Role of TRPM7 in cardiac fibrosis: A potential therapeutic target (Review)

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Abstract. Cardiac fibrosis is a hallmark of cardiac remodeling associated with nearly all forms of heart disease. Clinically, no effective therapeutic drugs aim to inhibit cardiac fibrosis, owing to the complex etiological heterogeneity and pathogenesis of this disease. A two-in-one protein structure, a ubiquitous expression profile and unique biophysical characteristics enable the involvement of transient receptor potential melastatin-subfamily member 7 (TRPM7) in the pathogenesis and development of fibrosis-related cardiac diseases, such as heart failure (HF), cardiomyopathies, arrhythmia and hyperaldosteronism. In response to a variety of stimuli, multiple bioactive molecules can activate TRPM7 and related signaling pathways, leading to fibroblast proliferation, differentiation and extracellular matrix production in cardiac fibroblasts. TRPM7-mediated Ca^{2+} signaling and TGF-β1 signaling pathways are critical for the formation of fibrosis. Accumulating evidence has demonstrated that TRPM7 is a potential pharmacological target for halting the development of fibrotic cardiac diseases. Reliable drug-like molecules for further development of high-affinity *in vivo* drugs targeting TRPM7 are urgently needed. The present review discusses the widespread and significant role of TRPM7 in cardiac fibrosis and focuses on its potential as a therapeutic target for alleviating heart fibrogenesis.

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1. Introduction

Cardiac fibrosis is a hallmark of cardiac remodeling associated with nearly all forms of heart disease, such as heart failure (HF), myocardial infarction (1), dilated and ischemic cardiomyopathies (ICMs) (2), arrhythmia (3) and hyperaldosteronism (4). The adult mammalian heart has negligible regenerative capacity, and cardiac repair is dependent on the clearance of dead cells and the formation of scar tissue to help preserve myocardial structural and functional integrity (5). Meanwhile, unrestrained tissue repair can result in pathological fibrosis, causing detrimental abnormalities (5,6). Pathological cardiac fibrosis is orchestrated predominantly by myofibroblasts, which are activated fibroblasts characterized by overproduction of growth factors, cytokines, chemokines, proteases and extracellular matrix (ECM) proteins. Moreover, myofibroblasts are particularly responsive to proinflammatory cytokines, including TNF-α, IL-1, IL-6 and TGF-β; vasoactive peptide angiotensin II (Ang II), endothelin-1, atrial natriuretic peptide and brain natriuretic peptide (5-7). The sources of these activated fibroblasts that accumulate in response to various pathological insults, such as myocardial injury, oxidative stress, mechanical stretch, autocrine-paracrine mediators

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and inflammatory stimuli, remain under active investigation (8). Lineage tracing has recently been utilized in cardiac fibrosis studies, and numerous cells, including resident fibroblasts (9), endothelial cells (ECs) (10) or epicardial cells (11), hematopoietic bone marrow-derived macrophages (12) and perivascular cells (13), have been proposed as precursors of the fibroblast population in the injured heart. Thus, the differentiation of quiescent fibroblasts into active matrix-producing myofibroblasts is a key step in disease progression (5). Cardiac fibrosis induces adverse structural remodeling of the myocardium, resulting in abnormalities in cardiac conduction, loss of contractility, and hardening of the ventricular walls (5). Thus, understanding the underlying mechanisms of cardiac fibrosis will facilitate the development of innovative treatment strategies to hinder pathological fibrosis.

The TRPM subfamily plays a crucial role in various physiological and pathological conditions such as those related to sensory or renal physiology, cancer, cardiac health and neuronal development (14). The subfamily contains eight isoforms that form four subfamilies of TRPM channels: TRPM1/3, TRPM2/8, TRPM4/5, and TRPM6/7. Among these, TRPM7, TRPM2 and TRPM6 are unique because they are bifunctional heteromeric ion channels containing functional enzymatic domains in their highly varied C-terminal segments (15). The TRPM2 channel has an enzymatic domain similar to Nudix hydrolase, cleaving monodinucleotidic polyphosphates (15). On the other hand, the TRPM6 and TRPM7 channels contain kinase domains and are classified as atypical alpha protein kinases (15). The double activity as channels and enzymes of these two proteins classifies them either as chanzymes or channel-kinases (16) (Fig. 1). TRPM7 and its close homologue TRPM6 are present in the tetrameric form, with each subunit consisting of six transmembrane segments, and are both permeable to calcium (Ca^{2+}), magnesium (Mg^{2+}) and zinc cations (Zn^{2+}) (17). The disruption of TRPM6 kinase phosphorylation activity reintroduces MgATP sensitivity to the heteromeric channel, which is similar to that of TRPM7, supporting the notion that TRPM6 kinase plays a critical role in the control of the TRPM7/6 channel complex (18).

