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Review Article

Cardiac Stem Cells at a Glance

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ABSTRACT

Myocardial cell loss is hypothesized to be irreversible but latest research indicates that human cardiac stem cells (hCSCs) may have a therapeutic use for cardiac repair; however, their properties and the regulatory mechanisms for their development have not been thoroughly explored. Stem cells are a population of cells with the potential for self-regeneration and differentiation into cell types that can play an important role in embryogenesis and embryonic development. So far, seven types of cardiac stem cells with different molecular phenotypes and differentiation potentials have been discovered and studied. Increased proliferation and differentiation of these cells in areas of cardiac ischemia is a significant factor in the repair and enhancement of heart injury. This role of endogenic cardiac stem cells can be regulated by factors. Numerous causes, such as paracrine and autocrine factors, extracellular matrixes and genetic factors. In general, it is well known that these variables could have an appropriate role to improve the effectiveness of the treatment of heart lesions. The work of cytokines and growth factors has been found to play an important role in increasing endogenous cardiac stem cell proliferation and migration capacity. One of the successful approaches is to use these factors along with cardiac stem cells to boost the repair efficiency of heart injury. Therefore, in the present review study, the types of cardiac stem cells and their molecular phenotype, cardiac stem cell genetics and their ability to treat heart injury have been studied. Understanding the molecular processes and messaging pathways involved in success Cardiac stem cells offer an effective approach to the production and presentation of cardiac lesion therapy using cardiac stem cells.

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INTRODUCTION

Stem cells are a population of cells with the potential for self-regeneration and differentiation into cell types that can play an important role in organ formation and embryo completion (Klimczak and Kozlowska, 2016; Ehnert *et al*., 2009). These cells themselves regenerate a population of similar cells and, in the process of differentiating, differentiate cells (Roobrouck *et al*., 2008). In 2000, with the discovery of cardiac stem cells, extensive research was conducted by various groups to understand the nature of the function and to identify specific antigens and molecular phenotypes of cardiac stem cells (Klimanskaya *et al*., 2008; di Giorgio *et al*., 2008). Studies showed that the formation of proliferation and extention of cardiac stem cells occurs during the mammalian developmental period before gastrulation. This process is necessary for heart formation (di Giorgio *et al*., 2008; Birket *et al*., 2015). According to studies, the ability of heart stem cells transplanted to infarcted areas to reduce the extent of damage and restore cardiac function has been determined (Masumoto *et al*., 2012; Huang *et al*., 2017). So far, seven types of cardiac stem cells have been discovered and studied, some of which are found only in animals and some in humans or both. The origin of markers and the ability of these cells to differentiate have been well studied. Until now two type of cardiac stem cells has been studied in the clinical stage and the promising results of transplantation of these cells in clinical studies have been significant (Passier *et al*., 2008). In the present study, the study of cardiac stem cell types, their molecular phenotype, biology and the possibility to use them in the treatment of heart damage is discussed.

TYPES OF CARDIAC STEM CELLS

C-kit+ cardiac stem cells

Beltrami *et al*. in 2003 succeeded in isolating and extracting stem cells from the heart of mice. Later, the presence of C-kit+ stem cells in the human heart muscle has been shown to have the same characteristics as C-kit+ mouse cardiac stem cells (Klimanskaya *et al*., 2008; Passier *et al*., 2008).

Studies have shown that in people with heart failure, C-kit+ stem cells play a key role in controlling the spread of damage, so that their endogenous levels around the myocardial infarction areas increase about 4-fold (Yaniz-Galende *et al*., 2012). In mammals, with aging, the number of C-kit+ cardiac stem cells in the myocardium decreases

