



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

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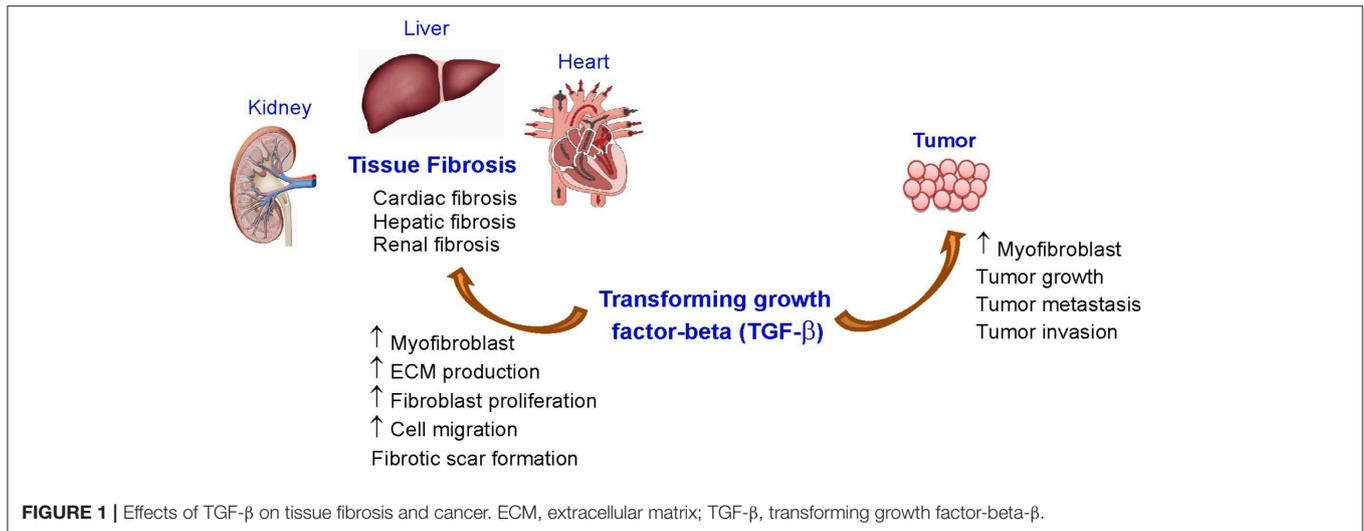
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Transforming growth factor- β (TGF- β) is a common mediator of cancer progression and fibrosis. Fibrosis can be a significant pathology in multiple organs, including the heart. In this review, we explain how inhibitors of TGF- β signaling can work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

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

INTRODUCTION

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



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

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

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

leading global cause of death (22, 23) and understanding the pathogenesis of fibrotic myocardial remodeling is crucial to identifying innovative treatment strategies for patients with cardiac fibrosis.

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

Introduction of Cancer

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

Therapeutic Targets for Treatment of Cancers

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

Introduction of Cardiac Fibrosis

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

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

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

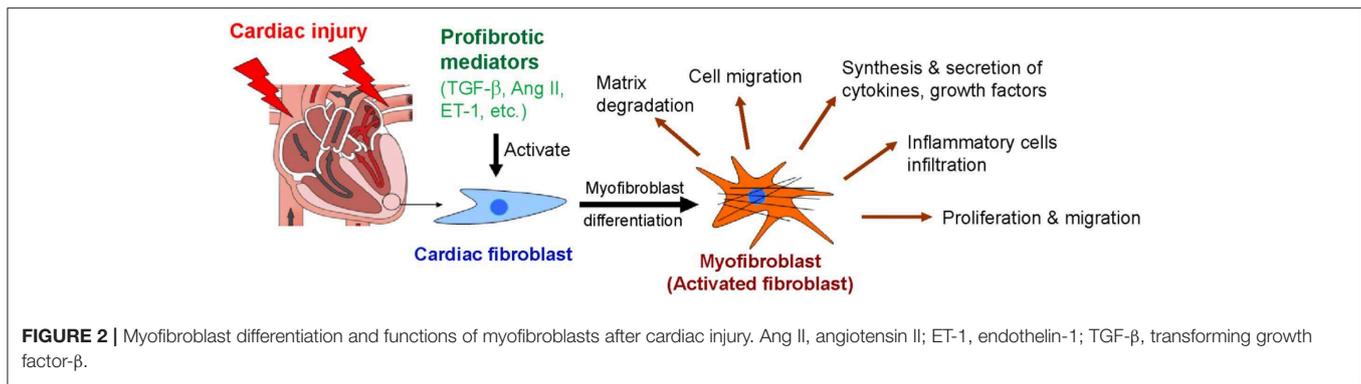
Classes	Example sub-classes	Representative drugs
Chemotherapy (25)	Alkylating agents	Cyclophosphamide, cisplatin
	Topoisomerase inhibitors	Irinotecan, etoposide, doxorubicin
	Mitotic inhibitors	Vincristine, paclitaxel
	Anti-metabolites	Methotrexate, cytarabine, hydroxyurea
	Others	Bleomycin, L-asparaginase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