The TRPM subfamily has been shown to have highly differing modes of activation, cation selectivity and tissue distribution (19) and play crucial roles in various physiological and pathological conditions such as those related sensory or renal physiology, cancer, cardiac health and neuronal development (14). TRPM6 is mainly expressed in intestinal and renal epithelia, and TRPM2 expression is highest in the brain and bone marrow (20). In contrast to TRPM6 and TRPM2, which have specific expression patterns, TRPM7 is widely distributed in the central nervous system as well as in the periphery, with the highest expression levels in the heart, pituitary, bone and adipose tissue (20). TRPM7 expression in cardiac fibroblasts (CFs) has been verified by immunocytochemistry (21). Two of the characteristics of TRPM7 channels, specifically, their non-voltage-gated design and Ca^{2+} permeability, suggest that they may have significant pathological and physiological functions in various cells, particularly in non-excitable cells such as CFs (21).

Their two-in-one protein structure, ubiquitous expression profile and unique biophysical characteristics that enable divalent ion transport results in the involvement of TRPM7 involvement in a number of pathophysiological processes (22-34), including Mg^{2+} (25,26) and Ca^{2+} (27) homeostasis, immune system homeostasis (28), embryonic development (25,29), hyperaldosteronism (4), cardiovascular inflammation and fibrosis (22), hypertension (23), diabetes (30,31), cerebral ischemia and hypoxia (32), airway remodeling (33) and tumorigenic activity (34,35). A lack of either TRPM6 or TRPM7 was found to be embryonically lethal, and several Mg-related diseases have been linked to mutations in these channels (22,23,29,36,37). Inhibition of TRPM2has been indicated to protect against renal fibrosis and inflammation (38), whereas chanzyme TRPM7 has been demonstrated to protect against cardiovascular fibrosis and inflammation (22). Since TRPM7 expression is highest in the heart, previous studies have demonstrated that TRPM7 plays a crucial role in cardiac fibrosis-related diseases (21,22). The present review will focus on recent developments in understanding the role of TRPM7 in cardiac fibrogenesis and the potential of TRPM7 as a therapeutic target for antifibrotic drug development.

2. Interacting proteins

TRPM7 represents a constitutively active ion channel that is heavily regulated by a variety of physiological feedback mechanisms. One of the most important regulatory factors of channel activity is free intracellular Mg^{2+} (39), which opens channels and thus result in ion movements. Detailed biophysical examination revealed that native TRPM7 in excised patches has two conductance states at 39 picosiemens (pS) and 186 pS, with both reversibly inhibited by Mg^{2+} (40).

Information on proteins interacting with TRPM7 remain scarce, even for the kinase domain. A study has shown that receptor-stimulated activation of phospholipase (PLC causes inhibition of TRPM7 channel activity through localized phosphatidylinositol 4,5-bisphosphate (PIP$_2$) hydrolysis (41). Furthermore, hypomagnesemic conditions increased TRPM7-kinase-regulated Ser/Thr phosphorylation in the C2 domain of PLCγ2, leading to reduced Ca^{2+} signaling (42), whereas the α-kinase domain activates downstream target proteins involved in cytoskeleton organization, cell proliferation, inflammatory responses and vascular contraction, among other properties (39,43). The involvement of TRPM7 kinase in cell motility and adhesion has been linked to its ability to phosphorylate the assembly domains of non-muscle myosin IIA, IIB, and IIC and ATP-dependent motor proteins involved in actomyosin-based cell motility (44-46). Annexin A1, a Ca^{2+}-dependent membrane-binding protein with the ability to promote membrane fusion, is also phosphorylated by the TRPM7 kinase, providing a possible link to TRPM7's known involvement in cell growth and apoptosis (47,48).

TRPM7 has autophosphorylation residues, and cleavage of the α-kinase results in the release of fragments that bind to transcription factors, leading to epigenetic modifications (49). Phosphomapping by mass spectrometry identified 47 autophosphorylation sites on TRPM7, the majority of which are located in the Ser/Thr-rich domain N-terminal of the kinase region (16). This part of the TRPM7 region is thought to control kinase substrate binding (16). The TRPM7 kinase specifically phosphorylates Ser and Thr residues
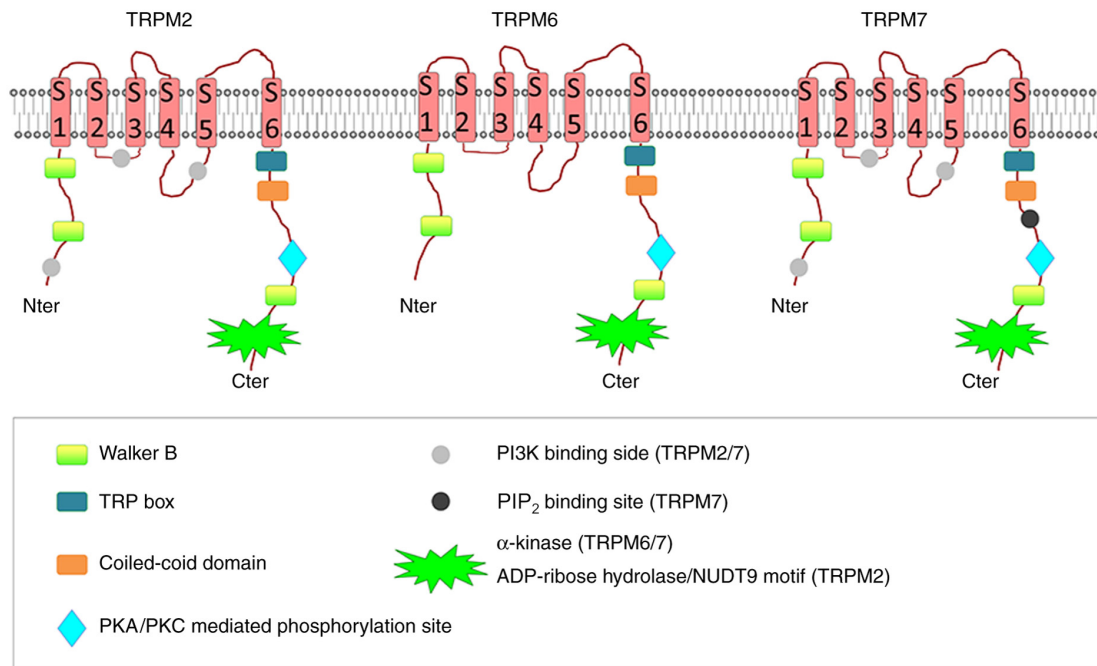
Figure 1. Schematic structure of TRPM2, TRPM6 and TRPM7 proteins. Nter, N-terminal; Cter, C-terminal; TRPM, transient receptor potential melastatin; PIP_2, phosphatidylinositol 4,5-bisphosphat; PKA, protein kinase A; PKC, protein kinase C; NUDT9, ADP-ribose pyrophosphatase.

