



# Wnt/ $\beta$ -catenin in ischemic myocardium: interactions and signaling pathways as a therapeutic target

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Published online: 12 December 2018  
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## Abstract

Cardiovascular disease (CVD) is still a factor of mortality in the whole world. Through canonical and noncanonical pathways and with different receptors, the Wnt/ $\beta$ -catenin signaling pathway plays an essential role in response to heart injuries. Wnt regulates the mobilization and proliferation of cells in endothelium and epicardium in an infarcted heart. Therefore, with its profibrotic effects as well as its antagonism with other proteins, Wnt/ $\beta$ -catenin signaling pathway leads to beneficial effects on fibrosis and cardiac remodeling in myocardium. In addition, Wnt increases the proliferation and differentiation of cardiac progenitors in an ischemic heart. Complex interactions and dual activity of Wnt, the changes in its expression, and mutations that can change its activity during heart development have an adverse effect on cardiac myocardium after injury. However, targeting the Wnt in myocardium with cellular and molecular pathways can be suggested to improve and repair ischemic heart. Given these challenges, in this review article, we deal with the role of Wnt/ $\beta$ -catenin signaling pathway as well as its interactions with other cells and molecules in an ischemic myocardium.

**Keywords** Wnt/ $\beta$ -catenin pathway · Ischemia · Myocardium

## Introduction

Cardiovascular disease (CVD) is a major factor causing the death of approximately 1.5 million people every year, which is

### Highlights

- Wnt/ $\beta$ -catenin interactions in ischemic myocardium are important both during fibrosis and repair of cardiac disorders
- The dual role of Wnt/ $\beta$ -catenin changes in the heart through its expression
- Targeting of Wnt/ $\beta$ -catenin pathway in ischemic patients is meant for better treatment of cardiovascular diseases

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responsible for one out of every four deaths. Acute ischemia and hypertrophy of myocytes are the most common injuries in the heart resulting from atherosclerotic plaque and thrombosis, which cause cardiac progenitor cell death [1, 2]. Several cell populations in the heart have various interactions and responses in heart injuries through cell-cell interactions, growth factors, and cytokines, and a number of signaling pathways are involved in cell-cell interactions in the heart [1–5]. Cardiac progenitor cells, fibroblasts, endothelial, epicardial, and muscle cells are found in normal adult heart [3, 6]. Wnt/ $\beta$ -catenin signaling pathway includes 10 types of frizzled (Fzd) receptors and related antagonists that play important roles in response to these injuries by canonical and noncanonical pathways [7]. In normal situation, there is a balance between canonical and noncanonical Wnt pathways in myocardium, which have a role in proliferation and differentiation of cardiac progenitors, respectively [8]. After heart injuries, apoptosis of cardiomyocytes and fibrosis lead to myocardium remodeling and heart failure [9]. Wnt can cause response in sub-epicardial region of epicardium and fibroblasts after cardiac injury. Also, endothelial and muscle cells in the infarcted region can be included in Wnt responsiveness (Table 1) (Figs. 1, 2, and 3) [3]. Wnt has a biphasic manner by negative or positive regulation in cardiac progenitors and by loss-of-function and gain-of-function mutations which can activate or

