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The double life of cardiac mesenchymal cells: Epimetabolic sensors and therapeutic assets for heart regeneration

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ABSTRACT

Organ-specific mesenchymal cells naturally reside in the stroma, where they are exposed to some environmental variables affecting their biology and functions. Risk factors such as diabetes or aging influence their adaptive response. In these cases, permanent epigenetic modifications may be introduced in the cells with important consequences on their local homeostatic activity and therapeutic potential. Numerous results suggest that mesenchymal cells, virtually present in every organ, may contribute to tissue regeneration mostly by paracrine mechanisms. Intriguingly, the heart is emerging as a source of different cells, including pericytes, cardiac progenitors, and cardiac fibroblasts. According to phenotypic, functional, and molecular criteria, these should be classified as mesenchymal cells. Not surprisingly, in recent years, the attention on these cells as therapeutic tools has grown exponentially, although only very preliminary data have been obtained in clinical trials to date. In this review, we summarized the state of the art about the phenotypic features, functions, regenerative properties, and clinical applicability of mesenchymal cells, with a particular focus on those of cardiac origin.

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1. Mesenchymal stem cells: a matter of definition

Mesenchymal stem cells (MSCs) are currently considered as one the most promising tools in regenerative medicine. These cells were first isolated by Friedenstein and coworkers, more than 45 years ago, from the bone marrow (BM) and identified as a plastic-adherent, colony-forming unit fibroblast (CFU-F) sub-population (hereafter defined as BM-MSC) (Friedenstein et al., 1968, 1970). After many years dedicated to their characterization, the presence of cells with mesenchymal properties is now recognized in virtually all postnatal organs and tissues

Abbreviations: bFGF, basic fibroblast growth factor; BM, bone marrow; BM-MSCs, bone marrow-derived mesenchymal stem cells; CMSCs, cardiac mesenchymal stem cells; CVD, cardiovascular disease; HAT, histone acetyltransferases; HDAC, histone deacetylase; iCMs, induced cardiomyocytes; MSCs, mesenchymal stem cells; MSC-DE, exosome derived from MSCs; Sirts, sirtuins.

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(da Silva Meirelles et al., 2006). In brief, MSCs have been successfully isolated from a variety of sources including adipose tissue, umbilical cord, saphenous and umbilical veins, lung, heart, endometrium, chorionic villi, peripheral blood, olfactory bulb, meniscus, dental pulp, and breast milk, to mention some of the best characterized sources (Zvaifler et al., 2000; De Bari et al., 2001; Zuk et al., 2001; Sabatini et al., 2005; da Silva Meirelles et al., 2006; Jang et al., 2014; Y.S. Huang et al., 2015). At present, it has been generally agreed that the biological properties of MSCs might differ according to their tissue of origin in vivo, although once isolated and cultured in vitro, all of them share common features such as fibroblast-like morphology, a typical immune phenotype, and the capacity to differentiate into a number of more specialized lineages. In 2006, the International Society for Cellular Therapy (ISCT) defined for the first time the MSCs as adult stem cells characterized by: (i) the ability to adhere to plastic; (ii) the presence of a number of defined surface makers, and (iii) the capacity of a multilineage differentiation (Dominici et al., 2006). Indeed, MSCs can easily differentiate into osteoblasts, chondrocytes, and adipocytes (Dominici et al., 2006). It has been recently demonstrated that under appropriate conditions, these cells can differentiate into several types of cells including neurons (Wakao et al., 2014; Kim et al., 2015) or skeletal and cardiac precursors (Toma et al., 2002; Gang et al., 2004; Vecellio et al., 2012). Therefore, in comparison to hematopoietic CD34+ stem cells, MSCs seem to be quite heterogeneous and difficult to define according to a unique set of markers or their biological features. However, it is commonly accepted that a cell population defined to be of mesenchymal origin should have at least 95% positivity for the surface antigens CD105, CD73, and CD90 and <2% positivity for immune-hematopoietic markers including CD45, CD34, CD14, CD79α, and HLA-DR (Dominici et al., 2006). Hence, there is a constant search for specific common molecular parameters to better define these cells. They include miRNAs or additional surface markers such as Sca-1, Stro-1, GD2, SSEA4, CD24, CD49a, CD146, CD200, CD271, MSCA-1 (W8B2), PDGFRα, and PDGFRβ (Buhring et al., 2007; Wong et al., 2015). The oldest evidence of their ability to adhere to plastic rapidly, however, remains probably one of the best features shared by all MSCs.

1.1. In vivo localization

For many years, the origin and distribution of MSCs have been much debated (da Silva Meirelles et al., 2008). As MSCs have been originally isolated from BM (Friedenstein et al., 1968, 1970), this body district was originally believed to be their unique source, implying an active recruitment to local or distal damaged tissues to exert their regenerative properties. Indeed, the plasma membrane of BM-MSC is enriched in chemokine receptors. This has been demonstrated by in vivo experiments that investigated the homing ability of these cells (Nagaya et al., 2004; Mouiseddine et al., 2007) and their biodistribution in nonhematopoietic tissues (Devine et al., 2003). However, the identification and characterization of other BM-derived stem cell populations weakened the original hypothesis about the BM as the sole source for these cells (da Silva Meirelles et al., 2006). The endothelial progenitor cells (EPCs) (Asahara et al., 1997) sharing common migration and biological properties with BM-MSCs are an example.

The cogent demand to understand more about their therapeutic potential prompted more research on the characterization of MSCs resident in specific organs (Zvaifler et al., 2000; De Bari et al., 2001; Zuk et al., 2001; Sabatini et al., 2005; da Silva Meirelles et al., 2006; Jang et al., 2014; Y.S. Huang et al., 2015). In normal conditions, organ-specific MSCs are quiescent, can self-renew at very low rate, and are considered as a sort of “health sensor” of the hosting tissue (Caplan, 1991). Located in the stroma, they are constantly exposed to environmental micro-changes. They react to these micro-changes by employing different types of interventions including the secretion of cytokines or the direct transdifferentiation into organ-specific cells (Zvaifler et al., 2000; Zuk et al., 2001; De Bari et al., 2001; Sabatini et al., 2005; da Silva Meirelles et al., 2006; Jang et al., 2014; Y.S. Huang et al., 2015).