in a Mg^{2+}-dependent manner (50). It autophosphorylates itself and phosphorylates myelin basic protein as well as histone H3 (51). At least two of the identified autophosphorylation sites (S1511 and S1567) do not seem to influence channel behavior (51). These proteolytically TRPM7-cleaved kinase fragments (M7CKs) translocate to the nucleus and bind multiple components of chromatin remodeling complexes, leading to epigenetic modifications and thereby influencing gene expression profiles (49). Furthermore, free cytosolic Zn^{2+} is TRPM7-dependent and regulates M7CK binding to transcription factors containing zinc-finger domains (49). These findings suggested that TRPM7-mediated modulation of intracellular Zn^{2+} concentration couples ion channel signaling to epigenetic chromatin covalent modifications that affect gene expression patterns (49). Deletion of TRPM7 in T-cells reduces apoptosis in response to Fas stimulation, while caspase-dependent cleavage at D1510 leads to separation of TRPM7's kinase domain from the channel without affecting the functionality of the kinase but enhancing ion channel activity (52).

The channel and/or enzyme domain of TRPM7 is relevant to cardiac fibrosis. TRPM7 is involved in Ang II-induced cardiac fibrosis development by mediating Mg^{2+} and Ca^{2+} influx (53). $TRPM7^{+/\Delta kinase}$ mice exhibit cardiovascular inflammation and fibrosis associated with $[Mg^{2+}]_i$ deficiency, abnormal macrophage activation, inflammatory cell infiltration, and increased signaling through Smad3, calpain-II and Stat1 (22). The kinase domain may influence the channel domain supported by the tissue, and cellular Mg^{2+} levels were lower, while TRPM7 channel phosphorylation was reduced in $TRPM7^{+/\Delta kinase}$ mice compared with control counterparts (22). Other studies have also suggested that the coexistence of the TRPM7 channel and kinase is functionally relevant; for example, channel-mediated Mg^{2+} influx is required for TRPM7 kinase activity, and thus

regulates RhoA activity and subsequent transcriptional activation in hepatocellular carcinoma cells (34). The TRPM7 kinase inhibitor TG100-115 suppressed ion channel activity in breast cancer cells (54).

3. Physiological roles of TRPM7 in the heart

TRPM7 is highly expressed in the adult heart and is first expressed in the embryonic myocardium (25). The physiological consequences of cardiac-targeted TRPM7 deletion depend on the timing of TRPM7 disruption during cardiogenesis (29). Early cardiac TRPM7 deletion before embryonic day 9 causes HF and death by embryonic day 11.5 due to myocardial fragility (29). Remarkably, mice with TRPM7 deletion late in cardiogenesis, at approximately embryonic day 13, exhibit normal adult ventricular structures and functions (29). Genetic deletion of TRPM7 at an intermediate developmental timepoint during mouse cardiogenesis alters the myocardial transcriptional profile and variably damages adult ventricular function, inducing atrioventricular block, impaired repolarization and ventricular arrhythmias (29). Interestingly, cardiac-targeted TRPM7 deletion during the intermediate and late stages (approximately embryonic days 11.5-13) results in significant interstitial ECM deposition and interstitial fibrosis in hearts at 6-8 months (29). Consistent with the cardiomyopathic phenotype, researchers also observed expected enrichment in genes upregulated in the ECM, ECM receptor interactions and pressure-overload murine heart disease (29). These results indicated that subtle differences in the timing of TRPM7 disruption led to significantly different cardiac phenotypes. TRPM7 is dispensable in the adult ventricular myocardium under basal conditions but is critical for myocardial proliferation during early cardiogenesis and fibrosis during intermediate-and-late cardiogenesis.

