Role of Signaling Pathways during Cardiomyocyte Differentiation of Mesenchymal Stem Cells

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Keywords

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Abstract

Multipotent stem cells, including mesenchymal stem cells (MSCs), represent a promising source to be used by regenerative medicine. They are capable of performing myogenic, chondrogenic, osteogenic, and adipogenic differentiation. Also, MSCs are characterized by the expression of multiple surface antigens, but none of them appear to be particularly expressed on MSCs. Moreover, the prospect of monitoring and controlling MSC differentiation is a scientifically crucial regulatory and clinical requirement. Different transcription factors and signaling pathways are involved in cardiomyocyte differentiation. Due to the paucity of studies exclusively focused on cardiomyocyte differentiation of MSCs, the present study aims at describing the roles of various signaling pathways (FGF, TGF, Wnt, and Notch) in cardiomyocyte differentiation of MSCs. Understanding the signaling pathways that control the commitment and differentiation of cardiomyocyte cells not only will expand our basic understanding of molecular mechanisms of heart development but also will enable us to develop therapeutic means of intervention in cardiovascular diseases.

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Introduction

Ischemic heart disease is a chief factor having impact on global mortality rates. The ischemic myocardium develops apoptosis and necrosis of cardiomyocytes quickly, resulting in left ventricular dilation and remodeling [1]. Treatments helping to regenerate damaged heart tissue have been considered as substantially ultimate strategy for myocardial infarction repairment. In this regard, therapeutic approaches related to stem cells address remarkable progresses in cardiac tissue engineering because stem cells can regenerate defective cardiac tissues with limited regenerative capacity [2].

Stem cells include embryonic stem cells (ESCs) and adult stem cells (ASCs). ESCs, which are pluripotent ones, can be differentiated into embryonic structures [3]. ASCs are derived from various adult tissues and can be differentiated into different cell types [4–7] (Fig. 1).

Mesenchymal stem cells (MSCs) should be effectively proliferative and adhesive fibroblastic-type cells with features of the unique expression profile of cell surface molecules and cell-cell interaction proteins. MSCs have self-renewal and multilineage differentiation displayed by ectodermal [8] and mesodermal differentiations [9–13] into, for example, muscles, bones, tendon, adipose tissue, and cartilages. MSCs can be extracted from multiple tissues such as the umbilical cord blood, adipose tissue, bone

Fig. 1. Schematic diagram showing differentiation potential of MSCs.

Table 1. CD marker expression and differentiation potential of mesenchymal stem cells extracted from different species and sources

Source of MSCs	Positive marker expression	Negative marker expression	Type of differentiation	References
Sternal hMSCs	CD29, CD44, CD90, CD105, CD51	CD7, CD34, CD45, AC-133	Cardiomyocytes	[22]
Rat BM-MSCs	CD29, CD90, CD44	CD34 and CD45	Cardiomyocytes	[23]
Ovine fetal BM-MSCs	CD44, CD166	CD34, CD45, and CD90	Cardiomyocytes	[24]
Human Wharton's jelly MSCs	CD73, CD90, CD105	CD34	Cardiomyocytes	[25]
Rat BM-MSCs	CD90, CD105	CD34, CD45	Osteocyte cells	[26]
hWJ-MSCs	CD73, CD90, Cd105	CD34	Chondrocyte cells	[27]

marrow, lungs, peripheral blood, and heart [6, 14, 15]. MSCs are considered confident and conceivable for cardiovascular therapy in clinical applications [16–18]. Ventricular action is significantly ameliorated after stem cell therapy mainly in association with the induction of angiogenesis [19], paracrine effects [20], or stimulation of endogenous cardiac progenitor cells [21]. Differential expression of CD markers by MSCs derived from various sources and species is shown in Table 1.