As the endothelium represents a ubiquitous component of the whole body, recent evidence assigned to the pericytes a fundamental role in the maintenance of the vascular homeostasis and architectural regeneration (Invernici et al., 2007; Campagnolo et al., 2010; Katare et al., 2011). The detailed relationship between MSCs and pericytes has been discussed in many review articles (Caplan, 2008; Wong et al., 2015; da Silva Meirelles et al., 2016a). In the present review, we report only the main aspects of these cell populations. Pericytes and MSCs share, indeed, several in vivo and in vitro characteristics including their morphology and the expression of some of the markers adopted by the International Society of Cellular Therapy, such as CD105, CD90, and CD73 (Dominici et al., 2006), as well as other less canonical mesenchymal markers including Sca-1, CD146, and PDGFRβ (Wong et al., 2015). Because of their mesenchymal nature, the pericytes may differentiate into osteoblasts, adipocytes, and chondrocytes and have a transcriptome (da Silva Meirelles et al., 2016b) and a secretome that largely overlap with that of BM-MSCs; this suggests that pericytes also have the capacity to synthesize immune modulators, inflammatory molecules, and trophic factors (da Silva Meirelles et al., 2015). Similarly to BM-MSCs, in fact, pericytes may be active players during regenerative processes. When a tissue-damaging injury occurs, the pericytes interrupt their physical association with endothelial cells, start proliferating, and move to the site of damage, releasing pro-angiogenic biomolecules (Wong et al., 2015). Indistinct from BM-MSCs or other MSCs, the pericytes appear to be involved in the regeneration of skeletal muscle (Diaz-Manera et al., 2012), lung (Johnson et al., 2015), and heart (Avolio et al., 2015a, 2015b). Therefore, BM-MSCs, tissue-specific MSCs, and pericytes may be considered to be phenotypically and functionally comparable, and the so-called “perivascular niche” might be their common site of localization (Ozen et al., 2012; da Silva Meirelles et al., 2015).

1.2. Biological functions

As described above, MSCs have been identified potentially in all tissues of the body. The molecular mechanisms responsible for the abilities of MSCs to maintain the mesenchymal pool in the tissue and simultaneously participate in tissue homeostasis and regeneration are still under investigation. Despite a similar antigenic repertoire, MSCs behave sometimes in different ways according to their origin (Kim et al., 2007; Hass et al., 2011; Rossini et al., 2011). In 2010, Collas gave an interesting explanation to this phenomenon (Collas, 2010). He stated that MSCs display a pre-determined epigenetic profile established at the embryonic stage. In fact, it has been identified that a specific histone modification pattern, the so-called “bivalent domain,” can regulate gene expression and consequently determine the fate of embryonic stem cells (ESCs) (Bernstein et al., 2006). Specifically, H3K27me3 and H3K4me enrichment at particular loci regulates stemness and commitment of ESCs. The same post-translational modifications together with H3K9me3 might influence the lineage specification and the signaling and metabolic processes of MSCs during adulthood. Their epigenetic state ensures a certain level of plasticity, which permits MSCs to overcome lineage barriers and to maintain tissue-specific identity, thus avoiding differentiation in unwanted lineages (Collas, 2010). Also, DNA methylation is associated with MSC plasticity. MSCs derived from different origin displayed similar but not identical methylation profile at the lineage specification promoter (Sorensen et al., 2010a, 2010b). In addition, as analyzed in detail in Section 2.1, alteration in the epigenetic landscape, including DNA methylation, was found in MSCs obtained from diseased organs (Vecellio et al., 2014).

However, the regenerative potential of MSCs mostly depends on their secretory activity exerting important paracrine effects on neighboring cells (Caplan & Dennis, 2006; Caplan, 2007). Nevertheless, MSCs have also been reported to physically interact with other stromal or parenchymal cells, exchanging exosomes or mitochondria (Spees et al., 2006;

Thery et al., 2009; Zoller, 2009; Pasquier et al., 2013; Liu et al., 2014; Yu et al., 2014). In this context, a large body of literature has emphasized on the characterization of their secretome and on the potential therapeutic application of MSC-derived bioactive molecules in regenerative medicine (Spaggiari et al., 2008; Meirelles Lda et al., 2009; Jang et al., 2014).

1.2.1. Secreted factors

Among the most commonly secreted factors, six different families of bioactive molecules have been reported as important for the regenerative properties of MSCs and recently classified as follows: I. homeostatic molecules; II. immunomodulatory cytokines; III. anti-apoptotic factors; IV. angiogenic factors; V. chemoattractants; and VI. anti-scarring bioagents (Meirelles Lda et al., 2009). Class I molecules are secreted by MSCs mostly to support the homeostasis of niches of tissue-specific precursors and to stimulate the activation of other bioactive cells such as macrophages (Caplan & Dennis, 2006; Caplan, 2007). Specifically, the stem cell factor (SCF), the leukemia inhibitory factor (LIF), the stromal cell-derived factor-1 (SDF-1), and the macrophage colony-stimulating factor (M-CSF) belong to this class.

The cross-talk between MSCs and the immune system, mediated by secretory compounds belonging to class II, is emerging as one of the main advantages of MSCs in cell therapy applications. Indeed, MSCs can positively regulate both the innate and adaptive immune response, recruiting cells into tissues by releasing inflammatory cytokines or other modulators such as LIF, interleukin 10 (IL-10), tumor growth factor β (TGF-β), hepatocyte growth factor (HGF), prostaglandin E2 (PGE-2), nitric oxide (NO), and indoleamine-pyrrole 2,3-dioxygenase (IDO). Furthermore, MSCs might exert an important immunosuppressive effect as they can act as suppressors of T- and B-cells (Bartholomew et al., 2002; Corcione et al., 2006), natural killer (NK) cells, (Spaggiari et al., 2008) and antigen-presenting dendritic cells (Jiang et al., 2005).

At the site of tissue damage, MSCs can recruit additional cell populations, stimulate angiogenesis, and counteract apoptosis by secretion of the growth factors grouped in classes III, IV, and V that include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), TGF-β, interleukin 6 (IL-6), insulin-like growth factor (IGF-1), and HGF. Finally, in several organs, including the heart and lungs, MSCs have been reported to play an important regulatory role during the fibrotic reaction occurring after an injury or in the presence of chronic inflammation (Usunier et al., 2014). Indeed, it has been reported that molecules of class VI, secreted by injected MSCs, may exert an anti-scarring activity due to the production of HGF and bFGF (L. Li et al., 2008) and, most importantly, by the secretion of matrix metalloproteinases (MMP-2 and MMP-9) (Mias et al., 2009).