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significantly, and the ability to differentiate these cells into muscle cell phenotypes is lost. The restorative function of C-kit + cardiac stem cells injected into the damaged myocardium appears to be paracrine / autocrine function of the injected cells through direct differentiation into cardiac cells. Tang *et al*. observed that intra-coronary injection of c-kit+ cardiac stem cells one month after induction of infarction in mice increased left ventricle function. Left ventricle transplantation of C-kit+ cardiac stem cells into infarcted areas increases the end systolic volume of the left ventricule and also improves the right ventricular outflow fraction (Tang *et al*., 2010). This demonstrates the ability of C-kit+ cardiac stem cells to improve heart failure (Passier *et al*., 2008). Not all C-kit+ cardiac cells are recognized as stem cells. C-kit+ cells with the CD45+ phenotype (a specific marker for mast cells) lack the characteristics of stem cells (Kirshenbaum *et al*., 1999). Accordingly, the population of cells with the CD45+/C-kit+ phenotype is known as mast cells and the cells with the CD45-/C-kit+ phenotype are known as stem cells (Kirshenbaum *et al*., 1999). Other features of cardiac stem cells include C-kit+ expression of transcription factors Nkx2.5 and GATA-4, which have been shown that cardiac stem cells express these factors in vitro in addition to the ability to differentiate myocardial cells into fibroblast cells of vascular endothelial and smooth muscle cells (Klimanskaya *et al*., 2008; Smith *et al*., 2014; Bao *et al*., 2017). Expression of vascular endothelial growth factor receptor in the C-kit+ cardiac stem cell population is an appropriate marker to identify and isolate these cells based on their differentiation orientation. KDR-/C-kit+ cardiac stem cells are more likely to differentiate into myocardial cells and KDR+/C-kit+ cardiac stem cells are more likely to differentiate into vascular endothelial cells, but both of these phenotypes are also differentiated into fibroblasts and smooth muscle cells (Yang *et al*., 2008).

Cardiosphere stem cells

In 2007, Lama *et al*. for the first time were able to isolate and extract multicellular, non-adherent clusters from endocardial tissue samples that had a high ability to differentiate into cardiac cell phenotypes. They called this cell mass the cardiosphere (Lama *et al*., 2007).

Cardiospheres are a diverse population of cardiac cells, the central part of which is composed of cells with these phenotypes: C-kit+, Sca-1+, CD105+, CD90+, CD29+ (Lama *et al*., 2007; Dutton *et al*., 2018), and the margin of the cardiosphere consists of differentiated cells which consists of differentiated cells that express markers of cardiovascular cells, endothelial cells, and mesenchymal cells (DeRuiter *et al*., 1997). Cardiosphere isolated from the heart of mice and humans have a high potential for regeneration and colonization and differentiation into myocardial cells. Another feature of the cardiospheres is the ability to maintain the microenvironment of the source of cardiac stem cells after successive divisions (Dutton *et al*., 2018; Davis *et al*., 2005). Numerous studies show that cardiosphere transplantation as well as its derived cells in cardiac ischemia models of mice (Yang *et al*., 2008; Aminzadeh *et al*., 2015) dogs (Bonios *et al*., 2011) and pigs (Gallet *et al*., 2017) have a good ability to repair and restore cardiac function. The number of colonies and the ability to repair cardiospheres transplanted into the heart muscle, like C-kit+ cardiac stem cells, decrease dramatically with age. Based on this, it has been determined that cells derived from neonatal cardiospheres have a higher ability to repair heart muscle than cells derived from adult cardiospheres. This function is due to the ability to express high levels of angiogenic cytokines and have a good level of cardiac stem cells (Bao *et al*., 2017). Also, during clinical studies, 6 months after injection of cardiosphere stem cells into patients with myocardial infarction, a significant improvement in scar rate, volume of live heart mass and systolic wall diameter of the heart was observed. Cardiosphere-derived stem cells are involved in increasing the rate of repair of heart failure. In this regard, scientists do not really agree on the fundamental nature of the cardiospheres. Spontaneous heart rate-derived cardiospheres in the neonatal mouse may be due to contamination of the cardiosphere with myocardial cells. In order to remove contaminating cells by filtering tissue fragments, they formed the cardiosphere without the ability to differentiate into cardiac cells (Mayfield *et al*., 2014). However, Davis *et al*. (2005) emphasized the fundamental nature of cardiospheres by culturing and purifying cardiospheric cardiac progenitor cells.