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

Myofibroblasts

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

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



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

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

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

Overproduction of ECM Proteins

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

Therapeutic Targets for Treatment of Cardiac Fibrosis

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

TGF- β SIGNAL TRANSDUCTION

TGF- β is a member of the TGF- β superfamily, which is comprised of TGF- β , bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), activin and inhibin (65). Members of this diversify superfamily are the pleiotropic multifunctional polypeptides that play a role in a wide range of physiological cellular activities such as growth, proliferation, differentiation, and apoptosis (65). Among these polypeptides, TGF- β has been proven to be one of the major factors driving the fibrotic response in most organs (2). In mammals, there are 3 isoforms of TGF- β : TGF- β 1, TGF- β 2, and TGF- β 3. These highly homologous polypeptides, encoded by various genes, are synthesized, processed and regulated in a similar fashion. However, these 3 isoforms are secreted by various types of cells and signals through the same receptors, but they exhibit distinct patterns of distribution in different tissues (3, 66). Even though any isoform can be found in fibrotic tissues, the progression of organ fibrosis, in particular cardiac fibrosis, is predominantly attributed to TGF- β 1 (67). To date, information on isoform-specific activities of various isoforms of TGF- β in a specific

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

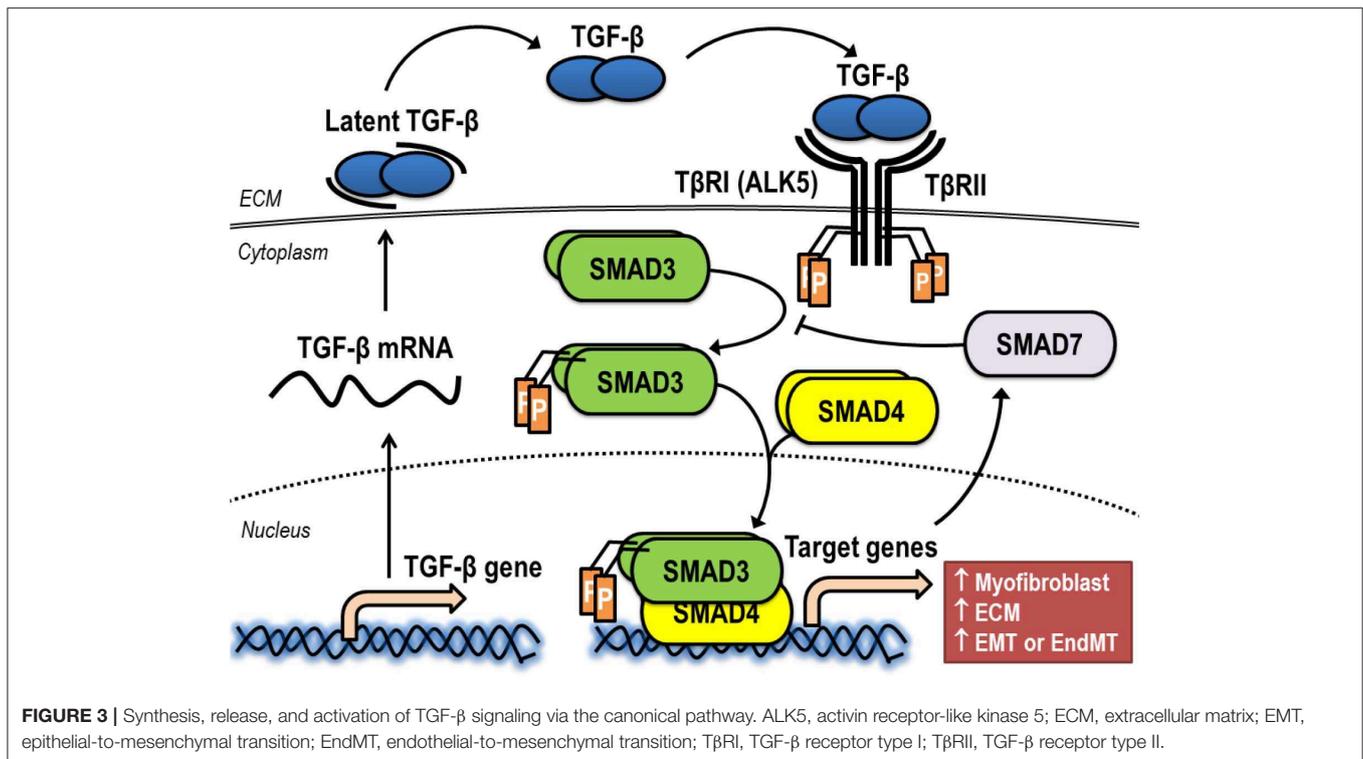
Targets/Strategies	Results	References
Inhibitors of TGF- β and its signaling pathway	Anti-TGF- β neutralizing antibody prevents myocardial fibrosis in pressure-overloaded hearts	(47)
	Blockade of TGF- β -activated kinase 1 (TAK1) inhibits TGF- β -mediated extracellular matrix (ECM) overproduction in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

