FINAL

Send Orders for Reprints to reprints@benthamscience.ae

Current Drug Targets, 2015, *16*, 000-000

Stem Cell Aging and Age-Related Cardiovascular Disease: Perspectives of Treatment by *Ex-vivo* Stem Cell Rejuvenation

Rosalinda Madonna[1], Felix B. Engel[2], Sean M. Davidson[3], Péter Ferdinandy[4,5], Anikó Görbe[5,6], Joost P.G. Sluijter[7] and Linda W. Van Laake [8]

[1]Center of Excellence on Aging, "G. d'Annunzio" University, Chieti, Italy; Heart Failure Research, Texas Heart Institute at St. Luke's Episcopal Hospital, Houston, Texas; Department of Internal Medicine, Cardiology, The University of Texas Health Science Center at Houston, Houston, Texas; [2]Experimental Renal and Cardiovascular Research, Department of Nephropathology, Institute of Pathology, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany; [3]The Hatter Cardiovascular Institute, University College London, United Kingdom; [4]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary; [5]Pharmahungary Group, Szeged, Hungary; [6]Cardiovascular Research Group, Department of Biochemistry, University of Szeged, Hungary; [7]University Medical Center Utrecht, The Netherlands; [8]University Medical Center Utrecht and Hubrecht Institute, the Netherlands

Abstract: Aging affects endogenous stem cells in terms of functionality and numbers. In particular, during aging, the stemness property can decrease because of enhanced apoptotic cell death and senescence. In addition, aging and aging-related co-morbidities affect the paracrine activity of stem cells and the efficiency of their transplantation. Collectively, this leads to a reduction of the capacity of organs to repair themselves, possibly due to a reduced functional capability of stem cells. Therefore, major efforts have been invested to improve the repair capability of stem cells in aged individuals by overexpressing antisenescence and antiapoptotic genes. In this review, we describe critical genes and signaling pathways in stem cell aging and discuss *ex vivo* genetic modification approaches aimed at stem cell rejuvenation that are of interest for the cardiovascular system.

Keywords: Aging, diabetes, gene therapy, Hippo, hyperlipidemia, myocardin, Notch, Pim-1, YAP, stem cell, rejuvenation, telomerase.

POTENTIAL OF STEM CELLS IN THE TREATMENT OF AGE-RELATED CARDIOVASCULAR DISEASES

Diseases of aging, such as metabolic syndrome, diabetes, hyperlipidemia, atherosclerosis, neurodegenerative diseases, osteoporosis and cancer, represent a major problem for all societies. Despite contemporary medical treatments, heart failure continues to be an important cause of morbidity and mortality amongst the elderly of developed countries [1]. In addition, the incidence of cardiovascular disease (CVD) is rapidly increasing in developing countries. Both type 1 and type 2 diabetes are associated with aging and increased risk of micro- and macrovascular disease, which can lead to ischemic heart disease, heart failure and critical limb ischemia. Hyperglycemia leads to and aggravates the reduction of blood flow in cardiovascular tissues. This is believed to occur in a cascade in which ischemia induces oxidative stress, which initiates fibrosis, thereby increasing the thickness of microvasculature walls [2]. Other consequences of oxidative stress associated with hyperglycemia include: alteration of energy metabolism, organ dysfunction, limited exercise tolerance, and greatly increased vulnerability to a super-imposed ischemic stressor (i.e., following atherosclerotic occlusion of a main artery).

Valid therapeutic strategies that repair damaged heart muscle and ischemic tissue have not yet been developed. Heart transplantation and mechanical support devices remain the only effective remedy for severe cardiac dysfunction. However, because of the existence of major limitations including the limited number of donor organs, immune rejection and infections, research interest has increased towards alternative treatments.

Additionally, critical limb ischemia represents an important cause of diabetes-associated cardiovascular complications [3], and still represents the most common cause of amputation in diabetic patients. Transplantation of stem cells, progenitor cells, or stem-cell derived engineered tissues could be an alternative treatment for tissue repair [4-6]. For the application of progenitor cells, different cell sources have been suggested, including pluripotent and adult cell populations. Unlike adult stem cells, concerns remain in regard to the harvesting of human embryonic stem cells, because of ethical concern, allogenicity, neoplasm and teratomas formation [7]. Muscle, bone marrow, blood, epidermis, brain, liver and adipose tissue are the major sources for adult stem cells

*Address correspondence to this author at the Center of Excellence on Aging, "G. d'Annunzio" University, Via Paolicchi, 66100 Chieti, Italy; Tel: 0871-41512; Fax: 0871-402817; E-mail: rmadonna@unich.it

1389-4501/15 $58.00+.00

[8, 9]. In addition, skin has been utilized to generate induced pluripotent stem cells [10]. Besides the transplantation of exogenous stem/progenitor cells, it might be possible to enhance the endogenous regenerative capacity of the heart. Recent data has suggested that the adult heart retains some capacity for self-healing and self-renewal due to the presence of resident cardiac stem (CSCs) and progenitor cells (CPCs) [11-14]. These observations suggest the opportunity to takea multilateral approach for cardiac regeneration, consisting of boosting the endogenous regenerative capability of the heart in addition to stem cell transplantation. Despite these remarkable advances, however, mortality and morbidity of patients with heart failure, remains high.