Sca-1+ cardiac stem cells

A number of studies have reported the presence of adult cardiac stem cells with the ability to express stem cell antigen-1(: Sca-1). Another feature of these cells is the lack of expression of structural genes in heart cells, including Nkx2.5.

However, in vitro, these cells have the ability to tend to heart cells in the presence of the substance azithidine as well as oxytocin (Hiroi *et al*., 2001; Prall *et al*., 2007), which indicates the fundamental nature of this group of cells. Studies have shown that Sca-1-expressing heart cells make up about 2% of all heart cells. In addition to expressing c-kit+, they also express markers such as CD29, CD44, CD31, and CD45 (Hass *et al*., 2011). Transplantation of Sca-1 stem cells into mice in the acute myocardial infarction model improved heart failure and increased repair of damaged tissue. The repair of

transplantation of these cells to myocardial infarction areas is due to the paracrine/autocrine function of these cells, which increases the rate of angiogenesis and increases the function of heart cells (Shafei *et al*., 2017). Cardiac stem cells sca-1 with secretion of stromal cell drived factor-1 (SDF-1) followed by cardiac STAT3 activity is one of the paracrine mechanisms induced by transplantation of Sca-1 + stem cells into infarct sites (Kwon *et al*., 2011). Takamiya *et al*. (1996) by purifying and transplanting cardiac Sca-1 + stem cells with Nestin +, Bcrp +, TERT +, Musashi1 and IS1-1 phenotypes into myocardial infarction sites, observed that these cells have the ability to differentiate directly into cardiac cells and improve left body function. So far, no similar human Sca-1 + stem cells have been identified and purified. Extensive studies are currently underway to obtain similar Sca-1 + cardiac stem cells in the human heart muscle (Barile *et al*., 2007).

Side population cardiac stem cells

Researchers isolated and purified side populations from cardiac cells between 2004 and 2006, characterized by a lack of staining as well as the expression of MDR 1, which has a high ability to differentiate into heart cells (Hadnagy *et al*., 2006; Masino *et al*., 2004). Abcg2 gene expression by these cells plays a very important role in maintaining the basic characteristics of the cells of the side population of the heart by increasing the survival and proliferation of these cells (Pfister *et al*., 2008). Cardiac stem cells have been shown to express high populations of Sca-1 (Hass *et al*., 2011).

Side cardiac population cells have two different populations with the phenotype CD31-/ Sca1+ and CD31+/ Sca1+. Cardiac side population cells with the CD31-/ Sca1+ phenotype are a group of cardiac stem cells that have the ability to repair heart tissue and improve cardiac function (Mayfield *et al*., 2014; Noseda *et al*., 2015). In the case of CD31+/Sca1+ side cardiac population cells, it appears in some animals, such as mice; act as vascular endothelial progenitor cells. After injecting CD31- / Sca1 + lateral cardiac population cells into the infarcted areas of rabbit heart, Liang *et al*. (2010) observed healthy and regular myocardial fibers in the infarcted injection sites. Studies also show that cardiac stem cells have a higher abundance of lateral populations than C-kit+ cardiac cells in the damaged human myocardium (Miyamoto *et al*., 2010). This indicates the important role of these cells in repairing heart damage.