Complex and versatile sources of MSCs along with the lack of a generally accepted method for the culture of MSCs create some different therapeutic effects on cardiovascular diseases. Different differentiation capabilities and immune-regulatory effects regarding cardiovascular

diseases are exhibited by MSCs derived from the bone marrow, adipose tissue, and umbilical cord [28, 29]. Different culture conditions may have different effects on MSC properties such as their activities and immunoregulatory abilities. Some part of MSCs' therapeutic effects is dependent on their transplantation and survival on the site of the graft. Nevertheless, stem cells are retained at a low rate, and hostile ischemic environment disturbs immunogenicity of transplanted cells, and as a result, only slight improvements are observed in cardiac functions [30, 31]. More improvements are needed to increase survival rates of MSCs because the rate is low in cardiac environment and because a large proportion of transplanted cells may disappear immediately after transplantation according to

Fig. 2. Different transcription factors during differentiation of MSCs.

some studies [32]. In order achieve successful proliferation and maintenance of MSCs to be employed in clinical applications, it is necessary to recognize the molecular mechanism by which MSCs are retained by regulatory factors mentioned above.

Based on the literature, different cardiac differentiation agents used to induce cardiomyocytes can be classified as (1) chemical analogs of cytidine such as 5-azacytidine; (2) multifunctional glycoproteins such as BMP2; (3) other compounds like DMSO and angiotensin II; (4) other methods, for example, co-culture with myocardial cells, cardiomyocyte lysis medium, and supernatants of cultured cardiomyocytes; and (5) other methods such as genetic modification [33]. Different transcription factors are involved in MSC differentiation including cardiomyocyte differentiation (Fig. 2). GATA4 was found to adjust differentiation and growth of various cell species. GATA4-transduced MSCs were indicated to have a higher level

of GATA4 expression during cardiomyocyte differentiation than the control MSCs, proposing that overexpression of GATA4 promoted potential of MSCs to differentiate into cardiomyocytes. Shen et al. [34] demonstrated that expression of miR-1-2 remarkably was enhanced after 5-azacytidine supplementation. In order to make obvious the functions of miR-1-2 in modulating cardiomyocyte differentiation, miR-1-2 imitations are transferred into BM-MSCs, and then these cells are induced to perform cardiomyocyte differentiation by the expression of cardiac-specific genes GATA binding protein 4 (GATA4), cardiac troponin I (cTnI), and Homeobox protein 2.5 (Nkx2.5).

Homeobox Nkx2-5 is one of the transcription factors preliminary expressed in the heart. Precise analysis of Nkx2-5's role in heart development has revealed that Nkx2-5 controlled many aspects of cardiomyogenesis, including cellular characteristics, differentiation, and proliferation [35, 36].

As a fundamental cardiomyogenic transcription factor, myocardin transactivates the serum response factor of the present transcription factor [37]. Myocardin controls the expression of plenty of growth-associated and muscle-restricted genes [38]. Overexpression of the longest splice variant of the human myocardin gene in hMSCs induced expression of smooth muscle and cardiac genes under in vitro conditions, without the expression of skeletal muscle genes [39].

The T-box gene family is extremely conserved during evolution and serves as a transcription factor regulating embryonic growth and development. The TBXS gene is an important member of the T-box gene family and regulates heart development and upper extremity formation during the primary embryonic period [40].

One of major challenges facing contemporary cellular and molecular biology is to understand molecular mechanisms governing cardiomyocyte differentiation of MSCs, which is generated by different signaling pathways. Comprehensive information and knowledge of signaling pathways were helpful for researchers to develop novel medical therapies. This review aims of summarizing the currently available information about signaling pathways involved in cardiomyocyte differentiation of MSCs.

Wnt Signaling Pathways

Signaling via the Wnt family of secreted glycolipoproteins through the transcription co-activator β -catenin regulates cell polarity, cell proliferation, and cell fate determination during embryonic development and tissue homeostasis [41]. Wnts are soluble glycoproteins that engage receptor complexes composed of Lrp5/6 and Frizzled proteins. A subgroup of Wnts induces a cascade of intracellular events that stabilize and facilitate β -catenin transport to nuclei where it binds to Lef1/Tcf transcription factors and alters gene expression to promote proliferation and differentiation [42]. Numerous studies have suggested that miR-1 plays a role in differentiating cardiomyocytes and maintaining the expression of muscle genes through the activity of Notch or Stat3 signaling [43, 44]. Compared to 5-azacytidine, overexpression of miR-1-2 in mouse bone marrow stem cells can increase the differentiation into cardiac cells more effectively by activating the WNT/ β -catenin signaling pathways [34]. In their study, use of LGK-974, an inhibitor of the Wnt/ β -catenin signaling pathway, significantly inhibited cardiac differentiation and partially blocked miR-1-2 actions [34].