**Table 1** Effect of Wnt signaling on angiogenesis factors in ischemic heart

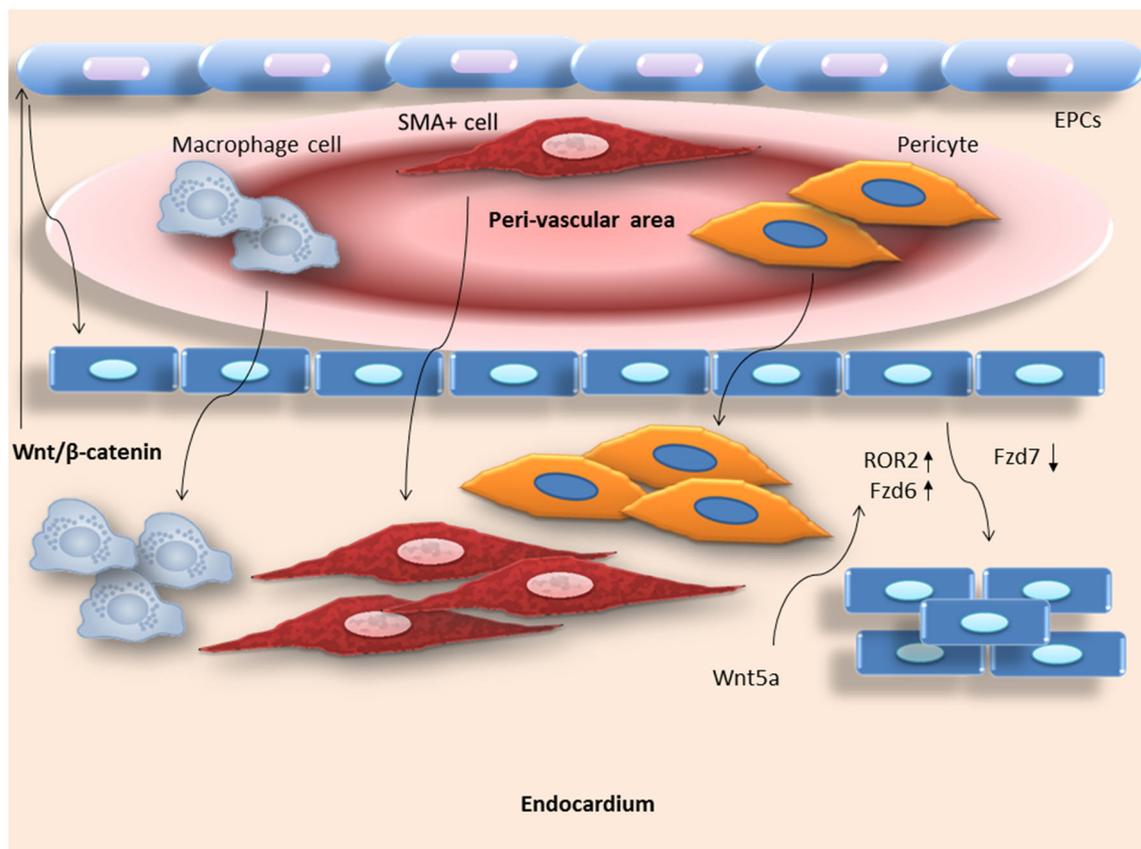
Cell	Wnt signaling pathway	Function	References
Endothelial cell	Canonical Wnt pathway	Increased proliferation in early and late stages of ischemia	[10, 11]
Pericytes cell	Canonical Wnt pathway	Increased proliferation in early stages of ischemia	[10, 11]
SMA <sup>+</sup> cell	Canonical Wnt pathway	Increased proliferation in early and late stages of ischemia	[12]
Macrophage cell	Canonical Wnt pathway	Increased proliferation in early stages of ischemia	[10]
EPC	Canonical Wnt pathway	Increased proliferation and differentiation after MI	[13]

EPC, endothelial progenitor cell; SMA<sup>+</sup> cell, smooth muscle actin-positive cell

inactivate it during heart development (Table 2). On the other hand, Wnt/ $\beta$ -catenin pathway has an adverse effect on ischemic heart remodeling, and the inhibition of Wnt can improve injuries in genetic models [18–20]. Dysregulation of Wnt has an important role in cardiac diseases such as hypertrophy, fibrosis, and ischemia [3]. Considering the complexity and dual features of Wnt/ $\beta$ -catenin signaling in myocardium, in this review, we examine the role of Wnt/ $\beta$ -catenin signaling pathway and its interactions in an ischemic myocardium.