In addition, TRPM7 in other organs can also affect heart function, especially in inflammation and fibrosis (4,22). TRPM7-dificient mice with deletion of the kinase domain (TRPM7$^{+/\Delta kinase}$) showed significant cardiac hypertrophy, fibrosis and inflammation (22). Furthermore, TRPM7$^{+/\Delta kinase}$ mice exhibit distinct pro-inflammatory and pro-fibrotic cardiovascular and renal phenotype linked to macrophage activation, increased signaling through Smad3, calpain-II and Stat1 and cellular hypomagnesaemia (22). The different physiological consequences between cardiac-targeted TRPM7 deletion and systemic deletion of the kinase domain alone may be due to macrophages from TRPM7$^{+/\Delta kinase}$ mice producing soluble factors that promote a pro-fibrotic phenotype in CFs through Mg^{2+}-dependent mechanisms (22).

Other studies suggested that TRPM7 activation causes dysregulated immune responses and inflammation, and that TRPM7 inhibition may have therapeutic potential in pro-inflammatory diseases and immune hypersensitivity (55,56). These discrepancies likely depend on the relative contributions of the TRPM7 channel vs. the kinase domain and highlight the complexity of the system. These results provided diagnostic insight into putative mutations in the TRPM7 gene in patients with unexplained structural or electrophysiological heart disease.

4. Pathological functions of TRPM7 in cardiac fibrosis

Pathological cardiac fibrosis in response to myocardial injury and/or chronic alterations of myocardial loading conditions increase myocardial wall stiffness and disrupt the order of myocardial structure, which is a requisite for normal cardiac output and electrical conduction, culminating in chamber dilatation, cardiomyocyte hypertrophy and apoptosis and ultimately leading to the development of HF and increased arrhythmogenicity (57). Atrial fibrosis is also a common pathological change in elderly people that is strongly associated with the perpetuation of atrial fibrillation (AF) (58). The biochemical mechanisms of cardiac fibrosis involve impaired Ca^{2+} or Mg^{2+} homeostasis, oxidative stress, hemodynamic abnormalities and activation of neurohormones, including Ang II, TGF-β1 and endothelin-1 (2,5,6,22,58,59). The production of increased quantities of ECM proteins by CFs leads to tissue fibrosis, which can impair both mechanical and electrical function of the heart, contributing to AF and HF (60). Previous studies have shown that TRPM7 is the only calcium channel expressed on the cell membrane of CFs (61), and that Ca^{2+} signaling is closely associated with the initiation of fibrosis (62-64). These findings suggested that TRPM7 may be the one of the most potent fibrosis factors, playing an important role in the molecular mechanism and pathological processes in cardiac fibrosis (Fig. 2).

TRPM7-mediated Ca^{2+} signaling during cardiac fibrosis. Ca^{2+}, which is influenced by TRPM7, is critically involved in controlling cell function including cell proliferation, growth, secretion, migration, differentiation and death (65). Ca^{2+} influx is necessary for the biological functions of CFs during collagen synthesis (66). The expression and current of TRPM7, but not of TRPM4, are increased in human atrial specimens from patients with AF compared with healthy controls (61). TRPM7-mediated Ca^{2+} influx is also significantly increased

in atrial fibroblasts from patients with AF (61). TRPM7 is the molecular basis of the main Ca^{2+} permeable channel in human AF, which is the hallmark of atrial structural remodeling (67). Atrial structural remodeling is one of the most important fundamental mechanisms underlying the perpetuation of AF and contributes synergistically with electrical and neural remodeling to the AF substrate (3,58). Previous studies have shown that TRPM7 is the only calcium channel expressed on the cell membrane of CFs (61). Knocking down TRPM7 largely eliminates endogenous TRPM7 currents and Ca^{2+} influx in AF (67).

Mechanistically, TRPM7-mediated Ca^{2+} influx mediates atrial fibroblasts differentiation into myofibroblasts, thus promoting atrial fibrosis (67). Myofibroblasts present with the characteristics of both smooth muscle cells and fibroblasts (66). In normal hearts, myofibroblasts are found only in valves (66). During tissue damage repair, myofibroblasts participate in two stages: Fiber formation and ECM remodeling in the fibrosis cascade reaction (66). TGF-β1 can induce the transformation of atrial fibroblasts into myofibroblasts, simultaneously upregulating TRPM7 (67). Inhibition of TRPM7-mediated Ca^{2+} influx renders fibroblasts less sensitive to TGF-β1-induced proliferation and differentiation, indicating that TRPM7-mediated Ca^{2+} signal is necessary for TGF-β1 elicited fibrogenesis (67). Inhibition of TRPM7 may prove to be an effective approach to reduce fibroblast differentiation and therefore attenuate fibrosis during AF.