Contrary to the results obtained by Shen et al. [34] showing positive effects of Wnt/ β -catenin signaling pathways during cardiac differentiation, Gao et al. [45] reported that cardiac differentiation of MSCs was made possible by the inhibition of β -catenin expression. In their study, addition of a combination of 5-azacytidine, salivarnic acid, and cardiomyocyte lysis medium to cardiac differentiation medium of BM-MSCs promoted expression of cTnT, α -cardiac actin, mef-2-c, Cx43, and GSK-3 β while it reduced β -catenin expression [45]. Regarding the role of Wnt/ β -catenin signaling pathways in the cardiac differentiation of MSCs, Zhang et al. [23] reported that induced overexpression of miR-499 in rat BM-MSCs promoted expression of cardiac-specific genes and activated this pathway by reducing the proportion of phosphorylated/dephosphorylated β -catenin in Wnt/ β -catenin signaling pathways. Also, they showed that knocking down the expression of Dvl, which is an adaptor molecule in the Wnt/ β -catenin signaling, partially blocked activity of miR-499 and reduced expression of cardiac-specific genes. Transduction of Wnt11 into MSCs increases their cardiac differentiation and ameliorates therapeutic effects of MSCs transplantation on the treatment of acute myocardial infarction [46]. Another study found that overexpression of Wnt11 and Akt1 by MSC transduction increased proliferation and cardiac differentiation of MSCs. Also, co-expression of Akt1 and Wnt11 reduced cell apoptosis of MSCs under hypoxia/reoxygenation treatments [47]. Different combined DNA methyltransferase and histone deacetylase were used by another study. Wharton's jelly MSCs differentiated into cardiomyocyte-like structures with upregulation of Wnt antagonists, sFRP3 and sFRP4, and Dickkopf (Dkk)1 and Dkk3 [25]. Wnt11 gene transduction enhances differentiation of MSCs into cardiomyocytes by upregulating GATA4 [48]. Much remains to be learned about how Wnt signaling regulates cardiomyocyte differentiation of MSCs.

FGF Signaling Pathways

The fibroblast growth factor (FGF) family includes 18 secreted proteins interacting with 4 signaling tyrosine kinase FGF receptors (FGFRs). Interactions between FGF ligands and their signaling receptors are controlled via protein or proteoglycan cofactors and by extracellular binding proteins. Activated FGFRs phosphorylate specific tyrosine residues that mediate interactions with cytosolic adaptor proteins and PI3K-AKT, PLC γ , RAS-MAPK,