1.2.2. Exosomes

The release of exosomes is another important mechanism by which MCSs exert their therapeutic effects. Exosomes are cell-derived microvesicles of 30–100 nm diameter that can transport nucleic acids and proteins, transferring them from one cell type to another (Yu et al., 2014). Exosomes can regulate important physiological and pathological processes such as antigen presentation, angiogenesis, inflammation (Thery et al., 2009), tumor progression, metastasis, and the distal diffusion of pathogens or oncogenes (Zoller, 2009). Purified exosomes, derived from MSCs (MSC-DE), have been injected and tested in several animal models. Briefly, Lai and coworkers showed the positive effect of MSC-DE in the heart after ischemia/reperfusion injury (Lai et al., 2010), while Xin and co-authors showed that the systemic administration of MSC-DE in a rat model of stroke could be beneficial by reducing tissue damage and accelerating recovery (Xin et al., 2013). Further, MSC-DE have been also proved to protect the kidney from damage (Reis et al., 2012), to enhance skeletal muscle regeneration (Nakamura et al., 2015), and to facilitate protective immune responses. It is noteworthy that MSC-DE have been recently linked to epigenetic and metabolic changes. For example, Vallabhaneni and co-authors showed that MSC-DE contained miRNAs, proteins, and metabolites and can regulate gene expression and tumor growth (Vallabhaneni et al., 2015). Consistently, Arslan and co-workers showed that intact MSC-DE increased ATP and NADH levels, decreased oxidative stress, and activated pro-survival signaling pathways, thereby enhancing cardiac function after myocardial ischemia/reperfusion injury (Arslan et al., 2013) further suggesting their therapeutic potential for cardiovascular disease (CVD) (L. Huang et al., 2015).

1.2.3. Mitochondria exchange

The physical evidence of an MSC-dependent active cell-to-cell transfer of the mitochondria clarified that MSCs might directly affect the intracellular metabolism of recipient organs/organisms. In 2006, Spees et al. described the ability of MSCs to restore the respiration chain in somatic cells depleted of mtDNA (Spees et al., 2006). More recently, the exchange of the mitochondria, through the actin-based extension of tunneling nanotubes and the presence of gap junctions enriched in connexin 43 (Cx43), has been thoroughly demonstrated (Pasquier et al., 2013; Liu et al., 2014). In the cardiovascular system, MSC-directed mitochondrial transfer induced vascular smooth muscle cell proliferation (Vallabhaneni et al., 2012), rescued injured endothelial cells in an in vitro ischemia/reperfusion model (Liu et al., 2014), and contributed to the reprogramming of adult cardiomyocytes toward a progenitor-like stage (Acquistapace et al., 2011).

2. Mesenchymal stem cells of the heart: origin, nature, and function

Many different cell types contribute to the maintenance of cardiac function and to its homeostatic equilibrium. Among them, those that have been more extensively characterized are cardiomyocytes, endothelial cells, leukocytes, and the so-called “cardiac MSCs” (Pinto et al., 2016). For years, the post-neonatal heart was considered a stable terminally differentiated post-mitotic organ, where myocardial cell proliferation does not normally occur and where the cells are kept indefinitely in the G_0 phase of the cell cycle (Poolman & Brooks, 1998). More recently, this vision has been changed by the evidence of a constant cellular turnover occurring in different cardiac districts albeit at a very low rate. The recent report from Bergmann and coworkers, in fact, well describes this process (Bergmann et al., 2015). On the basis of the residual amount of ^{14}C stably incorporated into the human genome, the authors showed the number of human cardiomyocytes to remain constant during the majority of the life span, with an exponential decrease occurring upon aging. On the contrary, the number of noncardiomyocytes cells such as endothelial cells and cardiac mesenchymal stem cells (CMSCs) increased during adulthood, revealing a high rate of turnover (Bergmann et al., 2015).

Intriguingly, Pinto and co-workers showed the impossibility to clearly distinguish cardiac pericytes and fibroblasts from CMSCs (Pinto et al., 2016). Transcriptome and fluorescence-activated cell sorting (FACS) analyses revealed only a limited number of variances, possibly justified by differences in isolation protocols and in the number of in vitro passages before assay (Halfon et al., 2011; Gaetani et al., 2014). On the basis of these findings, Bergmann and coworkers defined CMSCs according to negative criteria such as the absence of cardiomyocytes and endothelial specific markers (Bergmann et al., 2015). They reported CMSCs as negative for the expression of cardiac pericentriolar material 1 (PCM-1) and endothelial *Ulex europaeus* agglutinin I (UEA-I) (Bergmann et al., 2015). Unfortunately, from both a biological and a translational point of view, an unambiguous definition of CMSCs is still missing. The field is additionally complicated by a vast literature often referring to CMSCs as cardiac precursor cells (CPCs) (Barile et al., 2007; Ott et al., 2007; Bollini et al., 2011). These cells are identified by a specific immunophenotype, which includes epitopes associated with classical mesenchymal markers such as CD90 and Sca-1 (Barile et al., 2007; Bollini et al., 2011; Gambini et al., 2011). Interestingly, CPCs show an immune phenotype and exert functions typical of MSCs, including a multilineage differentiation potential characterized by the

ability to differentiate efficiently into cardiomyocytes. To simplify the topic, and because of a large number of shared functions and the common biomarkers expressed by all of them, in this review article, we will always refer to CMSCs as a general population including cells described in the literature as CPCs, pericytes, or cardiac fibroblasts, although the latter cell population showed a limited differentiation potential (Alt et al., 2011) with the ability to generate functional myocytes under appropriate conditions (Santiago et al., 2010; Driesen et al., 2014; Furtado et al., 2014).

2.1. Cardiac mesenchymal stem cells: sensors of the heart in health and disease

Despite the similarity with the mesenchymal component of the BM, CMSCs are mesodermal cells stemming out from the proepicardium a consequence of a process of intramyocardial migration and epithelial-to-mesenchymal transition (EMT) (Dettman et al., 1998; Smith et al., 2011; Germani et al., 2015). Indeed, good evidence of the proepicardial origin of mammalian CMSCs has been provided by Chong and co-workers who utilized the transgenic mice tracing technique to follow CMSC progenitors from different sources during embryonic development (Chong et al., 2011) (see Table 1).

During adulthood, CMSCs localize into the stroma of the heart, where, in a resting state, they play the role of homeostatic “sensors” (Caplan, 1991). When an injury occurs, the plasticity of CMSCs enables them to actively participate in the process of cardiac remodeling (Souders et al., 2009). In vivo, they can either repopulate the cardiac stem cell reservoir or directly contribute to the preservation of the physical and mechanical integrity of the cardiac structure through extracellular matrix (ECM) deposition/remodeling (Souders et al., 2009). Because of their interconnection with cardiomyocyte gap junctions enriched in Cx43 and Cx45, CMSCs might even participate in the reconstitution of the cardiac electrical conduction system (Gaudesius et al., 2003; Zeigler et al., 2015). In vitro, they can recapitulate all the mesodermal lineages (Rossini et al., 2011; Zhang et al., 2015), remaining, however, biochemically distinguishable from BM-MSCs, as indicated by the comparative analysis of their proteome, transcriptome, and miRNAome (Rossini et al., 2011; Meraviglia et al., 2014).

