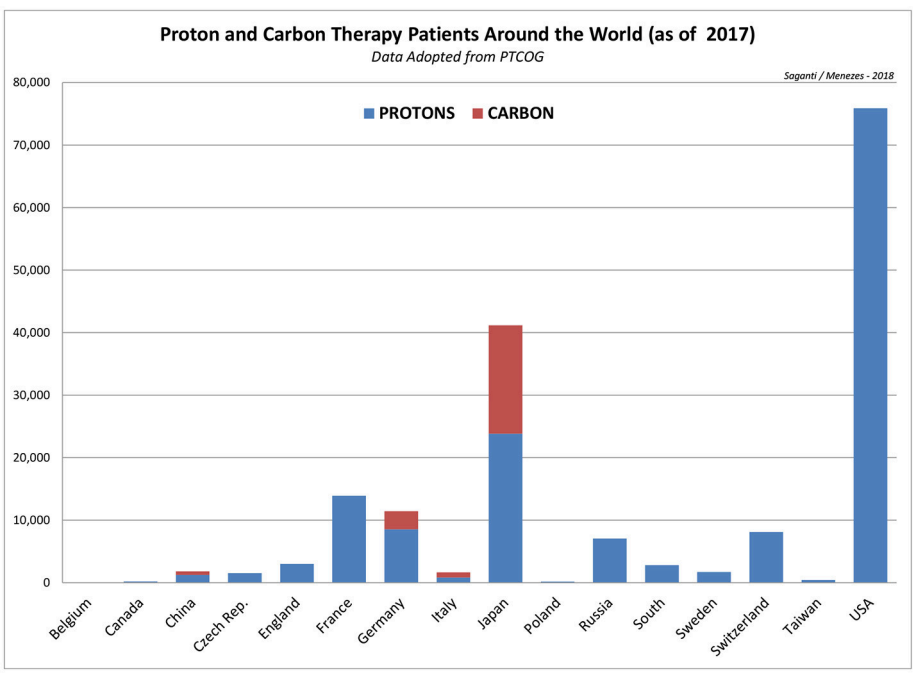

FIGURE 3 | Depiction of worldwide patients treated with protons and carbon ions as of 2017 indicating largest number patients treated with protons (75,896) in the US and patients treated with carbon ions (17,331) in Japan. Data is adopted from PTCOG, Particle Therapy Co-Operative Group (https://www.ptcog.ch/).

CONCLUSIONS

From various studies reviewed for this publication, it is evident that age at first radiation exposure plays a prominent role in cardiovascular related damage. The younger the age at first treatment, the greater the protection of the heart tissue and hence the lower is the risk. On the other hand, the older the age at first treatment the risk is significantly higher and the repercussions onset at an earlier time. It is also noted that by age 45–50 years, the risk of cardiovascular related damage risk increases by about 50%. This is of significant importance for general public and further studies and assessment by sex and treated conditions are to be published at a later time. We recommend more comprehensive long-term studies to be considered and evaluated as a function of time (up to ten years and beyond), sex (M/F), and radiation dose and type administered for various target sites.

A new class of radiation treatment procedures with particle therapy will be of greater challenge ahead in the years to come. At a rapid pace, nearly 20,000 patients per year during recent five years with ion therapy (protons and carbon) pose a potential challenge of cardio toxicity studies in near future. It is essential to establish the radiation related toxicity to the heart from particle therapy; it is believed that particle therapy is a rapidly growing approach for most cancer treatment protocols around the world.

Very likely it would be desirable for oncology research to encourage both medical and scientific explorations within the cardiac care and research communities to extend their follow-up for a greater period of time to discern any unforeseen cardiac complications which at present are most likely under-reported. Nonetheless, current radiation protocols far surpass the previous regimens in providing more radioprotection to critical organs including the heart. Much of the radiation related cardiotoxicity is associated with the use of traditional radiation approaches and older methods whereas the advanced modern therapies including particle therapy might reduce the immediate cardiac damage drastically. Advanced particle radiotherapy holds the promise for moving forward toward enhancing the efficacy of tumor cell killing and lowering the risk of cardiac complications from traditional radiation treatment approaches.

AUTHOR CONTRIBUTIONS

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

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Ionizing Radiation Induces Endothelial Inflammation and Apoptosis via p90RSK-Mediated ERK5 S496 Phosphorylation

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Background: Adverse cardiovascular events are a leading nonmalignant cause of morbidity and mortality among cancer survivors who have been exposed to ionizing radiation (IR), but the exact mechanism of the cardiovascular complications induced by IR remains unclear. In this study we investigated the potential role of the p90RSK-ERK5 module in regulating IR-induced endothelial cell inflammation and apoptosis.

Methods and Results: Whole body radiation of mice with 2 Gy γ-ray significantly increased endothelial VCAM-1 expression; especially in the disturbed flow area *in vivo*. *In vitro* studies showed that IR increased p90RSK activation as well as subsequent ERK5 S496 phosphorylation in cultured human endothelial cells (ECs). A specific p90RSK inhibitor, FMK-MEA, significantly inhibited both p90RSK activation and ERK5 S496 phosphorylation, but it had no effect on IR-induced ERK5 TEY motif phosphorylation, suggesting that p90RSK regulates ERK5 transcriptional activity, but not its kinase activity. In fact, we found that IR-induced NF-kB activation and VCAM-1 expression in ECs were significantly inhibited by the over-expression of S496 phosphorylation site mutant of ERK5 (ERK5 S496A) compared to overexpression of wild type ERK5. Furthermore, when ECs were exposed to IR, the number of annexin V positive cells increased, and overexpression of ERK5 S496A, but not wild type ERK5, significantly inhibited this increase.

Conclusions: Our results demonstrate that IR augmented disturbed flow-induced VCAM-1 expression *in vivo*. Endothelial p90RSK was robustly activated by IR and subsequently up-regulated ERK5 S496 phosphorylation, inflammation, and apoptosis in ECs. The EC p90RSK-ERK5 signaling axis can be a good target to prevent cardiovascular events after radiation therapy in cancer patients.

Keywords: Ionizing radiation, p90RSK, ERK5, EC inflammation, EC apoptosis

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INTRODUCTION

Radiation therapy (RT) is a critical component of the management of many malignancies, including primary thoracic malignancies, breast cancer, and lymphoma, but it is now known that RT causes various cardiovascular (CV) disorders such as coronary artery disease, congestive heart failure, and valvular disorders, resulting in morbidity and mortality among cancer survivors. For example, in survivors of Hodgkin lymphoma, RT to the chest area increases the risk of CV disease by up to 7-fold based on multivariate analyses controlling for other risk factors (1), and heart disease is the leading cause of non-oncologic death (2). Likewise, RT has been associated with significantly increased mortality due to CV disease in breast cancer survivors (3–5). In patients with locally advanced non-small cell lung cancer, the 2 year incidence of grade ≥3 CV toxicity after RT is 11% according to a combined analysis of 4 prospective studies (6), and deaths related to excess heart exposure may offset any benefit of increasing the radiation dose to control the disease (7). Thus, the cardiotoxic effects of RT are a serious concern. The cardiovascular effects of RT may be amplified in patients who also receive cardiotoxic chemotherapeutic agents, such as anthracyclines and trastuzumab. Although the role of IR in EC inflammation has been reported (8, 9), the exact molecular mechanisms remain unclear.

Shear stress is imposed directly on the luminal surface of blood vessels covered by a thin monolayer of ECs. There are two types of flow, which can differentially affect EC structure and function by regulating different cellular mechanosignaling pathways. Each of the mechanosignaling mechanisms will then activate shear stress response promoter elements and transcription factors in ECs residing in areas exposed to the two different types of flow and determine the cellular phenotype (10–13). For example, atherosclerosis (AS) lesions are rare in areas exposed to laminar flow. It is known that steady laminar flow (s-flow:10–20 dyne/cm^2) induces anti-inflammatory signaling and anti-atherogenic gene expression in cultured ECs (14, 15). S-flow suppresses the expression of inflammatory chemokines and adhesion molecules (VCAM-1, ICAM-1, and E-selectin) while maintaining the production of athero-protective factors such as nitric oxide (NO) and EC nitric oxide synthase (eNOS) (16–19). ERK5, a member of the mitogen-activated protein kinase family, is unique in that it is not only a kinase but also a transcriptional co-activator with a unique C-terminus transactivation domain (20, 21). It has been suggested that Krüppel-like factor 2 (KLF2) plays a crucial role in s-flow-induced anti-inflammatory effects via ERK5 activation (20, 22). Atherosclerotic plaques localize to areas of disturbed flow (d-flow) found in regions where vessels curve acutely, bifurcate, or branch. D-flow is pro-atherogenic; induces inflammation, apoptosis, and proliferation of ECs and reduces vascular reactivity. p90RSK is a unique serine/threonine kinase with two distinct functional kinase domains (23) that has been well-characterized by our group for its role in heart failure (24, 25). Our group has reported that d-flow induces phosphorylation of p90RSK, which then allows p90RSK to bind the C-terminus region of ERK5 (amino acids 571–807). This binding enables p90RSK to phosphorylate ERK5 at S496 (26) and inhibits the transcriptional activity of ERK5 (26). This inhibition ablates the promotor activity of KLF2 and eNOS activity. These series of molecular events lead to EC inflammation and dysfunction.

In this study, we found that IR enhanced EC inflammation, especially in the disturbed flow area compared to the laminar flow area *in vivo*. We also found that IR increased p90RSK activation and subsequent ERK5 S496 phosphorylation. Overexpression of an ERK5 S496A mutant, but not wild type ERK5, in ECs significantly inhibited the expression of NF-κB and VCAM-1 and apoptosis. To our knowledge, this is the first report that shows the crucial role of p90RSK-mediated ERK5 S496 phosphorylation in regulating IR-induced EC inflammation and apoptosis. The endothelial p90RSK-ERK5 axis can be a good target to prevent cardiovascular events after radiation therapy in cancer patients. In addition, since endothelial inflammation is a hallmark of radio-resistance, IR-induced activation of the endothelial p90RSK-ERK5 signaling cascade may contribute to radiation sensitivity of cancers.

METHODS

Antibodies and Reagents

Antibodies were purchased from the following vendors: anti-p90RSK1 (C-21, #SC-231) and anti-VCAM-1 (SC-8304) from Santa Cruz Biotechnology (Dallas, TX); anti-phospho-p90RSK (Ser380, #9341), anti-phospho-ERK5 (Thr218/Tyr220, #3371L), and anti-ERK5 (#3372) from Cell Signaling (Danvers, MA); anti-tubulin (T-5168) from Sigma (St. Louis, MO); anti-VE-cadherin (#555289) from BD Transduction Laboratory (San Diego, CA); and anti-phopho-specific ERK5 S496 antibody (#A02812) from Boster Bio (Pleasanton, CA).

Generation of Plasmids

Plasmids containing wild type (WT) p90RSK (WT-p90RSK) and dominant negative (kinase dead) p90RSK (DN-p90RSK) were generated as previously described (26). Gal4-ERK5 was created by inserting mouse ERK5 isolated from pcDNA3.1-ERK5 into the BamH1 and Not1 sites of the pBIND vector (Invitrogen, Carlsbad, CA). An adenovirus vector containing constitutively active (CA)-MEK5α was subcloned into the pENTR vector (Invitrogen) using specific enzyme sites, and then a recombinase reaction was performed to get a pDEST-based vector following manufacture's instruction (#K4930-00, ViraPower Adenoviral Expression System, Promega, Madison, WI). All constructs were verified by DNA sequencing by using vector specific primers.

Irradiation

Cells and mice were irradiated on the Cesium-137 Research-Irradiator "Mark-I Model M68A" (J. L. Shepherd and Associates, San Fernando, CA). The unit consists of sealed pencil-like (37 cm long) Radio-Active Source Cesium-137 producing 662 keV Gamma radiation. The Petri dishes were placed on an Acrylic stand, 10.8 cm above the upper floor-plate, to ensure dose-uniformity within 5% (95% iso-dose circle). C57BL/6 mice were placed in the cylindrical container (inner Diameter 20.2 cm, height 13.4 cm). Thin black plastic-strips were arranged to create wedge-shaped partitions for 8 mice. A round top-cover of clear plastic with a

wedged-cut was used to place and retrieve mice one at a time. The mice-loaded container was placed on an Acrylic stand 30.1 cm above the axis of pencil-like Cesium-Source. A dose of 2 Gy was delivered in this geometry. The mice container diameter (20.2 cm) ensured dose-uniformity within 5% (the 95% iso-dose circle has a diameter of 24 cm). In-air dose-rate at the irradiation height was measured with an ion-chamber employing the Task-Group Report 61 of the AAPM (American Association of Physicists in Medicine). The chamber had been calibrated by the M. D. Anderson's ADCL (Acredited Dosimetry Calibration-Laboratory). The chamber-calibration is traceable to NIST (National Institute of Standards and Technology). Experimental procedures were approved by the Institutional Care and Use Committee (IACUC) at Texas A&M Institute of Biosciences and Technology and the University of Texas MD Anderson Cancer Center.

En Face Staining of Mouse Aortas

C57Bl/6 male mice were used for this study. The disturbed flow (d-flow) and undisturbed laminar blood flow (l-flow) areas in the mouse aorta were identified based on generally accepted anatomical locations where such flow patterns are known to occur (27–29). We performed *en face* staining and confocal imaging as described in our previous reports (26, 28). Experimental procedures were approved by the Institutional Care and Use Committee (IACUC) at Texas A&M Institute of Biosciences and Technology and the University of Texas MD Anderson Cancer Center.

Transient Over-Expressions

For protein overexpression, ECs were transfected with Flag-tagged ERK5 WT, and ERK5-S496A mutant using GIBCO Opti-MEM reduced serum medium (cat. no. 31985070; Thermo Fisher Scientific, Waltham, MA) containing Plus and Lipofectamine reagents (cat. no. 11514015 and 18324020, respectively; Life Technologies, Carlsbad, CA) as we described previously (26).

PathDetect in Vivo Signal Transduction Pathway Reporting System

NF-κB activity was assayed using the PathDetect Signal Transduction Pathway trans-Reporting Systems (Stratagene, San Diego, CA) as we described previously (30).

