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REVIEW ARTICLE

Endothelial to Mesenchymal Transition in the Cardiogenesis and Cardiovascular Diseases

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Abstract: Today, cardiovascular diseases remain a leading cause of morbidity and mortality worldwide. Endothelial to mesenchymal transition (EndMT) does not only play a major role in the course of development but also contributes to several cardiovascular diseases in adulthood. EndMT is characterized by down-regulation of the endothelial proteins and highly up-regulated fibrotic specific genes and extracellular matrix-forming proteins. EndMT is also a transforming growth factor-β-driven (TGF-β) process in which endothelial cells lose their endothelial characteristics and acquire a mesenchymal phenotype with expression of α-smooth muscle actin (α-SMA), fibroblast-specific protein 1, *etc*. EndMT is a vital process during cardiac development, thus disrupted EndMT gives rise to the congenital heart diseases, namely septal defects and valve abnormalities. In this review, we have discussed the main signaling pathways and mechanisms participating in the process of EndMT such as TGF-β and Bone morphogenetic protein (BMP), Wnt#, and Notch signaling pathway and also studied the role of EndMT in physiological cardiovascular development and pathological conditions including myocardial infarction, pulmonary arterial hypertension, congenital heart defects, cardiac fibrosis, and atherosclerosis. As a perspective view, having a clear understanding of involving cellular and molecular mechanisms in EndMT and conducting Randomized controlled trials (RCTs) with a large number of samples for involving pharmacological agents may guide us into novel therapeutic approaches of congenital disorders and heart diseases.

Keywords: Endothelial to mesenchymal transition, cardiovascular disease, congenital heart diseases, cardiogenesis, TGF-β.

1. INTRODUCTION

Although great progress improved preventive, diagnostic and treatment measurements, cardiovascular diseases remain a leading cause of morbidity and mortality worldwide [1, 2]. The endothelial layer of blood vessels plays a key role in the maintenance of the homeostasis of the cardiovascular system by releasing several kinds of vasoactive factors. Endothelial to mesenchymal transition (EndMT) does not only play a major role during development but also contributes to several cardiovascular diseases in adulthood. Important mesenchymal cells include fibroblasts, which have a key role in atherosclerosis, as well as regulation of inflammation, matrix and collagen production [3]. EndMT process is characterized by down-regulation of the endothelial proteins and highly up-regulated fibrotic specific genes and extracellular matrix-forming proteins [4].

The epithelial to mesenchymal transition (EMT) is a biological process that induces the formation of cells involved both in tissue repair and in morbid conditions including tissue fibrosis and tumor angiogenesis. The endothelium is a specialized type of epithelium; hence, EndMT pertains to a sub-type of EMT [5].

EndMT is referred to a process by which endothelial cells transform from squamous cell monolayer type to fusiform; EndMT is also a TGFβ-driven process in which endothelial cells lose their endothelial characteristics and acquire a mesenchymal phenotype with expression of α-SMA, fibroblast-specific protein 1, *etc*. [6]. EndMT is a vital process during cardiac development where cardiac valves and septa arise at specific regions in the cardiac endothelium in embryonic stages [7].

In spite of this significance, however, the disrupted EndMT gives rise to the congenital heart diseases, namely septal defects and valve abnormalities [8, 9].

Thus, having a better understanding of the underlying molecular and cellular mechanisms of the formation of cardiac fibroblasts *via* EndMT will enable the control of EndMT and may provide an opportunity for therapeutic strategies to cure heart diseases [10]. In this article, we have discussed the mechanisms of the signaling pathways participating in the process of EndMT and studied the role of

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The name Wnt is a portmanteau created from the name Wingless and the name Int-1

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EndMT in physiological cardiovascular development and pathological conditions including myocardial infarction (MI), pulmonary arterial hypertension (PAH), congenital heart defects, cardiac fibrosis and atherosclerosis.