Several other potential cardiac progenitor cell types have been reported in recent years, such as c-kit(+) cells. However, the potential of c-kit(+) cells has become controversial [15, 16]. Recent lineage tracing experiment suggest that endogenous c-kit(+) cells contribute only minimally to the formation of new cardiac myocytes [17]. There are also data that epicardium-derived progenitor cells are capable of regenerating the adult heart when stimulated [18]. Although these different progenitor cell types appear to be distinct from each other in situ [19], cell culture expanded adult cardiac-derived cardiac progenitor cell populations exhibit a high degree of transcriptome similarity [20]. This suggests that more effort needs to be invested on optimizing the regenerative potential of these cells and less on the different isolation methods of these cells.

The use of adult stem cells is limited by the high degree of morbidity due to the isolation procedure itself. An example of this is represented by the isolation of bone marrow-derived stem cells, which requires general or spinal anesthesia associated with limited cell yield and further steps for *in vitro* cell expansion, which in turn introduce a risk of bacterial contamination [21, 22]. Some aspects of these problems have been solved by the use of more easily available stem cell types such as adipose tissue-derived stem cells [23], or by somatic reprogramming of fibroblasts into pluripotent stem cells [10]. These types of sources can be considered as the ideal stem cell source, since they allow easy stem cell isolation, higher cell yield and lower patient morbidity. However, the following limitations of current approaches have still to be solved: 1) inadequate recruitment of circulating or resident cardiac stem cells; 2) poor capability of adult stem cells to differentiate into cardiomyocytes; 3) elevated mortality of transplanted stem cells; 4) abnormal electromechanical behavior of transplanted cells after stimulation and the eventual onset of arrhythmias; 5) improper structure of newly formed heart tissue; and 6) diminished functionality and number of both resident and circulating stem/progenitor cells or even induced pluripotent stem cells [24, 25] with the onset of aging and age-related CVD [26-28].

Stem cell aging is of particular importance in patients with CVD. Even though CVD can affect people of all ages, the risk of CVD increases significantly with age [29]. Over the last decade, it has become clear that vascular wall integrity is maintained by circulating cells, named vascular stem/progenitor cells dedicated to endothelial repair and angiogenesis (reviewed in [30]). However, patients with severe obstructive vascular disease, usually caused by atherosclerotic plaque narrowing of arteries, are often aged and have tissue-resident and circulating vascular stem/progenitor cells with diminished functionality [31, 32]. These functional deficits may determine poor angiogenic activity in response to hypoxia or ischemia, associated with lower collateral vessel formation and impaired microcirculation [33]. Thus, the regenerative properties of cardiac stem cells might also deteriorate with age resulting in a decreased repair capacity upon injury. Furthermore, in aged tissues, myogenic or angiogenic stem cells may transform into fibroblasts, which contribute to enhanced fibrosis [34, 35]. These combined deficits associated with aging are likely to be the major cause of decreased muscle and weakened vessel regeneration after injury as well as facilitation of atherosclerosis and its sequelae in older individuals [26].

In recent years, evidence has accumulated that it might be possible to reverse stem cell aging at the organism level [36, 37]. Thus, replenishing the function of stem cells either by rejuvenating aged cells or by transplanting young stem/progenitor cells from young donors has been considered an appropriate therapy for age-related diseases. In this review, we describe critical genes and signaling pathways in stem cell aging that are of interest for the cardiovascular system, and discuss *ex vivo* genetic modification approaches aimed at stem cell rejuvenation.