Is11+ cardiac stem cells

In fact, Is11+ stem cells are known as cardioblasts or endogenous cardiac precursor cells. Co-culture of these cells with embryonic muscle cells increased the differentiation of these cells to the phenotype of mature cardiac cells with stable expression of myocardial cell markers (Laugwitz *et al*., 2005). The pure population of primary Is11 + stem cells has the ability to regenerate and differentiate into different types of heart cells. However, the volume and number of cells extracted from the myocardium were large enough to be used in animal studies (Ishizu *et al*., 1995). Is11+ stem cells appear to be a group of mature cardiac stem cells whose heart formation period plays an important role in the development of the primary cardiac and secondary cardiac field, each of which causes the formation of the left and right ventricles of the heart, respectively (Elliott *et al*., 2011; Klaus *et al*., 2012). IS11 + cells have been shown to be the only population of cardiac stem cells that have the ability to proliferate and regenerate themselves during heart development before they differentiate. Decreased expression of empty genes in mice leads to incomplete formation of the right ventricular outflow tract and a large part of the atria (Lindqvist *et al*., 2003).

Cardiac epicardial stem cells

In addition to the primary and secondary progenitor cells of the heart, there are two other types of epicardial progenitor cells, each identified by the T-box transcription factor as well as the Wt1 transcription factor (Cai *et al*., 2008; Limana *et al*., 2010).

In fact, there is no consensus on the stem cell nature of Tbx18 + epicardial cells (Christoffels *et al*., 2009). However, evidence suggests that Wtl+ cardiac epicardial cells have the ability to repair heart damage in experimental models (Ahmed, 2008). Recent studies have shown that the peptide that binds to the actin thymosinβ4 stimulates the division and regeneration of ancestral epicardial cells (Poss *et al*., 2003).

Cardiac SSEA-1 stem cells

Stage-specific embryonic antigen-1 or CD15 is a type of embryonic antigen that is expressed by SSEA-1+ cells. The pure population of SSEA-1+ stem cells has the ability to differentiate into smooth muscle and heart endothelial cells. Cardiac SSEA-1 + stem cells transplanted into the ischemic regions of myocardial ischemia mice have been shown to regenerate and form cardiovascular cells (Kucia *et al*., 2006).

FACTORS THAT CONTROL THE FUNCTION OF CARDIAC STEM CELLS

Cardiac muscle cells are able to survive throughout life; however, the death of these cells during the pathological process is inevitable (Maron *et al*., 1975).

Throughout life, the heart muscle has a population of cardiac stem cells that proliferate and function significantly during heart damage (Li *et al*., 2019; Ohnishi *et al*., 2007). The presence and formation of endogenous cardiac stem cells around the infarcted areas is a key factor in repairing damage and restoring cardiac function. Cardiac stem cell function is controlled by several factors such as paracrine in the microenvironment (Kusuma *et al*., 2017), cytokines and chemotaxis (Matsushima and Oppenheim, 1989) as well as the function of microRNAs (Fabbri *et al*., 2008; Baek *et al*., 2008). The rate of diffusion and secretion of stroma-derived and protease-resistant factor-1 appears to increase the migration of cardiac stem cells to infarcted areas, thereby improving heart function. So far, several studies have been performed to investigate the function of cytokines and growth factors in increasing the proliferation and migration capacity of endogenous cardiac stem cells (Kilian *et al*., 2004). The use of hepatocyte growth factor and insulin-like growth factor-1 seems to be a suitable way to increase the endogenous function of these cells (Gemberling *et al*., 2013; Duerr *et al*., 1995). It plays a key role in many biological processes such as stimulation of the mammary glands, stimulation of the uterine wall at delivery, limiting tumor growth, and the formation and development of the mammalian heart.

Oxytocin receptor levels have been shown to increase significantly in the primary myocardium of rodents in the first 7 days of gastrulation (Kimura *et al*., 1996; Szeto *et al*., 2008), indicating a key role in the differentiation and formation of heart cells from cardiac stem cells. Treatment of Sca-1+ cardiac stem cells with oxytocin differentiates the population of these cells from pulsating cardiac cells, which is accompanied by the expression of cardiac transcription factors, cardiac contractile proteins, and the expression of the sarcomere phenotype by the differentiated cell population (Shim *et al*., 2004). Nitric oxide messaging pathway seems to play an important role in repairing and protecting the heart muscle from damage (De Palma and Clementi, 2012). The cardiac protective role of nitric oxide messaging pathway through oxytocin receptor stimulation has been well studied (Szeto *et al*., 2008; Pournajafi-Nazarloo *et al*., 2007). Alizadeh *et al*. (2012) showed that oxytocin has the ability to protect heart cells against ischemic reperfusion damage through pathways related to mitochondrial ATP-dependent potassium channels and increased permeability of transition pores.