According to their method of isolation, CMSCs can be distinguished into two subgroups: unselected CMSCs and selected CMSCs. While selected CMSCs are isolated by a purification protocol based on the expression of specific markers, for several years, unselected CMSCs have been isolated and characterized by the nonspecific criterion of plastic adherence (Chong et al., 2011; Rossini et al., 2011; Zhang et al., 2015). Independent of the procedures mentioned above, CMSCs cultured *ex vivo* express the typical mesenchymal markers (CD105, CD73, CD29, and CD44), including the pericyte marker CD146, and are positive for the fibroblast protein vimentin as well as for several other surface antigens such as CD31, c-Kit, or CD144 (Rossini et al., 2011; Vecellio et al., 2012). Notably, selected CMSCs can be obtained from unselected CMSCs by a subsequent round of FACS performed using a specific marker such as CD44 (Carlson et al., 2011), W8B2 (Zhang et al., 2015), or Sca-1 (Ryzhov et al., 2014). In addition, cells obtained from the human atrium and purified according to the expression of the CPC marker c-Kit (c-Kit+), once cultured in vitro, showed an immunophenotype similar to that of unselected CMSCs (Gambini et al., 2011). In these experiments, the percentage of c-Kit + cells decreased progressively with the number of passages, although the most common mesenchymal markers remained stable. Remarkably, this process occurred without compromising their properties of cardiovascular precursor cells (Gambini et al., 2011). Accordingly, although still under debate (Hatzistergos & Hare, 2016), recent studies have shown that c-Kit + cells *per se* minimally differentiate into cardiomyocytes (van Berlo et al., 2014) and are mostly prone to differentiate into endothelial cells (Sultana et al., 2015) (see Table 1.). Indeed, a number of studies showed no difference between the regenerative properties of unselected CMSCs (Rossini et al., 2011; Zhang et al., 2015) and those of selected CMSCs, purified according the presence of Sca-1 or CD44 (Oh et al., 2003) and directly injected into the heart of animal models with myocardial infarction (MI) (Wang et al., 2006). Indeed, both populations were reported to contribute to either scar formation or tissue repair (Wang et al., 2006; Carlson et al., 2011; Fioret et al., 2014).

CMSCs can sense both the physiological and pathological states of the heart. CMSCs obtained from type II diabetic patients (D-CMSC) were impaired in proliferation and differentiation and underwent senescence faster than CMSCs derived from nondiabetic patients (Vecellio et al., 2014). In this condition, D-CMSCs showed an altered epigenetic landscape with the prevalence of repressive histone marks and an enrichment in repressive DNA methylation (Cencioni et al., 2014; Vecellio et al., 2014). Accordingly, Bastianelli and coauthors, after exposure of CMSCs to high glucose, found molecular alterations involving the function of transcription factor Egr-1, leading to reduced level of histone acetylation (Bastianelli et al., 2014). Moreover, it has been reported that the level of O-GlcNAcylation is increased in diabetes (Ngoh et al., 2010)

Table 1
Stem cells derived from mesodermal lineage.

Cell type	Origin	Feature	Marker	Reference
Cardiac mesenchymal stem cells (CMSCs)	Mesoderm	Multilineage differentiation potential. Homeostatic “sensors.” Cardiac remodeling.	PCM-1 negative, UEA I negative, CD90, Sca-1, CD105, CD73, CD29, CD44, CD146, CD31, c-Kit, CD144, W8B2	Bergmann et al., 2015 Barile et al., 2007 Bollini et al., 2011 Ryzhov et al., 2014 Gambini et al., 2011 Rossini et al., 2011 Vecellio et al., 2012 Carlson et al., 2011 Zhang et al., 2015
Cardiac precursor cells (CPCs)	Mesoderm	Multilineage differentiation potential. Differentiation into cardiomyocytes.	CD90, Sca-1, c-kit	Barile et al., 2007 Bollini et al., 2011 Gambini et al., 2011
Cardiac fibroblasts	Mesoderm	Physical and mechanical preservation of the heart. Scar formation. Tissue repair. Differentiation into myocytes.	Vimentin, CD44, CD73, CD105	Souders et al., 2009 Pinto et al., 2016 Rossini et al., 2011 Vecellio et al., 2012
Pericytes	Mesoderm	Release of pro-angiogenic biomolecules. Regeneration of skeletal muscle, lung, and heart.	CD105, CD90, CD73, Sca-1, CD146, PDGFRβ	Dominici et al., 2006 Wong et al., 2015
Hematopietic stem cells (HSCs)	Mesoderm	Hematopoiesis.	CD34, CD45, CD14, CD79, HLA-DR	Dominici et al., 2006

and affects the function of CMSCs (Zafir et al., 2015). Aging and heart failure can alter the in vivo pool and the normal homeostasis of CMSCs. In light of this finding, studies by Cesselli and Avolio demonstrated that CMSCs obtained from aged and failing explanted hearts showed in vitro senescence and impairment in molecular signaling fundamental to drive CMSC activation and function compared with controls (Cesselli et al., 2011; Avolio et al., 2014). In a recent study, Sommariva and coworkers showed that arrhythmogenic cardiomyopathy (ACM) modulates CMSC (Sommariva et al., 2015) epigenetics. In this genetic disease, the cardiac tissue is progressively substituted by the fibro-adipose tissue (Asimaki et al., 2015), and CMSCs undergo adipogenic differentiation by a plakophilin 2 (PKP2)-dependent mechanism, actively contributing to the pathological conversion of the cardiac tissue (Sommariva et al., 2015) (see Table 1.). This evidence opens up new potential interventions for arrhythmogenic cardiomyopathy treatment specifically targeted to CMSCs (Sommariva et al., 2015).

The following section will explore in detail the most commonly reported epigenetic signatures of CMSCs that have potential consequences on their physiology and therapeutic potential.

3. Cardiac epimetabolic alterations of cardiac mesenchymal stem cells

In this section, we will limit our analysis to some of the most recent observations linking epigenetics and metabolism with special attention paid to their association with adult stem cells and cardiac diseases. Specifically, we will consider the impact that epimetabolic alterations may have on MSCs with particular emphasis on those present in the heart.