Western Blotting

We applied an equal amount of proteins from control and treatment samples into each well of SDS-PAGE gels and performed Western blotting using specific antibodies for the indicated molecules in the figure as we described previously (31). Nitrocellulose membranes were incubated with primary antibodies diluted 500–1,000 times overnight at 4°C. After blocking with 3% BSA for one hour, the membranes were incubated with HRP-conjugated appropriate (goat anti-mouse or anti-rabbit) secondary antibodies diluted 4,000–5,000 times. Blots were developed using an ECL reagent, imaged using Film Processor SRX-101A (Konika Minolta Medical Graphics, Tokyo, Japan), and signal intensities quantified by densitometry using ImageJ software as we described (26).

Cell Culture and Transfection

We purchased primary human aortic endothelial cells (HAECs) from Invitrogen (Cat no-C0065C) and were grown in complete Endothelial Cell Medium (ECM, Sciencell Research Laboratories, Carlsbad, CA) supplemented with endothelial growth factors along with 5% FBS and 1% penicillin/streptomycin. Human umbilical vein ECs (HUVECs) were obtained from collagenase digested umbilical cord veins (32) and collected in M200 medium supplemented with LSGS (Cascade Biologics Inc., Portland, OR) and 2% fetal bovine serum (FBS) (Atlanta Biologicals Inc., Lawrenceville, GA) as we described previously (26). HUVECs were cultured on 0.2% gelatin pre-coated dishes. All the cells were maintained at 37°C in a humidified atmosphere of 5% CO2. For transient expression experiments, 70 to 80% confluent cells were transfected with cDNA using Opti-MEM containing Plus-Lipofectamine as we previously described (33). After 4 h of transfection, Opti-MEM was replaced with the complete M200 medium.

EC Apoptosis and VCAM-1 Expression

After transfection with flag-tagged ERK5 wild type or the S496A mutant, ECs were exposed to d-flow (24 h) and harvested by incubating with EDTA (10 mM in PBS). Cells were stained with annexin V-fluorescein isothiocyanate (Annexin V-FITC Apoptosis Detection Kit, cat. no. ab14085, Abcam, Cambridge, MA) according to manufacturer's instructions. Annexin V-positive cells were quantified using a BD Accuri C6 Flow Cytometer and the FlowJo software program. For detecting VCAM-1 expression, each sample was divided into 2 parts: one part was probed with an Alexa fluor ® 488-conjugated mouse monoclonal antibody against human VCAM-1 (RD systems FAB5649G) while the other was probed with Alexa fluor ® 488-conjugated isotype-matched control mouse IgG2a (RD systems: #IC003G). Both were subjected a BD Accuri C6 Flow Cytometer and assayed according to manufacturer's instructions.

Statistics

Differences between two independent groups were determined using the Student *t*-test (two-tailed) and, when applicable, one-way ANOVA followed by Bonferroni post hoc testing for multiple group comparisons using GraphPad Prism (GraphPad Software, SanDiego) was employed. When groups exhibited unequal variances, Welch's ANOVA was used to perform multiple group comparisons. *P* values less than 0.05 were considered statistically significant and are indicated by asterisks in the figures. *P* values <0.01 are indicated by two asterisks.

RESULTS

IR Induced EC Inflammation, Especially in Disturbed Flow Area *in Vivo*

Mancuso et al. have reported that atherosclerotic lesion formation in irradiated athero-prone ApoE$^{-/-}$ mice was accelerated, especially in the d-flow area (34). Therefore, to determine the role of IR in EC inflammation *in vivo*, aortas from male C57BL/6 mice exposed to IR (0–2 Gy) were isolated 19 h after IR, and

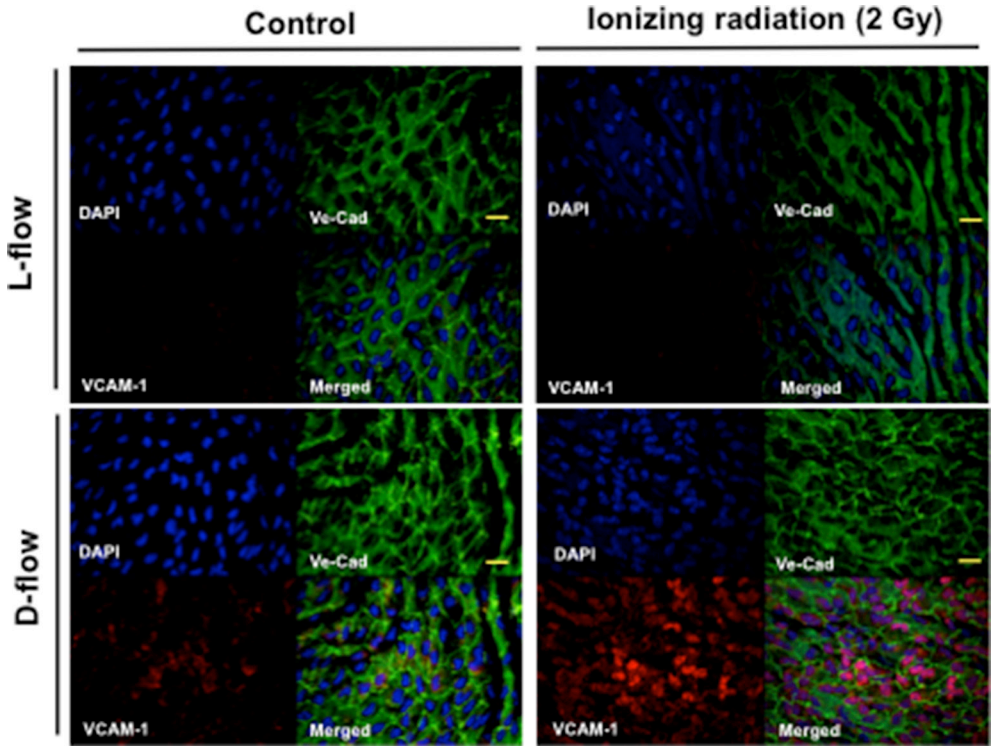

FIGURE 1 | IR enhanced d-flow-induced VCAM-1 expression. En face preparations of the aortic arch after 19 h of IR 0 (control) and 2 Gy-treated mice were tripple-stained with anti-Ve-cadherin (Ve-Cad as an EC marker), DAPI, and anti–VCAM-1. Representative confocal images from three independent experiments are shown. Scale: 20 μm

en face preparations were stained with anti-VCAM-1. 2 Gy of whole-body radiation did not have any significant detrimental life threating effects on mice. As we have reported previously, we found that VCAM-1 expression was higher in the d-flow area compared with the laminar flow (l-flow) area. Interestingly, we found that IR treatment significantly enhanced VCAM-1 expression only in the area exposed to d-flow, and we could not find any significant increase in VCAM-1 expression after IR treatment in the l-flow area (**Figure 1**). These data suggest the interplay between d-flow and IR in EC inflammation and support the idea that IR initially affects EC inflammation at the athero-prone area.

IR Increased ERK5 S496 Phosphorylation via p90RSK Activation

Previously, we have reported the key role of p90RSK activation in regulating ERK5 S496 phosphorylation (26). We found that p90RSK activation was dose-dependently increased by IR (**Figure 2A**). In addition, IR (2Gy) significantly increased phosphorylation of ERK5 S496, but we found no change in ERK5 TEY motif phosphorylation after IR (**Figure 2B,C**), which is directly related to ERK5 kinase activation. In addition, we also found that the pre-treatment of cells with a p90RSK specific inhibitor, FMK-MEA, significantly inhibited ERK5 S496 phosphorylation, but not TEY motif phosphorylation (**Figure 2B,C**). These data support the critical role of p90RSK activation in regulating IR-induced ERK5 S496 phosphorylation.

EC Inflammation Was Regulated by ERK5 S496 Phosphorylation

To determine the role of ERK5 S496 phosphorylation in EC inflammation, we overexpressed either wild type ERK5 or the ERK5 S496A mutant in ECs and examined whether IR can increase NF-kB activation and VCAM-1 expression (**Figure 3**). We found that IR significantly increased both NF-kB and VCAM-1 expression in cells overexpressing wild type ERK5 but that these increases were not observed in cells overexpressing the ERK5 S496A mutant.

IR-Induced EC Apoptosis Was Inhibited by Overexpression of ERK5 S496A Mutant

The anti-apoptotic role of ERK5 has been reported (33). Therefore, we examined the role of ERK5 S496 phosphorylation in IR-induced EC apoptosis. A significant increase of annexin V expression after IR was observed, and overexpression of ERK5 S496A mutant significantly inhibited IR-induced apoptosis (**Figure 4**). These results are consistent with the crucial role played by ERK5 S496 phosphorylation in IR-induced EC apoptosis. All the data obtained in this study indicate that IR causes ERK5 S496

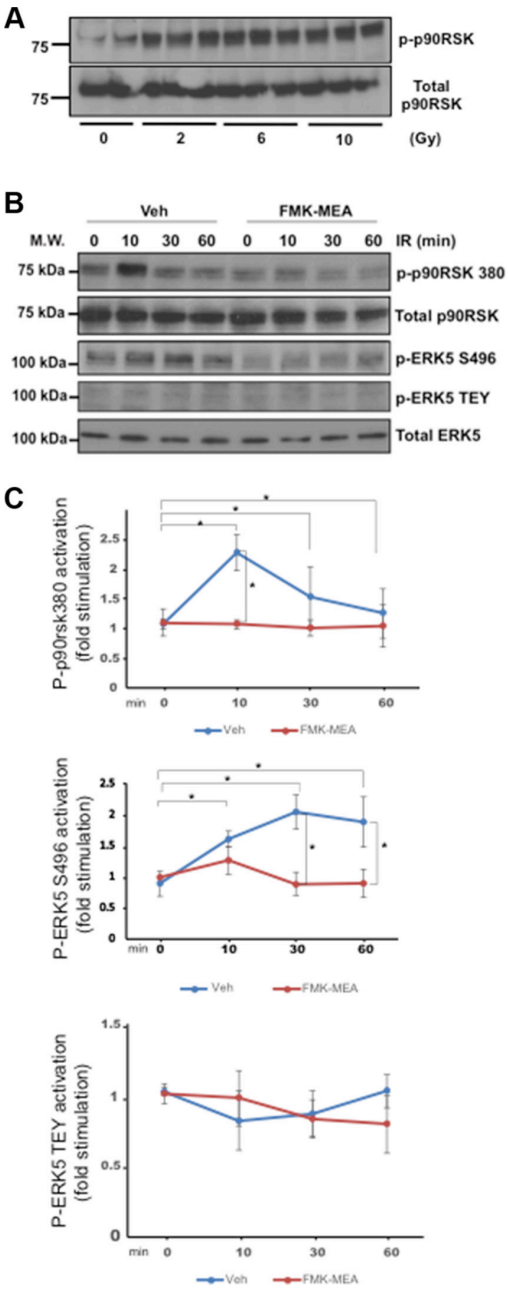

FIGURE 2 | IR-induced p90RSK activation lead to ERK5 S496 phosphorylation, but not ERK5 TEY motif phosphorylation. **(A)** HUVECs were treated with IR by the indicated doses, and 30 min after IR, ECs were collected and p90RSK activity was detected by Western blotting with anti–p-p90RSK and anti-p90RSK antibodies. Representative images from three independent experiments are shown. **(B)** HAECs were pre-treated with FMK-MEA (5 μM) for 30 min and treated by IR (2 Gy) for the indicated times. ECs were collected, and total p90RSK, p90RSK phosphorylation, ERK5 S496 phosphorylation, ERK5 TEY motif phosphorylation, and total ERK5 were detected by Western blotting with anti-p90RSK, anti-phospho-p90RSK (S380), anti-phospho-ERK5 (S496), anti-phospho-ERK5 (TEY motif), and anti-ERK5. Representative images from three independent experiments are shown. **(C)** Quantification of IR-induced p90RSK activation (S380 phosphorylation; top), ERK5 S496 phosphorylation (middle), and ERK5 activation (TEY motif phosphorylation; bottom) is shown after normalization by total protein levels. Data represent mean ± SEM (n = 3).

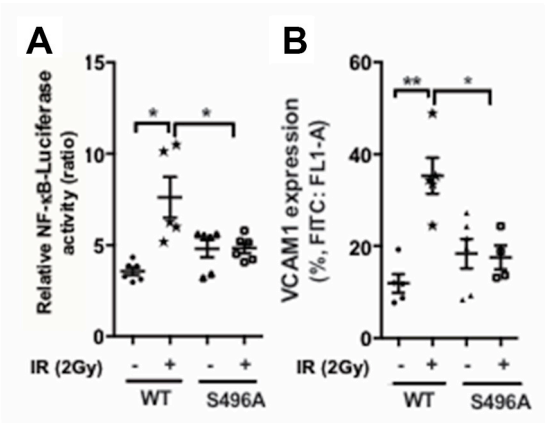

FIGURE 3 | ERK5 S496 phosphorylation is critical for IR-induced EC inflammation. HUVECs were co-transfected with the NF-κB-Luc luciferase reporter vector and the pRL-CMV vector (renilla luciferase activity, internal control) together with the expression vector encoding the flag-tagged ERK5 wild type (WT) or the S496A mutant. Transfected cells were then exposed to gamma radiation (2 Gy) and harvested after 24 h. **(A)** NF-κB activation was quantified by measuring relative luciferase activity using a dual-luciferase reporter system. Shown are relative luciferase activity, presented as firefly luciferase/renilla luciferase activity ratio. (n = 6) **(B)** VCAM1 expression was determined by measuring percentage of positive VCAM1-staining cells using the BD Accuri C6 Flow Cytometer system using the FL1 533/30 nm filter. (n = 5)

phosphorylation and that this phosphorylation plays a major role in inducing EC inflammation and apoptosis.