Similar to the aforementioned roles in AF, TRPM7-mediated Ca^{2+} signaling may also mediate ventricular fibrosis. Previous studies have shown that TRPM7 expression and currents were upregulated in isoproterenol (ISO)-induced cardiac fibrosis *in vitro* and *in vivo* (68). Recently, TRPM7 was demonstrated to be increased in left ventricular tissue samples isolated from the explanted hearts of end-stage patients with HF (69). HF is characterized by impaired Ca^{2+} homeostasis/contraction, markedly prolonged Ca^{2+} transients and impaired restoration of low diastolic Ca^{2+} concentrations (70). Increased internal flow of extracellular Ca^{2+} to the cell activates and initiates biological signaling cascades, leading to the secretion of a large quantity of pro-inflammatory and pro-proliferative cytokines and ECM proteins, ultimately giving rise to cardiac interstitial fibrosis (70). In adult rat ventricular CFs, 2-aminoethyl diphenylborinate (2-APB, an TRPM7 inhibitor) or TRPM7 knockdown by short hairpin RNA abolished Ca^{2+} influx induced by Ang II (53). Furthermore, 2-APB inhibited the increase of myocardial connective tissue growth factor, α-smooth muscle actin (SMA) expression and CF proliferation induced by Ang II (53). To date, the mechanism of TRPM7-mediated Ca^{2+} signaling in cardiac fibrosis in HF has not been reported and requires further investigation.

TRPM7 is involved in hypomagnesemia during cardiac fibrosis. Mg^{2+} is the second-most abundant cation in mammalian cells and an essential cofactor in numerous enzymatic reactions. Mg^{2+} influences cell growth processes associated with remodeling and fibrosis, which are characteristic features of vascular damage in hypertension, atherosclerosis and diabetes (71). At the subcellular level, these effects occur at least partly via Mg^{2+}-dependent regulation of mitogen-activated protein kinases, tyrosine kinases and reactive oxygen
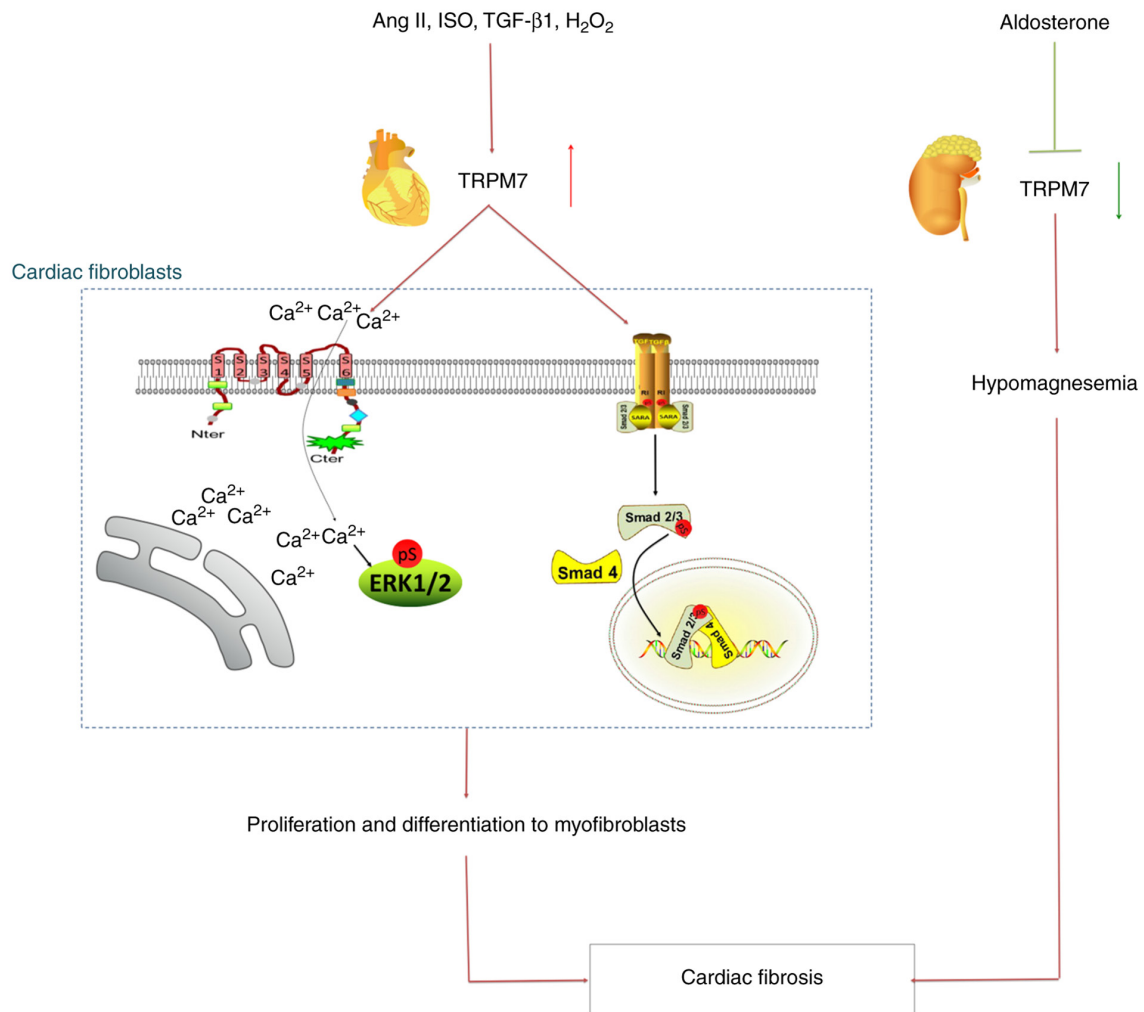
Figure 2. Schematic diagram illustrating the role of TRPM7 in the development and pathogenesis of cardiac fibrosis. TRPM, transient receptor potential melastatin; Ang II, angiotensin II; Nter, N-terminal; Cter, C-terminal; ISO, isoproterenol; pS, phosphorylation.