LIMITATIONS OF STEM CELLS IN THE TREATMENT OF AGE-RELATED CARDIOVASCULAR DISEASE

Aging determines the reduction in the capacity for organs, including the heart, to undergo self-repair [31, 38, 39]. Aged organs become compromised after ischemic injury, partially due to the reduced functional capabilities of stem cells [31]. Major obstacles for stem cell therapy in aged people are the decreased functionality of autologous stem cells and difficulties in the engraftment and survival of transplanted stem cells in the pathological microenvironment of the host tissue. A plausible reason for this unfavorable microenvironment is the ischemic host tissue with acidic pH, scar formation, as well as a vascularisation [40]. Moreover, stem cells may be more susceptible to ischemia/reperfusion injury than more developed cells; e.g. cells of the embryonic stem cell-derived cardiac myocyte lineage have been shown to be more sensitive to ischemia/reperfusion-induced injury than neonatal cardiac myocytes [41, 42]. In addition, aging determines the reduction in number, function and paracrine activity of both resident and circulating stem/progenitor cells, as well as the reduction of stem cell resistance to senescence and apoptosis [43]. Furthermore, aging and aging-related cardiovascular risk factors such as diabetes [44] and hyperlipidemia negatively influence endogenous cardioprotective pathways [45, 46] and somatic reprogramming [47, 48]. Collectively, this results in the problem that transplantation of autologous stem cells into adult patients, as well as the transplantation of non-autologous stem cells from young donors into old recipients, is often affected by age-related disease. Moreover, aged or diseased people might not be good donors as aging and/or disease may lead to a poor quality of the stem cell preparation, and may impair the source for stem cells [48]. In this context, the reintegration of stem cell function and paracrine activity by stem cell rejuvenation or transplantation of functional competent

stem/progenitor cells, or by injecting factors that are usually secreted by the cells, can represent possible strategies to treat age-related CVD.

CRITICAL GENES AND SIGNALING PATHWAYS IN STEM CELL AGING AND REJUVENATION

Parabiotic pairings between mice at different ages via shared circulatory system, which expose old mice to factors present in young serum, indicated in 2005 that aging of skeletal muscle stem cells can be reversed [36, 37, 49]. In recent years, it has been suggested that aging can also be reversed in cardiovascular stem cells. For example, Pim-1 kinase has been identified as an anti-senescence factor in cardiac stem cells. Pim-1 enhances proliferation [50], metabolic activity [51] and differentiation [52, 53] of CSCs and mesenchymal stem cells (MSCs) in neovessels and new cardiac myocytes. Pim-1 also serves as a pro-survival mediator by preserving mitochondrial integrity [54] and antagonizing intrinsic apoptotic cascades [55]. Moreover, Pim-1 preserves telomere length and telomerase activity of CSCs [56]. Finally, Mohsin and colleagues have shown that genetic modification of aged human CPCs with Pim-1 kinase results in remarkable rejuvenation of the CPCs associated with enhanced proliferation, increased telomere lengths, and decreased susceptibility to replicative senescence [51] (Fig. **1**).

Several signaling pathways have been identified that revert the process of cardiac senescence. For example, the activation of Notch has been shown to restore the myogenic differentiation capacity of satellite cells, bringing it to a level similar to that of young cells from 20 year old humans [57]. Notch also plays important roles in cardiac differentiation and regeneration. In mice it has been shown that Notch is required for cardiac development and promotes the expansion of cardiac precursor cells [58-60]. Moreover, in zebrafish it has been demonstrated that Notch signaling is required for cardiac regeneration [61] (Fig. **1**). Another pathway involved in stem cell aging is the telomere-telomerase axis. In 1998 it was suggested that the life-span of human cells can be extended by telomerase activation [62]. Since then great effort has been invested to translate this finding to cardiac cells [63, 64]. Telomerase has been demonstrated to maintain telomere length, to contribute to cell survival and proliferation, and to prevent cellular senescence [65, 66] (Fig. **1**). A subpopulation of adipose tissue–derived mesenchymal stromal cell MSCs (AT-MSCs) was recently identified that expresses high levels of myocardin (MYOCD) and the catalytic subunit of telomerase (i.e., telomerase reverse transcriptase or TERT) with antisenescence properties [67, 68]. MYOCD is a key regulator of cardiovascular myogenic development [67, 69-71] and nuclear co-transcription factor for myogenic genes, as well as genes involved in muscle regeneration and protection against apoptosis [69, 72, 73]. AT-MSCs have been shown to contain a population of adult multipotent mesenchymal stem cells with high cardiovascular regenerative potential [67, 74-77]. AT-MSCs that co- express TERT and MYOCD are characterized by high endogenous levels of octamer-binding transcription factor 4(Oct-4), MYOCD, myocyte-specific enhancer factor 2c (Mef2c), and homeobox protein Nkx2.5 [67, 68, 78], as well as decreased cell death both in the form of spontaneous cell death and Fas-induced apoptosis [48]. Therefore TERT and MYOCD may act together to protect AT-MSCs from apoptosis and to enhance their cardiovascular myogenic development [48, 68, 78].