DISCUSSION

In this study, we found the crucial role of p90RSK-mediated ERK5 S496 phosphorylation in IR-induced EC inflammation and apoptosis. Although it has been well established that IR can induce inflammation, the details of mechanistic insights into this inflammatory response are lacking. We found that p90RSK activity was very sensitive to radiation. This IR-induced p90RSK activation led to increased ERK5 S496 phosphorylation. Previously, we have reported that ERK5 S496 phosphorylation decreased ERK5 transcriptional activity and subsequently inhibited KLF2/4 expression, which can up-regulate NF-kB activation and inflammatory gene expression (26). In the current study first we found that a p90RSK specific inhibitor, FMK-MEA, significantly inhibited IR-induced NF-kB activation and subsequent VCAM-1 expression as well as EC apoptosis. Next, to determine the role of p90RSK-mediated ERK5 S496 phosphorylation in IR-induced EC inflammation and apoptosis, we generated adenovirus containing ERK5 S496A mutant and found that overexpression of ERK5 S496A mutant inhibited not only IR-induced inflammatory responses but also apoptosis in endothelial cells. Taken together, these data support the critical role of p90RSK-mediated ERK5 S496 phosphorylation in EC inflammation and apoptosis. The IR-induced EC apoptosis

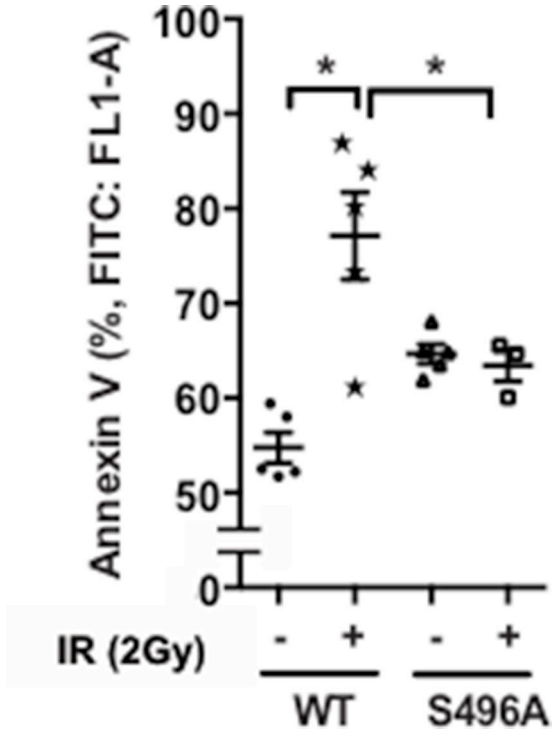

FIGURE 4 | ERK5 S496 phosphorylation is critical for IR-induced apoptosis. HUVECs were transfected with the expression vector encoding the flag-tagged ERK5 wild type (WT) or the S496A mutant. Transfected cells were then exposed to gamma radiation (2 Gy), and harvested after 60 h. Cell apoptosis was determined by measuring percentage of Annexin V- positive cells (Abcam's Annexin V-FITC Apoptosis Detection Kit) using the BD Accuri C6 Flow Cytometer system with the FL1 533/30 nm filter. Shown are mean ± SEM, n = 3–6.

appears to take place via a new signaling pathway, which is responsible for mediating IR-induced EC dysfunction that leads to atherosclerotic plaque formation.

As shown in **Figure 1**, we found that IR increased EC inflammation *in vivo*. Interestingly, we found that the extent of increase of VCAM-1 expression by IR was more evident in the disturbed flow area than in the laminar flow area. Previously, we have reported that disturbed flow, but not laminar flow, increased p90RSK activation (35). Importantly, we also reported that p90RSK expression in the disturbed flow area was higher than that in the laminar flow area (35). Therefore, disturbed flow area is more sensitive to IR, which may explain why VCAM-1 expression is considerably higher in the disturbed flow region than in the laminar flow area. Our studies have raised several new areas of investigation. First, it is important to find out in what way p90RSK is involved in IR-induced VCAM-1 expression. For determining the exact molecular mechanism of IR-induced EC dysfunction *in vivo*, it is crucial to understand what determines the differential IR responses in ECs exposed to two different types of flow and how the signaling pathway(s) activated by IR and those activated by different flow types interact.

The crucial role of reactive oxidative species (ROS) induced by IR in regulating cancer development and the surrounding microenvironment has been reported (36). Since we have reported that p90RSK is a redox-sensitive kinase (37), it is possible that endothelial p90RSK activation is up-regulated by IR-induced ROS. IR can generate ROS via radiolytic hydrolysis and mitochondrial dysfunction (36). Hydrolysis results in decomposition of water by ionizing radiation to hydrogen peroxide, superoxide, hydroxyl radicals and singlet oxygen. These radicals can, in turn, react with organic molecules to generate secondary radicals. These primary and secondary radicals can cause protein oxidation, lipid peroxidation and oxidative DNA damage all of which can be potentially lethal to cells (38). IR can also decrease electron transport chain complex 1 activity and produce ROS persistently (39). It is well known that these ROS production mechanisms can cause EC dysfunction and consequently enhance plaque formation (40, 41). Of note, IR-induced ROS production can generate inflammatory tumor micro-environments and decrease the effectiveness of radiotherapy (36). Therefore, determining the molecular mechanisms of IR-induced EC inflammation is crucial for understanding not only the process of atherosclerosis and cardiovascular events in cancer survivors but also radio-resistance. Further investigation is necessary to clarify these issues.

In this study, we focused on the direct effects of IR on ECs, but it is possible that the contents in tumor cells such as electrolytes and DNA are released after cancer treatment, which then indirectly causes metabolic disturbance and cardiovascular toxicity (42). Not only cancer treatment itself but also the contents of tumor cells after tumor lysis will be important to determine the whole picture of EC activation after IR.

ETHICS STATEMENT

Experimental procedures were approved by the Institutional Care and Use Committee (IACUC) at Texas A&M Institute of Biosciences and Technology and the University of Texas MD Anderson Cancer Center.

AUTHOR CONTRIBUTIONS

HV and N-TL performed experiments, interpreted data and wrote the manuscript. SK, KK, YF, YT, JM, TT performed experiments and interpreted data. PS performed radiation study and interpreted data. MH, AKS, SM, SK, KF and J-IA interpreted data, wrote and edited the manuscript.

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Ponatinib Activates an Inflammatory Response in Endothelial Cells via ERK5 SUMOylation

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Ponatinib is a multi-targeted third generation tyrosine kinase inhibitor (TKI) used in the treatment of chronic myeloid leukemia (CML) patients harboring the Abelson (Abl)-breakpoint cluster region (Bcr) T315I mutation. In spite of having superb clinical efficacy, ponatinib triggers severe vascular adverse events (VAEs) that significantly limit its therapeutic potential. On vascular endothelial cells (ECs), ponatinib promotes EC dysfunction and apoptosis, and inhibits angiogenesis. Furthermore, ponatinib-mediated anti-angiogenic effect has been suggested to play a partial role in systemic and pulmonary hypertension via inhibition of vascular endothelial growth factor receptor 2 (VEGFR2). Even though ponatinib-associated VAEs are well documented, their etiology remains largely unknown, making it difficult to efficiently counteract treatment-related adversities. Therefore, a better understanding of the mechanisms by which ponatinib mediates VAEs is critical. In cultured human aortic ECs (HAECs) treated with ponatinib, we found an increase in nuclear factor NF-kB/p65 phosphorylation and NF-kB activity, inflammatory gene expression, cell permeability, and cell apoptosis. Mechanistically, ponatinib abolished extracellular signal-regulated kinase 5 (ERK5) transcriptional activity even under activation by its upstream kinase mitogen-activated protein kinase kinase 5α (CA-MEK5α). Ponatinib also diminished expression of ERK5 responsive genes such as Krüppel-like Factor 2/4 (*klf2/4*) and *eNOS*. Because ERK5 SUMOylation counteracts its transcriptional activity, we examined the effect of ponatinib on ERK5 SUMOylation, and found that ERK5 SUMOylation is increased by ponatinib. We also found that ponatibib-mediated increased inflammatory gene expression and decreased anti-inflammatory gene expression were reversed when ERK5 SUMOylation was inhibited endogenously or exogenously. Overall, we propose a novel mechanism by which ponatinib up-regulates endothelial ERK5 SUMOylation and shifts ECs to an inflammatory phenotype, disrupting vascular homeostasis.

Keywords: ponatinib, vascular adverse events, ERK5 SUMOylation, EC inflammation, tyrosine kinase inhibtor (TKI)

INTRODUCTION

CML and Ph^+ALL involve the reciprocal translocation of the Abl oncogene on chromosome 9 and the Bcr on chromosome 22 (1–3). The resulting chromosomal fusion produces a constitutively active Bcr-Abl tyrosine kinase (4) that promotes dysregulated proliferation and survival signaling, leading to leukemogenesis (1, 5). Therefore, Bcr-Abl kinase is the primary therapeutic target for CML patients. Newly diagnosed CML patients commonly receive imatinib (first generation TKI), a small molecule that binds the ATP pocket on the Bcr-Abl tyrosine kinase, as a first line of treatment (6, 7). Because CML patients often develop Bcr-Abl point mutations conferring resistance to imatinib (8), dasatinib, and nilotinib (second generation TKIs) were generated (9). T315I is a specific point mutation present in ∼15% of relapsed CML patients (10) that causes therapeutic resistance to all currently approved first and second generation TKIs (11–13). Ponatinib (a third generation TKI) was specifically designed to circumvent the sterical hindrance warranted by the T315I mutation (14–17). Since it is the only drug effective against this mutation, ponatinib has become the treatment of choice for CML patients harboring T315I Bcr-Abl (18–23).

In spite of having superb clinical efficacy, ponatinib treatment comes with an array of adverse side effects attributable to the broad-spectrum inhibition of multiple kinase families in addition to Bcr-Abl (3). Common secondary effects of ponatinib treatment are xerostomia, abdominal pain, and cytopenia. Specifically in the cardiovascular system, ponatinib treatment induces substantial arterial and venous VAEs (24) including peripheral arterial occlusive disease (25), ischemic heart disease (26), cerebrovascular accident, venous thrombo-embolism (27), pulmonary hypertention (28), platelet dysfunction, and hyperglycemia (26–30). In a prospective analysis of 19 patients who received ponatinib therapy, 42% developed arterial cardiovascular events after 8.5 months. A phase I trial showed a significant percentage of vascular occlusive events (24, 31) and a phase II trial (PACE) demonstrated a strong correlation between ponatinib administration and serious arterial thrombotic events (10, 32). A randomized, opened-label phase III trial (EPIC) designed to compare efficacy between ponatinib and imatinib as first line treatments in newly diagnosed CML patients was terminated early due to serious VAEs observed in the ponatinib treated group (33). Ponatinib-associated VAEs are a serious clinical challenge in CML patients subjected to this therapeutic regime (34). A broad comparative profiling analysis of ponatinib and other TKIs showed that ponatinib inhibits VEGFRs with greater potency (26), through which it reduces viability, function, migration, and tube formation in ECs, thus causing vascular toxicity (35). Ponatinib-associated VEGFR2 inhibition has also been implicated in hypertension (26).

Even though ponatinib-mediated VAEs have been documented (36, 37) the exact molecular mechanism by which this drug induces VAEs remains obscure. Interestingly, despite promoting arterial thrombotic events (26, 38), ponatinib inhibits platelet activation, aggregation, spreading, and granule secretion (39). These observations suggest that ponatinib-associated thrombotic events are not due to the activation of platelets (40), but rather of other cell types. Because ponatinib treatment increases EC dysfunction and apoptosis (35), both of which are associated with a higher rate of VAEs (41), it is plausible that ponatinib-associated VAEs are related to EC inflammation, dysfunction and apoptosis.

In ECs, ERK5 plays an important role in maintaining vascular homeostasis (42). Similar to ERK1/2, ERK5 has the activation loop (T-x-Y sequence) on its dual phosphorylation sites (T218/Y220) (43) as well as a kinase domain on the NH2-terminus. Uniquely, ERK5 contains two transcriptional activation domains on the COOH-terminus (44–46), giving it a different function and regulatory mechanism from ERK1/2 (45, 47, 48). ERK5 is activated by a wide range of stimuli, among which is laminar flow with anti-inflammatory and anti-atherogenic properties (43). In its inactive form, the intra-molecular interaction between NH2-terminus and COOH-terminus of ERK5 inhibits ERK5 transcriptional activity (47). The activation of ERK5 by upstream regulators such as MEK5α and laminar flow (49, 50), disrupts this intra-molecular interaction and triggers T218/Y220 phosphorylation and subsequent transcriptional activation, conferring anti-inflammatory, anti-apoptotic, and anti-atherogenic properties (51–59). Under pro-inflammatory conditions, such as reactive oxygen species and disturbed flow, ERK5 is SUMOylated at K6/22 (60) and phosphorylated at S496 (52). These two post-translational modifications inhibit ERK5 transcriptional activity, resulting in EC inflammation and apoptosis (52, 61).

In the current study, we tested the hypothesis that ponatinib triggers an endothelial inflammatory response by promoting ERK5 SUMOylation.

METHODS

Antibodies used in this study are listed in **Table 1**.

Ponatinib Preparation and Treatment

Ponatinib was obtained from ARIAD pharmaceuticals. Following the manufacturer's instructions, ponatinib was dissolved in citrate buffer 25 mM pH 2.75 [2.5 mM sodium citrate (CAS no. 6132-04-3); 25 mM citric acid (CAS no. 77-92-9)]. Confluent, quiescent Human Umbilical Vein ECs (HUVECs) or Human Aortic ECs (HAECs) were treated with ponatinib or vehicle and incubated for the indicated times at 37°C.

Generation of Plasmids and Adenoviruses

Constitutively active form of MEK5α (CA-MEK5α) plasmid, adenoviruses expressing ERK5 wild type (Ad-ERK5-WT), ERK5 non-SUMOylatable mutant (Ad-ERK5-K6/22R), ERK5

Abbreviations: VAEs, Vascular adverse events; TKI, Tyrosine kinase inhibitor; Pona, Ponatinib; CML, Chronic myeloid leukemia; ERK5, Extracellular signal regulated kinase 5; VCAM1, Vascular cell adhesion molecule 1; ICAM1, Intercellular adhesion molecule 1; TNF, Tumor necrosis factor; SUMO, Small ubiquitin-like modifier; ECs, Endothelial cells; Ad, Adenovirus; HUVECs, Human umbilical vein ECs; HAECs, Human Aortic ECs; CAMEK5α, Constitutive active form of MEK5α; LF, Laminar flow; p90RSK, 90 kDa ribosomal protein S6 kinase; SENP2, Sentrin/SUMO-specific protease 2; KLF2/4, Krüppel-like Factor 2/4; eNOS, Endothelial nitric oxide synthase.

TABLE 1 | List of antibodies.