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

The synthesis, release, and activation of TGF- β is a complex process (**Figure 3**). Following intracellular biosynthesis, a dimer of TGF- β is secreted as an inactive protein complex (latent TGF- β), which is retained in the ECM. Active TGF- β 1 can be liberated from ECM by multiple activators such as reactive oxygen species (ROS), plasmin, thrombospondin-1, and α v β 6 integrin (68). Once active TGF- β is released from ECM, it binds to transmembrane TGF- β receptor type II (T β RII) of a target cell. This receptor-ligand interaction induces serine/threonine kinase activity of T β RII for autophosphorylation (69). The canonical pathway of TGF- β signaling is initiated after phosphorylated T β RII forms a stable heteromeric complex with TGF- β receptor type I (T β RI), also known as activin receptor-like kinase 5 (ALK5), for the transphosphorylation of residual phosphate to T β RI (70). This receptor binding complex, which is a heterotetrameric combination between two molecules of T β RII and another two of T β RI, recruits and phosphorylates the downstream signaling proteins Smad2 or Smad3, which are called receptor-activated Smads. After phosphorylation, Smad2 or Smad3 is released and forms an intracellular complex with Smad4, the mediator Smad. This intracellular complex between Smad2/4 or Smad3/4 moves from the cytoplasm into the nucleus, where it binds to promoter regions of the genes involved in physiological process of induction of specific gene expression (71). For an example of fibrogenesis, gene

encoding α -SMA, collagens, and fibronectin are significantly upregulated via the Smad3-dependent pathway (72). The expression of these fibrosis-related genes plays a pivotal role in the cellular transdifferentiation that generates myofibroblasts and the production/deposition of ECM by myofibroblasts in fibrotic tissue (72). In addition to fibrogenesis, the Smad-mediated signaling pathway is also a significant intracellular process activated by TGF- β that increases genes associated with carcinogenesis (73). Furthermore, the activation of TGF- β signaling results in the expression of Smad7, an inhibitory SMAD, which acts as a negative regulator by interacting with Smad2 or Smad3, thereby mitigating signaling through receptor-activated Smads and further decreasing TGF- β actions (74).