3.1. Histone-modifying enzymes

Stress signals, including oxidative stress or hypoxia, can trigger cardiac hypertrophy, a process typically paralleled by the activation of molecular pathways normally present during the cardiac embryonic development (Chien, 1999; Lowes et al., 2002). Notably, several epigenetic enzymes have been implicated in these processes and are often reported to play a protective role (Zhang et al., 2002; Delgado-Olguin et al., 2012; Matsushima & Sadoshima, 2015). In this regard, Zhang and coworkers provided the evidence that class II histone deacetylases ($HDAC_{II}$) protect from the initiation of transcriptional programs associated with cardiac hypertrophy and heart failure. Specifically, the authors found that hypertrophic stimuli induced the HDAC kinase PKD1 (Trivedi et al., 2007), leading to phosphorylation and consequent inactivation of HDAC5 and HDAC9. The resulting increase in the acetylation of fetal cardiac genes contributed to the heart damage (Zhang et al., 2002). Furthermore, class I HDACs ($HDAC_I$), including HDAC2, have been reported as positive regulators of some hypertrophic responses by inhibiting anti-hypertrophic signals such as those provided by the e Inpp5f–Akt-Gsk3β axis (Trivedi et al., 2007). Interestingly, both intracellular and extracellular cues regulate HDAC activity. Endogenously, the intracellular level of the β-hydroxybutyrate, a metabolite derived from liver during fasting, can inhibit HDACs, protecting cells from the consequences of excessive oxidative stress (Shimazu et al., 2013). Zou and coworkers demonstrated that in fasting rats, the increase in β-hydroxybutyrate levels protects from ischemia/reperfusion injury by reducing cardiac cell apoptosis and the myocardial infarct size after coronary artery occlusion (Zou et al., 2002). Additional work highlighted the protective role of HDAC inhibitors such as trichostatin A (TSA), valproic acid (VPA), and suberoylanilide hydroxamic acid (SAHA), which act as blockers of the cardiac hypertrophic response (Antos et al., 2003; Kook et al., 2003; Kee et al., 2006; Kong et al., 2006; Ooi et al., 2015). Class III HDACs ($HDAC_{III}$), known as Sirtuins (Sirts), have also been the subject of intensive studies for their effect on the heart. Sirts activity is strictly interconnected to cellular metabolism, as they are dependent on nicotinamide mononucleotide (NAD+) levels. Similarly to $HDAC_{II}$, Sirts play an anti-hypertrophic and protective role in the myocardium, perhaps because of their ability to contrast oxidative stress (Cencioni et al., 2015; Matsushima & Sadoshima, 2015). Yamamoto and coworkers demonstrated the protective role of NAD+ administration, which activates Sirts function, in a mouse model of ischemia/reperfusion injury (Yamamoto et al., 2014). The pharmacological modulation of $HDAC_{III}$ in the context of CVD has been extensively discussed in a previous review (Cencioni et al., 2015). Summarizing here the role of HDACs, the cited literature shows a negative role of $HDAC_I$ and a positive role of $HDAC_{II}$ and $HDAC_{III}$ in the prevention of CVD. Hence, effective therapeutics should be aimed at inhibiting $HDAC_I$- and activating $HDAC_{II}$- and $HDAC_{III}$-dependent pathways.

The detrimental increase in acetylation can also be due to an excessive activation of histone acetyltransferases (HATs). Similarly to HDACs, HATs are linked to metabolism. HAT activity depends on the intracellular level of acetyl-CoA; it catalyzes protein acetylation, that is, the reaction of addition of the acetyl group to the lysine's ε-amino group (Langer et al., 2002). Miyamoto and coworkers demonstrated the activation of HAT p300 by phosphorylation during the left ventricular remodeling occurring after MI. Specifically, phosphorylated p300 acetylates the transcription factor GATA-4 with consequent activation of a cardiac reprogramming, leading to heart damage (Miyamoto et al., 2006). This evidence is further supported by the protective effect of the poly-phenolic HAT inhibitor curcumin, present in the turmeric spice, which inhibits cardiac hypertrophy, inflammation, and fibrosis, as demonstrated in a series of experiments conducted in mice (H.L. Li et al., 2008) and in a rat model of heart failure (Morimoto et al., 2008).

Among other modifying complexes, special attention has been paid recently to the role of enzymes devoted to histone methylation. These classes of enzymes are divided into two groups, depending on the metabolite used during their enzymatic reaction. Specifically, they can catalyze oxidation by flavin adenine dinucleotide (FAD) reduction or function as dioxygenases using iron (FeII) and α-ketoglutarate as cofactors (Klose & Zhang, 2007). The methylation of histone 3 (H3) at lysine (K) residue 4 (H3K4) can be catalyzed by the K-specific methyltransferase 2C/D (KMT2C/D). Of note, one of the critical subunits of the KMT2C/D complex is the PAX interacting protein 1 (PTIP), which is involved in the regulation of transcription in cardiomyocytes. Indeed, Stein and coworkers demonstrated that PTIP deletion impairs the conduction system leading to ventricular arrhythmias (Stein et al., 2011). Lysine demethylase 1 (LSD1) is responsible for histone H3 lysine 4 (H3K4) demethylation; it has been reported to be genetically altered, with consequent loss of function, in both a mouse model and a cohort of patients affected by salt-sensitive hypertension (Williams et al., 2012). The histone H3 lysine 27 (H3K27) can be methylated by the histone methyltransferase Ezh2 member of polycomb repressor complex 2 (PRC2). Indeed, Ezh2-deficient hearts showed right ventricular wall enlargement with hypertrophic cardiac myocytes associated with the presence of a disorganized and fibrotic tissue (Delgado-Olguin et al., 2012). In humans, some cases of hypertrophic cardiomyopathy have been associated with an upregulation of the histone demethylase jumonji D2A (JMJD2A), responsible for the demethylation of H3 at K residues 9 and 36. Hypertrophic stimuli may lead JMJD2A to bind the promoter of the four-and-a-half LIM domains 1 (FHL1) protein with its consequent activation and positive regulation of fetal genes (Zhang et al., 2011). Overall, these reports suggest that several different epigenetic changes characterize cardiac diseases. This structural modification of the chromatin regulates the expression of genes responsible for hypertrophy, fibrosis, and heart failure, indicating the presence of an epigenetic signature. Heart failure is associated mostly with H3K4me3 and in some cases, also with H3K9me3 (Kaneda et al., 2009). The cardiac hypertrophy signature is characterized by both active histone marks such as H3K9Ac, H3K27ac, H3K4me3, and H3K79me2, and repressive histone marks such as H3K9me2, H3K9me3, and H3K27me3 (Kaneda et al., 2009; Papait et al., 2013) (see Table 2).

Table 2
Epigenetic modulator involvement during cardiac dysfunction.