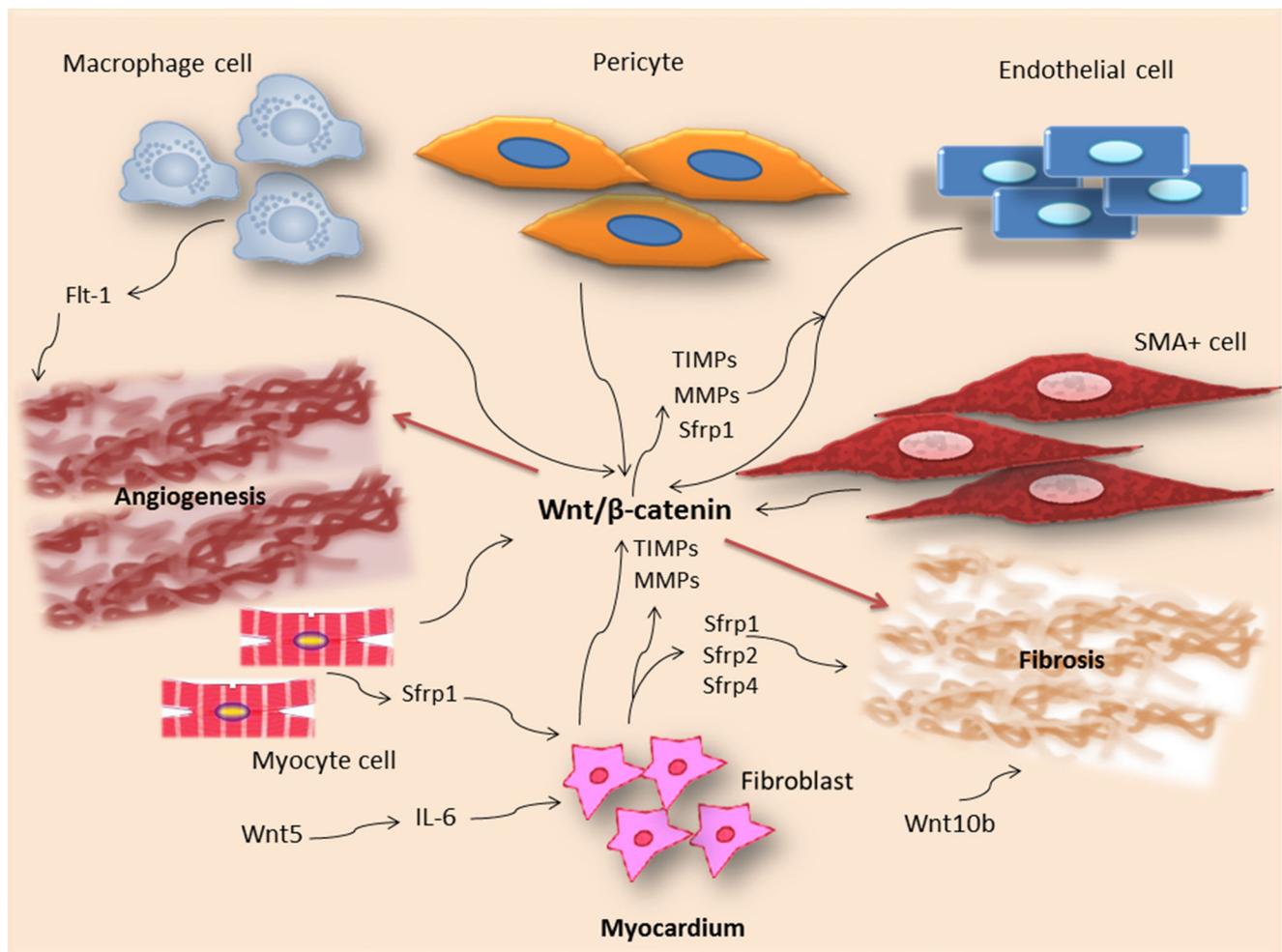
### Wnt signaling in endothelial cells: dual role in development and repair

Canonical Wnt signaling is active in endothelial cells and pericytes of an uninjured heart. Four days after myocardial infarction (MI), the activity of canonical Wnt is increased in the cells around perivascular areas of the heart. Seven days after MI, the cells with positive canonical Wnt expression in infarcted region are expanded [10, 11], which indicates that