EX VIVO GENE MODIFICATION APPROACH FOR STEM CELL REJUVENATION

Ex vivo cell-based gene delivery represents the most-used strategy to augment regeneration of old and diseased cardiovascular tissues. It consists of removing the cells of interest from the donor, infecting them with a viral vector encoding

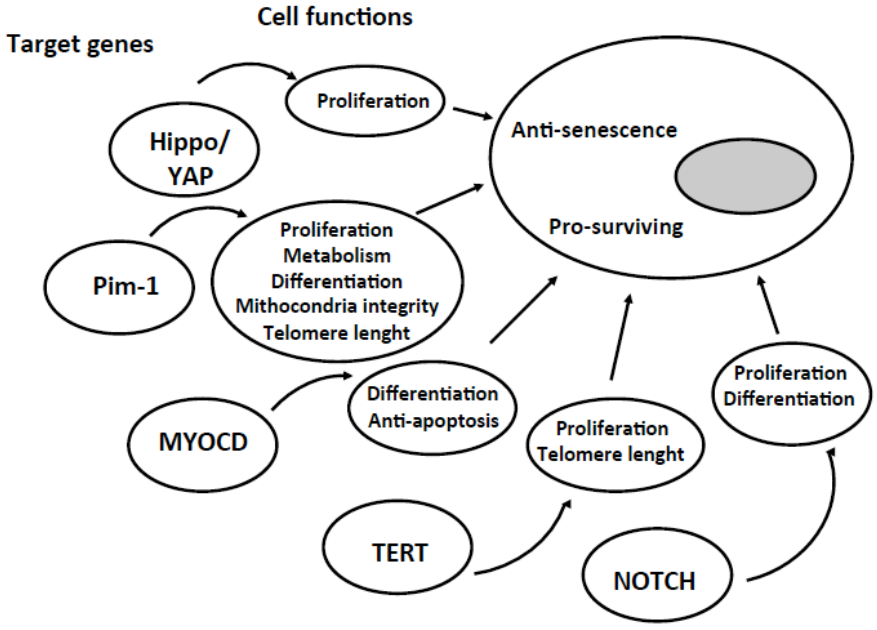

Fig. (1). Candidate genes and cell functions involved in anti-senescence and cell survival. Abbreviations: TERT, telomerase reverse transcriptase; MYOCD, myocardin; Pim-1, pro-viral integration site 1; Hippo, protein kinase Hpo; YAP, Yes-associated protein

Table 1. Pre-clinical studies of stem cell rejuvenation.

Cell Signaling	Rejuvenation Method	*In vivo*/*In vitro* Model	Effect	Ref.
Pim-1 kinase	Lentiviral Pim-1 overexpression	Human CSCs	Anti-sentences, anti-apoptosis, proliferation, differentiation	[51,53,54]
Notch	Notch hyperactivation	Cardiac ampulation in double Tg (hsp70:Gal4); Tg(UAS:NICD) Zebrafish	Cardiac development, differentiation	[58-62]
TERT	Lentiviral-mediated TERT expression	Murine CSCs	Anti-senescence, proliferation	[63,64-67]
MYOCD	Lentiviral-mediated MYOCD overexpression	Murine CSCs	Anti-apoptosis	[68-78]
YAP	Adeno-associated-mediated YAP overexpression	AMI in adult murine myocardium	Cariomyocyte proliferation	[97]
Hippo	Hippo down-regulation	AMI or ampulation in Hippo-deficient adult mouse heart	Cariomyocyte proliferation	[98]
VEGF and bFGF	Porous collagen scaffolds releasing cytokines	Human CSCs	Proliferation	[99]

Legend: VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; TERT, telomerase reverse transcriptase; MYOCD, myocardin; YAP, Yes-associated protein; Hippo, protein kinase Hpo; CSCs, cardiac stem cells; MSCs, mesenchymal stem cells; AMI acute myocardial information; Pim-1, pro-viral integration site 1.

for the therapeutic transgene and then injecting them into the recipient organ [50, 79]. Although this approach requires more cell manipulation with some risk of cell contamination, accumulation of mutations during *in vitro* culture or insertional activation of other genes, it avoid the direct injection of viral vectors *in vivo* [80]. Therefore, transient modulation of cell specification and thereby enhancing myogenic differentiation via e.g. microRNAs could be beneficial approaches. For this, miR-1 and 499 are excellent candidates that enhance both differentiations *in vitro* [81] as *in vivo* and thereby enhance cardiac performance [82]. However, after the overexpression of the gene encoding for the rejuvenating factor followed by *in vitro* proliferation and expansion, genetically modified cells may secrete high amount of the regenerating factor, either transiently or permanently, in the site where they have been transplanted [83, 84]. Recently, the research group of Madonna examined the interplay in MSCs rejuvenation between TERT and MYOCD [48]. They determined the role of TERT and MYOCD in the rejuvenation of aged MSCs [48]. It was found that delivery of the *TERT* and *MYOCD* genes can rejuvenate MSCs from aged mice by increasing cell survival, proliferation, and smooth muscle myogenic differentiation *in vitro* [48]. Furthermore, the improved efficacy of these rejuvenated cells was demonstrated in an *in vivo* hindlimb ischemia model [48].