Epigenetic mechanisms	Epigenetic modulator	Function	Metabolite/drug	Physio/pathological effect	Reference
Histone modifications	$HDAC_I$	Lysine deacetylation. Chromatin condensation.	TSA; SAHA;VPA; β-Hydroxybutyrate (inhibitor)	Hypertrophic responses. Negative role in CDV prevention.	Zhang et al., 2002 Trivedi et al., 2007 Zou et al., 2002 Antos et al., 2003 Shimazu et al., 2013
	$HDAC_{II}$	Lysine deacetylation. Chromatin condensation.	TSA; SAHA; Valproic acid; β-Hydroxybutyrate (inhibitor)	Protection from cardiac hypertrophy and heart failure. Positive role in CVD prevention.	Kee et al., 2006 Kong et al., 2006 Kook et al., 2003 Ooi et al., 2015
	Sirts/$HDAC_{III}$	Lysine deacetylation, succinylation, malonylation. Mono-ADP-ribosyl transfer. Demyristoylation, depalmitoylation.	NAD+ (oxidative cofactor)	Anti-hypertrophic and protective. Positive role in CVD prevention.	Yamamoto et al., 2014 Cencioni et al., 2015 Matsushima & Sadoshima, 2015
	HAT (p300)	Lysine acetylation. Transcription factors acetylation. Chromatin relaxation.	Acetyl-CoA (metabolite, cofactor) Curcumin (inhibitor)	Cardiac reprogramming associated with heart damage.	Langer et al., 2002 H.L. Li et al., 2008 Morimoto et al., 2008
	KMT2C/D	Lysine methylation (H3K4me3).	SAM (metabolite, cofactor)	Ventricular arrhythmias. Heart failure.	Kaneda et al., 2009 Stein et al., 2011
	LSD1	Lysine demethylation (H3K4me1/2).	FAD (oxidative cofactor)	Hypertension.	Williams et al., 2012
	Ezh2	Lysine methylation (H3K27me3).	SAM (metabolite, cofactor)	Cardiac hypertrophy, inflammation, and fibrosis.	Kaneda et al., 2009 Delgado-Olguin et al., 2012 Papait et al., 2013
	JMJD2A	Lysine demethylation (H3K9me3, H3K36me3).	FeII, O_2 (trace elements) α-Ketoglutarate (metabolite)	Hypertrophic cardiomyopathy. Positive regulation of fetal genes.	Klose & Zhang, 2007 Zhang et al., 2011
Noncoding RNA	miRNAs	mRNA degradation. Translation inhibition.		Biomarkers. Therapeutic targets.	D'Alessandra et al., 2012 Greco et al., 2014 Boon & Dimmeler, 2015 Vegter et al., 2016
	lncRNAs: MIAT; aHIF ANRIL; KCNQ1OT1; MALAT1; CARL; CHRF; Mhrt	Guides transcriptional regulators. Decoys. mRNA mimics. Sponges.		Biomarkers. Therapeutic targets.	Hu et al., 2012 Ishii et al., 2006 Vausort et al., 2014 Wang, Liu et al. 2014 Han et al., 2014
DNA methylation	DNMT1 DNMT3a-3b-3L	DNA methylation maintenance. *De novo* DNA methylation.	SAM (metabolite, cofactor) Genomic imprinting SAM (metabolite, cofactor)	Biomarkers. Cardiac development and homeostasis maintenance.	Bestor & Verdine, 1994 Guarrera et al., 2015 Agardh et al., 2015 Gilsbach et al., 2014 Sim et al., 2015 Babu et al., 2015

HDACI, class I histone deacetylases; HDACII, class II histone deacetylases; HDACIII, class III histone deacetylases; TSA, trichostatin A; SAHA; suberoylanilide hydroxamic acid; VPA, valproic acid; Sirts, sirtuins; NAD+, nicotinamide mononucleotide; HAT, histone acetyl transferases; FAD, flavin adenine dinucleotide; KMT2C/D, K-specific methyltransferase 2C/D; LSD1, lysine demethylase 1; JMJD2A, demethylase jumonji D2A; SAM, S-adenosylmethionine; miRNAs, small noncoding RNAs; lncRNAs, long noncoding RNAs; MI, myocardial infarction; MIAT, MI-associated transcript; aHIF, hypoxia inducible factor 1A antisense RNA 2; ANRIL, cyclin-dependent kinase inhibitor 2B antisense RNA 1; KCNQ1OT1, potassium voltage-gated channel, KQT-like subfamily, member 1 opposite/antisense transcript 1; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; CARL, cardiac apoptosis-related lncRNA; CHRF, cardiac hypertrophy-related factor; Mhrt, myosin heavy chain-associated RNA transcripts.

3.2. Noncoding RNAs

In addition to histone or protein modifications, several CVDs have been associated with the expression of specific noncoding RNAs. For example, a large number of small noncoding RNAs (miRNAs) have been proposed as biomarkers and therapeutic targets or agents in cardiac ischemia and failure (D'Alessandra et al., 2012; Greco et al., 2014; Boon & Dimmeler, 2015; Vegter et al., 2016). We will focus here on some of the most recent studies regarding the role of long noncoding RNAs (lncRNAs) (Hu et al., 2012) during the onset of CVD. An altered expression of lncRNAs has been clearly associated with MI. Specifically, the MI-associated transcript (MIAT), the hypoxia-inducible factor 1A antisense RNA 2 (aHIF), the cyclin-dependent kinase inhibitor 2B antisense RNA 1 (ANRIL), the potassium voltage-gated channel, KQT-like subfamily, member 1 opposite/antisense transcript 1 (KCNQ1OT1) and the metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (Ishii et al., 2006; Vausort et al., 2014) have been proposed to play a role in MI. Their mechanism of action remains, however, largely not understood. Interestingly, some lncRNAs have been seen to act as sponges of miRNAs involved in CVD. For instance, the cardiac apoptosis-related lncRNA (CARL) blocks miR-539 maintaining the physiological dynamics of the mitochondria and is usually dysregulated during the onset of cardiomyopathies (Wang, Long et al. 2014). Similarly, the cardiac hypertrophy-related factor (CHRF) prevents miR-489 from downregulating its target Myd88 involved in cardiac hypertrophy (Wang, Liu et al. 2014). This condition can also be efficiently counteracted by the action of myosin heavy chain-associated RNA transcripts (Mhrt) that prevent the binding of the chromatin-remodeling factor Brg1 to its genomic targets involved in the progression of cardiomyopathy (Han et al., 2014) (see Table 2).