**Fig. 1** Endocardium and Wnt/ $\beta$ -catenin signaling in ischemic heart. After heart injury, Wnt is increased in endocardium in endothelial cells, pericytes, SMA<sup>+</sup>, and macrophage cells and they expand around perivascular region and then the epicardium to cause angiogenesis. Wnt increases EPC proliferation to expand endothelial cells. ROR2 and Fzd6

in endothelial cells are activated after MI by Wnt5a and Fzd7 expression is downregulated which leads to the expansion of endothelial cells to the infarcted region. EPCs, endothelial progenitor cells; SMA<sup>+</sup> cells, smooth muscle actin (SMA)-positive; Fzd, frizzled; ROR2, receptor tyrosine kinase-like orphan receptor 2



**Fig. 2** Wnt/β-catenin signaling in myocardium of ischemic heart. Wnt makes a link between fibrosis and angiogenesis in the myocardium. The epicardium with epithelial cells includes fibroblasts and myocyte cells. After heart injury, it expands fibroblasts and myocytes with Wnt1 expression which leads to fibrosis in myocardium. Fibroblasts express Sfrp 1, Sfrp2, and Sfrp4 and myocytes express Sfrp1 that affect Wnt signaling and fibroblasts and increase fibrosis. Fibroblasts also express

MMPs and TIMPs that regulate Wnt/β-catenin and also contact with endothelial cells for angiogenesis and proliferation of these cells. Macrophage cells also decrease angiogenesis by (Flt-1) expression. SMA<sup>+</sup> cells, smooth muscle actin (SMA)-positive; Sfrp, secreted frizzled-related protein; MMPs, metalloproteinases; TIMPs, tissue inhibitor of metalloproteinases; Flt-1, FMS-related tyrosine kinase 1

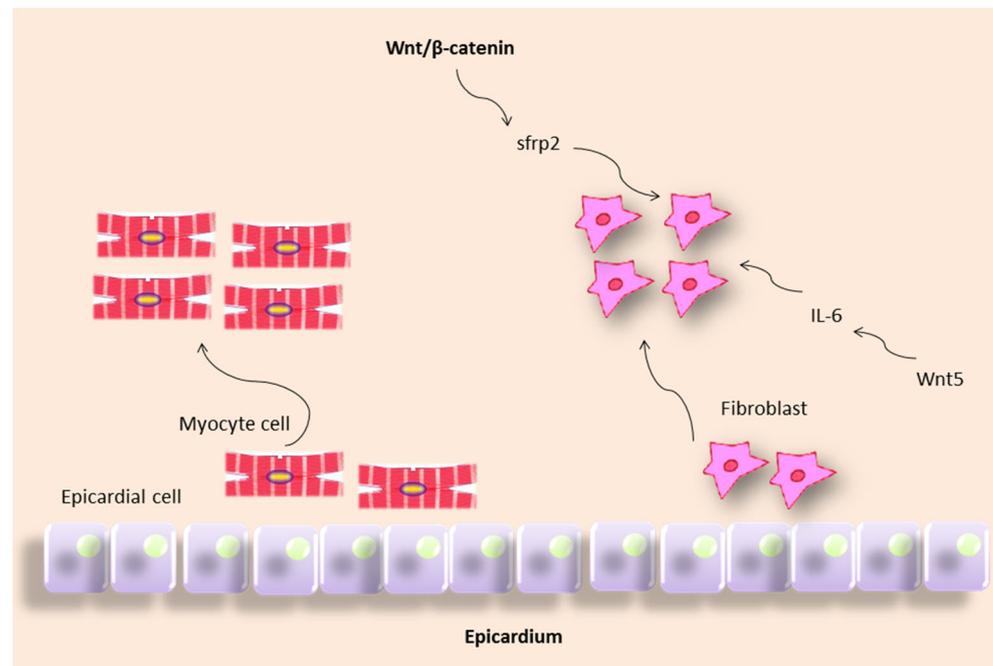
canonical Wnt cannot be active in early stages of ischemia and inflammation but is activated in late processes of granulation tissue in smooth muscle as well as in endothelial and macrophage cells [10]. In the early stages of injury before canonical Wnt expansion in cells, endothelial and smooth muscle actin-positive (SMA<sup>+</sup>) cells are increased around the injury region. In addition, endothelial and smooth muscle cells can be present in late stages of canonical Wnt activation in the heart. Thus, it seems that these two cell types have the same origin in endothelium. Studies have shown that half of SMA<sup>+</sup> cells have endothelial origin [10, 12]. Also, canonical Wnt signaling provides a link between fibrosis and angiogenesis in the myocardium. There is a connection between cells and molecular pathways involved in the repair of injury, but this repair has two opposing results of fibrosis and vascularization,

indicating that the cardiac injury can be improved with cells in which Wnt signaling has been activated [10, 21] (Table 1).