2. EndMT SIGNALING PATHWAYS IN CARDIOGENESIS AND CARDIOVASCULAR DISEASES

As mentioned above, EndMT is a sub-group of EMT. It has been recently demonstrated that the involved mechanism of both share similar pathways and are interrelated, which are discussed as follows (Fig. **1**).

EndMT is referred to a process by which endothelial cells transform from squamous cell monolayer type to fusiform; EndMT is also a transforming growth factor-β (TGF-β)-driven process in which endothelial cells lose their endothelial characteristics and acquire a mesenchymal phenotype with expression of α-smooth muscle actin (α-SMA), fibroblast-specific protein 1, *etc*.

EC: Endothelial cells, EndMT: Endothelial to mesenchymal transition, MC: mesenchymal cells, TGFβ: transforming growth factor-β, CD31: cluster of differentiation 31, Ve-Cad: vascular endothelial cadherin, FSP-1: fibroblast specific protein 1, α -SMA: α -smooth muscle actin.

2.1. TGF-β and BMP Signaling Pathway

TGF-β and its superfamily BMP play vital activity in endothelial-to-mesenchymal transition (EndMT). TGF-β consists of three isoforms (β1 to β3) all of which are crucial in angiogenesis and may induce EndMT, a process involved in embryonic heart development, angiogenesis and fibrosis [11]. TGF-β superfamily members are known to contribute to the cell plasticity during EndMT. TGF-β1 induces the cells of endothelium to undergo the transition, whereas BMP-7 preserved the endothelial phenotype [12]. Beside three isoforms of TGF-β1–β3, several receptors are also involved in cardiac EndMT in various contexts. TGF-β1 acts in the EMT of the murine atrioventricular canal (AVC); in the avian models, TGF-β ligands and receptors have special activity throughout EMT. TGF-β2 induces endothelial cell-cell activation and segregation. It has been shown that TGF-β2 plays a role in the formation of endocardial cushion, and TGF-β3 mediates cellular invasions into the extracellular matrix [13].

Recent studies have linked atherosclerosis and pathological fibrosis to EndMT mainly through the TGFβ/Smad signaling pathways although their exact roles remain unclear [14]. Some herbal ingredients such as Cinnamaldehyde may delay the progression of cardiac fibrosis [15]. TGF-β activates EndMT *via* both Smad-dependent and non-Smad-dependent pathways. TGF-β1 is the principle inductor of endothelial fibrosis, acting through the TGF-β1/activin receptor-like kinase 5 (ALK5)/Smads intracellular signaling pathway [4]. Losartan inhibits cardiac fibrosis through blocking EndMT *via* TGF-β/Smad2 [16, 17].

Non-Smad-dependent pathways such as p-ERK pathway in TGF-β-mediated EndMT in mitral valve endothelial cells (VECs) [18], phosphoinositide 3-kinase and Rho-like GTPase p38 MAPK signaling pathways are also involved in TGF-β-stimulated EndMT [19-21]. Additionally, other cytokines are involved in TGF-β-related EndMT, for instance, basic fibroblast growth factors (b FGF) exerts strong inhibitory properties on many TGFβ-regulated genes but acts in accordance with TGFβ to upregulate others [22].

Other findings identified FGFR1 as the key regulator of TGF-β signaling and EndMT development [6]. Akt/nuclear factor-κB involves in inflammatory cytokine-induced EndMT in valve endothelium in both embryonic and adult stages, using TGF-β signaling pathway [23]. Hepatocyte growth factor (HGF), a growth factor for epithelium and endothelium, which is released by various cell types. HGF has proangiogenic effects. *In vitro*, HGF prohibits EMT and