PERSPECTIVE AND OPEN QUESTIONS

Ex-vivo genetic modification of stem cells may offer an effective strategy for rejuvenating aged stem cells and diseased organs. Additionally, lack of cellular retention is an ongoing problem [85] that needs attention but might be tackled via cardiac tissue engineering and 3D bioprinting [86], as highlighted elsewhere [87, 88]. Further studies, particularly more bench-to-bedside translational work, are needed to clarify the impact of aging and aging-related cardiovascular risk factors on stem cell regeneration and help identifying the genetic as well as pharmacological tools that can rescue aged/sick stem cells as part of personalized medicine. In particular, future research in this field should aim at achieving the following goals:

(1) add fundamental novel information on the pathophysiology of aged stem cells, isolated from aged, atherosclerosis-prone or cardiac infarct patients; (2) design new protocols for stem cell rejuvenation that allow improved preparation and clinical application of stem cells harvested from aged tissues and their products, and (3) design new protocols for *in vivo* application of rejuvenated stem cell therapies.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by grants from the Italian Ministry of University and Scientific Research (PRIN), from the CARIPLO Foundation, and from the Istituto Nazionale Ricerche Cardiovascolari (INRC).

REFERENCES

[1] MERIT-HF Study Group. Effect of metoprolol CR/XL in chronic heart failure: metoprolol CR/XL randomised intervention trial in congestive heart failure (MERIT-HF). Lancet 1999; 353: 2001-7.

[2] Tooke JE. Possible pathophysiological mechanisms for diabetic angiopathy in type 2 diabetes. J Diabetes Complications 2000; 14(4): 197-200.

[3] Hirsch AT, Haskal ZJ, Hertzer NR, *et al.* ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and

Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. Circulation 2006; 113(11): e463-654.

[4] Forrester JS, Price MJ, Makkar RR. Stem cell repair of infarcted myocardium: an overview for clinicians. Circulation 2003; 108(9): 1139-45.

[5] Zimmermann WH, Didie M, Doker S, *et al.* Heart muscle engineering: an update on cardiac muscle replacement therapy. Cardiovasc Res 2006; 71(3): 419-29.

[6] Sharma R, Raghubir R: Stem cell therapy: a hope for dying hearts. Stem Cells Dev 2007; 16(4): 517-36.

[7] Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al.* Embryonic stem cell lines derived from human blastocysts. Science 1998; 282(5391): 1145-7.

[8] Moore KE, Mills JF, Thornton MM. Alternative sources of adult stem cells: a possible solution to the embryonic stem cell debate. Gend Med 2006; 3(3): 161-68.

[9] Zuk PA, Zhu M, Mizuno H, *et al.* Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001; 7(2): 211-28.

[10] Takahashi K, Tanabe K, Ohnuki M, *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131(5): 861-72.

[11] Bergmann O, Bhardwaj RD, Bernard S, *et al.* Evidence for cardiomyocyte renewal in humans. Science 2009; 324(5923): 98-102.

[12] Nadal-Ginard B, Ellison GM, Torella D. The cardiac stem cell compartment is indispensable for myocardial cell homeostasis, repair and regeneration in the adult. Stem Cell Res 2014.

[13] Goumans MJ, de Boer TP, Smits AM, *et al.*TGF-beta1 induces efficient differentiation of human cardiomyocyte progenitor cells into functional cardiomyocytes *in vitro*. Stem Cell Res 2007; 1(2): 138-49.

[14] Smits AM, van Laake LW, den Ouden K, *et al.* Human cardiomyocyte progenitor cell transplantation preserves long-term function of the infarcted mouse myocardium. Cardiovasc Res 2009; 83(3): 527-35.

[15] Servick K. Top heart lab comes under fire. Science 2014; 345(6194): 254.

[16] Couzin-Frankel J. The elusive heart fix. Science 2014; 345(6194): 252-7.

[17] van Berlo JH, Kanisicak O, Maillet M, *et al.* c-kit+ cells minimally contribute cardiomyocytes to the heart. Nature 2014; 509(7500): 337-41.

[18] Smart N, Bollini S, Dube KN, *et al.* De novo cardiomyocytes from within the activated adult heart after injury. Nature 2011; 474(7353): 640-4.

[19] Dey D, Han L, Bauer M, *et al.* Dissecting the molecular relationship among various cardiogenic progenitor cells. Circ Res 2013; 112(9): 1253-62.

[20] Gaetani R, Feyen DA, Doevendans P, *et al*. Different types of cultured human adult Cardiac Progenitor Cells have a high degree of transcriptome similarity. J Mol Cell Cardiol 2014, in press.