Studies have shown that the function of genetic factors such as miRNA play an important role in the function of endogenous cardiac stem cells and the complete repair of areas of neonatal heart damage in rodents (Li *et al*., 2019). Lack of miRNA expression of miR-15 and miR-195 families in early infancy is a key factor in the proliferation and differentiation of endogenous cardiac stem cells. Excessive expression of miR-15 and miR-195 in the heart of neonatal rats inhibits myocardial repair, increases migration and proliferation of endogenous cardiac stem cells (Qiu *et al*., 2017).

Based on this, it was found that the expression of miR-15 and miR-195 family microRNAs is one of the main factors preventing the proliferation and extension of heart cells in adults (Fabbri *et al*., 2008; Baek *et al*., 2008). Hosoda *et al*. (2011) also investigated the relationship and role of microRNA expression in the differentiation of human C-kit+ stem cells and found that miR-449 plays an important role in the differentiation of these cells into myocardial cells.

The transfer of miR-449 to cardiac stem cells from myocardial cells by open junction seems to be one of the factors controlling the differentiation of endogenous cardiac stem cells (Parekh *et al*., 2020).

MOLECULAR PATHWAYS THAT CONTROL THE FUNCTION OF CARDIAC STEM CELLS

The tendency for survival and self-regeneration of cardiac stem cells is largely controlled by messages from factors in the microenvironment of the heart. The Wnt/β-catenin, Akt, and Notch signaling pathways are among the molecular pathways that control the function of cardiac stem cells. Studying these pathways gives us an understanding of how heart stem cells function in generalizing heart damage.

Wnt/β-catenin

Wnt is a protein ligand rich in the amino acid cysteine, which binds to g-protein receptors to induce their function. The Wnt messaging path typically operates from two different paths. The normal pathway is dependent on β-catenin activity and the unconventional pathway is dependent on JNK and PKC activity. Binding of Wnt protein to its receptors at the cell surface (frizzled and LRP5/6) leads to activation of dishevelled protein (DSH) and thus inhibits the function of the Axin / GSK-3 / APC complex, which is a limiting factor for Wnt (Farr III *et al*., 2000; Harwood, 2001). Increased activity of the normal Wnt pathway and consequently inactivation of GSK-3β function leads to a decrease in β-catenin phosphorylation, its accumulation in the nucleus. Studies show that the Wnt/β-catenin messaging pathway plays an important role in embryonic stem cell differentiation during the mesoderm development process.

It seems that at the beginning of the process of completing the vertebrate heart, the Wnt/β-catenin

pathway prevents the formation and expansion of the fetal myocardium by inhibiting the function of bone morphogenic protein (Bastakoty *et al*., 2016; Daskalopoulos *et al*., 2013). In contrast, the unconventional pathway of Wnt with Wnt11 activity plays an important role in inducing the formation of vertebrate fetal heart (Garriock *et al*., 2005). The Wnt signaling pathway in cardiac ISL-1 stem cells via banding to FGF plays an important role in the formation of the right part of the heart and the simultaneous expression of FGF3, FGF10, FGF16 and FGF20 during Wnt signaling pathway activity plays an important role in the formation of the right heart in ISL-1 stem cells (Langdon, 2008). The normal Wnt pathway, with the function of the IGFBP3 protein downstream of the signaling pathway, reduces the proliferation of cardiac stem cells in the side population and stops the regeneration of cardiac damage (Urbanek *et al*., 2005).