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



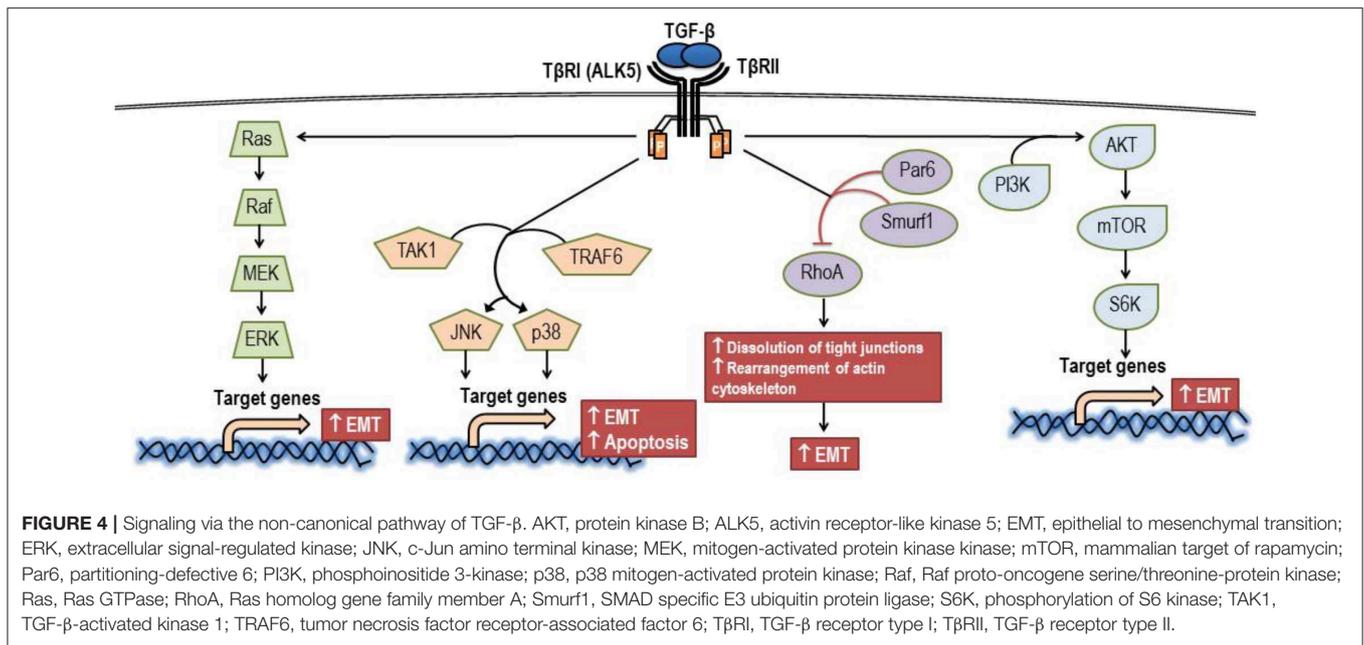
of TNF receptor-associated factor 6 (TRAF6). TRAF6 is capable of recruiting TGF- β -activated kinase 1 (TAK1) to subsequently allow the activation of c-Jun amino terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38-MAPK) (77). In addition, the TRAF6-TAK1-JNK/p38 pathway is believed to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

TGF- β Signaling in the Development of Cancers

For the ultimate outcome of TGF- β -mediated responses in any pathological condition, it is apparent that a combination of canonical and non-canonical pathways are coordinated (1). Cancers and fibrotic diseases are the most common pathologies associated with the activity of TGF- β . Currently, most putative

drugs affecting TGF- β for the treatment of cardiac fibrosis were initially developed for the management of cancer; therefore, we next discuss the signaling of TGF- β in carcinogenesis.

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



activation of TGF- β (88). Interestingly, TGF- β also regulates the functions of various immune cells, including the modulation of cytokines released from these cells. Impairment of TGF- β signaling pathways leads to immune dysregulation, fibrosis, and cancer [Reviewed in (7)]. TGF- β is produced as a complex with latency associated peptide (LAP). This complex associates with ECM by binding to latent TGF- β binding protein (LTBP) or glycoprotein A repetitions predominant (GARP) expressed on T cells, especially on Tregs, or platelets. Integrins bind to the complex and stimulate the release of TGF- β from the complex. The release of active TGF- β promotes oncogenesis and immune tolerance in breast cancer (89). Inhibition of α v β 8 integrins potentiates cytotoxic T cell responses and recruitment of immune cells to tumor centers. Cancer cells can evade host immunity by mobilizing active TGF- β 1 through α v β 8 integrins (90). Thus, TGF- β acts as a significant suppressor of immune responses during tumor progression.