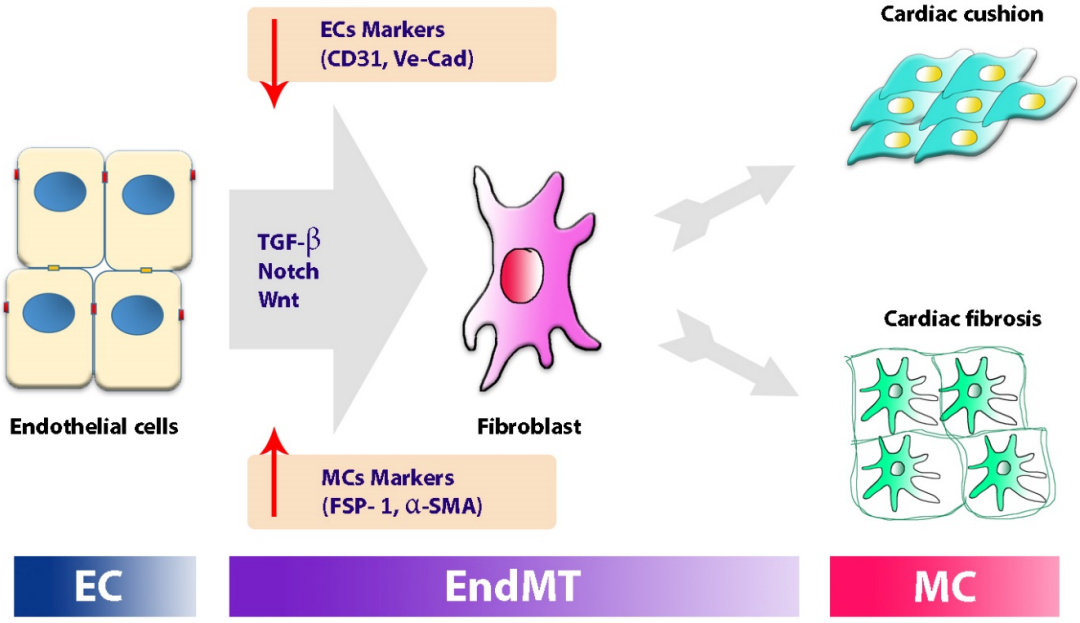

Fig. (1). a Schematic view of physiologic and pathologic role of EndMT. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

induces the apoptosis of myofibroblasts. *In vivo*, HGF possessed antifibrotic activity indicated in experimental models of kidney, lung, liver and heart fibrosis [24]. BMP is also necessary for EndMT [25]. Throughout the formation of endocardial cushion, BMP2 and BMP4 are expressed in atrioventricular canal and outflow tract (OFT) myocardium [26]. Myocardial BMP2 signal integration produces a valve-forming region between two cardiac chambers [27].

Several BMPs and their receptors (BMPRs) are necessary factors needed for EndMT and aid to the formation of cardiac valves and septation [28]. An early stage in cardiac valve formation is the EMT of a subpopulation of endothelial cells in particular areas of the heart tube (the cardiac cushions). The Type III TGFβ receptor (TGFβR3) is needed for TGFβ2- or BMP-2-stimulated EMT in atrioventricular endocardial cushion [29].

BMP4 and BMP7 deficiency resulted in defective EMT and reduced cardiac neural crest ingress, resulting in permanent truncus arteriosus. The results of some studies showed that vascular endothelial growth factor A (VEGFA) was upregulated in the BMP4 and BMP7 mutant hearts [30]. Cell-specific expression of BMPR1a is required for endocardial cushion formation. BMPR1a-induced signaling is a critical pathway involved in the pathogenesis of atrioventricular septal and valve abnormalities, which have been reported as one of the most prevalent cases of human congenital heart defects [28]. In some studies, the pulmonary endothelial cells collected from mice with endothelial cell-specific loss of BMPR2 indicated analogous gene and protein changes. It was concluded that enhanced high Mobility Group AT-hook 1 (HMGA1) in Pulmonary artery endothelial cells (PAECs) resulting from abnormal BMPR2 signaling may transit endothelium to SM-like cells associated with PAH [31].

2.2. Wnt Signaling Pathway

The canonical Wnt/β-catenin pathway accounts for many features of angiogenesis, vascular remodeling, and differentiation in different species and organ systems [32]. Beside its main contribution to brain angiogenesis and barrier formation, the Wnt/β-catenin pathway affects vasculature. Additionally, canonical Wnt signaling contributes to the heart valve formation by inducing EndMT [33]. In zebrafish and mouse, the Wnt/β-catenin signaling pathway is vital for valve genesis, yet the exact details are to be elucidated [34].