Antibody	Vendor	Cat #
Tubulin	Sigma	T9026
ERK5	Cell Signaling	3372
SUMO2/3	Abgent	Ap1224a
pERK5-S496	Abnova	PAB15918
pERK5-T218/Y220	Cell Signaling	3371
SENP2	Novus	NBP1-31217
VCAM1	Cell Signaling	13662
p-p65 NFkB	Cell Signaling	3033
p-65 NFkB	Cell Signaling	8242
β-Actin	Novus	NB600-532
p-p90RSK-S380	Cell Signaling	9341
RSK	R&D Systems	MAB2056

phosphorylation resistant mutant (Ad-ERK5-S496A), and Sentrin/SUMO-specific protease 2 (SENP2) were generated previously (33, 41, 44, 48–50). Where indicated, an adenovirus containing β-galactosidase (Ad-LacZ) was used as a control (33, 41, 44, 48–50).

Cell Culture

HUVECs were purchased from Lifeline cell technology (C-12200, cat. no. 10171-906). HAECs were a kind gift from Dr. Lusis (UCLA, David Geffen School of Medicine). HUVECs and HAECs were cultured in Petri dishes or flasks coated with 0.2% gelatin type A (cat. no. 901771; MP Biomedicals, Santa Ana, CA, USA), in Endothelial Cell Medium (ECM, Cat.no. 1001, ScienCell, Carlsbard, CA. USA) containing 465 mL of basal medium, 25 mL of fetal bovine serum (FBS, Cat. no. 0025, ScienCell, Carlsbard, CA, USA), 5 mL of Endothelial Cell Growth Supplement (ECGS, Cat. no. 1052, ScienCell, Carlsbard, CA, USA) and 5 mL of penicillin/streptomycin solution (P/S, Cat. no. 0503, ScienCell, Carlsbard, CA, USA). Only HUVECs with less than 6 passages and HAECs with <15 passages were used in this study.

NF-κB Activity Assay

NF-κB activity was measured using a luciferase assay with a reporter gene containing five NF-κB-binding sites as an enhancer [pLuc-MCS with five repeated NF-κB–binding sites (TGGGGACTTTCCGC); Stratagene, La Jolla, CA, USA]. A transfection mixture was made using GIBCO Opti-MEM Reduced Serum Medium (cat. no. 31985070**;** Thermo Fisher Scientific, Waltham, MA, USA) to which DEAE-DEXTRAN (final concentration, 0.375 μg/μl, cat. no. D9885; Sigma, St. Louis, MO, USA), a reporter vector, and a pRL-CMV vector (Promega, Madison, WI, USA) were added, and the mixture was incubated for 10 min at 37°C. pRL-CMV was used as an internal control for Renilla luciferase activity. Next, culture medium was removed, cells were washed with PBS and the transfection mixture was added. After 90 min of incubation, cold Opti-MEM Reduced Serum Medium containing 5% dimethyl sulfoxide was added to the cells, and the mixture was incubated for an additional 5 min. Cells were then washed once with PBS and cultured in a normal ECM culture medium. At the completion of experiments, cells were harvested in a passive lysis buffer (cat. no. E1960; Promega, Madison, WI, USA), and the NF-κB activity was determined by using a GloMax 20/20 Luminometer (Promega, Madison, WI, USA) to measure luciferase activity in resulting cell lysates (dual-luciferase reporter assay system, cat. no. E1960; Promega, Madison, WI, USA), as we have described previously (41, 50)**.** Relative NF-κB activity was calculated by normalizing firefly luciferase activity to Renilla luciferase activity (firefly: renilla luciferase activity ratio).

qRT-PCR

At the end of experiments, ECs were washed three times with PBS, and lysed in RLT Plus RNeasy lysis buffer (cat. no. 74136; QIAGEN, Germantown, MD, USA). The resulting cell lysates were loaded onto a QIAshredder column (cat. no. 79656; QIAGEN, Germantown, MD, USA), and spun down to collect the eluted lysates. Total RNA was then isolated from this lysate using an RNeasy Plus Mini Kit (cat. no. 74136; QIAGEN, Germantown, MD, USA) following the manufacturer's instructions. cDNA reverse transcription was performed with a 50 μl reaction mixture containing 1 μg of purified RNA, 5 μl of 10X buffer, 11 μl of $MgCl_2$, 10 μl of dNTPs, 2.5 μl of a random hexamer, 1.25 μl of oligo-dT, 1 μl of RNase inhibitor, and 0.75 μl of a reverse transcriptase enzyme using TaqMan Reverse Transcription Reagents (cat. no. N808-0234; made for Applied Biosystems by Roche Molecular Diagnostics, Pleasanton, CA, USA). First-strand cDNA was reverse-transcribed from total RNA by incubating reaction mixtures at 25°C for 10 min followed by 37°C for 60 min, 42°C for 60 min, and 95°C for 5 min before soaking at 4°C in a PCR cycler. Target cDNA levels were quantified using a CFX Connect Real-Time System (Bio-Rad, Hercules, CA, USA). Each reaction mixture (10 μl) contained cDNA synthesized from 20 ng of total RNA, 5 μl of iQ SYBR Green Supermix (cat. no. 1708882; Bio-Rad, Hercules, CA, USA), and 0.5 μmol/l each forward and reverse primer (see **Table 2** for primer sequences). RT-PCR was carried out at 95°C for an initial 3 min followed by 40 cycles of denaturation at 95°C for 10 s and annealing at 65°C for 45 s (ICAM1, KLF2), at 56°C for 45 s (VCAM1, TNF). The ΔΔCt method was used to calculate fold changes in expression of target RNAs (51): ΔCt = Ct (target gene)–Ct (housekeeping gene), ΔΔCt = ΔCt (treatment)–ΔCt (control), and fold change = $2^{(-\Delta\Delta Ct)}$.

ERK5 Transcriptional Activity Assay (Mammalian One-Hybrid Assay)

Sub-confluent ECs plated on 6-well-plate were incubated in Opti-MEM medium (Invitrogen, Carlsbad, CA, USA) containing Plus-Lipofectamine transfection reagents, the pG5 luciferase (pG5-Luc) and pBIND-ERK5 plasmids with pcDNA3.1-CA-MEK5α or control pcDNA3.1 vector, as we performed and described previously (41), for up to 4 h. Then, the transfection mixture was removed, ECs were washed, and ECM was added. Next, cells were treated with ponatinib at the concentrations indicated in the figures, for 24 h. Finally, cells were harvested, lysed, and luciferase activity was measured by a TD-20/20 Luminometer (Turner Designs, Sunnyvale, CA, USA), using

TABLE 2 | List of primers.

Primers	Sequences
GAPDH-Fwd	ggt ggt ctc ctc tga ctt caa
GAPDH-Rev	gtt gct gta gcc aaa ttc
VCAM1-Fwd	ccg gat tgc tgc tca gat tgg a
VCAM1-Rev	agc gtg gaa ttg gtc ccc tca
ICAM1-Fwd	gtc ccc tca aaa gtc atc c
ICAM1-Rev	Aac ccc att cag cgt cac c
TNF-Fwd	ccc agg gac ctc tct cta atc
TNF-Rev	atg ggc tac agg ctt gtc act
KLF2-Fwd	gca cgc aca cag gtg aga ag
KLF2-Rev	acc agt cac agt ttg gga ggg
KLF4-Fwd	acc agg cac tac cgt aaa cac a
KLF4-Rev	ggt ccg acc tgg aaa atg ct
eNOS-Fwd	gtg gct gtc tgc atg gac ct
eNOS-Rev	cca cga tgg tga ctt tgg ct
SENP2-Fwd	agc ctg gtg gtg att gac cta aga
SENP2-Rev	agc tgt tga ggga atc tcg tgt ggt

dual-luciferase reporter reagents (Promega, Madison, WI, USA). The pG5-Luc plasmid has five Gal4 binding sites upstream of a minimal TATA box, which in turn, is upstream of the firefly luciferase gene. The pBIND-ERK5 plasmid has Gal4 fused with ERK5. Because pBIND vector also contains the Renilla luciferase gene, the expression and transfection efficiency were normalized to the Renilla luciferase activity. Therefore, relative ERK5 transcriptional activity was calculated by normalizing firefly luciferase activity according to Renilla luciferase activity (firefly:renilla luciferase activity ratio).

Flow Study

Confluent HAECs cultured in 100-mm dishes were exposed to laminar flow using a cone-and-plate apparatus placed in an incubator at 37°C and 5% CO_2 for 24 h, as we previously described (52).

KLF2 Promoter Activity

Sub-confluent HAECs were transfected with Flag-ERK5, a reporter gene encoding KLF2 promoter ($-924 \sim +14$) and the luciferase control reporter vector pRL-CMV, using an OPTI-MEM/Plus-Lipofectamine mix, as we previously described (52). After incubating 3 h at 37°C, the transfection mix was removed and replaced with complete ECM. Next day, complete ECM was replaced with low serum ECM (0.2% FBS, 1% P/S, no ECGF). After 1 h, ponatinib (150 nM) was added to the medium and cells were incubated an additional 24 h. KLF2 promoter luciferase activity was assayed using a dual-luciferase reporter system.

Immuno-Precipitation (IP, to Detect ERK5-SUMOylation), SDS/PAGE and Immuno-Blotting (IB)

At the end of experiments, ECs were washed three times in ice-cold PBS and lysed by adding a sufficient volume of 1X cell lysis buffer (cat. no. 9803S; Cell Signaling Technology, Danvers, MA, USA) or modified RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate (SDS), 0.25% sodium deoxycholate) supplemented with a mammalian protease inhibitor cocktail (cat. no. p8340; Sigma, St. Louis, MO, USA), 1 mM phenylmethylsulfonyl fluoride (cat. no. 36978; Thermo Fisher Scientific, Waltham, MA, USA), and 20 mM N-ethylmaleimide (cat. no. E3876; Sigma, St. Louis, MO, USA). The resulted cell lysates were centrifuged at 15,000 rpm for 15 min, and supernatants were collected. Protein concentrations were determined using a standard BCA protein assay. For IP, anti-ERK5 was added to the cell lysates followed by overnight incubation in cold room, with rocking. Next, a mixture of protein A/G agarose [1:1 ratio protein A agarose (cat. no. 15918-014; Invitrogen, Carlsbad, CA, USA) and recombinant protein G agarose (cat. no. 15920-010; Invitrogen, Carlsbad, CA, USA)] was added and incubated further. Beads were then washed three times with ice-cold lysis buffer, and bound proteins were released in 2X SDS sample buffer and analyzed by IB with anti-SUMO to detect SUMOylated ERK5. For IB, we loaded equal protein amounts from control and treated samples in each well of SDS-polyacrylamide gel, and proteins were resolved using SDS-polyacrylamide gel electrophoresis and electro-transferred onto Immobilon polyvinylidene fluoride transfer membranes (cat. no. IPVH00010; EMD Millipore, Darmstadt, Germany). The membranes were then immunoblotted with an antibody against each indicated protein. We incubated with the primary antibodies at 1:1,000 and at 1:5,000 dilutions for goat anti-mouse or anti-rabbit secondary antibodies conjugated with HRP. Resulted membranes were visualized using an enhanced chemiluminescence detection reagent (cat. no. 170-5060; Bio-Rad, Hercules, CA, USA) following the manufacturer's instructions.

Automated Capillary Electrophoresis Western Analysis

Whole cell lysates were collected in modified RIPA buffer as described in the IP and IB section. A total of 5 μL of 0.4–1 mg/mL protein was loaded into plates and capillary electrophoresis western analysis was carried out following the manufacturer's instructions (Protein simple WES, part no. 004-600, ProteinSimple, San Jose, CA) using the 12–230 kDa Separation Module (part no. SM-W003, ProteinSimple, San Jose, CA) and either Rabbit (part no. DM-001, ProteinSimple, San Jose, CA) or Mouse (part no. DM-002, ProteinSimple, San Jose, CA) Detection Modules. Briefly, whole cell lysates were mixed with 5X fluorescent master mix containing 200 mM DTT followed by heating at 95°C for 5 min. Cell lysates, blocking buffer (antibody diluent), primary antibodies (in antibody diluent), HRP-conjugated secondary antibodies, and luminol-peroxide were then dispensed onto the separation plate. Antibodies against β-actin served as loading controls and were multiplexed with the primary antibodies for all samples. Capillary electrophoresis was performed using the instrument default settings: separation

time 25 min, separation voltage 375 V, blocking 5 min, primary and secondary antibodies 30 min. Finally, automatically detected standards and peaks were manually inspected, and the data were analyzed with the inbuilt Compass software (ProteinSimple) (62).

Assessment of Barrier Function by Transepithelial/Transendothelial Electrical Resistance (TEER) Measurements

TEER values of HAEC monolayers treated with ponatinib was assessed in real-time by ECIS system using 8W10E+ array chambers. Briefly, the array chambers were treated with 10 mM L-Cysteine solution (room temperature, 15 min) followed by washing twice with ultra-pure water. The treated chambers were then coated with 0.2% gelatin type A. HAECs were seeded into the chambers and grown in complete ECM overnight to produce a confluent monolayer. Next day, complete ECM was replaced with low serum ECM (0.2% FBS, 1% P/S, no ECGF) and baseline resistance measurements were taken. Upon stabilization, ponatinib was added, and change in TEER values were recorded by an ECIS-Zθ instrument (Applied BioPhysics Inc., Troy, NY, USA) connected with a Dell personal computer equipped with ECIS software (Applied Biophysics). Figures illustrate normalized TERR values (where the value of 1.0 represents the basal TEER measurement immediately before adding ponatinib). Decrease in TEER indicates increased permeability (63).