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

TGF- β Signaling in the Development of Cardiac Fibrosis

During tissue injury, TGF- β expression is increased to play a role in the tissue repair process and scar formation. In the heart tissue following MI, TGF- β signaling plays an important role in reparative, angiogenetic, and fibrotic responses by modulating inflammation (93). Studies on mice and dogs have revealed that TGF- β 1 and TGF- β 2 were upregulated in the early phase after MI, and then TGF- β 3 was increased in a later stage post-infarction myocardium (94). Among various cells that release TGF- β , a significant amount of TGF- β might be released from infiltrated macrophages that migrate to the injured area to engulf the damaged cardiomyocytes, as shown in a mouse model (95). On the other hand, a study using a porcine model of chronic coronary constriction revealed that cardiomyocytes were a significant source of TGF- β (96). Another study suggested that TGF- β was found in the extracellular fluid of ischemic canine myocardium tissue (97). Multiple pathways involving integrins and thrombospondin-1 were found to be associated with the release of TGF- β from the cardiac ECM-bound TGF- β (98, 99). Following the release of active TGF- β , TGF- β binds to the receptors, as described earlier, to activate intracellular responses in the infarcted tissue. The TGF- β -mediated effects can be classified into 4 actions in the following order: cardiomyocyte survival, immune cell-related action, formation of myofibroblasts, and production/deposition of ECM, all of which modulate the effects on myocardial endothelial cells.

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

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

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

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

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

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

Inhibitors of TGF- β Signaling for the Treatment of Cancers

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

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

Trabedersen (AP12009)

Preclinical data

Trabedersen (AP12009, Antisense Pharma) is a synthetic, 18-oligomer phosphorothioate antisense oligonucleotide (ASO). It was developed as an ASO specifically targeting human TGF- β 2 mRNA, which leads to a reduction in TGF- β 2 expression, cellular proliferation, and cellular migration in various types of tumors *in vitro* and *in vivo*, including gliomas (119), melanoma (120), pancreatic carcinomas (121, 122), and colorectal cancer (123). Trabedersen has been shown to reduce cell proliferation, tumor growth, cell migration or metastasis, and vascularization

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

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

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

Clinical data

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

Belagenpumatucel-L Vaccine

The principle of anti-TGF- β cancer vaccines is to deliver antisense molecules of TGF- β into cancer cells and overturn the effects of immunosuppression in host cells, as well as to enhance antitumor immunity (9). Belagenpumatucel-L

(Lucanix, NovaRx) is a TGF- β 2, antisense, gene-modified non-viral based allogenic tumor cell vaccine. It was developed from non-small cell lung cancer (NSCLC) and modified to express ASO, which leads to suppression of the immunosuppressive activity implicit in TGF- β 2 overexpressing cancer cells (141).

Clinical data

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

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

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

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

Fresolimumab (GC1008)

Clinical Data

Fresolimumab (GC1008, Genzyme/Sanofi) is a fully human monoclonal antibody blocking pan-TGF- β (TGF- β 1, TGF- β 2, and TGF- β 3) [Reviewed in (150)]. Fresolimumab demonstrated acceptable safety and preliminary evidence of antitumor activity in a phase I trial on patients with previously treated malignant melanoma or renal cell carcinoma (151). In a phase II trial on 13 patients with malignant pleural mesothelioma, 3 patients showed stable disease for at least 3 months, and those who produced antitumor antibodies had an increased median OS. However, treatment with fresolimumab had no effect on the expression of NK, CD4⁺, or CD8⁺ T cell activating and inhibitory markers, other than a decrease in the expression of CD244 (also known as 2B4) and CD266 (best known as DNAM1) on NK cells (144). A phase II trial on 23 patients with metastatic breast cancer undergoing radiotherapy has reported that fresolimumab in combination with focal radiotherapy significantly increased OS and was well-tolerated in a dose-dependent manner. Higher doses of fresolimumab correlated with an improved CD8⁺ pool, leading to a favorable systemic immune response and longer median OS (145).

Galunisertib (LY2157299)