The Wnt/β-catenin pathway is initiated and EndMT is induced after MI which may help cardiac tissue repair [35, 36]. This pathway is also up-regulated in adult valves with calcific aortic stenosis [37]. Some studies reported contradictory effects of Wnt on EndMT. For example, a released Wnt inhibitor Dkk1 increased EndMT, whereas Wnt7b preserved endothelial phenotype in aortic endothelial cells culture [38]. Interactions between these pathways enhance the complexity of the process, which has to be elucidated in further studies [35].

2.3. Notch Signaling Pathway

There are four Notch receptors (Notch1–Notch4) for the formation of cardiac valve in mammals, which are involved in the activation of Notch signaling pathway. The activation of this signaling pathway requires the binding of ligands (Dll1, Dll3, Dll4, Jagged1, and Jagged2) with adjacent Notch receptors; thus the Dll4-Notch1 signaling leads to EMT and cushion formation [39].

Notch signaling pathway is of great importance as a key signaling system for embryonic cardiovascular development [40]. Additionally, mutations within the Notch signaling pathway have been established to be associated with congenital cardiovascular defects in man [41, 42]. It has been demonstrated that bicuspid aortic valve and associated aortic aneurysm are associated with failure in the regulation of the whole Notch signaling pathway [43]. Mutations in Jagged1 and Notch1 prohibit EndMT and result in valve formation defects and even fatalities [8, 43].

Notch is a critical mediator of EndMT for endocardial cushion formation. A transcriptional repressor, called Slug, is a Notch target, which is an important Notch effector of EndMT in the endocardial cushion [44]. In addition to these data, Notch-Jagged signaling within a second cardiac field progenitors is responsible for some types of congenital and adult heart diseases [45].

Notch receptors (especially Notch1 and Notch3) are important players in foramen ovale closure, which are highly expressed in the endocardium of this region during EndMT-mediated fibrosis [46].

In murine models of cardiac fibrosis, inhibition of Notch1 and Jagged-1 proteins caused decrease in EndMT and cardiac fibrosis. For instance, relaxin (RLX) may inhibit the cardiac fibrosis *via* EndMT by Notch-mediated signaling [47].

3. EndMT IN CARDIOGENESIS AND DISEASES

3.1. EndMT in Cardiogenesis and Congenital Heart Defects

Approximately 1-5 % of human newborns are born annually with congenital heart defects, and of these cardiac defects 20-30 % are due to heart valve abnormalities [47]. Abnormal development of the valves and membranous septa gives rise to the majority of congenital heart defects. The same key factors and signaling pathways involved in heart development are also engaged in congenital defects of the heart [48]. In the embryonic stage of heart development, valvulogenesis initiates through the formation of endocardial cushion in the regions of AVC and OFT. EndMT has a key role in cardiac development [49].

In the course of cardiac development, the endocardial endothelial cells (inner-endocardium), lining the atrioventricular canal, undergo an EndMT to form the cardial mesenchymal cushion that later form the septum and also tricuspid and mitral valves [50, 51]. EndMT is one of the complex developmental events, affecting the transformation of the early embryonic OFT into the aorta, semilunar valves, interventricular septum, and pulmonary trunk [52].

In order to initiate EndMT in cardiogenesis three signaling pathways, including TGF-β, BMP and Notch are needed. Any inhibitions or absences in these pathways may cause the failure of valvar and septum formation, eventually leading to

congenital heart defects [11, 27, 41, 45, 52]. These pathways cooperate with each other. BMPs released from the myocardium provide the environment to activate the endocardium; Notch signaling triggers EMT, and both BMP and TGF-β signaling synergize with Notch to accelerate the transition of endothelia to mesenchyme [53].