[21] Rickard DJ, Kassem M, Hefferan TE, Sarkar G, Spelsberg TC, Riggs BL. Isolation and characterization of osteoblast precursor cells from human bone marrow. J Bone Miner Res 1996; 11(3): 312-24.

[22] Pittenger MF, Mackay AM, Beck SC, *et al.* Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284(5411): 143-7.

[23] Madonna R, Geng YJ, De Caterina R. Adipose tissue-derived stem cells: characterization and potential for cardiovascular repair. Arterioscler Thromb Vasc Biol 2009; 29(11): 1723-29.

[24] Rohani L, Johnson AA, Arnold A, Stolzing A. The aging signature: a hallmark of induced pluripotent stem cells? Aging Cell 2014; 13(1): 2-7.

[25] Wang B, Miyagoe-Suzuki Y, Yada E, *et al.* Reprogramming efficiency and quality of induced Pluripotent Stem Cells (iPSCs) generated from muscle-derived fibroblasts of mdx mice at different ages. PLoS Curr 2011; 3: RRN1274.

[26] Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, *et al.* Aging, progenitor cell exhaustion, and atherosclerosis. Circulation 2003; 108(4): 457-63.

[27] Wu KH, Mo XM, Han ZC, Zhou B. Stem cell engraftment and survival in the ischemic heart. Ann Thorac Surg 2011; 92(5): 1917-25.

[28] Pavo N, Charwat S, Nyolczas N, *et al.* Critical review and summary of the clinical experiences. J Mol Cell Cardiol 2014; 75C: 12-24.

[29] Go AS, Mozaffarian D, Roger VL, *et al*. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. Circulation 2014; 129(3): e28-e292.

[30] Madonna R, De Caterina R. Cellular and molecular mechanisms of vascular injury in diabetes--part II: cellular mechanisms and therapeutic targets. Vascul Pharmacol 2011; 54(3-6): 75-9.

[31] Zenovich AG, Taylor DA. Atherosclerosis as a disease of failed endogenous repair. Front Biosci 2008; 13: 3621-36.

[32] Park JA, Kwon YG. Could circulating progenitor cell count be a barometer for coronary artery disease progression? Circ J 2010; 74(9): 1804-5.

[33] Werner N, Kosiol S, Schiegl T, *et al.* Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005; 353(10): 999-1007.

[34] Trensz F, Haroun S, Cloutier A, Richter MV, Grenier G. A muscle resident cell population promotes fibrosis in hindlimb skeletal muscles of mdx mice through the Wnt canonical pathway. Am J Physiol Cell Physiol 2010; 299(5): C939-47.

[35] Brack AS, Conboy MJ, Roy S, *et al.* Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 2007; 317(5839): 807-10.

[36] Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 2005; 433(7027): 760-4.

[37] Rando TA, Chang HY. Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. Cell 2012; 148(1-2): 46-57.

[38] Porrello ER, Mahmoud AI, Simpson E, *et al.* Transient regenerative potential of the neonatal mouse heart. Science 2011; 331(6020): 1078-80.

[39] Andersen DC, Ganesalingam S, Jensen CH, Sheikh SP. Do Neonatal Mouse Hearts Regenerate following Heart Apex Resection? Stem Cell Reports 2014; 2(4): 406-13.

[40] Don CW, Murry CE. Improving survival and efficacy of pluripotent stem cell-derived cardiac grafts. J Cell Mol Med 2013; 17(11): 1355-62.

[41] Gorbe A, Giricz Z, Szunyog A, Csont T, Burley DS, Baxter GF, Ferdinandy P. Role of cGMP-PKG signaling in the protection of neonatal rat cardiac myocytes subjected to simulated ischemia/reoxygenation. Basic Res Cardiol 2010; 105(5): 643-50.

[42] Gorbe A, Varga ZV, Paloczi J, *et al.*Cytoprotection by the NO-donor SNAP against ischemia/reoxygenation injury in mouse embryonic stem cell-derived cardiomyocytes. Mol Biotechnol 2014; 56(3): 258-64.

[43] Madonna R, Renna FV, Cellini C, *et al.* Age-dependent impairment of number and angiogenic potential of adipose tissue-derived progenitor cells. Eur J Clin Invest 2011; 41(2): 126-33.

[44] Madonna R, Geng YJ, Shelat H, Ferdinandy P, De Caterina R. High glucose-induced hyperosmolarity impacts proliferation, cytoskeleton remodeling and migration of human induced pluripotent stem cells via aquaporin-1. 2014; 1842(11): 2266-75.

[45] Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. Pharmacol Rev 2007; 59(4): 418-58.