and STAT intracellular signaling pathways [49]. FGF family proteins are secreted as endocrine and paracrine. They have several activities, including the development of heart tissue. There is limited information about the role of this pathway in regulating MSC differentiation into cardiac cells. Activation of extracellular signal related kinases (ERK) as a part of downstream of the FGF signaling pathway during cardiac differentiation of human umbilical cord-derived MSC was evaluated by Qian et al. [50]. To do that, they used 5-azacytidine as a cardiac differentiation agent. In addition, they evaluated cardiac genes and proteins expression. They revealed that ERK was activated in MSCs extracted from the human umbilical cord (hUC-MSCs) while proteins on kinase C pathways were not expressed in response to the cardiac differentiation factor, namely, 5-azacytidine. Another interesting point in the study by Qian et al. [50] was using the inhibitor of ERK, U0126. After the addition of U0126 to their medium, action of 5-azacytidine as cardiac differentiation was inhibited. Also, Soltani et al. [10] evaluated cardiac differentiation of ovine fetal BM-MSCs after the pretreatment with reversine as a de-differentiation factor, followed by the treatment with 5-azacytidine as a cardiac differentiation agent. They observed some improvements in cardiac differentiation of BM-MSCs and an increase in the expression of *Fgfr1* and *ERK2* during cardiomyocyte differentiation. Combined therapy of mouse adipose-derived MSCs (Ad-MSCs) and rosuvastatin reduced fibrosis and cardiomyocyte apoptosis and preserved heart functions through PI3K/Akt and MEK/ERK1/2 signaling pathways after infarction [51]. Zhang et al. [52] assumed that inositol hexakisphosphate kinase (IP6K) inhibition might increase Akt activation in MSCs, resulting in enhanced cardiac protective effects after transplantation. For this purpose, they evaluated functions of IP6Ks to improve MSC functional survival and cardiac protective effects after performing transplantation into infarcted hearts of mice. Their results showed that inhibition of IP6Ks increased MSCs engraftment and paracrine effects on infarcted hearts at least in part by downregulating IP7 production and enhancing Akt activation, which might contribute to the preservation of myocardial functions after MI [52]. Protective effects of ghrelin on engrafted Ad-MSCs and its benefits with cellular therapy in mouse MI were investigated by Han et al. [53]. Their results indicated that ghrelin improved AD-MSC survival under hypoxia/serum deprivation conditions. Also, ghrelin promoted AKT phosphorylation both in vivo and in vitro, reduced the proapoptotic protein Bax, and enhanced the antiapoptotic protein Bcl-2 in vitro while these effects were eliminated by the

PI3K inhibitor LY294002. In a similar study, Tongxinluo protected MSCs against hypoxia/serum deprivation-induced injury at least partially through AMPK/eNOS pathways [54]. Pretreatment of Ad-MSCs with sphingosine 1-phosphate (S1P) exhibited increased potencies to inhibit cardiomyocyte apoptosis and fibrosis and to stimulate angiogenesis and cardiac function protection. Mechanics-based studies showed that S1P promoted AT-MSCs migration via activation of ERK1/2-MMP-9 and protected AT-MSCs against apoptosis via Akt activation [54, 55]. PI3K-Akt pathways could play a vital role in directing dental stem cells into cardiomyocytes [56]. Rosuvastatin supplementation ameliorates efficacy of stem cell transplantation on infarcted hearts by activating JAK2-STAT3 signaling pathways [57]. Given the important roles of FGF signaling pathways in cardiomyocyte differentiation of MSCs, further investigation of FGF sources and mechanisms of differentiation will provide new knowledge that can be applied to therapeutic strategies (Table 2).

TGF Signaling Pathways

The TGF- β family has been confirmed to regulate a wide variety of biological activities [64]. TGF- β can enhance or prevent cell growth depending on the cell types. TGF- β is a multifunctional cytokine involved in survival, growth, and differentiation of different cells in vitro [65, 66].

In the canonical pathway of TGF- β signaling, T β RI employs serine/threonine kinase activity to direct intracellular signaling through phosphorylation of cytoplasmic proteins Smad2 and Smad3 which combine with Smad4 and form a heteromeric complex being able to migrate to nuclei [67]. The nuclear Smad complex binds to TGF- β effector genes for transcriptional regulation [68].

TGF- β induces differentiation of cardiomyocytes, smooth muscle cells, and osteoblasts [69–71]. TGF- β signaling is essential for the suppression of sedition gene synthesis in healing infarcted cells. The cardiac-specific growth factor TGF- β may act a significant function in modulating fibroblast phenotype and gene expression, assisting extracellular matrix elimination in infarcted cells by promoting collagen and fibronectin synthesis and by reducing matrix deprivation via addition of enzymes called protease inhibitors [72]. One of TGF- β applications, as a cytokine, is to act as a cardiac differentiation factor. Combination of 5-azacytidine with TGF- β in mouse

Table 2. Status of signaling pathways in cardiac differentiation of mesenchymal stem cells