Flow Cytometric Analysis of Apoptotic Cells by Annexin V Staining

Following treatment (as indicated in the figures), cells were washed twice with PBS, harvested passively using 10 mM Ethylenediaminetetraacetic acid (EDTA, pH 8.0) solution at room temperature, and stained for apoptotic marker Annexin-V using Annexin V-FITC Apoptosis Detection Reagent (cat. no. ab14082; Abcam; Cambridge, MA, USA) as per the manufacturer's instructions. Briefly, cell pellets were re-suspended in 1X Annexin V Binding Buffer (cat. no. ab14084; Abcam; Cambridge, MA, USA) and baseline measurements were taken (unstained controls). Then, cells were stained with Annexin V-FITC (cat. no. ab14083; Abcam; Cambridge, MA, USA) at room temperature for 5 min in the

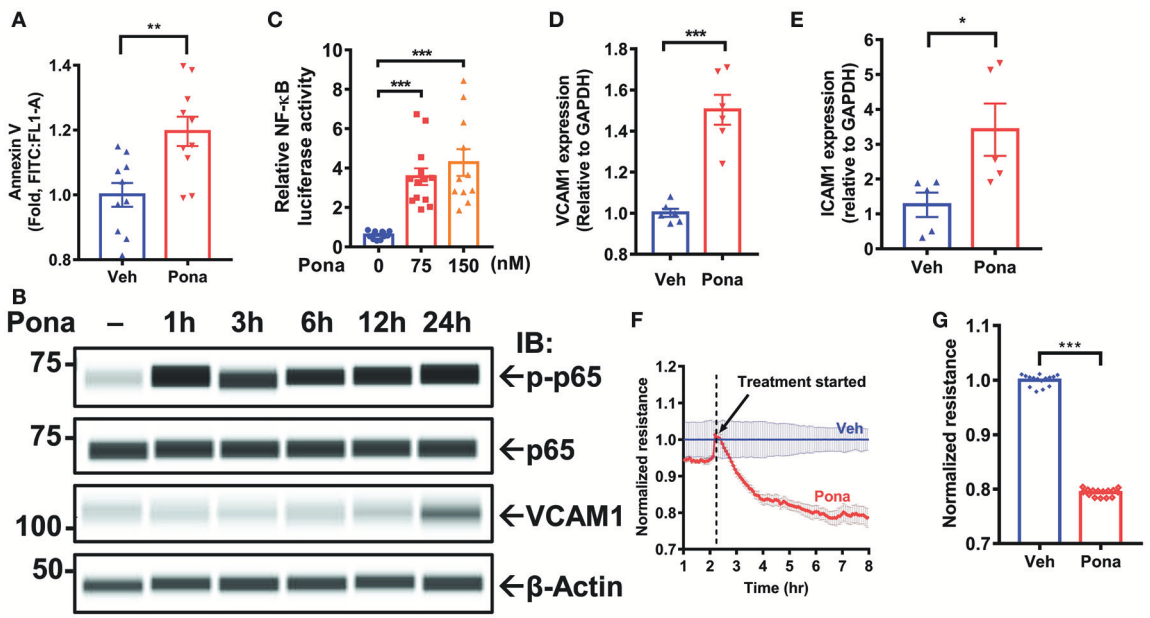

FIGURE 1 | Ponatinib mediates endothelial inflammatory responses **(A)** Flow cytometric analysis of Annexin V staining in HAECs treated with ponatinib (150 nM, 24 h). Graph shows the fold increase of apoptosis in ponatinib treated cells compared to control cells. Data is sourced from two independent experiments, each contains 4–5 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using student's *t*-test (two-tailed). $^{**}p < 0.01$. **(B)** Expression of phospho-p65, total p65, VCAM1, and β-actin (loading control) in HAECs treated with ponatinib was assessed by Protein simple WES system (capillary electrophoresis western analysis). Protein bands are shown as pseudoblots. **(C)** Graph demonstrates relative NF-kB activity, as measured by promoter-driven luciferase reporter gene assay in HAECs treated with phamacological concentrations of ponatinib for 24 h, and presented as ratio of firefly/renilla luciferase activity. A representative data set of three independent experiments is shown, contains 11–13 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using ANOVA followed by Bonferroni *post hoc* testing for multiple group comparison. $^{***}p < 0.001$. **(D-E)** qRT-PCR analysis of relative *vcam1* and *icam1* expression in HAECs treated with ponatinib (150 nM, 24 h). A representative data set of three independent experiments is shown, contains 5–6 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using student's *t*-test (two-tailed). $^{***}P < 0.001$; $^{*}P < 0.05$ vs. vehicle control (Veh) **(F)** Ponatinib (150 nM) effect on transcellular electrical resistance was assessed using ECIS system as described in methods. The dashed line indicates addition of ponatinib. Graph shows normalized resistance measured approximately every 4 min for 8 h, contains 4 technical replicates. Error bars represent mean ± SEM. **(G)** Graph demonstrates normalized resistance after 7 h of ponatinib treatment relative to Veh, contains 15 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using student's *t*-test (two-tailed). $^{***}P < 0.001$.

dark. Measurements for all samples were carried out using Accuri C6 flow cytometer (BD Biosciences, CA, USA). Ten thousand cells were acquired based on forward and side scatter characteristics. Results were analyzed using FlowJo software (version 10.5.0, FlowJo LLC, USA).

"Scratch" Wound Closure Assay

Confluent HAECs transduced with Ad-ERK5-WT, Ad-ERK5-K6/22R or Ad-LacZ were wounded using a 1000 μL microtip. Complete ECM medium was replaced with low serum ECM, and cells were treated with ponatinib. 48 h later, cells were photographed and wound closure ability was assessed by comparing the wound size of ponatinib and veh-treated cells.

Statistics

Differences between two independent groups were determined using the student's *t*-test (two-tailed). Differences between multiple groups were determined using one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* testing for multiple group comparison by GraphPad Prism (GraphPad Software, San Diego, CA, USA). *P* values < 0.01 were considered statistically significant and are indicated by two asterisks in the figures. *P*-value < 0.001 is indicated by three asterisks.

RESULTS

Ponatinib Triggers an Inflammatory Response in ECs

Cell viability, migration, and functionality are decreased in HUVECs treated with ponatinib (35). To examine if the observed effect is similar across different types of ECs, we treated HAECs with ponatinib to assess apoptosis. Flow cytometric analysis of Annexin V staining indicated increased cell apoptosis in ponatinib treated group (**Figure 1A**). Because dying cells trigger an inflammatory response (64), we asked if ponatinib-associated apoptosis triggers an endothelial inflammatory response. In HAECs treated with pharmacologically relevant concentrations of ponatinib (75 nM, 150 nM) (35, 65), we noted a significant increase on NF-kB p65 phosphorylation (**Figure 1B**) and NF-κB activity (**Figure 1C**). Expression of inflammatory genes including vascular and intercellular cell adhesion molecule 1 (*vcam1* and *icam1*) was also increased, both at mRNA (**Figures 1D,E**) and protein levels (**Figure 1B**) in ponatinib treated cells. EC apoptosis

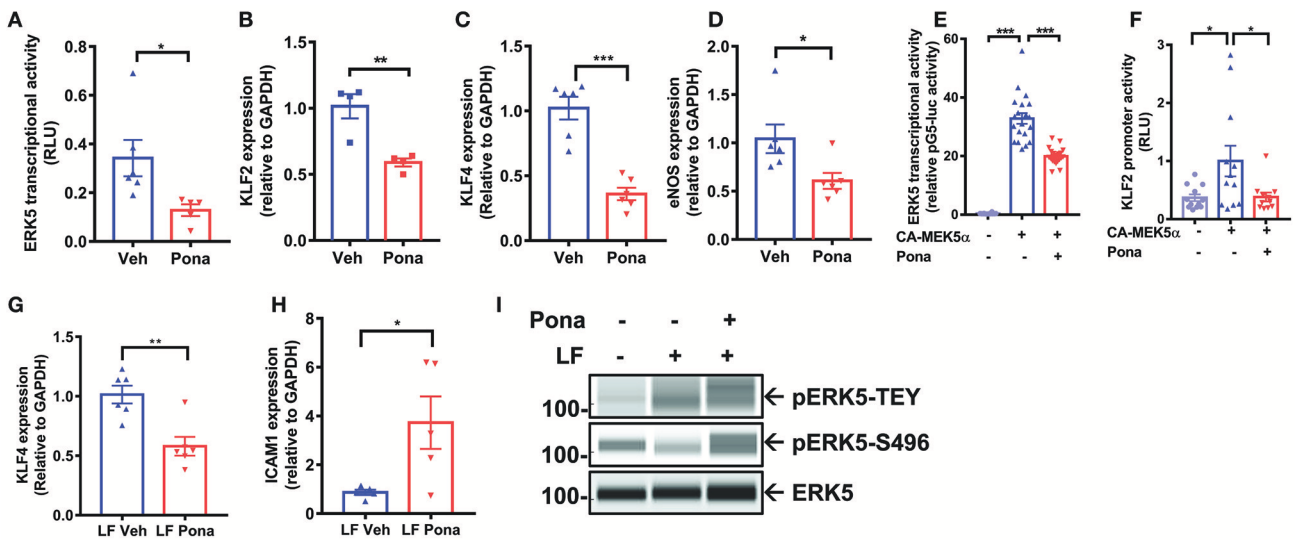

FIGURE 2 | Ponatinib inhibits ERK5 transcriptional activity **(A)** Graph demonstrates relative ERK5 transcriptional activity, as measured by mammalian one-hybrid assay, in HAECs treated with ponatinib (150 nM, 24 h), and presented as ratio of firefly/renilla luciferase activity. A representative data set of three independent experiments is shown, contains 6 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using student's *t*-test (two-tailed). $^{*}P < 0.05$ vs. Veh control. **(B-D)** qRT-PCR analysis of relative *klf2, klf4,* and *enos* expression in HAECs treated with ponatinib (150 nM, 24 h). A representative data set is shown, contains 4–6 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using student's *t*-test (two-tailed). $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$ vs. Veh control. **(E)** Graph demonstrate relative ERK5 transcriptional activity, as measured by mammalian one-hybrid assay in HAECs treated with ponatinib (150 nM, 24 h). Results are presented as ratio of firefly/renilla luciferase activity. As indicated, some cells were also transfected with CA-MEK5α. A representative data set of three independent experiments is shown, contains 11–20 technical replicates. Error bars represent mean ± SEM. Statistical significant was assessed using ANOVA followed by Bonferroni *post hoc* testing for multiple group comparison. $^{***}P < 0.001$ vs. Veh control. **(F)** Graph demonstrates relative KLF2 promoter activity, as measured by promoter-driven luciferase reporter gene assay in HAECs treated with ponatinib (150 nM, 24 h), and presented as ratio of firefly/renilla luciferase activity. As indicated, some cells were also transfected with CA-MEK5α. Error bars represent mean ± SEM. Data set contains 10–12 technical replicates. Statistical significance was assessed using student's *t*-test (two-tailed). $^{*}P < 0.05$ vs. Veh control. **(G-H)** qRT-PCR analysis of relative *klf4* and *icam1* expression in HAECs treated with ponatinib (150 nM, 24 h) in the presence of laminar flow. Data set contains 5–6 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using student's *t*-test (two-tailed). $^{*}P < 0.05$; $^{**}P < 0.01$; vs. Veh control. **(I)** Expression of phospho-ERK5 at T218/Y220 (TEY), S496, and total ERK5 in HAECs treated with ponatinib (150 nM, 30 min) was assessed by Protein simple WES system (capillary electrophoresis western analysis). Protein bands are shown as pseudoblots.

can lead to disruption of the EC barrier that results in vascular leakage (64, 66). Using electric cell-substrate impedance sensing (ECIS) system, we assessed the effect of ponatinib on EC barrier function by measuring transendothelial electrical resistance (TEER) of cell monolayers. TEER values revealed increased EC permeability in ponatinib treated cells (**Figures 1F,G**). Taken together, our results suggest that ponatinib induces apoptosis along with and inflammatory response in ECs.

Ponatinib Inhibits ERK5 Transcriptional Activity

Endothelial ERK5 plays a crucial role in vascular homeostasis, and its reduction leads to an accelerated inflammatory response in ECs (52). We asked if ponatinib triggers an endothelial inflammatory response via reducing ERK5 function. Employing a mammalian-one-hybrid assay, we noted decreased ERK5 transcriptional activity in ponatinib treated cells (**Figure 2A**). Similarly, expression of ERK5 responsive genes, including *klf2/4* and *eNOS*, was also inhibited (**Figures 2B–D**). In HAECs over-expressing a constitutive active form of MEK5α (CAMEK5α), ERK5 transcriptional activity (**Figure 2E**, bar 2 from the left) and KLF2 promoter activity (**Figure 2F**, bar 2 from the left) were activated, both of which were inhibited upon ponatinib treatment (**Figures 2E,F**, bar 3). Ponatinib also inhibited the increase of *klf4* (**Figure 2G**) and decrease of *icam1* expression (**Figure 2H**) induced by laminar flow (43). These results indicate that ponatinib reduces ERK5 transcriptional activity. Since ERK5 transcriptional activity is regulated by activation of its kinase domain, we studied the effect of ponatinib on ERK5 T218/Y220 phosphorylation (pERK5-TEY). We found that ponatinib did not affect laminar-flow induced ERK5 T218/Y220 phosphorylation (**Figure 2I**, first lane) suggesting that ponatinib-mediated reduced ERK5 transcriptional activity is independent of ERK5 kinase activity. Interestingly, we found that after ponatinib treatment, ERK5 S496 phosphorylation was significantly increased, even in the presence of protective laminar flow (**Figure 2I**, second lane). Because ERK5 S496 phosphorylation promotes an inflammatory response in EC (52), ponatinib-mediated phosphorylation at this residue might play a crucial role in ponatinib-associated endothelial inflammatory response.