[46] Ferdinandy P, Hausenloy D, Heusch G, Baxter GF, Schulz R. Interaction of risk factors, comorbidities and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. Pharmacol Rev 2014, in press.

[47] Sluijter JP, Condorelli G, Davidson SM, *et al*. Novel therapeutic strategies for cardioprotection. Pharmacol Ther 2014; 144(1): 60-70.

[48] Madonna R, Taylor DA, Geng YJ, *et al.* Transplantation of mesenchymal cells rejuvenated by the overexpression of telomerase and myocardin promotes revascularization and tissue repair in a murine model of hindlimb ischemia. Circ Res 2013; 113(7): 902-14.

[49] Sinha M, Jang YC, Oh J, *et al.* Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. Science 2014; 344(6184): 649-52.

[50] Fischer KM, Cottage CT, Wu W, *et al.* Enhancement of myocardial regeneration through genetic engineering of cardiac progenitor cells expressing Pim-1 kinase. Circulation 2009; 120: 2077-87.

[51] Mohsin S, Khan M, Nguyen J, *et al.* Rejuvenation of human cardiac progenitor cells with Pim-1 kinase. Circ Res 2013; 113(10): 1169-79.

[52] Choudhery MS, Khan M, Mahmood R, *et al.* Mesenchymal stem cells conditioned with glucose depletion augments their ability to repair-infarcted myocardium. J Cell Mol Med 2012; 16(10): 2518-29.

[53] Zippo A, De Robertis A, Bardelli M, Galvagni F, Oliviero S. Identification of Flk-1 target genes in vasculogenesis: Pim-1 is required for endothelial and mural cell differentiation *in vitro*. Blood 2004; 103(12): 4536-44.

[54] Borillo GA, Mason M, Quijada P, *et al.* Pim-1 kinase protects mitochondrial integrity in cardiomyocytes. Circ Res 2010; 106(7): 1265-74.

[55] Yan B, Zemskova M, Holder S, *et al.* The PIM-2 kinase phosphorylates BAD on serine 112 and reverses BAD-induced cell death. J Biol Chem 2003; 278(46): 45358-67.

[56] Mohsin S, Khan M, Toko H, *et al.* Human cardiac progenitor cells engineered with Pim-I kinase enhance myocardial repair. J Am Coll Cardiol 2012; 60(14): 1278-87.

[57] Carlson ME, Suetta C, Conboy MJ, *et al.* Molecular aging and rejuvenation of human muscle stem cells. EMBO Mol Med 2009; 1(8-9): 381-91.

[58] Zhao C, Guo H, Li J, *et al.* Numb family proteins are essential for cardiac morphogenesis and progenitor differentiation. Development 2014; 141(2): 281-95.

[59] Nemir M, Metrich M, Plaisance I, *et al.* The Notch pathway controls fibrotic and regenerative repair in the adult heart. Eur Heart J 2014; 35(32): 2174-85.

[60] Yang J, Bucker S, Jungblut B, *et al.* Inhibition of Notch2 by Numb/Numblike controls myocardial compaction in the heart. Cardiovasc Res 2012; 96(2): 276-285.

[61] Zhao L, Borikova AL, Ben-Yair R, *et al.* Notch signaling regulates cardiomyocyte proliferation during zebrafish heart regeneration. Proc Natl Acad Sci USA 2014, 111(4): 1403-08.

[62] Bodnar AG, Ouellette M, Frolkis M, *et al.* Extension of life-span by introduction of telomerase into normal human cells. Science 1998; 279(5349): 349-52.

[63] Brandt S: TERT over-expression affects the growth of myocardial tissue derived from mouse embryonic stem cells. Differentiation 2010; 79(1): 1-8.

[64] Wong LS, Oeseburg H, de Boer RA, van Gilst WH, van Veldhuisen DJ, van der Harst P. Telomere biology in cardiovascular disease: the TERC-/- mouse as a model for heart failure and ageing. Cardiovasc Res 2009; 81(2): 244-52.

[65] Jan HM, Wei MF, Peng CL, Lin SJ, Lai PS, Shieh MJ. The use of polyethylenimine-DNA to topically deliver hTERT to promote hair growth. Gene Ther 2011; 19(1): 86-93.

[66] Qu Y, Duan Z, Zhao F, *et al.* Telomerase reverse transcriptase upregulation attenuates astrocyte proliferation and promotes neuronal survival in the hypoxic-ischemic rat brain. Stroke 2011; 42(12): 3542-50.

[67] Madonna R, Willerson JT, Geng YJ. Myocardin a enhances telomerase activities in adipose tissue mesenchymal cells and embryonic stem cells undergoing cardiovascular myogenic differentiation. Stem Cells 2008; 26(1): 202-11.