species, which are important signaling molecules involved in vascular smooth muscle cell proliferation, fibrosis and inflammation (72). Human microvascular endothelial cell growth inhibition by low magnesium is correlated with an increase in P_{21} or P_{27} kip, which are inhibitors of cyclin-dependent kinase (73).

TRPM7 is a key modulator of Mg^{2+} homeostasis, whose basal activity is regulated by intracellular levels of Mg^{2+} and MgATP (37). The role of TRPM7-mediated Mg^{2+} signaling in vascular homeostasis has been widely reported (74) but is less reported in the heart, especially in cardiac fibrosis. Recently, researchers utilized a particular DMEM (Ca^{2+}- and Mg^{2+}-free DMEM) supplemented with only calcium or only magnesium. The increase in α-SMA and fibronectin expression induced by Ang II were abolished without calcium or magnesium, and the same outcome was observed for CF proliferation, indicating that both cations are required for the fibrosis response induced by Ang II, and that neither cation can be omitted (53). However, the association between TRPM7 and Mg^{2+} signaling in CFs requires further investigation.

In TRPM7 kinase-deficient mice, Ang II-induced cardiac hypertrophy, interstitial fibrosis and left ventricular dysfunction are amplified and associated with hypomagnesemia,

inhibited TRPM7 kinase expression/signaling and pro-inflammatory vascular responses (23). Unlike the pro-cardiac fibrosis role of TRPM7 in mediating Ca^{2+} signaling, findings from animal models indicated that TRPM7 activation and increased Mg^{2+} influx protected against vascular and cardiac fibrosis (75). Hyperaldosteronism is associated with hypertension, cardiovascular fibrosis and electrolyte disturbances, including hypomagnesemia (76). Aldosterone modulates renal TRPM7 expression, which could be important in altered Mg^{2+} homeostasis associated with hyperaldosteronism (76). In a hyperaldosteronistic mouse model, aldosterone decreased the expression of renal TRPM7 without affecting TRPM6 and mediated blood pressure-independent renal and cardiovascular fibrosis and inflammation via Mg^{2+}-sensitive pathways (4). Mg^{2+} supplementation normalizes TRPM7 mRNA expression and attenuates cardiac fibrosis (4). Therefore, Mg^{2+} supplementation may have an underlying therapeutic benefit in aldosterone-associated cardiac fibrosis.

TRPM7 is involved in oxidative stress during cardiac fibrosis. TRPM7 can be activated by oxidative stress-related pathologies, such as Alzheimer's disease, anoxia, ischemia/reperfusion injury and diabetes (24,32,77). Reactive

oxygen species-mediated oxidative stress is also well known to be the main contributor to cardiac injury and is involved in cardiac remodeling (78). In response to pathological stimuli such as oxidative stress and inflammation, CFs can differentiate into myofibroblasts to initiate myocardial fibrogenesis (79). In explanted human hearts with idiopathic dilated cardiomyopathy, ventricular tachycardia is associated with greater cardiomyocyte hypertrophy, myocardial fibrosis, oxidative stress and increased expression of TRPM7 (80). Previous studies have shown that TRPM7 is upregulated in rat models of myocardial ischemia and reperfusion (81). TRPM7 mRNA levels are downregulated in left atrial and left ventricular samples from patients with ICM, and this change has an inverse relationship with ventricular dysfunction (82). TRPM7 also contributed to H_2O_2-induced cardiac fibrosis by mediating Ca^{2+} influx and ERK1/2 activation in rat primary cardiac fibroblasts, and the blockade or silencing of TRPM7 inhibited myocardial fibrogenesis (83). Wu *et al* (68) found that microRNA (miR)-135a protects against ISO-induced cardiac fibrosis by downregulating TRPM7 expression and currents. Astragaloside IV treatment also inhibited ISO-induced cardiac fibrosis by targeting the miR-135a-TRPM7-TGF-β/Smad pathway (84,85). These results provided a better understanding of the potential roles of TRPM7 in oxidative stress-induced cardiac fibrosis and suggested that TRPM7 channels can be regarded as a therapeutic target.