Ponatinib Increases ERK5 SUMOylation in ECs

ERK5 SUMOylation regulates endothelial inflammatory response via repressing laminar flow-mediated ERK5 transcriptional activation (60). We tested if ponatinib inhibits ERK5 transcriptional activity by promoting ERK5 SUMOylation. Following ponatinib treatment, we collected cell lysates for IP studies to determine the level SUMOylated ERK5 in ECs. We found that ERK5 SUMOylation was significantly increased in both HUVECs (**Figure 3A,B**) and HAECs (**Figures 3C,D**) treated with ponatinib, and that this increase was reversed in cells

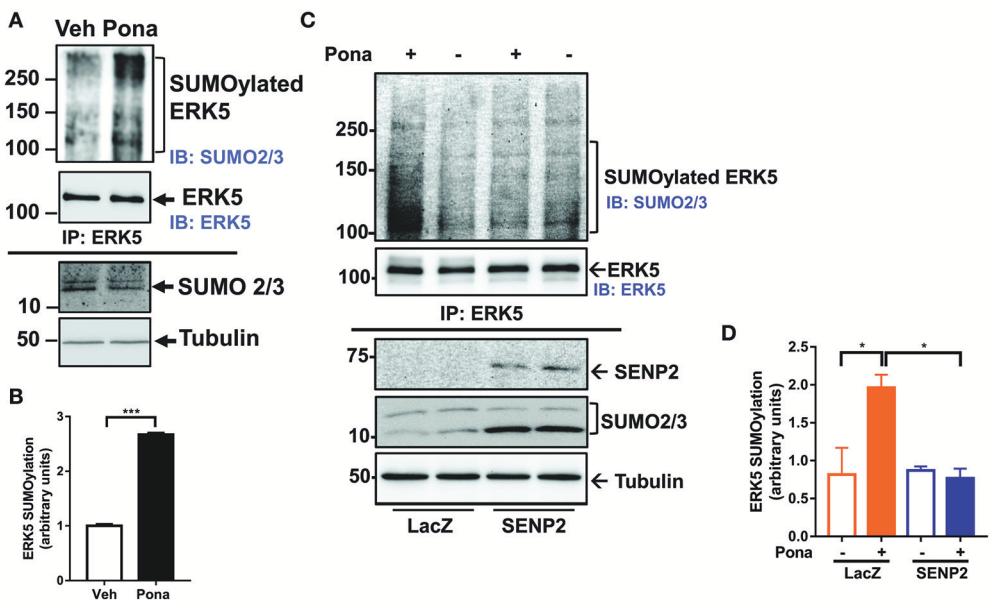

FIGURE 3 | Ponatinib increases ERK5 SUMOylation. **(A,B)** Western blotting analysis of immuno-precipitated samples to detect SUMOylated ERK5 and ERK5 in HUVECs treated with ponatinib (150 nM, 60 min). A representative data set of two independent experiments is shown **(A)**. Densitometric quantification of SUMOylated ERK5. Data is sourced from two independent experiments, each contains 2 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using student's *t*-test (two-tailed). ***$p < 0.001$ **(B)**. **(C,D)** Western blotting analysis of immuno-precipitated samples to detect SUMOylated ERK5 and ERK5 in HAECs, transduced with Ad-LacZ or Ad-SENP2, treated with ponatinib (150 nM, 60 min). A representative data set of three independent experiments is shown **(C)**. Densitometric quantification of SUMOylated ERK5. Data is sourced from three independent experiments, each contains 2 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using ANOVA followed by Bonferroni *post hoc* testing for multiple group comparison. *$P < 0.05$ vs. control.

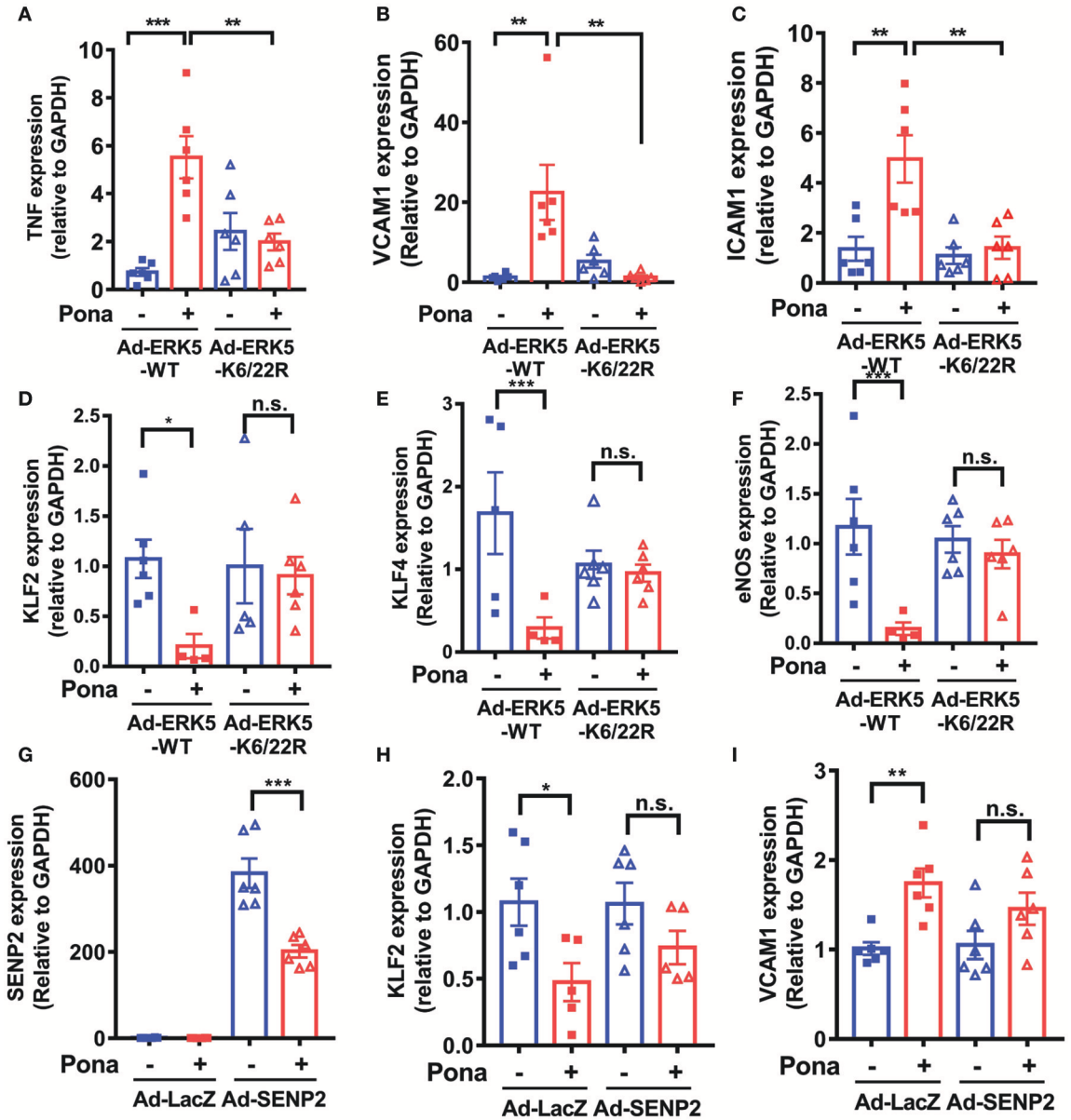

FIGURE 4 | Ponatinib elicits endothelial inflammatory responses via promoting ERK5 SUMOylation. qRT-PCR analysis of relative *tnf, vcam1, icam1, klf2, klf4, eNOS,* and *senp2* in HAECs expressing ERK5-WT or K6/22R mutant **(A–F)**, and SENP2 **(G–I)** treated with ponatinib (150 nM, 24 h). Data set contains 5–6 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using ANOVA followed by Bonferroni *post hoc* testing for multiple group comparison. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ vs. control.

overexpressing deSUMOylation enzyme Sentrin/SUMO-specific protease 2 (SENP2) (**Figures 3C,D**).

Ponatinib Elicits Endothelial Inflammatory Responses via Promoting ERK5 SUMOylation

To verify the involvement of ERK5 SUMOylation in ponatinib-associated endothelial inflammatory response, we transduced HAECs with either an adenovirus expressing ERK5 wild type (Ad-ERK5-WT) or ERK5 non-SUMOylatable mutant (Ad-K6/K22R). The cells were then treated with ponatinib for 24 h, and expression of various genes were determined. qRT-PCR analysis revealed that the increased *tnfα*, *vcam1*, and *icam-1* as well as the decreased *klf2/4* and *enos* expression by ponatinib seen in ECs expressing ERK5-WT was reversed in ECs expressing the ERK5 K6/22R mutant (**Figure 4A–F**). It is possible that ERK5-WT over-expression might skew the involvement of ERK5 SUMOylation in ponatinib-mediated endothelial inflammatory response. Thus, in an independent experiment,

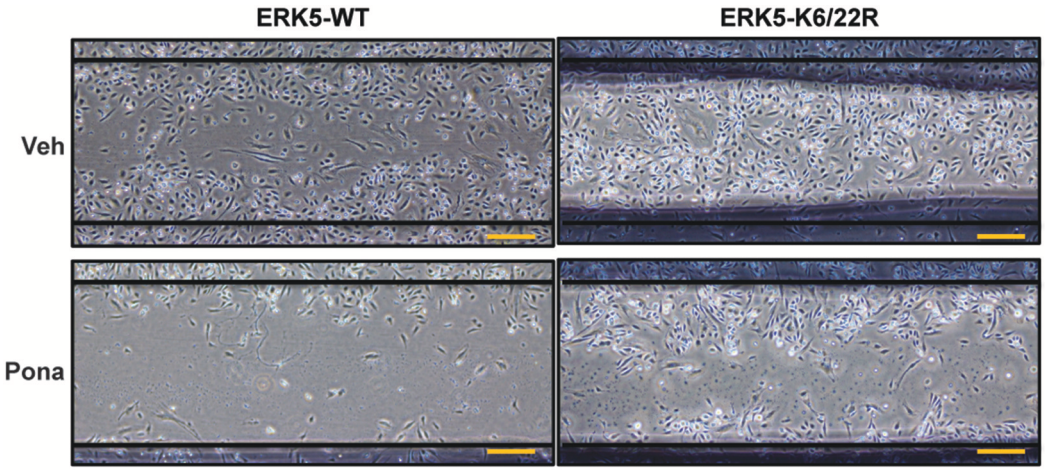

FIGURE 5 | Delayed migration of cells toward the wounded region seen in ECs expressing ERK5-WT was rescued in ECs expressing the ERK5 K6/22R mutant: Confluent monolayers of HAECs expressing Ad-ERK5-WT or Ad-ERK5-K6/22R mutant were serum starved for 1 h, and then wounded with a 1000 μL pipette tip followed by ponatinib treatment (150 nM, 48 h). Cells were photographed and assessed for wound closure. Scale bar represent 100 μM. A representative data set of two independent experiments is shown.

we used SENP2 overexpression to inhibit endogenous ERK5 SUMOylation (**Figures 3C**, **4G**), and found that the reduced ERK5 SUMOylation by SENP2 could partially reverse ponatinib's effect on *klf2* and *vcam1* expression (**Figures 4H,I**).

A functional characteristic of ECs is their ability to migrate. During physiological processes, EC migrate during vasculogenesis and angiogenesis whereas in pathological process, such as vessel damage, EC migrate to restore vessel integrity (67). To investigate the role of ponatinib-mediated ERK5 SUMOylation in EC function, we performed an *in vitro* scratch wound healing assay. Of note, we minimized the contribution of cell proliferation in this process by: (1) wounding a confluent and quiescent monolayer of cells and (2) maintaining the cells in reduced-serum culture medium for the duration of the assay. In ECs expressing ERK5-WT, cell migration toward the wounded region seen in the veh-treated group was inhibited by ponatinib treatment (**Figure 5**, left panel). However, in ECs expressing ERK5-K6/22R mutant, ponatinib-mediated delayed cell migration was rescued (**Figure 5**, right panel). Taken together, our data suggests the importance of endothelial ERK5 SUMOylation in ponatinib-associated endothelial inflammatory response, and, to a greater extent, vascular adverse events.

DISCUSSION

Previously, we reported that p90RSK activation increases SENP2 T368 phosphorylation that inhibits SENP2 deSUMOylation activity, leading to increased ERK5 SUMOylation. Additionally, p90RSK activation increases ERK5 S496 phosphorylation. Both, SUMOylation and S496 phosphorylation reduce ERK5 transcriptional activity that accelerates EC inflammation, dysfunction, apoptosis, and subsequent atherosclerotic plaque formation (52, 61). In the current study, we identified a novel role for ERK5 SUMOylation in ponatinib-mediated endothelial inflammatory response. Interestingly, we also detected increased p90RSK phosphorylation by ponatinib (**Supplementary Figure 1A**). This signaling pathway elicited by ponatinib resembles that of ECs exposed to atheroprone stimuli, such as disturbed flow, reactive oxygen species, or advanced glycation end products (52, 61), suggesting that ponatinib-mediated ERK5 SUMOylation might be involved in ponatinib-associated atherosclerosis, VAEs and subsequent cardiovascular complications. Thus, inhibition of endothelial ERK5 SUMOylation can be viewed as a novel approach to mitigate VAEs resulting from ponatinib treatment.

Because we also found decreased SENP2 expression in ECs expressing SENP2 treated with ponatinib (**Figure 4G**), we speculate that ponatinib elicits an endothelial inflammatory response not only by reducing SENP2 activity but also by reducing SENP2 at the protein expression level. This might be a unique feature of ponatinib, compared to other pro-inflammatory stimuli where effects on SENP2 are only on its enzymatic activity.

Endothelial ERK5 can be phosphorylated at multiple sites, each of which confers different biological functions (51, 52, 68–70). Among them, ERK5 S496 phosphorylation plays a crucial role in EC inflammation. ERK5 S496 phosphorylation has a similar effect to that of ERK5 SUMOylation in inhibiting ERK5 transcriptional activity (52). We reported previously that ERK5 phosphorylation at S496 is induced not only by disturbed flow and reactive oxygen species (52, 61) but also by radiation (IR) and that it plays a crucial role in IR-mediated EC inflammation (69). In the current study, we found that ERK5 S496 phosphorylation is increased by ponatinib (**Figure 2I** and **Supplementary Figure 1A**). Furthermore, ponatinib-mediated increased *tnfα* expression was reversed in ECs expressing ERK5 S496A phosphorylation resistant mutant (**Supplementary Figure 1B**). Similarly, flow cytometric analysis of Annexin V staining revealed the contribution of ERK5

S496 phosphorylation in ponatinib-induced EC apoptosis (**Supplementary Figure 1C**).

It is noteworthy that the reduction of ERK5 transcriptional activity by ponatinib via ERK5 SUMOylation and, probably, S496 phosphorylation occurs independently of kinase activation, highlighting the predominance of these posttranslational modifications on ERK5 function and, subsequently, EC integrity. If and how ponatinib-induced ERK5 SUMOylation and S496 phosphorylation interact and/or coordinate to control ERK5 transcriptional activity requires further investigation. To the best of our knowledge, this is the first study to demonstrate the role of endothelial ERK5 SUMOylation in ponatinib-regulated EC inflammation.