Endothelial progenitor cells (EPCs) can be used for the treatment of patients after ischemia and MI in order to increase the cardiac function [13]. In this regard, *CXCL-12/CXC-R4* axis increases the expression of *CXCL-12* and *CXC-R4* chemokines that cause the mobilization of EPCs to the injury site [22]. Studies have shown that Wnt1 increases EPCs function in vascular diseases and that the use of human EPCs with Wnt1 overexpression improves blood flow in ischemic patients. Wnt signaling can also increase the proliferation and differentiation of EPCs (Table 1) (Figs. 1 and 2) [23, 24].

Endothelial-mesenchymal transition (EndoMT) is a process after heart injury, which induces the conversion of endothelial cells to SMA<sup>+</sup> cells and fibroblasts by canonical Wnt signaling

**Fig. 3** Wnt/ $\beta$ -catenin signaling and epicardium after heart injury. The epicardium with epithelial cells includes fibroblasts and myocyte cells. After heart injury, it expands fibroblasts and myocytes with Wnt1 expression which leads to fibrosis in myocardium. Wnt5a induces IL-6 and causes the interaction of fibroblasts and inflammation after MI. Sfrp, secreted frizzled-related protein; IL, interleukin



pathway [10]. Similar to epicardial cells that are activated in injury, endothelial cells can also be a source of cells for the repair of myocardium after injury. Therefore, different cells with various responses to injury are involved in heart repair. It is not well understood whether these cells have separate functions or their activation during response to injury can imply an increased speed of repair [25]. In addition, receptor tyrosine kinase-like orphan receptor 2 (ROR2) and Fzd6 as the receptors of noncanonical Wnt pathway in endothelial cells can be activated after MI [26]. This activation is done by Wnt5a which leads to the disruption of adherence junction of endothelial cells. Moreover, the expression of Fzd7 as a receptor of canonical Wnt is downregulated which causes the disruption of endothelial and inflammatory cells adherence junction and their expansion to the infarcted region [27, 28].

Therefore, canonical Wnt signaling causes the proliferation of endothelial progenitor cells in early stages of heart

development as well as affecting angiogenesis in endothelial and smooth muscle cells during late stages of injury. On the other hand, noncanonical Wnt signaling can also be activated after MI in endothelium. Therefore, it is suggested that targeting the canonical Wnt signaling by in vivo interactions with other cells and signaling pathways can increase endothelial progenitor cell proliferation and be used for the treatment of ischemic patients. In addition, targeting the noncanonical Wnt signaling by in vivo interactions in order to decrease the inflammatory responses after MI can be suggested for the better management of ischemic patients after injury.

### Complex interactions of Wnt pathway in profibrotic activity of ischemic heart

Epicardium is a layer with epithelial cells around the heart that originates from the pro-epicardium. The epicardium has an

**Table 2** Mutations of Wnt/ $\beta$ -catenin pathway genes in CVD

Gene	Mutation	Locus	Functional outcome	Normal function	References
CTNNB1	Gain of function	10q21	Activation of canonical Wnt signaling	Key downstream component of the canonical Wnt signaling pathway	[14]
GSK3b	Loss of function	3q13	Suppression of canonical Wnt/ $\beta$ -catenin signaling	Negative regulator of Wnt signaling pathway	[6]
TCF7L2	Loss of function	10q25	Suppression of canonical Wnt/ $\beta$ -catenin signaling	Encodes a high mobility group box-containing transcription factor of Wnt signaling pathway	[6, 14]
DSP	Loss of function	6q24	Suppression of canonical Wnt/ $\beta$ -catenin signaling	Component of functional desmosomes	[15]
PKP2	Loss of function	12p11	Reduction of Wnt signaling	Regulate the signaling activity of Wnt	[16]
JUP	Gain of function	17q21	Activation of canonical Wnt signaling	Component of functional desmosomes	[17]

essential role in heart development and includes fibroblasts and smooth muscle cells in coronary arteries [29, 30]. After heart injury, the epicardium expands Wnt1-expressing fibroblasts and myofibroblasts, which causes profibrotic epicardial function. Therefore, Wnt/ $\beta$ -catenin pathway is inevitable to regulate the epicardial-derived cells (EPDCs) following ischemia [31, 32].