TRPM7 is involved in neurohormone activation during cardiac fibrosis. Ang II, a pro fibrogenic cell growth factor, plays a significant role in the occurrence and development of cardiac fibrosis (86). The relationship between TRPM7 and Ang II is complex. Ang II reduced TRPM7 kinase expression in the heart and aorta of mice (23), whereas it increased TRPM7 expression in rat ventricular CFs in a concentration-dependent manner (53). In TRPM7 kinase-deficient mice, Ang II-induced cardiac hypertrophy, interstitial fibrosis and left ventricular dysfunction are amplified (23). However, downregulation of TRPM7 attenuated Ang II-induced CF proliferation, differentiation, ECM production and accumulation (53,87). The TRPM7 Ca^{2+} current, as well as the protein expression levels of TRPM7 and collagen III, initially increases and then later decreases under optimal Ang II concentrations for inducing cardiac fibrosis (88). Therefore, in different stages of Ang II-induced cardiac fibrosis, the role of TRPM7 may be different.

Similar effects in fibrotic changes in a rat sick sinus syndrome (SSS) model have been observed (89). The sinoatrial node and atria of SSS rats exhibit more fibers and higher expression levels of Ang II, TRPM7 and phosphorylated (p)-Smad2 and produce more collagen than sham rats (89). TRPM7 small interfering RNA (siRNA) inhibited Ang II-induced p-Smad2 expression and collagen synthesis in cardiac fibroblasts *in vitro* (89). The considerable inward flow of Ca^{2+} activated signaling pathways such as the TGF β/Smad pathway, causing inflammation and cellular differentiation and thereby resulting in an acceleration of fibrosis (67). When exposure to Ang II is prolonged, the induction of cardiac fibroblast apoptosis and necrosis increases, possibly generating ulterior inflammatory reactions and fibrogenesis.

TGF-β1 is a profibrotic factor which can stimulate the proliferation of fibroblasts. TGF-β/Smad signaling is regarded to be the main pathway leading to tissue fibrosis in a number of diseases (90-92). TGF-β1 can promote cardiac fibrosis by phosphorylating downstream Smad2/3, while activated Smad7 can ameliorate fibrosis by triggering TGF-β receptor I and Smad protein degradation (93). Studies have found that the TRPM7 channel is also an important downstream target of TGF-β1 induced liver (92), lung (94) and atrial fibrosis (67). TGF-β1 induced differentiation of cultured human atrial fibroblasts is correlated with an increase of TRPM7 expression induced by TGF-β1 (67). In addition, inhibition of TRPM7-mediated Ca^{2+} influx renders fibroblasts less sensitive to TGF-β1 induced proliferation and differentiation (67), indicating that TRPM7-mediated Ca^{2+} signal is necessary for TGF-β1 elicited fibrogenesis. Interestingly, latest research confirmed that TRPM7 gene silencing significantly suppressed the expression of TGF-β1 and p-smad3, while the expression of Smad7 protein was increased (84). In addition, SB431542 (a TGF-β1 blocker) significantly inhibited the expression of TRPM7 protein and currents in CFs (84). Thus, positive feedback occurs between enhanced TRPM7 function and TGF-β/Smad pathway activation, which promotes cardiac fibrosis progression.

5. Modulators for TRPM7

Due to the wide range of physiological and pathophysiological roles ascribed to TRPM7, reliable drug-like molecules allowing distinction of the channel versus kinase activity *in situ* and under *in vivo* conditions are urgently needed.

Several potent inhibitors of the TRPM7 channel have been identified, including a group of non-specific channel blockers such as SKF-96365 and 2-APB, natural compounds and metabolites including waixenicin A, quinine and sphingosine and several synthetic drug-like compounds (95). In adult rat ventricular CFs, 2-APB inhibited Ang II-induced Ca^{2+} influx and the expression of myocardial connective tissue growth factor, α-SMA and CF proliferation (53). Pharmacological targeting of the TRPM7 channel in conjunction with genetic silencing of the entire TRPM7 protein or the comparative analysis of effects induced by structurally unrelated TRPM7 modulators were shown to be instrumental in uncovering new cellular functions of TRPM7 and assessing the therapeutic potential of anti-TRPM7 drugs (95,96).

The first positive gating modulator of the TRPM7 channel, naltriben, an antagonist of δ-opioid receptors, reversibly activated the TRPM7 channel without prior depletion of intracellular Mg^{2+} and even under conditions of low PIP_2 (96). Naltriben is the prototype of type 1 activators, allowing induction of the TRPM7 channel independently of $[Mg^{2+}]_i$ (95). Moreover, mibefradil is able to stimulate TRPM7-mediated Ca^{2+} entry as well as TRPM7 currents with an EC_{50} of 53 M (97). Mibefradil is a type 2 agonist acting on the TRPM7 channel in a $[Mg^{2+}]_i$ dependent manner (97). Type 1 agonists (naltriben) will stimulate Mg^{2+} and Ca^{2+} influx irrespective of cytosolic Mg^{2+} levels, whereas type 2 agonists (mibefradil) will act preferentially on cells with reduced intracellular Mg^{2+} content. Hence, it is worth investigating whether putative endogenous TRPM7 ligands act in a similar manner (95).