AUTHOR CONTRIBUTIONS

JP-M, AC, RA, EH, YT, and N-TL performed experiments, interpreted data. JP-M, SK, BD, M-CH, and N-TL wrote and edited the manuscript. All authors have read and agreed to the content of the 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.2018.00125/full#supplementary-material

Supplementary Figure 1 | Ponatinib-mediated ERK5-S496 phosphorylation in endothelial inflammatory responses. **(A)** Expression of phospho-ERK5 at S496, ERK5, phospho-p90RSK at S380, and p90RSK in HAECs treated with ponatinib (150 nM) was assessed by Protein simple WES system (capillary electrophoresis western analysis). Protein bands are shown as pseudoblots. **(B)** qRT-PCR analysis of relative *tnf* expression in HAECs expressing ERK5-WT or S496A mutant treated with ponatinib (75 nM, 24 h). Data set contains 6 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using ANOVA followed by Bonferroni *post hoc* testing for multiple group comparison. $^{***}P < 0.001$ vs. control. **(C)** Flow cytometric analysis of Annexin V staining in HAECs expressing ERK5-WT or S496A mutant treated with ponatinib (150 nM, 24 h). Graph shows percentage of apoptotic cells. Data set contains 3–6 technical replicates. Error bars represent mean ± SEM. Statistical significance was assessed using student's *t*-test (two-tailed). $^{***}p < 0.001$.

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Cardiotoxicity of Anticancer Therapeutics

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As cancer therapeutics continues to improve and progress, the adverse side effects associated with anticancer treatments have also attracted more attention and have become extensively explored. Consequently, the importance of posttreatment follow-ups is becoming increasingly relevant to the discussion. Contemporary treatment methods, such as tyrosine kinase inhibitors, anthracycline chemotherapy, and immunotherapy regimens are effective in treating different modalities of cancers; however, these reagents act through interference with DNA replication or prevent DNA repair, causing endothelial dysfunction, generating reactive oxygen species, or eliciting non-specific immune responses. Therefore, cardiotoxic effects, such as hypertension, heart failure, and left ventricular dysfunction, arise posttreatment. Rising awareness of cardiovascular complications has led to meticulous attention for the evolution of treatment strategies and carefully monitoring between enhanced treatment effectiveness and minimization of adverse toxicity to the cardiovasculature, in which psychological assessments, early detection methods such as biomarkers, magnetic resonance imaging, and various drugs to reverse the damage from cardiotoxic events are more prevalent and their emphasis has increased tremendously. Fully understanding the mechanisms by which the risk factors action for various patients undergoing cancer treatment is also becoming more prevalent in preventing cardiotoxicity down the line.

Keywords: cardiotoxicity, anticancer therapies, signaling pathway inhibitors, chemotherapy, immunotherapy, cardiotoxicity early detection and prevention

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INTRODUCTION

In the past few decades, anticancer treatment has achieved remarkable progress in improving the quality of life and survival rates of cancer patients. The exploration and application of novel cancer therapeutics have increased tremendously, paralleled with the growth and abundance of literature surrounding the mechanisms underlying cancer metastasis. Novel drug therapies focusing on targeting signaling pathways pertaining to angiogenesis to prevent cellular proliferation *via* kinase inhibitors have especially been promising. Other methods, including anthracycline chemotherapy, have dramatically improved the outcomes of cancer treatment over the last 10 years (1). However, accompanying with the significant improvements toward cancer treatment, cardiotoxicity-related adverse effects caused by these anticancer therapies, specifically on deleterious cardiovascular effects, such as hypertension, heart failure, QT interval prolongation, and left ventricular dysfunction (LVD) (2–5), as well as heart failure with preserved preserved left ventricular ejection fraction (HFpEF), are increasingly reported (6). The development of cardiotoxicity has also been associated with patient

age, existing health conditions (at risk of cardiac dysfunction), treatment dosage, and other risk factors (7). Thus, carefully monitoring the development, early detection and prevention of cardiotoxicity, as well as understanding of the interaction between cancer and the cardiovascular system, thereby promoting the development of safer cancer therapeutics, without or with minimized cardiotoxicity, are urgently needed (5).

In this review, we discuss contemporary methods of cancer therapy and the related signaling pathways, which are promoting heart dysfunction and are affected through inhibitory drug treatments that are often compounded with chemotherapy and radiotherapy. We also discuss the importance of the novel therapeutic detection approaches for cardiotoxicity, namely, early detection *via* biomarkers and cardiovascular imaging, including, but not limited to, magnetic resonance imaging. These alternative treatment routes may provide more insight into the efficacy of cancer treatment strategies and cancer diagnostic tools, which highlight the importance of early detection to avoid later onset of adverse cardiotoxic effects.

CARDIOTOXICITY BY ANTICANCER TREATMENT

The National Cancer Institute defines cardiotoxicity as "toxicity that affects the heart." Cardiotoxicity may be acute, which occurs during or soon after treatment and is transient, or chronic, and can be categorized into type I (early onset) and type II (late onset) (5). Type I is irreversible cardiac cell injury and usually caused by anthracyclines and chemotherapeutics; type II is typically caused by novel biological-targeted antibodies (8). Chemotherapy and metabolic pathway inhibition has been shown to create adverse side effects, predominantly focusing on myocardial damage and the risks associated with heart failure posttreatment, although the newly emerged targeted drugs such as tyrosine kinase inhibitors and antibodies induce toxicities different from chemotherapy. Often, cardiotoxicity is commonly associated with LVD and other symptoms of systemic heart failure. Furthermore, LVD condition has several facets, which can be related to myocardial toxicity but also to other cardiovascular toxicities, namely, QTc prolongation, arrhythmia, myocardial ischemia induced through atherosclerosis, and pulmonary hypertension. Especially, HFpEF (also called diastolic heart failure) occurs when the lower left ventricle is unable to properly fill with blood during the diastolic phase, and increasingly arises in patients undergoing chemotherapy, and may become predominant HF. Because the pathophysiology underlying HFpEF is heterogeneous, has different phenotypes, and is poorly understood, the etiological definition of HFpEF is variable. Thus, accurate diagnosis is challenging, and currently there is no effective therapy for HFpEF (6, 9–11). However, recently identified novel biomarkers, such as protein biomarker of cardiac stress (ST2), matrix metalloproteinase-2, and growth differentiation factor-15, for the risk stratification of HFpEF may be used for development of significant therapeutic targets for the treatment of HFpEF (12). Furthermore, recent advancements in imaging techniques and exploration into biomarkers have raised the important issue of the multiple comorbidities of cardiotoxicity, many of which are not agglomerated through drug therapies.

Currently, there is no consensus definition of cardiotoxicity. The Cardiac Review and Evaluation Committee of trastuzumab-associated cardiotoxicity defines cardiotoxicity as symptoms of heart failure, decline of left ventricular ejection fraction (LVEF), symptomatic fall in LVEF $\geq$5 to <55% or an asymptomatic reduction of LVEF $\geq$10 to <55% (13). The American Society of Echocardiography and European Association of Cardiovascular Imaging define cardiotoxicity as global longitudinal strain (GLS) with a 10–15% early reduction (http://asecho.org/wordpress/wp-content/uploads/2015/12/MLM-Revised-Strain-Code-11-12-15.pdf). The Food and Drug Administration defines LVEF drop <40–45% or is 40–49% with a $\geq$10% absolute decrease below baseline with anti-HER2 targeted therapy as being necessary to be monitored (14).

The Common Terminology Criteria for Adverse Events (CTCAE) is a descriptive terminology that is used for adverse event reporting based on a grading scale ranging from 1 to 5, with 1 being abnormal elevations in biomarker expression or imaging abnormalities, to 5, death due to cardiotoxicity (CTCAE, version 4.0 National Cancer Institute, June 14, 2010). However, the current reporting scale may be confusing and depends highly on symptomology, which may not represent a clear image of LVD abnormalities, for example, with patients who are asymptomatic. Finally, attributing signs and symptoms of cardiotoxicity prove to be difficult, as many symptoms are may not be induced or attributable to the drug therapy itself. This is especially challenging in older patients, whom often have common comorbidities.

Although the current scale may be deficient in some ways, it is important to address the limitations of potential definitions. Research of this magnitude requires consistency in defining the issue to share a common language and reinforce validity in the research.

CONTEMPORARY THERAPEUTICS FOR NEOPLASIA

Currently, immunotherapies use the immune system to enhance their antitumor immunity and further immune responses by employing immune checkpoint inhibitors, chimeric antigen receptor (CAR) T cell therapy, and adaptive cell transfer (ACT) (using the patient's own T-cells, engineered to specifically target cancer cells) have been promising in certain cancer treatments (15). Other targeted therapies, specifically focusing on signaling pathway inhibition to prevent certain cell processes from occurring, namely, angiogenesis, also improve clinical outcomes. Angiogenesis, a normal process in which blood vessels are created through currently existing vessels, is a vital process in wound healing, growth, and development. However, growing tumors hijack this process to feed and proliferate tumor cells, thereby creating malignant tumor vessels within the body, making angiogenesis a key factor for tumor growth and survival.

One of the most prominently used and evaluated strategies is the inhibition of the vascular endothelial growth factor (VEGF) signaling cascade. VEGF plays a critical role in angiogenesis

through binding VEGF receptors and activating the VEGF signaling pathway. Inhibition of angiogenesis, thereby, prevents growing tumors from hijacking the body's natural process (16). Another popular target for cancer therapies is the human epidermal growth factor receptor 2 (HER2). The HER2 protein, also called ERBB2, is commonly overexpressed in patients with breast cancer, which accounts for approximately 15–30% cases in breast cancer (17, 18). Normally, HER2 helps in growth, proliferation, and repairing of abnormal cells within the body. However, similar to the VEGF signaling pathway, tumorigenesis takes control of the cellular process and promotes the proliferation of cancer cells (19, 20).

Trastuzumab (Herceptin), a monoclonal antibody, specifically targets HER2. Treatment regimens with Trastuzumab, coupled with chemotherapy, have shown remarkable outcomes in patients with breast cancer after 1 year (21). Anthracycline antibodies, including doxorubicin (DOX), have also been a consistent and prominent form of chemotherapy schemes for nearly half a century. By limiting proliferation of cancer cells *via* preventative interference with its DNA or RNA structure, DOX is able to halt tumorigenesis and ultimately stop cancer cell proliferation and division (8, 22).

TARGETING THE VEGF SIGNALING PATHWAY

Vascular endothelial growth factor and its corresponding receptors, VEGFR, are one of the most important tyrosine kinase pathways. VEGF includes seven members in its family—VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PIGF. VEGF-A is the most representative component. VEGF-A mRNA is expressed in various tissues in the body, such as the lung, kidney, and heart (16). Consequently, the VEGF signaling pathway plays a central role in other signaling pathways which also affect the vasculature, and any alterations to this pathway have been shown to exhibit deleterious effects (23).

Mechanistically, VEGF binds to three receptor sites—VEGFR-1 (Flt-1), VEGFR-2 (Flk-1), and VEGFR-3 (24). Binding of VEGF to VEGFR-2 initiates the tyrosine signaling cascade, which then facilitates cell migration, proliferation, growth, and vasodilation, all of which are important and contribute to the development of angiogenesis (25). Historically, the VEGF pathway can be targeted using several different methods, including the molecule itself (monoclonal antibodies), receptors (recombinants), or downstream signaling pathways and inhibiting downstream expression (tyrosine kinase inhibition) (4).

Current antiangiogenesis therapies, including the inhibition of VEGF, have shown to have adverse effects on the cardiovascular system (2, 7, 16). VEGFR-1 and VEGFR-2 are both expressed naturally in endothelial cells (EC) (26). Due to the nature of inhibitory drug therapies, affecting the expression and interaction between VEGF and its receptor sites affects the entire circulatory system and primarily induces proliferation of EC and promoting vascular integrity. Anti-VEGF therapies, such as the tyrosine kinase inhibitor, imatinib, dasatinib, nilotinib, bosutinib, sunitinib, sorafenib, axitinib, and ponatinib, exacerbate LVD, as well as incidences of hypertension, ischemic events, rapid acceleration of atherosclerosis, and other vascular toxic events (27). Inhibition of the VEGF pathway, therefore, can lead to endothelial dysfunction, arising from the disruption of normal endothelial homeostasis mediated through nitric oxide (23).

Meta-analyses with sunitinib have shown increased relative risk for cardiovascular complications. In a meta-analysis of 9,387 patients, the risk of myocardial ischemia caused by sunitinib was 3.03-fold higher compared with placebo (28). Sunitinib has also been speculated to induce LVD risk in over 20% of patients (28). Ponatinib has been associated with significant cardiovascular toxicity, with an indication of 10% cardiovascular, 7% cerebrovascular, and 7% peripheral adverse events by 28 months after treatment. Hypertension was reported in 26% of patients treated with this drug (28).

TARGETING THE HER2 SIGNALING PATHWAY

Trastuzumab is a humanized monoclonal antibody that directly inhibits the HER2 signaling pathway. It is used as first-line therapy with chemotherapy and has been shown to be effective in 25–30% of breast cancer cases (5, 29). However, anti-HER2 therapy was reported to increase the risk for asymptomatic decreased left ventricular ejection fraction, leading to complications such as systemic heart failure. Interestingly, the cardiotoxic effects are shown early (usually within weeks of initial treatment) and are independent of dosing regimens. The cardiotoxic effects are also type II, or reversible, and often not severe in terms of cell death or damage (3).

Mechanistically, Trastuzumab competes for binding sites on the extracellular domain of HER2 and inhibits the activation of the tyrosine kinase signaling pathway (30, 31). It has also been speculated that Trastuzumab also induces cell death *via* antibody treatment, although the data supporting the claim are not yet clear (32). Regardless, HER2 inhibition prevents cell repair and significantly limits proliferation. However, it is speculated that because Trastuzumab's effect is established through targeting the HER2 signaling pathway, which regulates cell differentiation, survival, and repair in healthy tissues, the body's myocytes cannot depend on the repair mechanism either, and that leads to cardiotoxicity when in the presence of anthracyclines (33). Supporting data also come from a study of 179 breast cancer patients, in which 44% of patients developed a cardiac event (e.g., he art failure or decreased ejection fraction) and a tenth developed a second event (34, 35).