Fibroblasts are the most frequent cells in the heart, which can migrate and relocate in the cardiac interstitium to interact with other cell populations [33]. Wnt1/ $\beta$ -catenin has profibrotic activity and can repair injury by cardiac fibroblasts response. Fibroblasts express Wnt1 in the first few days of MI, which induces activation, proliferation, and upregulation of genes involved in fibrogenesis (such as collagen and endothelin) by autocrine and paracrine mechanisms [31]. Studies have indicated that the secreted frizzled-related protein (Sfrp) 1, Sfrp2, and Sfrp4 are expressed by fibroblasts 7 days after cardiac injury, which can increase the activation of pro-collagen C-proteinase as well as collagen formation from pro-collagen [34]. Studies on Sfrps are conflicting and have shown that Sfrp1 overexpression leads to adverse effects on cardiac disease and reverses the benefits of treatment for patients [35, 36]. Overexpression of Sfrp1 in myocytes can affect Wnt signaling in these cells but has less effect on other cells such as fibroblasts, which can confirm these discrepancies. These features show the complexity of Wnt signaling in different cells, which needs a better understanding of cellular interactions between different cell populations. So, Wnt signaling system can affect both myocytes and fibroblasts during infarction and ischemia [3].

Wnts induce the expression of metalloproteinases (MMPs) by fibroblasts, which play a crucial role in the migration of endothelial cells after injury [37]. Fibroblasts can express tissue inhibitor of metalloproteinases (TIMPs) that regulates the effects of Wnt/ $\beta$ -catenin signaling. Direct fibroblast–endothelial contact is necessary for angiogenesis through the expression of TIMP [38, 39]. It has been shown that after MI,  $\beta$ -catenin is accumulated and activated in endothelial cells. Sfrps have a dual feature and increase pro- and anti-angiogenic effects [40]. Sfrp1 also causes the proliferation of endothelial cells, and it is not known how Sfrp1 is involved in angiogenesis of heart disease [41]. Therefore, Wnt and Sfrps can be expressed by myocytes and fibroblasts and influence the endothelial response, which depends on the type of Wnt, affinity to receptors, and presence of other antagonists [3]. In vitro studies have shown that Sfrp2 can activate Wnt signaling and remodeling of extracellular matrix in fibroblasts. Also, in vivo studies on rat models indicated Sfrp2 as a cause of fibrosis reduction and extracellular matrix deposition [42, 43]. Macrophages and myeloid cells lead to angiogenesis via migration of endothelial cells in ischemic area. On the other hand, macrophages can limit angiogenesis through the expression of FMS-related tyrosine kinase 1 (Flt-1) as an anti-angiogenic molecule by noncanonical Wnt pathway;

therefore, indirect and direct contacts by cell-cell interactions can affect angiogenesis in the heart [3, 39]. Canonical Wnt/ $\beta$ -catenin pathway has an important role in cardiac diseases, is involved in fibrogenesis, and can promote the expression of genes involved in fibroblasts [30, 34, 44]. Deletion of  $\beta$ -catenin in fibroblasts decreases fibroblast proliferation and wound improvement in the heart [30].

Wnt3a as canonical Wnt protein and Wnt5a as noncanonical Wnt protein increase collagen I in myelofibroblast cells. In addition, Wnt5a causes the induction of interleukin (IL)-6 which results to the interaction of fibroblasts with other cells and inflammation after MI. On the other hand, expression of Wnt10b is cardiomyocyte-specific and decreases the fibrosis in ischemic injury (Figs. 2 and 3) [45–47].