Pathway	Type of MSCs	Observations	References
FGF	Ovine fetal BM-MSCs	Addition of 5-azacytidine after pretreatment with reversine increased cardiomyocyte differentiation Increased expression of Fgfr1 and ERK2 genes after induction	[10]
	Human umbilical cord-derived MSCs	Cardiomyocyte differentiation after addition of 5-azacytidine and activated ERK protein Supplementation of U0126 as ERK inhibitor suppressed cardiomyocyte differentiation	[50]
TGF	Mouse BM-MSCs	5-Azacytidine and TGF- β in MSCs culture medium induced cardiac differentiation	[58]
	Ovine fetal BM-MSCs	Cardiomyocyte differentiation after addition of 5-azacytidine Expression level of Smad2 was promoted	[59]
	BM-MSC	Upregulation of TGF- β BMP signaling pathway genes, including TGFB1, ACVR2B, and phosphorylated Smad2 and Smad3, within 24 h	[60]
Notch	hUC-MSCs	High-level expression of DLL4 and Notch genes after induction with 5-azacytidine	[61]
	hBM-MSCs	Overexpression of the Notch1 and specific cardiac mRNA after induction with 5-azacytidine	[62]
	rBM-MSCs	Upregulates Notch1 signaling after cardiac differentiation	[63]
Wnt	rBM-MSCs	Overexpression of mir-499 induced cardiomyocyte differentiation in MSCs Reduced the ratio of phosphorylated/dephosphorylated β -catenin in the Wnt/ β -catenin pathway	[23]
	Human Wharton's jelly MSCs	Upregulation of Wnt antagonists, sFRP3 and sFRP4, and Dickkopf (Dkk)1 and Dkk3 after cardiomyocyte differentiation	[25]

BM-MSCs culture medium induced cardiac differentiation, as shown by high-level expression of GATA4 and cTnT genes although signaling pathway genes and proteins associated with TGF- β signaling pathways were not evaluated [58]. Another study revealed that combinations of low concentrations of TGF- β and 5-azacytidine reduced rat BM-MSC apoptosis and had strong effects on levels of expression of troponin I and α -actin proteins. That study reported high-level phosphorylation of extracellular signal-regulated protein kinases 1 and 2 (p-Erk1/2) [73].

TGF- β 1 induced BM-MSCs to differentiate into cardiomyocyte-like cells. Also, BM-MSCs increased within 1–3 days after the administration of treatments with TGF- β expression of cardiac transcription factors, GATA4 and NKX2.5. High levels of cardiac myosin, troponins, and ANP expressions were observed within 3–14 days after the induction, with regard to cardiac-specific genes. In addition, upregulation of TGF- β BMP signaling pathway genes, including TGFB1, ACVR2B, and phosphorylated Smad2 and Smad3, was observed within 24 h by Li et al. [60]. In another study, we found that after treatment of ovine fetal BM-MSCs with 5-azacytidine, expression of cardiac-specific genes increased and expression levels of Smad2 were promoted [60]. These results highlight the necessity of investigating TGF- β signaling pathways in order to evaluate stem cell differentiation into cardiomyocyte lineage.

Notch Signaling Pathways

Notch signaling, which is substantial in cell fate specification during embryogenesis, has formerly been implicated in controlling differentiation of stem cells in adults [74]. Several research studies have proved that Notch signaling controls different types of processes during embryonic and postnatal development, including cell fate decisions, apoptosis, and proliferation [75, 76]. Notch signaling involves (1) cell-to-cell contacts that enable Notch receptor and Delta/Serrate/Jagged ligand (henceforth DSL) interactions; (2) a receptor-ligand force generation system that unfolds a protective domain within the Notch receptor (the negative regulatory region [NRR]) to thereby allow sequential proteolytic activation and release of the Notch intracellular domain (NICD); and (3) NICD translocation from membrane to nucleus where it binds to a conserved transcription factor (CSL; CBF1/RBPJ, Su[H], and Lag-1) to upregulate Notch target genes [77].