[68] Madonna R, Wu D, Wassler M, De Caterina R, Willerson JT, Geng YJ. Myocardin-A enhances expression of promyogenic genes without depressing telomerase activity in adipose tissue-derived mesenchymal stem cells. Int J Cardiol 2012; 167(6): 2912-21.

[69] Madonna R, Geng YJ, Bolli R, *et al.* Co-Activation of Nuclear Factor-kappaB and Myocardin/Serum Response Factor Conveys the Hypertrophy Signal of High Insulin Levels in Cardiac Myoblasts. J Biol Chem 2014; 289(28): 19585-98.

[70] Ueyama T, Kasahara H, Ishiwata T, Nie Q, Izumo S. Myocardin expression is regulated by Nkx2.5, and its function is required for cardiomyogenesis. Mol Cell Biol 2003; 23(24): 9222-32.

[71] Wang Z, Wang DZ, Pipes GC, Olson EN. Myocardin is a master regulator of smooth muscle gene expression. Proc Natl Acad Sci USA 2003; 100(12): 7129-34.

[72] Cao XL, Hu XM, Hu JQ, Zheng WX. Myocardin-related transcription factor-A promoting neuronal survival against apoptosis induced by hypoxia/ischemia. Brain Res 2011; 1385: 263-74.

[73] Huang J, Min Lu M, Cheng L, *et al.* Myocardin is required for cardiomyocyte survival and maintenance of heart function. Proc Natl Acad Sci USA 2009; 106(44): 18734-9.

[74] Miranville A, Heeschen C, Sengenes C, Curat CA, Busse R, Bouloumie A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. Circulation 2004; 110(3): 349-55.

[75] Fraser JK, Schreiber R, Strem B, *et al.* Plasticity of human adipose stem cells toward endothelial cells and cardiomyocytes. Nat Clin Pract Cardiovasc Med 2006; 3 Suppl 1: S33-37.

[76] Gaustad KG, Boquest AC, Anderson BE, Gerdes AM, Collas P. Differentiation of human adipose tissue stem cells using extracts of rat cardiomyocytes. Biochem Biophys Res Commun 2004; 314(2): 420-7.

[77] Traktuev DO, Merfeld-Clauss S, Li J, *et al.* A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. Circ Res 2008; 102(1): 77-85.

[78] Madonna R, De Caterina R, Willerson JT, Geng YJ. Biologic function and clinical potential of telomerase and associated proteins in cardiovascular tissue repair and regeneration. Eur Heart J 2011; 32(10): 1190-6.

[79] Cho HC, Marban E. Biological therapies for cardiac arrhythmias: can genes and cells replace drugs and devices? Circ Res 2010; 106: 674-85.

[80] Gansbacher B, European Society of Gene Therapy. Report of a second serious adverse event in a clinical trial of gene therapy for X-linked severe combined immune deficiency (X-SCID). Position of the European Society of Gene Therapy (ESGT). J Gene Med 2003; 5: 261-2.

[81] Sluijter JP, van Mil A, van Vliet P, *et al.* MicroRNA-1 and -499 regulate differentiation and proliferation in human-derived cardiomyocyte progenitor cells. Arterioscler Thromb Vasc Biol 2010; 30(4): 859-68.

[82] Hosoda T, Zheng H, Cabral-da-Silva M, *et al.* Human cardiac stem cell differentiation is regulated by a mircrine mechanism. Circulation 2011; 123(12): 1287-96.

[83] Rando TA, Wyss-Coray T. Stem cells as vehicles for youthful regeneration of aged tissues. J Gerontol A Biol Sci Med Sci 2014; 69 Suppl 1: S39-42.

[84] Siddiqi S, Sussman MA. Cell and gene therapy for severe heart failure patients: the time and place for Pim-1 kinase. Expert Rev Cardiovasc Ther 2013; 11(8): 949-57.

[85] Feyen D, Gaetani R, Liu J, *et al.* Increasing short-term cardiomyocyte progenitor cell (CMPC) survival by necrostatin-1 did not further preserve cardiac function. Cardiovasc Res 2013; 99(1): 83-91.

[86] Gaetani R, Doevendans PA, Metz CH, *et al.* Cardiac tissue engineering using tissue printing technology and human cardiac progenitor cells. Biomaterials 2012; 33(6): 1782-90.

[87] Gaetani R, Rizzitelli G, Chimenti I, *et al.* Cardiospheres and tissue engineering for myocardial regeneration: potential for clinical application. J Cell Mol Med 2010; 14(5): 1071-7.

[88] Patra C, Boccaccini A, Engel F. Vascularisation for cardiac tissue engineering: the extracellular matrix. Thromb Haemost 2014; 113(1).

Received: October 09, 2014 **Revised: November 05, 2014** **Accepted: December 01, 2014**