Sfrps can have beneficial effects on the repair of cardiac disease; however, the exact mechanisms of these effects and antagonized activity of Wnt are not clear. Therefore, the question of how Wnt signaling can be different in various cell populations after injury should be answered, and the cellular mechanisms in these effects for the development of treatment strategies on the application of Wnt signaling in heart disease should be investigated.

## Wnt and cardiac progenitor interactions

Wnt/ $\beta$ -catenin signaling has been shown to cause the expansion of cardiac progenitor cells [48]. Mesoderm posterior bHLH transcription factor 1 (MesP1) can activate the transcription of cardiac cells, and dickkopf-1 is the target of MesP1 for cardiac cell fate. Studies have indicated that Wnt/ $\beta$ -catenin upregulation is necessary for MesP1 activation to increase the proliferation of different precursor cells in the heart [49, 50]. Inhibition of canonical Wnt pathway by dickkopf-1 leads to early differentiation of cardiac precursor cells from cardiac stem cells with MesP1 expression [51]. In cardiac development, Wnt/ $\beta$ -catenin plays a biphasic role [52]. Studies have shown that insulin-like growth factor-binding protein 4 (IGFBP-4) can inhibit Wnt signaling by binding to Fzd8 and lipoprotein receptor-related protein (LRP6) on cell membrane [7, 53]. In vitro studies on stem cell cultures have confirmed that the inhibition of Wnt/ $\beta$ -catenin and bone morphogenetic protein (BMP)-1 pathways increase the differentiation of cardiomyocyte. Also, in vivo studies have shown that the upregulation of Wnt/ $\beta$ -catenin is necessary to increase the cardiomyogenic precursor cells in the heart [8, 54]. Positive regulation of Wnt/ $\beta$ -catenin is required for the proliferation of precursor cells; in turn, negative regulation of Wnt/ $\beta$ -catenin is needed for the differentiation of precursor cells in the heart [7]. Noncanonical Wnt signaling pathway relates Wnt signaling with other signaling pathways such as Ras homolog gene family, member A (RhoA), and Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) in cardiac remodeling among adults. The canonical Wnt/ $\beta$ -

catenin pathway regulates the differentiation of cardiac precursor cells and heart muscles [7, 55]. According to the biphasic role of Wnt signaling, targeting the noncanonical pathway to increase the proliferation of cardiac progenitors and the canonical pathway to increase the differentiation of these cells can be suggested to be used for increasing the proliferation and differentiation of cardiac progenitors in an ischemic heart and better management of the heart disease.

Wnt signaling promotes cardiac myocardium proliferation during heart development. In the myocardium,  $\beta$ -catenin is active and is expressed in most cardiac myocytes. Furthermore, a loss-of-function mutation in  $\beta$ -catenin inhibits the proliferation of cardiac myocytes. Differential activation of  $\beta$ -catenin affects the differential proliferation of cardiac myocardium compartments [56], and the overexpression or loss-of-function mutations of  $\beta$ -catenin inhibit these differences (Table 2). This suggests that signal induction or suppressive signals from the epicardium regulates the differential activation of  $\beta$ -catenin and therefore controls the differential proliferation in myocardium compartments [56, 57]. Therefore, understanding the Wnt signaling mechanism in cardiac progenitors of CVD cases is essential for proper design of drugs [2].

## Discussion

Different signaling pathways play central roles in interactions between different cell populations to affect cardiac response after injury. Detection of these cellular interactions can be helpful to find better strategies for therapeutic targets in the heart [3]. In addition, the complex processes of fibrosis and angiogenesis can be effective on heart responses [3, 58]. However, some questions should be answered in this regard, such as molecules that play a role in downregulation of Wnt/ $\beta$ -catenin. Also, the relationship between Wnt and other signaling pathways should be investigated in CVD development [3]. Many signaling pathways such as Notch, transforming growth factor- $\beta$  (TGF- $\beta$ ), and BMP-1 can be activated in the heart after injury, and the Wnt signaling system can interact with these pathways to induce a response in the heart. Notch signaling can bind to Wnt components and inhibit Wnt/ $\beta$ -catenin signaling pathway [59]. Both of these signaling pathways can also activate the formation of fibroblasts. Moreover, as a Wnt antagonist, Sfrp2 affects fibrosis by inhibiting BMP-1. Thus, signaling systems can interact with one another in cardiac myocardium to induce a response in the heart [43].