Moreover, Ding et al. [78] indicated that genetically modified MSCs with an adenovector overexpressing Notch1 intracellular domain (NICD) represented enhanced differentiation potential of MSCs favoring differentiation toward cardiomyocytes [78]. In order for cardiomyocytes to differentiate, levels of cTnI expression increased

due to 5-azacytidine-induced hUC-MSCs. After, 5-azacytidine-caused induction, expression of DLL4 and Notch genes increased at different times [61]. Cardiomyocyte differentiation of hBM-MSCs treated with 5-azacytidine led to observation of overexpression of the Notch1 and specific cardiac mRNA [62]. Co-culture of MSCs with neonatal cardiomyocytes showed that Jagged1-dependent Notch activation enhances production of MSC-derived cardiomyocytes [79]. H₂O₂- or glucose oxidase-mediated oxidative stress upregulates Notch1 signaling, which increases expression of cardiac-specific genes in rBM-MSCs, possibly involving Wnt11. Modulating the balance between Notch activation and H₂O₂-mediated oxidative stress may lead to ameliorated adult stem cell-based therapies for cardiac repair and regeneration [63].

Other Signaling Pathways

Overexpression of miR-124 in BM-MSCs downregulated myogenic-specific markers including ANP, TNT, and alpha-MHC proteins via targeting STAT3 mRNA [80]. Downregulation of miR-199b-5p induced differentiation of mBM-SCs treated with 5-azacytidine toward cardiomyocyte-like cells partly via HSF1/HSP70 signaling pathways [81]. The nuclear factor NF-κB pathway has long been investigated and identified as a prototypical proinflammatory signaling pathway, chiefly based on functions of NF-κB in the expression of proinflammatory genes such as chemokines, adhesion molecules, and cytokines [82].

Conclusions

Among different types of stem cells, MSCs are a strong candidate to be used by regenerative medicine and to investigate cellular differentiation. They represent an absorbing cell source for the transplantation because they may be derived from various tissues. MSCs can differentiate into cells and tissues with mesodermal origins such as adipocytes, cartilages, bones, and skeletal muscles. Based on this advancement, various clinical concepts can be extremely promising for cell therapy and tissue engineering. MSCs could be induced into cardiomyocytes, which migrate to damaged locations to exert therapeutic functions. Currently, mechanisms facilitating proliferation and differentiation of cardiomyocytes from MSCs are not understood well, which is an

obstacle to the generation of therapeutically effective cells. To recognize signaling pathways with a major impact on MSC differentiation is a prerequisite for designing cell therapies in order to replace damaged tissues, helping to integrate signal inputs initiating the differentiation into specific lineage [83]. Cardiac development and cardiomyocyte differentiation have been related to an important role the signaling pathways play. The present study summarized some signaling pathways being activated during cardiomyocyte differentiation of MSCs. In addition, no information is available about preclinical and clinical data supporting important roles the different signaling pathways play in MSCs related to cardiac cell therapies. Probably, the lack of sufficient information on in vitro culture systems is one of reasons why roles of signaling pathways during cardiotherapy by MSCs remained unknown. Physiologically precise functions and action mechanisms of such proteins remained confusing although most of the cardiomyocyte differentiation agents were discovered in the past decade. Future research should discover the signaling pathways activated after the addition of cardiomyocyte differentiation agents. An important point which has to be investigated in the future is that how such agents modulate cardiomyocyte differentiation. In conclusion, it is necessary to gain more scrutinized understanding of information signaling pathways originating from in vitro heart development and of MSC interactions with microenvironments along with deeper analyses of cell-intrinsic mechanisms governing proliferation- versus differentiation-inducing signaling pathways in order to better recognize mechanisms of highly efficient cardiomyocyte differentiation under in vitro conditions.

Conflict of Interest Statement

None of the authors have any conflicts of interest to declare.

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Author Contributions

Soltani and Mahdavi screened the literature and selected relevant data included in the review. Soltani and Mahdavi wrote the manuscript. Soltani and Mahdavi edited the manuscript in terms of intellectual content.

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