3.3. DNA methylation

DNA cytosine methylation, realized by specific enzymes through the addition of a methyl group to the carbon 5´ of a cytosine, is an additional epigenetic modification that alters the transcriptional potential of cardiac cells. This modification is the consequence of the action of specific DNA methyltransferases (DNMT1-3a-3b-3 L) that uses the metabolite S-adenosylmethionine (SAM) to transfer a methyl group to the targeted cytosine generating methylcytosine and S-adenosylhomocysteine (SAH) as reaction products (Bestor & Verdine, 1994). Of note, the availability of the methyl donor SAM is strictly interconnected to that of other metabolites such as folic acid, vitamin B12, choline, and betaine (Waterland, 2006). The reaction end-product SAH can be measured in the plasma, and it has been proposed as a predictor of CVD (Kerins et al., 2001). Indeed, specific methylation profiles of blood cells have been recently associated with the risk of MI (Guarrera et al., 2015) and proliferative diabetic retinopathy (Agardh et al., 2015). In this direction, it has been recently pointed out that DNA methylation is a crucial event in cardiac development, homeostasis maintenance, and the onset of certain pathophysiological conditions (Gilsbach et al., 2014; Sim et al., 2015). Specifically, the study conducted by Sim and coworkers identified two postnatal waves of DNA methylation in the heart of rodents: a hypermethylation wave occurring early between postnatal days 1 and 14, followed by one of global demethylation (Sim et al., 2015). Bioinformatics analysis revealed that among the genes affected by these two DNA methylation/demethylation rounds, there were Notch, DMRs, Wnt, and Hedgehog, all known as fundamental for cardiomyocyte differentiation and cardiac development (Sim et al., 2015). Accordingly, Gilsbach and coauthors unraveled a pattern of DNA methylation in isolated cardiomyocytes that controls normal postnatal development, but may also be detected in failing hearts (Gilsbach et al., 2014). In this regard, a recent work from Ylä-Herttuala's group showed that the impairment in neovascularization occurring after an ischemic insult in hyperlipidemia or type 2 diabetes mouse models is associated with altered DNA methylation in specific promoter regions of macrophage cells, leading them to mature into the pro-inflammatory and anti-angiogenic class M1 (Babu et al., 2015).

In a wider vision, the epigenetic machinery, through some of its components may shed new light on molecular pathways at the basis of the onset of CVDs. Remarkably, they may represent direct targets for therapy. However, they can also be exploited to cooperate with existing therapeutic approaches, including those based on adult stem cells and in particular CMSCs (see Table 2).

Table 3
Genetic and epigenetic enhancement strategies.

Intervention	Outcome	Reference
Genetic reprogramming		
Gata4, Mef2c, and Tbx5 (GMT)	cTnT + iCM generation	Ieda et al., 2010
Gata4, Mef2c, and Tbx5 (GMT)	In vivo iCM generation	Inagawa et al., 2012
Gata4, Mef2c, and Tbx5 (GMT)	Cardiomyocyte-like cell generation	Qian et al., 2012
Gata4, Tbx5, and Baf60c	Beating cardiomyocyte generation	Takeuchi & Bruneau, 2009
Gata4, Mef2c, Tbx5, and Hand2 (GHMT)	Beating cardiac-like myocyte generation in vitro	Song et al., 2012
Nkx2.5, Srf, Smartcd3, Mesp1, Myocd, Gata4, Mef2c, and Tbx5	Fibroblast reprogramming	Addis et al., 2013 Christoforou et al., 2013
miR-1, miR-133, miR-208, and miR499	Fibroblast conversion into cardiomyocyte-like cells Cardiac contractility and performance improving after injury	Jayawardena et al., 2012 Jayawardena et al., 2015
miR-1, miR-133, miR-208, miR499, Gata4, Mef2c, and Tbx5	Cell reprogramming enhancement	Nam et al., 2013
Epigenetic reprogramming		
ATRA, PB, DETA/NO (EpiC), and 5% FBS	Competent cardiovascular precursor generation	Vecellio et al., 2012
VPA, CHIR9921, RepSox, Forskolin	iCM generation	Fu et al., 2015
AA 2-P, and rdrHA	cTnT + iCM generation in vitro	Burridge et al., 2014
CHIR99021, A83-01, SC1, OAC2, Y27632, BIX01294, AS8351, SU16F, and JNJ10198409	Cardiomyocyte-like cell generation similar to embryonal cardiomyocytes	Cao et al., 2016
Combined genetic and epigenetic reprogramming		
Gata4, Mef2c, Tbx5, Hand2, Nkx2.5, and TGFβ inhibitor	iCM generation	Ifkovits et al., 2014
Oct4, CHIR99021, Forskolin, Parnate, and SB431542	iCM generation	H. Wang et al., 2014

cTnT +, cardiac troponin T-positive cells; iCM, induced cardiomyocyte; ATRA, all-trans retinoic acid; PB, phenyl butyrate; DETA/NO, diethylenetriamine/nitric oxide; AA 2-P, L-ascorbic acid 2-phosphate; rdrHA, rice-derived recombinant human albumin.