In contrast to the normal Wnt pathway, the induction of the Wnt/β-catatin pathway in C-kit+ cardiac stem cells increases the proliferation of C-kit+ cardiac stem cells by increasing the stem cell factor secreted by cardiac muscle cells (Lutz *et al*., 2008). This suggests that the Wnt and Wnt/β-catenin pathways play an important role in myocardial repair and development (Fu *et al*., 2019).

Akt

Akt's messaging pathway plays an important role in controlling important biological mechanisms such as glycogen metabolism, cell division, and cell survival. In the mammalian genome, there is an Akt encoding gene in the form of Akt1 / PKBα, Akt2/PKBβ, and Akt3 / PKBγ. The Akt protein consists of a hydrophobic motif at the end of a central domain of kinase function and a pleckstrin-homology domain (ph) at its amino terminus. Coating of the second central with the hydrophobic domain of the motif at the carboxyl terminus of the Akt protein inhibits Akt kinase activity in unstimulated cells. Akt protein binds to plasma membranes via the domain in response to growth factor stimulation, and the tyrosine 308 and serine 470 amines in Akt protein bind via phosphoinositide-dependent protein kinase-1 and hydrophobic motif kinase, respectively, which are upstream kinases of Protein Akt, will be phosphorylated and activated (Wang and Ceresa, 2012; Nicholson and Anderson, 2002). Phosphorylation of these amino acids causes the formation and transmission to the Akt messaging pathway. In this regard, serine/threonine protein kinase Akt, as a mediator in phosphatidyl 3-kinase (PI3K) signaling, plays an important role in the development and differentiation of a wide range of cells, including heart muscle cells (Son *et al*., 2007; Crackower *et al*., 2002). Blocking the PI3K/Akt signaling pathway in PI9CL6 embryonic carcinoma stem cells by stopping the function of the Wnt/β-catenin signaling pathway increased Glycogen Synthase Kinase activity and β-catenin breakdown in the cytoplasm of cells, which stopped Embryonic stem cells differentiate into heart cells (Popova *et al*., 2012).

Studies show that increasing VEGF expression plays an important role in increasing stem cell differentiation and increasing the repair capacity of these cells (Lee *et al*., 2009).

Activation of the PI3K/AKT signaling pathway increases the migration of endogenous cardiac stem cells to infarct sites in infarct mice. Among these, C-kit+ cardiac stem cells show the highest rate of migration compared to other cardiac stem cells (Shi *et al*., 2018). The PI3K/Akt signaling pathway also plays an important role in inducing differentiation of cardiac stem cells into vascular endothelial cells, a process that results from increased VEGF expression and function (Xiao *et al*., 2014). However, not many studies have been performed on the role of the Akt messaging pathway in cardiac stem cell function.

Notch

This messaging pathway is an intracellular and highly protected pathway that plays an important role in the formation of formation and homeostasis of various organs or the development of some diseases (Wynn *et al*., 2013; Wynn, 2007). In mammals, four types of Notch receptors have been identified, including Notch1, Notch2, Notch3, and Notch4. The ligands of these receptors also consist of two different classes, including the delta-like proteins DLL1, DDL3 and DLL4, as well as JAG1 and JAG2 proteins.

Notch receptor is a membrane-permeable protein with the second extracellular consisting of repetitive epidermal growth factor-like sequences and the second intracellular NICD The ligands of this receptor activate the Notch messaging pathway through cell-cell connections. After binding of the second extracellular ligand to the second extracellular domain, the Notch receptor, ADAM, (metalloprotease and disintegrin) and γ-secretase are activated, and after two proteolytic cleavages, the second NICD is released from the Notch receptor. Normally, the Notch messaging path transmits NICD to the kernel.

The NICD then binds to MAML1, MAML2, and MAML3 proteins to activate CSL/RBPJ transcription factors and eventually transcribe target genes such as Hairy/Enhancer of split-related family (HES). PI3K, AKT, NF-KB, PPAR, CyclineD1, p21, p27 are the downstream targets of this route. CSL/RBPJ is not performed in the absence of a Notch message by inhibitors and transcripts (Mirza-Aghazadeh-Attari *et al*., 2019).