EndMT is also involved in the angiogenesis. It was shown that endothelial cells of the endocardium are progenitors of pericytes. EndMT occurs in the endocardial cells and they convert into primitive mesenchymal progenitors [54].

It is reported in some studies that NF-κB regulates EndMT in lungs, providing new insights into the induction of PAH and right ventricular hypertrophy and cerebral cavernous malformation (CCM) [55].

CCMs are vascular malformations occurring within the central nervous system usually causing cerebral bleeding. CCM cavernomas are lined by endothelial cells undergoing EndMT. This change in phenotype is due to the activation of the TGFβ/BMP signaling [56]. In one study, CCM proteins are shown to keep the function of endothelial cells and impede angiogenesis by modulating the β1 integrin-KLF2-EGFL7-signaling pathway [57], whether the CCM complex results in disease remains controversial because numerous signaling pathways (including SMAD, Rho and Wnt/β-catenin) are involved [58].

Today, there is no effective treatment for valve abnormalities, therefore, targeting the EndMT pathway components may be regarded as a therapeutic strategy in heart congenital disease [31].

3.2. EndMT in Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a severe, progressive, devastating and incurable pulmonary vascular disease [59]. The main feature of PAH is pulmonary arterial remodeling (PAR) that enhances pulmonary arterial pressure and pulmonary vascular resistance. The endothelial dysfunction appears as a pathological response of PAR in the form of thickening and stiffness in the intimal and medial layers [60]. This process is also characterized by recruitment of circulating progenitors, fibroblast, smooth muscle cell activation, and endothelial dysfunction [61]. In one study, it was shown that EndMT is a key signaling pathway that promotes pulmonary vascular remodeling [60].

Additionally, it has been shown in another study that myofibroblast-like cells create the microenvironment promoted EndMT; this process in turn induced endothelial cell dysfunction and increased intimal remodeling in chronic thromboembolic PAH. In some studies, the features of EndMT have been described as loss of endothelial and gain of mesenchymal marker expression, increased TGF-β1 and Smad expression [62]. One study reported that bone morphogenetic protein receptor 2 (BMPR2) is primarily localized on the endothelium of normal pulmonary artery [63]. Expression and function of BMPR2 decrease PAH [31]. One study showed that DNA methylation analysis identified a group of genes primarily engaged in lipid transport pathway which may account for the pathophysiology of PAH [59].

It was reported that the upregulation of BMPR2 partially reduced right ventricular hypertrophy and pulmonary arterial pressure *via* amelioration of EndMT [64].

Higher expression of the chromatin architectural factor High Mobility Group AT-hook 1 (HMGA1) has been reported in pulmonary arterial endothelial cells (PAECs) from patients who had idiopathic PAH compared with controls. In PAH, decreased BMPR2 activates EndMT through HMGA1 and its target slug [31].

PAH therapies are limited; therefore novel approaches are urgently needed for the treatment of PAH. In recent studies Ponatinib was introduced as a multi-targeted tyrosine-kinase inhibitor, which reverses TGF-β1-induced EndMT in human pulmonary microvascular endothelial cells and alleviates the severity of PAH by regulating the Wnt signaling [65]. In addition, the key role of nuclear factor-κB (NF-κB) in cardiac pathologies has been studied although the role of NF-κB remains limited in PAH [55].

3.3. EndMT in Atherosclerosis

EndMT has been reported to play a key role in the development of atherosclerosis [14]. Low shear stress-induced EndMT is shown to be the underlying pathogenesis of early stage atherosclerosis [66]. Snail has been demonstrated to be induced in the setting of low shear stress as a transcription factor [67]. It is reported that EndMT development through TGFβ signaling is regulated by fibroblast growth factor receptor 1 (FGFR1), eventually promoting progression of atherosclerosis [6]. In one study, it was shown that several subtypes of FGFs (*e.g.* 1, 2, 4, 8, 9, and 18) contribute to tubulogenesis [68]. Additional to TGF-β signaling, it has been confirmed that EndMT is brought about by oxidative stress and hypoxia, which both are the hallmarks of atherosclerosis [3]. As the atherosclerotic plaques undergo rupturing, the vascular calcification occurs. This mineralization process is promoted by EndMT through BMP-Wnt signaling pathway [69].