ANTHRACYCLINES

Anthracyclines, a class of chemotherapy drugs, are traditional cancer therapies that have been effective in treating many forms of cancer for the last half century. One of the most prominently used and readily identifiable is the anthracycline DOX. DOX-induced cardiotoxicity is classified as type I, or irreversible. It directly contrasts with Trastuzumab and HER2 inhibition by the fact that cardiotoxicity from DOX is dose dependent (usually doses $\geq$450 mg/m^2) (36). Moreover, the risk for future

complications significantly increases with cumulative doses (37, 38). Although it was originally speculated that the risks for immediate, on-treatment events decreased with lower doses, it is becoming more apparent that late onset complications can be verified regardless of dose. Therefore, there is no dosage regimen of DOX that is completely safe. The immediate cardiotoxic effects can range from a few weeks to years after treatment, even after the treatment has been discontinued. Incidences and reporting of LVD complications range from 1 to 20%, although the rate may be much higher (39). Some studies estimate that over half of the patients exposed to anthracyclines will develop some forms of LVD within 6 years (40). This claim is further supported by recent studies reporting the incidence of cardiotoxicity in 17.9 and 6.3% of patients for subclinical and overt cardiotoxicity, respectively, in 9 years (41). Other studies have also shown LVEF in 98% of patients in a cohort of 9% ($n = 226$) overall incidence for cardiotoxicity within 1 year after chemotherapy (42).

Chemically, DOX has both hydrophilic and hydrophobic regions, allowing it to bind to both plasma proteins and cell membranes. DOX also has both acidic and base functions, its characteristics of being reoxidized results in the production of reactive oxygen species (ROS) and gives DOX an antineoplastic and antibiotic capabilities (8, 43). DOX has several different channels in which it affects the overall homeostasis in the body. One of the most important is its interaction with topoisomerase II. Topoisomerases are isomerase enzymes that participate in the overwinding or underwinding of DNA, allowing DNA to replicate by binding to the double strand DNA and overcome the tangles caused by the double helix. Topoisomerase II has two nuclear localized isoforms: topoisomerase IIα and IIβ. DOX inhibits topoisomerase interaction with DNA by directly binding to both IIα and IIβ, forming a DNA cleavage complex that increases double strand breakage (8, 22). Although DOX prevents cancer cells from replicating, it also serves as the primary cytotoxic reagent to induce cell apoptosis. DOX affects calcium homeostasis as well. It directly interferes with calcium storage capacity of the mitochondria by activating the selective CsA-sensitive calcium channel, causing calcium overload (8, 44) and leads to mitochondrial dysfunction, as well as apoptosis. The decreased calcium levels from the mitochondria have been shown to be irreversible. DOX, therefore, is highly versatile in terms of its uses, as well as its impact on the vasculature, and the cardiovascular system as a whole.

CANCER IMMUNOTHERAPY

Cancer immunotherapy is a newly emerging treatment method which bases itself on the deeper understanding of the mechanism of antitumor immune responses, discoveries of novel anticancer molecules (peptides and vaccines), and development of innovative technologies of gene transfer (45). Current popular cancer immunotherapy employing inhibitory effects to immune checkpoint receptors has proven to be very effective in several malignancies and has shown very promising clinical outcomes in various types of cancers in the past 10 years. This revolutionized strategy has brought anticancer treatment into a new era (8, 45). Anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and anti-programmed cell death protein-1 (PD-1) have especially been beneficial to ailments, such as melanoma (46). Normally, cancer cells can utilize these receptors to avoid destruction *via* T cells in the immune system by binding to CLTA-4 and reducing naïve T cell activation or by expressing the cell death protein ligand-1 (PD-L1), which then binds to PD-1 and mediates T cell downregulation and apoptosis. Inhibitors, such as ipilimumab (anti-CTLA-4 monoclonal antibody) and pembrolizumab and nivolumab (anti-PD-1 monoclonal antibodies), bind to and block the inhibitory effects of the receptor sites, thus enhancing the cytotoxic immune response to the cancer cells (45).

Adoptive T cell transfer (ACT) is another novel and a promising method for a wide spectrum of solid cancer treatment. ACT uses a patient's T cells to specifically target tumor cells. Mechanistically, tumor infiltrating lymphocytes (TILs) are collected and genetically engineered into a patient's microenvironment along with systemic interleukin-2 (IL-2) to stimulate their survival and expansion (45). Successful implementation of ACT as a treatment modality for patients with metastatic melanoma has established the basis for multiple modifications and improvements of this strategy for targeting many different cancers (47). It has been reported that in 50% of patients with metastatic melanoma exhibit objective tumor regression by ACT using autologous TILs (47). As an innate cytokine in the immune system with essential and wide spectrum of immunological functions, IL-2 has long been used for cancer treatment alone and has shown great tumor regression (48, 49). IL-2 directly regulates T cell differentiation into different T cells in response to antigens to enhance the defense of the immune system (48, 49). A recent report of a clinical study with patients treated with lymphodepleting conditioning chemotherapy followed by infusion of autologous TILs and high-dose IL-2 showed regression of metastatic uveal melanoma in 7 out of 20 patients, which had been believed to be resistant to immunotherapy previously (50). Similarly, chimeric antigen receptor (CAR)-T therapy has had valuable benefits to certain deficiencies, such as B cell acute lymphoblastic leukemia (B-ALL). Mechanistically, CAR-T functions very similar to ACT, wherein native T-cells are engineered to express a chimeric antigen receptor on the cell membrane. The receptor is coupled with an external binding domain to specifically recognize and bind to tumor antigens. CAR-T also has an internal activation domain that then activates the T-cell once CAR-T binds to its target. In patients with B-ALL, CAR-T therapy is effective in 70–90% of cases (51).

However, the therapeutic effects are also counterbalanced with similar cardiotoxic effects (52–54). This is especially the case with immunotherapies, since activated T cell responses may be non-specific to cancer cells, thereby targeting normal tissue as well, leading to frequent immune-related adverse events (IRAEs) such as colitis, endocrinopathies, hepatitis, and pneumonitis. Other events, such as cardiomyopathy, myocardial fibrosis, myocarditis, and acute heart failure, were also reported in single anti-CTLA-4 or anti-PD-1 treatment (54), and lethal myocarditis accompanying with myositis was seen in combinatorial treatment (55), although such cases are rare. Patients using the monoclonal antibody ipilimumab have an IRAE frequency rate of 64–80% (54, 56, 57). Patients treated with pembrolizumab have an IRAE

frequency rate of up to 79% (54, 58). Combination therapies using both ipilimumab and nivolumab have been shown to have tremendous therapeutic efficacy and response rates in patients with melanoma from 19% using ipilimumab alone to 58% with the combination (46, 52). However, the frequency rate of IRAEs also increased parallel to the combination therapy, reaching as high as 96% in patients using both ipilimumab and nivolumab (59). CAR-T therapy and ACT both have associated risks with cardiotoxicity. CAR-T therapy, specifically, has links with cytokine release syndrome (CRS), an inflammatory response that is correlated with the activation of CAR-T cells. Manifestations of CRS include arrhythmias, decreased LVEF, and QT prolongation (60).

PREVENTION OF CARDIOTOXICITY AND FUTURE DIRECTIONS

Reversing certain effects of cancer drug therapies may not always be possible. However, current research into managing and monitoring cardiotoxicity and the various side effects arising from both modern and traditional therapeutics have been very promising. Dexrazoxane, a most promising cardioprotective agent, has been shown to be effective in reducing both acute and chronic cardiotoxicity induced by anthracycline therapy (8, 61). Dexrazoxane has been shown to interfere with iron-dependent redox reactions, thereby reducing ROS originating from DOX (62). Dexrazoxane also directly inhibits topoisomerase IIβ, thereby preventing anthracycline binding and DNA double strand breaks (63). However, the ability of Dexrazoxane in reducing anthracycline-induced cardiotoxicity has been limited in its clinical use because of adverse side effects (5). A recent clinical study indicated that Dexrazoxane, in addition to DOX, resulted in higher rates of bone marrow suppression, more febrile neutropenia events and dose reductions (64). Seeking novel protection of cardiotoxicity drugs is thereby more essential and in urgent demand.

Treatments modalities employed either before, after, or before and after cancer therapeutics have proven effective when dealing with certain cardiotoxic effect risk factors, such as heart failure. The use of beta blockers (BBs), angiotensin-converting enzyme (ACE) inhibitor, angiotensin inhibitors, and mineralocorticoid receptor antagonists have all shown promising results in preventing cardiac damage and is an active ongoing investigation area (7). BBs have antioxidant properties and were utilized for chemotherapy-induced LVEF and other systolic LV dysfunction. One of the new generation BBs and a most commonly used agent, carvedilol, showed strong antioxidant characteristics and greater protective effect on anthracycline-induced cardiomyopathy (6, 8). A recent report demonstrated that favorable effects of ACE inhibitors and BBs on preventing cardiotoxicity and improving survival of breast cancer patients treated with trastuzumab and/or anthracyclines (65). Moreover, beta-adrenergic blockade with nebivolol, metoprolol, lisinopril, etc., has also been proven to be effective for cardiomyopathy (66, 67). However, reports on some BBs demonstrating beneficial cardioprotective effects in patients with LVEF remains controversial (68). Novel modalities, such as lcz696 (valsartan/sacubitril) (69, 70) and ivabradine can also be used to treat heart failure and subsequent impaired myocardial systolic dysfunction (6, 71). Furthermore, the cardioprotective role of ranolazine (inhibition of late INa elevation) and phosphodiesterase-5 inhibitors has been reported (66). In addition, nutrition supplements and exercise have shown positive effects on cardiomyopathy (66). However, significant supportive clinical data are still needed to prove the protective function of these novel agents in patients affected by cardiotoxic effects (7).

The role of biomarkers has also been important in early detection. Elevated or abnormal expression levels of several biomarkers can be used as indicators for screening and assess the risk factors for future cardiotoxicity complications. Interleukin-6 (IL-6), a cytokine produced by adipose tissues, increases blood pressure and induces inflammation. Overexpression of IL-6 inhibits cell apoptosis, stimulates angiogenesis, and plays a role in drug resistance (72). There have also been promising data exploring biomarkers that pertain to type I and type II cardiac events. Increases in troponin-I, brain-type natriuretic peptide (BNP), and N-terminal-pro-BNP have all been linked to drops in LVEF (5, 73). Plasma myeloperoxidase also predicts a decrease in cardiac function (7). MicroRNA has emerged as a potential marker for early onset of heart failure. miR-1, miR-133b, and miR-146a were all upregulated corresponding to DOX chemotherapy (74).

Early detection is vital when considering the deleterious effects that drug therapeutics may have. One facet in which early detection has been outlined is through the risk factors for both cardiovascular diseases and cancer. Shared risk factors, such as obesity, carcinogenic agent usage (e.g., tobacco, smoking, and alcohol), previous history of diabetes, hypertension, non-modifiable risk factors (e.g., age and sex), and physical activity, should all be taken into consideration, especially when pertaining to drug therapeutics, thereby minimalizing the risk of future cardiotoxicity events (75).

Current exploration into psychological stress as an additional risk factor is promising (76). The mechanism in which psychological distress plays in cardiovascular dysfunction is twofold, focusing on behavioral and pathophysiological. In terms of behavioral mechanisms, it is best understood that personality types, temperament, anxiety, and depression can influence and are related to other risk factors, such as unhealthy lifestyles, lack of exercise, smoking, and alcohol consumption (77). In terms of pathophysiological mechanisms, being under psychological distress may form complications such as platelet dysfunction, autonomic nervous system dysregulation, hypothalamic–pituitary–adrenal axis (HPA-axis) dysregulation, cellular aging, and inflammatory activation. Platelet activation has shown increases in patients with depression (78). The HPA-axis has a known role and association in cardiovascular dysfunction *via* cortisol regulation (79). Patients with depression, anxiety, and fatigue often show elevated cortisol levels (80). Patients with depression and anxiety also have shorter telomere length, a biomarker for cellular aging and also an associated risk factor for cardiovascular dysfunction (81).

Takotsubo cardiomyopathy (TC), known as stress cardiomyopathy or broken heart syndrome, may be caused by emotional stress and long-term anxiety (82). TC shows similar images as

that of acute coronary syndrome and is characterized by dynamic electrocardiographic changes along with transient, severe, and reversible LVD, but unclear pathophysiological mechanisms (82, 83). It is more common in elder women and was taken into account only in recent years in cancer patients treated with anticancer drugs suggesting that chemotherapy can induce TC (83–85).

Detection and early screening of cardiotoxicity *via* imaging techniques have become more prevalent. Two-dimensional echocardiography (2DE) has been the standard for quite some time. However, assessment of LVEF and GLS *via* three-dimensional echocardiography (3DE) and speckle-tracking echocardiography, respectively, has been shown to be a valuable asset in early detection and is able to overcome many challenges that affect traditional 2DE methods (7, 86). Moreover, GLS seems to predate LVEF decreases, and impairment of GLS in HEpEF has been reported (87). Thus, GLS seems a better indicator of myocardial dysfunction prior heart failure progression. Cardiac magnetic resonance imaging has emerged in recent years and is used as the gold standard parameter. It is accurate, reproductive, and reliable and has higher sensitivity than 2DE and 3DE in detecting early changes in global and regional cardiac function, and its high contrast-to-noise ratio provides excellent structural characteristics (88, 89).

CONCLUSION

Cardiotoxicity is one of the most deleterious effects arising from cancer therapeutics and a major barrier to survivorship. However, today's cancer patients should not be tomorrow's cardiovascular disease patients. Recent research has shed the light of optimism with an emphasis on early prevention. Continued research and discussion will further advance our literature pertaining to cardiotoxicity, opening up additional avenues for safer treatment strategies. Further investigation into alternative therapeutics, as well as the increasing information and understanding of modern technologies in screening and detecting at-risk patients will be helpful in developing and evaluating different modalities for cancer therapeutics. Moreover, such research will be able to fill in the gaps in understanding cardiotoxicity and explore other avenues of research that are often overlooked.

Understanding the importance of risk factors and associated risks, such as the role of psychological distress, are necessary to advance the care of cancer patients. Minimizing the risk of cardiovascular complications induced through various therapies is vital to treatment and care. This, again, is an emphasis on the importance of early detection and risk assessment when considering drug administration to certain patients.

AUTHOR CONTRIBUTIONS

JD and HC wrote the manuscript and finalized the manuscript.

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