Observations show that the blockage of the Notch signaling pathway in the mesoderm of the Common fruit fly's heart during gastrulation induces myocardial formation. During this process, the Notch / Su (H) messaging pathway, also known as the CBF-1 / RBPJk pathway, prevents the expression of cardiac transcription factors, preventing myocardial formation during the common fruit fly gastrulation period (Bray and Furriols, 2001). The second N1ICD in the Notch1 receptor appears to increase the expression of the Nkx2.5 proteins in cardiac stem cells and the formation and differentiation of myocardial cells. Cardiac C-kit+ stem cells in the myocardium are surrounded by other heart cells expressing the Jagged1+ marker (CD339).

Stimulation of C-kit+ cardiac stem cell Notch receptor by Jagged1 increases the expression of Nkx2.5 in cardiac C-kit+ stem cells and thus increases the differentiation and function of cardiac stem cells. Boni *et al*. (2010) showed that blocking the Notch1 pathway in myocardial infarction mice significantly reduced the repair of cardiac stem cell function by inhibiting the activity of these stem cells. In fact, the function of the Notch messaging pathway in cardiac stem cells is not exactly known. The proliferation and differentiation resulting from this messaging pathway is precisely a time-dependent interval between the type and location of cardiac stem cells so that it can function properly in cardiac stem cells (Assis *et al*., 2010).

TARGETED TREATMENT WITH HEART STEM CELLS

Studies show that about 90% of transplanted heart stem cells die one week after transplantation due to ischemia, inflammation and apoptosis and eventually about 1% of transplanted cells remain in the fourth week after transplantation.

The development and presentation of optimal and efficient methods to protect transplanted cells and increase cell survival in cardiac tissue is essential in targeted stem cell therapy. In this regard, in order to obtain a suitable strategy for therapeutic purposes, a large number of heart cells are needed. For this purpose, simple and cost-effective methods for isolation and culture of cardiac stem cells have been proposed. Using enzymatic analysis, a higher number and percentage of human C-kit + cardiac stem cells can be purified from samples isolated from the atrium within four weeks, using the magnetic cell isolation method (Vicinanza *et al*., 2017).

Also, to increase the survival of C-kit+ cardiac stem cells extracted from adults, signals from factors such as insulin-like growth factor-1 can be used. The use of genetic engineering is an efficient way to increase the efficiency and survival of transplanted stem cells (Boni *et al*., 2010). Modified genetic stem cells with modified Pim-1 kinase gene have a significant function in regenerating heart damage by inducing differentiation, increasing proliferative capacity and survival of cardiac stem cells (Assis *et al*., 2010). The Pim-1 kinase gene increases the viability of cardiac stem cells by lengthening the telomere (Siddiqi and Sussman, 2013).

Subsequent studies have shown that increasing the expression of Pim-1 kinase in human cardiac stem cells significantly increases the efficiency and function of these cells in animal myocardial infarction models (Fischer *et al*., 2009). However, due to the high cost and high risk of viral uses, this method is not recommended. On the other hand, using physical methods to induce differentiation and increase the viability of cells at the transplant site is a safe and effective method for clinical studies. Today, three general methods are proposed for this purpose:

1. Mechanical stresses

Although the induction of mechanical stress is a barrier to the growth and proliferation of cardiac stem cells, this stress induces the tendency of cardiac stem cells to become cardiac by increasing the secretion of inflammatory cytokines and angiogenic factors (Nagaya *et al*., 2004).

2. Magnetic field

Low frequency electromagnetic field by regulating the function of Ca2+ ion in cardiac stem cells increases the ability of cardiac stem cells to differentiate into cardiac cells (Li *et al*., 2007).