3.4. EndMT in Myocardial Infarction

In spite of ongoing treatment, MI remains a leading cause of death worldwide [70, 71]. It has been revealed that canonical Wnt activation and EndMT are molecular and cellular responses to MI. Mesenchymal cells derived from EndMT participate in cardiac tissue repair after MI, through canonical Wnt signaling pathway [35]. These mesenchymal cells produce non-functional buildup of fibrosis. Therefore, prevention of EndMT development followed by MI may be considered as a therapeutic end [72].

In some studies, the presence of numerous cardiac stem cells in the subepicardium of the human heart has been reported and it has been suggested that epicardially-derived cells may contribute to the population of cardiac stem cells in the adult heart [73]. Ischemic mitral regurgitation is a post-MI complication, which is associated with EndMT through TGF-β1 signaling pathway (*ex vivo*), and the expression of CD45-positive ($CD45^+$) endothelial cells (*in vivo*) [73]. On the other hand, it has been proven that inhibition of CD45 protein-tyrosine phosphatase may decrease the fibrosis formation [74].

It was shown that mitral VECs have angiotensin II type 1 and 2 receptors, which angiotensin II type 2 has a predominant effect on the non-canonical p-ERK pathway in TGFβ-mediated EndMT. As a result, angiotensin receptor blocker agents, *e.g*. Losartan may be useful in manipulating EndMT to prevent excessive growth and fibrosis that occurs after myocardial infarction [18] although the formation of fibrotic tissue in the early stage is necessary for wound healing and preventing the heart rupture after MI [75].

3.5. EndMT in Heart Fibrosis

Cardiac fibrosis (CF) is secondary to any injuries followed by almost all types of heart diseases. CF is associated with augmented stiffness of ventricles and diastolic dysfunction [76, 77]. CF eventually leads to heart failure. Fibroblasts/myofibroblasts primarily account for CF. They are formed by cardiac fibroblast differentiation, fibrocyte differentiation, EMT, and EndMT, modulated by cytokines, namely TGF-β, angiotensin II and Platelet-derived growth factor (PDGF) [78].

Current shreds of evidence have indicated that TGF-β-mediated EndMT plays a critical role in CF [17]. Two resident fibroblast lineages, including epicardial population and a population of endothelial origin mainly account for cardiogenesis and CF [79]. EndMT is mediated by TGF-β1 influenced by Smad and may be mainly prohibited by recombinant BMP7 [12].

The results provided by some studies suggest that diabetes mellitus-induced CF is associated with the formation of fibroblasts from endothelial cells and that this EndMT process is initiated by endothelin-1 (ET-1). Thus, targeting ET-1 derived from endothelial cells might be effective in the diabetic cardiomyopathy prophylaxis [80]. Cytokine-like 1 (Cytl1) is a secreted protein that is involved in diverse biological processes, and it is structurally and functionally similar to monocyte chemoattractant protein 1 (MCP-1). MCP-1 plays an important role in cardiac fibrosis (CF) and heart failure (HF). Similarly, the heart failure may lead to upregulation of Cytl1. By contrast, in another study it was shown that cytl1 knock-out mice showed significantly decreased CF induced by pressure overload, compared to wild-type mice [81]. Angiotensin II (Ang II) is also an inducer of CF. Ang II induces endothelial NOX2 activation, which in turn has profound pro-fibrotic effects in heart. Endothelial NOX2-induced EMT and resulted pro-inflammatory effects may have an important role in CF development during enhanced renin-angiotensin activation [82]. Reported results of the studies revealed that Losartan and Irbesartan (Ang II receptor type 1 blockers) prevented the hypertensive CF through the inhibition of EndMT *via* classical TGF-β/Smad pathway [83, 84]. CF is predominantly augmented in patients with chronic kidney disease (CKD). CKD results from an enhancement in NO inhibitors and circulating angiogenesis, which affects the proliferation and apoptosis of endothelial cells in the heart and induces EndMT, leading to CF and reduced capillary density [85]. EndMT triggers cardiac fibrosis in acute myocarditidis, which may be mediated by TGF-β1 [86]. In addition to cardiac fibrosis, the EndMT is a cellular mechanism that is responsible for fibrotic stages of several other organs, such as lungs, skin, kidney and liver. [87, 88]. EndMT is involved in myofibroblast formation during fibrotic process, especially it has been implicated as a key source of cancer-associated fibroblasts (CAFs), facilitating tumor progression and metastasis [89, 90], for instance, pancreatic adenocarcinoma and breast cancer [22].