Preclinical Data

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

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

Clinical data

Among small molecule inhibitors, galunisertib is one of the most advanced. It has shown promising results in clinical trials due to its safety profile, with no cardiac potential toxicity in humans, which was a primary concern with first-generation TGF- β inhibitors (154). A phase I study on 28 patients with Grade IV glioma showed galunisertib was well-tolerated. The dose limiting toxicities included pulmonary embolism and thrombocytopenia, but no cardiotoxicities were observed (155). In addition, the safety of galunisertib was confirmed by a first-in-human dose study with 79 cancer patients with glioma and solid tumors treated with galunisertib as monotherapy or in combination with lomustine. No medically relevant cardiac toxicity or signs of cardiovascular injury were found, including increased blood pressure, troponin I, BNP, or hs-CRP or reductions in cystatin C levels (156). Likewise, no safety concerns or dose limiting toxicities was observed after treatment with galunisertib in patients with glioblastoma based on a pharmacokinetic/pharmacodynamic (PK/PD) model (157). Galunisertib as monotherapy and as second-line therapy after sorafenib failure in a subset of 109 patients with hepatocellular carcinoma yielded a median OS of 8.3 months in a phase II trial (147). Interestingly, patients who had decreased expression levels of specified blood biomarkers [e.g., alpha-fetoprotein (AFP), TGF- β 1, and CDH1] had improved clinical outcomes, indicating that the effects of galunisertib might be more pronounced in patients with a poor prognosis due to elevated AFP at baseline (147). Similarly, galunisertib in combination with gemcitabine improved OS with minimal added toxicity in a phase II study on patients with locally advanced or metastatic pancreatic adenocarcinoma who were considered candidates for first-line chemotherapy with gemcitabine (146).

Vactosertib (EW-7197) and EW-7195

Preclinical Data

Vactosertib (EW-7197 or TEW-7197), a novel small molecule inhibitor of ALK5, has been recently developed as a more potent and specific antitumoral compound than galunisertib. Vactosertib and EW-7195 expressed potent antimetastatic activity *in vivo* via an inhibition of TGF- β 1-induced Smad/TGF β signaling, cell migration, invasion, EMT, and breast tumor metastasis to the lung in xenografted nude mice and transgenic MMTV/cNeu mice (134, 135). In addition, vactosertib expressed the potential to boost cytotoxic T lymphocyte function in 4T1 orthotopic-grafted mice and prolonged the lifespan of 4T1 breast tumor-bearing mice (134).

Clinical data

Vactosertib is currently being tested in phase I/II clinical trials for several cancer types in combination with chemotherapy or antibodies against immune checkpoints. A phase I study is evaluating the safety and tolerability of the drug in combination with paclitaxel in 12 metastatic gastric cancer patients (NCT03698825). The phase Ib/IIa trials include a study

of vactosertib in combination with durvalumab in patients with advanced NSCLC who progressed following platinum-based chemotherapy ($N = 63$) (NCT03732274). A combination with pembrolizumab is being employed for metastatic or locally advanced colorectal or gastric/gastroesophageal junction adenocarcinoma ($N = 67$) (NCT03724851), and a combination with imatinib is being employed for patients with advanced desmoid tumors ($N = 24$) (NCT03802084). The latest phase II trial aims to determine whether administration of vactosertib with durvalumab will provide meaningful increases in the overall response rate in patients with urothelial cancers that fail to achieve a CR with anti-PD-1/PD-L1 based regimens ($N = 48$) (NCT04064190).

Remarkably, given TGF- β signaling plays a crucial role in fibrotic states, vactosertib has recently been investigated as an antifibrotic agent to delay the development of fibrosis in primary organs including the liver, kidney, and lung. Vactosertib was found to suppress fibrosis-induced accumulation of ROS and ECM proteins (collagen, α -SMA, fibronectin, and integrins) in the liver, lungs, and kidneys of mice due to its antifibrotic mechanism via inhibition of both TGF- β 1/Smad2/3 and ROS signaling (158). A study on a rat model of Peyronie's disease showed that vactosertib suppressed phospho-Smad2 expression and recruitment of inflammatory cells, leading to a decline in fibrotic plaques (159). Thus, vactosertib and EW-7195 could be a promising antifibrotic compound for the treatment of fibrotic diseases.

Tasisulam (LY573636)

Clinical Data

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

M7824 (MSB0011359C)

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

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

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

Anti-TGF- β 1 neutralizing antibodies have also been under investigation as potential antifibrotic agents by interfering with TGF- β signaling. Administration with anti-TGF- β 1 antibody attenuated cardiac fibrosis and diastolic abnormalities in a rat model of pressure overload (47) (Table 2). Although these antibodies attenuated fibroblast activation and collagen synthesis, no improvements in overall cardiac functions were found in pressure-overloaded rats (47). Furthermore, anti-TGF- β neutralizing antibody inhibited ECM proteins synthesis and reduced cardiac fibrosis in a rat model induced by a chronic blockade of nitric oxide synthesis (162). However, in a mouse model of MI, a neutralizing anti-TGF antibody administered before or after coronary artery ligation resulted in increased mortality rates and left ventricular (LV) dilation after MI (163).