Cardiac hypertrophy is defined as an increase in the size of cardiomyocyte, fibrosis, and induced expression of myocyte genes in the heart. According to investigations, the expression of Wnt is upregulated after cardiac hypertrophy. On the other hand, Wnt signaling is decreased in patients with heart failure, and the reason for these changes is still not understood [60,

61]. The canonical Wnt signaling is quiescent in normal conditions, but it can be activated in pathological situations such as hypertrophy and ischemia [62]. On the other hand, canonical Wnt signaling in myocytes causes cardiac hypertrophy, and its antagonism can be a pharmacological target [3]. Glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) is a regulatory molecule for canonical Wnt, and it has been shown that GSK3 $\beta$  overexpression decreases cardiac hypertrophy and that GSK3 $\beta$  inhibition increases hypertrophy in response to heart disease. Moreover,  $\beta$ -catenin is increased in cardiac progenitor cells due to hypertrophy [3, 63, 64].

Identifying Wnt signaling modulators to treat CVD is essential given the adverse effects of Wnt such as fibrosis. Until now, the drugs developed for this goal target several molecules: the ligand, the ligand–receptor, and the intracellular targets [65]. Some molecules have shown good results by targeting Wnt or destroying  $\beta$ -catenin. GNF-6231 is an inhibitor of porcupine protein, which is necessary for Wnt activity. It inhibits the canonical and noncanonical Wnt pathways, which decrease the profibrotic activities and increase the improvement of injury in the heart [2]. Antagonists of tankyrase increase Axin, which is an inhibitor of Wnt canonical pathway that results in decreased function of canonical Wnt pathway [66]; therefore, a number of molecules that are used for cancer treatment can also be useful for CVD. In addition, some proteins or neutralizing antibodies such as recombinant Sfrps can influence canonical and noncanonical pathways to be used for clinical applications in future. Therefore, cardiovascular improvement by these molecules should be investigated on Wnt signaling for treatment strategies of patients with heart disease [67, 68].

In vitro and in vivo studies have shown that Wnt signaling can be a target for cardiac precursor cells to induce the response of these cells for repair and treatment of injury. Some studies suggest that the proliferation of cells by Wnt stimulation in vitro and injection of cells can be a good model for treatment of heart diseases [69]. On the other hand, on in vivo studies, Wnt pathway plays a vital role in cardiac remodeling, which can have application for therapeutic purposes. Studies have indicated that Wnt inhibition can scavenge Wnt and prevent its binding to Fzd receptors via injection of bone marrow mononuclear cells that release Sfrp. Wnt inhibition causes left ventricle (LV) remodeling and reactivates the remodeling program of LV from heart progenitor embryonic cells [7, 70]. Although, Wnt signaling activation and modulation on in vivo studies for treatment of CVD have shown the beneficial results, but the exact clinical outcome of this pathway must be evaluated in future. Therefore, further investigations for understanding of CVD pathogenesis and Wnt mutations and their interactions with other pathways should be designed for better management of ischemic patients.

## Conclusion and future perspectives

Wnt/ $\beta$ -catenin can cause hypertrophy, but the overexpression of it leads to adverse effect on the heart during injury. Loss-of-function and gain-of-function mutations of  $\beta$ -catenin can be a solution for control of this signaling pathway as well as targeting the activation or inactivation signals from the myocardium (Table 2). It is suggested that in vitro Wnt/ $\beta$ -catenin pathway targeting in cardiac progenitors and especially in vivo Wnt targeting with more beneficial results can improve and repair ischemic heart. As a result, further studies on Wnt/ $\beta$ -catenin signaling pathway are needed to find better strategies for the treatment of patients with ischemia and heart failure in future.

**Acknowledgments** We wish to thank all our colleagues in Golestan Hospital and Allied Health Sciences School, Ahvaz Jundishapur University of Medical Sciences.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Research involving human participants and/or animals** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent** Informed consent is not required for this type of study.

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