CONCLUSION AND PROSPECTS

The endothelial-mesenchymal transition has been approved as a principle in the embryonic period for cardiogenesis and as a predisposing factor for congenital heart disease and in adults as pathogenesis of cardiac disease like CF and PAH. Generally, EndMT has been accepted in the embryonic period as a physiological phenomenon and any mutation or inhibition in its involving pathways may trigger congenital heart disease. For instance, mutations within the Notch signaling pathway have been established to be associated with congenital cardiovascular defects in man [41].

Since 1-5% of the human birth defects including congenital cardiovascular have no approved therapy, therefore targeting the EndMT pathway components may be regarded as a therapeutic strategy in congenital heart disease [31]. Contrarily, it has been implicated as a pathological phenomenon and its inhibition has been considered as a therapeutic target in several heart diseases especially in post-myocardial infarction fibrosis.

Precedently, three principle signaling pathways have been detected for EndMT in cardiogenesis and cardiac diseases. These pathways, however, are not independent of each other, they have several interactions among them. They act sometimes synergistically and sometimes as inhibitory, for example, TGF-β1 induces the cells of endothelium to undergo the transition, whereas BMP-7 preserved the endothelial phenotype. Additionally, different cytokines such as ET-1, FGF, NF-κB, PDGF and HGF possess different roles in the induction of EndMT. It has been shown that angiotensin-blockers, *e.g*. losartan, play an important role in the prohibition of EndMT-induced cardiac disease in adults, hence, it may be considered as a significant therapeutic target in the future researches.

Having a clear understanding of involving cellular and molecular mechanisms in EndMT and conducting RCTs with a large number of samples for involving pharmacological agents such as Losartan and Irbesartan may guide us into novel therapeutic approaches of congenital disorders and heart diseases [83, 84].

LIST OF ABBREVIATIONS

Ang II	=	Angiotensin II
AVC	=	Atrioventricular Canal
b FGF	=	Basic Fibroblast Growth Factors
BMP	=	Bone Morphogenetic Protein
BMP-7	=	Bone Morphogenic Protein 7
BMPRs	=	BMPs and their Receptors
CAFs	=	Cancer-associated Fibroblasts
CCM	=	Cerebral Cavernous Malformation

CD45+ = CD45-positive
CF = Cardiac Fibrosis
CKD = Chronic Kidney Disease
Cytl1 = Cytokine-like 1
EMT = Epithelial to Mesenchymal Transition
EndMT = Endothelial to Mesenchymal Transition
HF = Heart Failure
HGF = Hepatocyte Growth Factor
HMGA1 = High Mobility Group AT-hook 1
MCP-1 = Monocyte Chemoattractant Protein 1
MI = Myocardial Infarction
OFT = Outflow Tract
PAECs = Pulmonary Arterial Endothelial Cells
PAH = Pulmonary Arterial Hypertension
PAR = Pulmonary Arterial Remodeling
PDGF = Platelet-derived Growth Factor
RCTs = Randomized Controlled Trials
RLX = Relaxin
TGF-β = Transforming Growth Factor-β-driven
TGFβR3 = Type III TGFβ Receptor
VECs = Valve Endothelial Cells
VEGFA = Vascular Endothelial Growth Factor A
α-SMA = α-smooth Muscle Actin

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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