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

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

while overexpression of p38-MAPK induces myofibroblast differentiation in cardiac fibroblasts (49).

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

GW788388

Preclinical data

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

Pirfenidone

Preclinical data

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

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

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

Agents	Targets	Experiments/Models	References
GW788388	ALK5 and T β R11	<i>In vivo</i> : murine Chagas disease	(170)
		<i>In vivo</i> : <i>Scn5a</i> ^{+/-} mouse model of cardiac conduction disease	(171)
		<i>In vivo</i> : rat model of heart failure (HF) following myocardial infarction (MI)	(50)
Pirfenidone	TGF- β	<i>In vivo</i> : Deoxycorticosterone acetate (DOCA)-salt hypertensive rats	(172)
		<i>In vivo</i> : rat MI model	(173)
		<i>In vivo</i> : Transverse aortic constriction (TAC)-induced left ventricular (LV) remodeling mouse model	(174)
		<i>In vivo</i> : TAC-induced pressure-overloaded HF model	(175)
		<i>In vivo</i> : Streptozotocin (STZ)-induced diabetic rats	(176)
Tranilast	TGF- β	<i>In vivo</i> : STZ-induced diabetic (mRen2)27 rats	(177, 178)
		<i>In vivo</i> : DOCA/salt and renovascular hypertensive rats	(179, 180)
		<i>In vivo</i> : LV remodeling post-MI rats	(181)
		<i>In vivo</i> : hypertensive (mRen2)27 rats	(182)

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

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

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

Clinical data

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

Tranilast

Preclinical data

Tranilast has been used to treat allergic disorders (e.g., allergic rhinitis, asthma, and atopic dermatitis); however, tranilast

might also be useful for other medical conditions due to its ability to suppress TGF- β expression and activity. The molecular mechanisms underlying its antifibrotic actions are not completely understood, but tranilast might inhibit several profibrotic growth factors such as TGF- β and platelet-derived growth factor (PDGF) (22). The effects of tranilast on inhibition of cardiac fibrosis have also been supported by multiple animal models of cardiomyopathy. In STZ-induced (mRen2)27 diabetic rats, tranilast treatment attenuated cardiac matrix deposition in association with reductions in phospho-Smad2 of the heart (177). In a similar model, administration of tranilast attenuated cardiac dysfunction and structural abnormalities in diabetic cardiomyopathy with improved LV systolic and diastolic function, while tranilast did not affect Smad phosphorylation but it significantly attenuated TGF- β -induced p44/42 MAPK phosphorylation (178).

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

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

Clinical data

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

CONCLUSION

TGF- β is a multifunctional cytokine regulator acting through transmembrane serine/threonine kinase receptors and intracellular Smad transcriptional regulators. Once TGF- β is activated, it regulates ECM remodeling and promotes a fibroblast to myofibroblast transition, which is essential for fibrotic processes. Given TGF- β plays a major role in various stages of cancer progression and in the development of cardiac fibrosis, TGF- β and its signaling pathway offer opportunities for novel treatment strategies in patients with cancer and

cardiac fibrosis. Research on the underlying mechanisms and the therapeutic targets of TGF- β inhibitors for cancer and cardiac fibrosis has advanced significantly in recent decades. The inhibitors of TGF- β signaling for cancer and fibrosis have been extensively studied in animal models and clinical studies; however, translation of these findings into human pathologic conditions has been limited due to the broad range of responses to TGF- β and its role in tissue homeostasis. Currently, various types of TGF- β inhibitors are challenged and tested their efficacies in patients with cancers. A few of TGF- β inhibitors are subjected into the clinical studies for treatment of cardiac fibrosis. The development of more specific agents targeting TGF- β signaling pathways such as M7824, a bifunctional fusion protein composed of TGF- β trap, and a monoclonal antibody against programmed death ligand 1 (PD-L1) are likely to help minimize potential side effects and enhances efficacy for treatment of cancers. Furthermore, the combination of anti-TGF- β therapies with various mechanisms of action might have greater efficacy against cancer and cardiac fibrosis.

AUTHOR CONTRIBUTIONS

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

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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