A set of small organic modulators of TRPM7 have been identified (95-97). These new compounds allow for the activation or inhibition of TRPM7 currents or modulate

Table I. Role of TRPM7 in cardiac fibrosis.

Modulators	TRPM7 alteration			Experimental models	Signaling pathway	Refs.	Authors (year)
	Expression	Current	Divalent cations				
None	/	I_{to} ↓; I_f ↓	Unchanged myocardial Mg^{2+} and Zn^{2+}	TRPM7 deletion mice at an intermediate developmental time point (approximately embryonic day 11.5 to 13) during mouse cardiogenesis	TGF-β1, SMAD6, SMARCE1 and PDGF pathways	(29)	Sah et al (2013)
None	/	/	Hypomagnesemia	TRPM7+/Δkinase mice; coculture of cardiac fibroblasts with TRPM7+/Δkinase macrophages	Calpain-II and TGF-β1 pathways	(22)	Rios et al (2020)
None	↑	↑	Ca^{2+} influx ↑	Atrial fibroblasts from patients with AF	TGF-β1	(68)	Du et al (2010)
Ang II	↑	/	/	CFs from rat Ang II-induced SAN fibrosis tissues	Ang II/TRPM7/Smad pathways	(90)	Zhong et al (2018)
None	↑	/	/	Left ventricular free wall samples from patients with NIDCM and VT	/	(81)	Parajuli et al (2015)
No	↓	/	/	Left ventricular samples from patients with ICM	/	(83)	Ortega et al (2016)
No	↑	/	/	Left ventricular samples from patients with end-stage HF	/	(70)	Dragún et al (2019)
H$_2$O$_2$	↑	/	Ca^{2+} influx ↑	Neonatal rat CFs	ERK1/2 pathway	(84)	Guo et al (2014)
Isoproterenol	↑	↑	/	Neonatal rat CFs	miR-135a-TRPM7-TGF-β/ Smad pathway	(69), (85), (86)	Wu et al (2018), Wei et al (2020), Lu et al (2017)
Ang II	↑	↑	Ca^{2+} and Mg^{2+} influx ↑	Neonatal rat CFs	/	(54), (85), (86)	Yu et al (2014), Li et al (2017), Zhou et al (2015)
Ang II	/	/	Hypomagnesemia	Ang II-infused TRPM7+/Δkinase mice	Calpain and annexin-1	(23)	Antunes et al (2016)
Aldosterone	Renal ↓	/	Hypomagnesemia	Aldosterone-infused mice	Mg^{2+}-sensitive pathways	(4)	Sontia et al (2008)

I_{to}, transient outward potassium currents in Trpm7-deleted ventricular myocytes; I_f, pacemaker current in Trpm7-deleted atrioventricular node cells; SMARCE1, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1; PDGF, platelet-derived growth factor; AF, atrial fibrillation; angiotensin II, Ang II; CFs, cardiac fibroblasts; SAN, sinoatrial node; NIDCM, nonischemic dilated cardiomyopathy; VT, ventricular tachycardia; ICM, ischemic cardiomyopathy; ↑, upregulation; ↓, downregulation; /, unknown.

TRPM7 kinase activity (95-97). The newly identified TRPM7 modulators will undoubtedly be instrumental in deciphering the cellular functions of TRPM7. Small molecules that have been identified to date might be regarded as lead structures for further development of high-affinity *in vivo* drugs targeting TRPM7 (95).

6. Implications and conclusion

Myocardial fibrosis is a hallmark of cardiac remodeling and functionally contributes to the development of HF, a leading cause of death worldwide (98). Clinically, no valid therapeutic agents target activated cardiac fibroblasts, owing to the extraordinary etiological heterogeneity and sophisticated pathogenesis of this disease. Blockade of TGF-β1 signaling through pharmacological inhibition and magnetic nanoparticle targeted delivery of TGF-β1 siRNA to ECs can relieve pathological cardiac fibrosis and hypertrophy in EC-forkhead box P1 deletion mice (6).

This growing evidence demonstrated that TRPM7 plays a nonredundant and vital regulatory role in activated cardiac fibroblasts through its ion channel function and/or kinase activity (Table I). In addition, the heart is a very compact organ comprising diverse cell types, and its pluricellularity offers the opportunity for intercellular communication within the heart. Fibroblasts create and sustain the biochemical and mechanical environment of the heart through their complex interactions with cardiomyocytes. Further investigations are needed to assess the interaction between TRPM7 in cardiac fibroblasts and cardiomyocytes or coronary microcirculatory ECs under different pathological disease models related to cardiac fibrogenesis.

In conclusion, these *in vitro* and *in vivo* findings suggested that TRPM7 is a potential pharmacological target for halting the development of fibrotic cardiac diseases. Future studies will provide more mechanistic insights into the role of TRPM7 in cardiac fibrosis.

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Authors' contributions

FHu participated in the review design and drafted the manuscript. ML revised the manuscript critically for important intellectual content and illustrated the figures. FHa revised the manuscript critically for important intellectual content. QZ and YZ contributed to preparation, editing and review of the manuscript. WZ and XC participated in review design and contributed to quality control of data and images. XC gave final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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