3. Hypoxia

Induction of cardiac stem cell hypoxia through the SDF-1α/CXCR4 pathway and downstream anti-apoptosis pathway appears to increase human cardiac stem cell function (Lama *et al*., 2007; Yan *et al*., 2012). Also, using some drugs to control the function of heart stem cells is considered a safe and effective method.

It seems that the use of statins and ascorbic acid by regulating the factors in the microenvironment protects the heart from damage (De Palma and Clementi, 2012; Sorice *et al*., 2014) and controls the differentiation of cardiac stem cells (De Palma and Clementi, 2012; Sorice *et al*., 2014; Hosoda *et al*., 2011). Concomitant use of paracrine factors with cardiac stem cells is also a good way to improve the function of these cells. Paracrine factors have a high ability to induce angiogenesis, reduce apoptosis, increase proliferation and differentiation of cardiac stem cells in various ways (Behfar *et al*., 2002). Collagen deposition in myocardial tissue significantly

increases left ventricular function (Trueblood *et al*., 2001). Zeng *et al*. (2008) observed that increasing the expression of angiopitin-1 through induction of angiogenesis induced by cardiac stem cell differentiation increases myocardial repair in myocardial infarction mice.

1. Modulation of microenvironment

The use of fibroblast growth factor BFGF after myocardial infarction by regulating the myocardial microenvironment and creating appropriate conditions leads to increased function of transplanted cardiac stem cells (Miao *et al*., 2017). Utilization of protease-resistant stromal cell derivative factor (SDF-1) also increases cardiac function after myocardial infarction (Miao *et al*., 2017). Subsequent studies have shown that by increasing the expression of SDF-1α through the CXCR4/PI3K pathway, the migration of cardiac stem cells to myocardial infarction areas increases (Siddiqi and Sussman, 2013). It seems that part of the cardiac repair function is due to endogenous expression of vascular endothelial growth factor (VEGF) with SDF-1α/CXCR4 activity in cardiac stem cells (Tang *et al*., 2011).

2. Induction of proliferation and differentiation

Studies show that transplantation into myocardium and cardiac stem cells together with insulin-like growth factor-1 increases the proliferation and survival of transplanted cells (Duerr *et al*., 1995). IGF1 also increases survival and controls the differentiation of cardiac stem cells into cardiac lines by increasing the level of connexin-43 (Duerr *et al*., 1995). Transfer of factor-1 gene derived from stroma cells to myocardial infarction areas increases the number of myocardial cells in myocardial infarction areas by increasing the proliferation of C-kit+ cardiac stem cells (Lutz *et al*., 2008). Numerous studies have demonstrated the positive role of paraclinical factors such as growth factor-transformingβ, neurogline-1, bone-forming protein-10 (BMP-10) and periosteum in stimulating the proliferation of cardiac stem cells (Hatzistergos *et al*., 2010).

The data from these studies indicate an important role of cell-cell interaction between C-kit + cardiac stem cells and this is fundamental in increasing the ability to repair heart damage (Kajstura *et al*., 2008; Bolli *et al*., 2011).

However, the direct use of growth factors is not effective. Growth factors in vivo have a short half-life and direct use of these factors increases the likelihood of neoplasm formation by inducing the epithelial transition process of mesenchyme (De Herreros *et al*., 2010).

CONCLUSION

The use of adult stem cells to increase the repair efficiency of heart damage is one of the effective methods. Cardiac stem cells in the myocardium have a high ability to differentiate into different types of cells in the myocardial microenvironment, which has the potential to differentiate into vascular endothelial cells and cardiomyocytes, indicating its high ability to increase repair of damage to the myocardium.

It has also been observed that these cells induce their restorative function by secreting a variety of paracrine/autocrine factors into the myocardial microenvironment. Therefore, it seems inevitable to use one or more simultaneous factors such as vascular endothelial growth factor, stromal cell derivative factor and oxytocin along with cardiac stem cells to increase proliferation, differentiation and repair.

Statement of conflict of interest

The authors have declared no conflict of interests.

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