4. Therapeutic potential of mesenchymal stem cells

4.1. Genetic and epigenetic interventions to enhance the therapeutic potential of mesenchymal stem cells

Tissue loss follows heart damage in consequence of the activation of a remodeling process, in which the original cardiac tissue is replaced by a scar (Leask, 2010). Cardiac nonmyocyte cells, including CMSCs, currently represent candidate targets for novel therapeutic strategies to counteract heart diseases (Gourdie et al., 2016). As aforementioned, the cardiac stroma, in which CMSCs reside, is directly involved in remodeling. Once activated, CMSCs contribute to the re-establishment of all the cardiac cell subpopulations, including cardiomyocytes (Gaudesius et al., 2003; Souders et al., 2009; Rossini et al., 2011; Zeigler et al., 2015; Zhang et al., 2015). Indeed, because of their plasticity, reduced immunogenicity, and the relative simplicity of isolation and culture *ex vivo*, CMSCs are a suitable adult stem cell population to consider for therapies based on cardiac regeneration. Recently, a significant effort has been made to enhance CMSC differentiation toward the cardiac lineage. As an example, the generation of induced pluripotent stem cells (iPS) starting from fibroblasts opened a new era for cell manipulation (Takahashi & Yamanaka, 2006). In 2010, Ieda and coworkers obtained for the first time induced cardiomyocytes (iCMs) from murine adult cardiac and skin fibroblasts by co-transduction of well-characterized cardiomyogenic transcription factors Gata4, Mef2c, and Tbx5 (GMT), (Ieda et al., 2010). By using this approach, the authors detected the rapid formation of cardiac troponin T positive cells (cTnT+) and the formation of contractile cells at a later time point (Ieda et al., 2010). Once injected into the heart, GMT-transduced fibroblasts underwent in vivo reprogramming leading to the generation of new cardiomyocytes within 2 weeks (Ieda et al., 2010). Qian et al. demonstrated that nonmyocyte cells resident in the murine heart can be successfully reprogrammed in vivo into cardiomyocyte-like cells by the local delivery of GMT after coronary ligation (Qian et al., 2012). Song et al. improved the effect of GMT transduction by adding Hand2, a transcription factor crucial for reprogramming of adult mouse cardiac fibroblasts into beating cardiac-like myocytes in vitro, through the “so-called” the GHMT cocktail. Nkx2.5, Srf, Smartcd3, Mesp1, and Myocd have also been described as other factors that can further increase fibroblast reprogramming when used in combination with GMT factors (Addis et al., 2013; Christoforou et al., 2013). In addition to the expression of coding genes, microRNAs have also been described as a powerful tool for non-myocyte cell reprogramming. Indeed, Jayawardena and coauthors found a combination of microRNAs suitable for cardiac fibroblast reprogramming both in vitro and in vivo (Jayawardena et al., 2012; Jayawardena et al., 2015). This combination included miR-1, miR-133, miR-208, and miR499. This set of microRNAs induced the expression of cardiac markers and also efficiently converted fibroblasts into functional cells with cardiomyocyte-like features such as the expression of ion channels, the presence of spontaneous calcium oscillations, and the in vivo improved contractility and cardiac performance after injury (Jayawardena et al., 2012; Jayawardena et al., 2015). Of note, in 2013, Nam at al. combined microRNAs with transcription factors to further enhance cell reprogramming (Nam et al., 2013). Recently, small molecules have also been tested in reprogramming protocols to obtain iCMs or cardiovascular precursors from CMSCs. In 2012, Vecellio and coworkers described a novel active epigenetic cocktail (EpiC) composed of all-trans retinoic acid (ATRA); phenyl butyrate (PB), a known HDAC inhibitor; and a nitric oxide donor (diethylenetriamine/nitric oxide – DETA/NO) as HDAC modulator (Illi et al., 2008; Illi et al., 2009; Spallotta et al., 2010; Rosati et al., 2011), which when combined with low serum (5% FBS) could reprogram human CMSCs into functionally competent cardiovascular precursors (Vecellio et al., 2012). HDAC inhibitors such as VPA have been further used by Fu and coworkers in combination with three compounds, CHIR9921 (GSK3 inhibitor), RepSox (TGFβR1 inhibitor), and Forskolin (adenilyl cyclase activator), to generate iCMs from fibroblasts without any transcription factor (Fu et al., 2015). Notably, CHIR9921 is one of the three inhibitors also responsible for the so-called “ground state” in mouse embryonic stem cells (Ying et al., 2008), and it has been recently used in combination with transcription factors and other small molecules to obtain iCMs (Ifkovits et al., 2014; H. Wang et al., 2014). The possibility to design an optimal culture medium to generate in vitro iCMs by a combination of small molecules (Burridge et al., 2014) represents a tool of unlimited potential and a big challenge for the future. In light of this finding, Cao and coworkers have recently reported the reprogramming of somatic fibroblasts into cardiomyocyte-like cells by a combination of nine chemicals. The chemical cocktail opens chromatin of the cardiac developmental genes, permitting their transcription and thus the acquisition of a phenotype similar to that of embryonal cardiomyocytes (Cao et al., 2016) (see Table 3).

According to all these evidences, the opportunity to generate iCM in vivo opens new possibilities to the so-called “enhancement strategies,” providing new powerful tools to counteract cardiac diseases and in particular heart failure. Currently, this approach combines the enhancement strategies based on small molecules or genetic manipulation with the activation of the injured tissue to specifically increase signals for cellular homing and survival (Seeger et al., 2007). Although less efficient, the in vivo reprogramming takes advantage of intrinsic cardiac features such as the ability of its specific metabolic environment to directly affect resident cell epigenome. In fact, whereas in vitro reprogramming permits to generate only immature iCM, only in vivo strategies can produce mature iCM (Takeuchi & Bruneau, 2009; Inagawa et al., 2012; Fu & Srivastava, 2015). The design of the best reprogramming viral vectors and the best delivery strategies represents the trickiest and the newest aspect of all these enhancement strategies for in vivo iCM generation. Moreover, the elucidation of the epi-metabolic mechanism controlling iCM formation is presently poorly characterized and requires specific detailed studies. In light of this, recently Liu and coauthors have evaluated the trend of H3K27me3, H3K4me, and DNA methylation during iCM generation (Liu et al., 2016), revealing a time-dependent differential re-patterning of histone modifications and DNA methylation at cardiac- and fibroblast-specific loci during iCM reprogramming (Liu et al., 2016).

4.2. Application of mesenchymal stem cells in cardiovascular cell therapy

The therapeutic potential of MSCs for the treatment of CVDs has been previously described (Williams & Hare, 2011). A number of preclinical studies in large animal models of MI pointed out significant positive effects on cardiac function and on the reduction of scar size (Amado et al., 2005; Qi et al., 2008; Quevedo et al., 2009; Hatzistergos et al., 2010). In these studies, MSCs have been administered by intravenous or intracoronary injection or through direct injection into the cardiac tissue (A. R. Williams & Hare, 2011). It is noteworthy that in some of these studies, MSCs have been injected in combination with other precursor cells (Avolio et al., 2015b). Of note, as discussed previously in this review, MSCs can sense epigenetic and metabolic changes altering their plasticity and functions. In fact, the stochastic lifetime accumulation of alterations consequential to DNA and mitochondrial damage, metabolic changes, oxidative stress, and inflammation, coupled with other environmental risk factors, may directly influence the homeostasis of mesenchymal niches (Schimke et al., 2015). Consistently, adipose tissue-derived MSCs isolated from aged patients present a reduced potential regarding viability, proliferation, and multilineage differentiation ability (Maredziak et al., 2016). These impairments may be partially overcome by specific interventions, for example, those realized with epigenetically active drugs aimed at preventing senescence and accumulating molecular alterations, making the cells suitable again for cell therapy (Wang et al., 2013; Vecellio et al., 2014; Linares et al., 2016).

Although MSCs can be virtually isolated from the stroma of every organ, the ongoing clinical trials are predominantly focused on BM-

MSCs or those derived from adipose tissues because of the relative simplicity of their harvesting procedure and the limited number of manipulations required before re-injection. The clinical outcomes of these trials indeed point out some positive effects of the MSC-based therapy, but the exact mechanism(s) at their origin remain(s) unclear. A so-called "paracrine effect" has often been claimed as the most important mechanism by which BM-MSCs exert their beneficial effect (Squillaro et al., 2016; Tao et al., 2016). In this review, we will report on the outcome of some recent clinical trials describing the therapeutic potential of adipose-, BM- or cardiac-derived MSCs in CVD treatment (www.clinicaltrials.gov). APOLLO (ClinicalTrials.gov Identifier: NCT00442806_Phase 1), ADVANCE (NCT01216995_Phase 2), and MyStromalCell (NCT01449032_Phase 2) are the acronyms of three clinical trials aimed at investigating the therapeutic potential of MSCs isolated from the adipose tissue. In 2007, APOLLO established the safety and feasibility of adipose-derived MSCs infused intracoronary in patients with MI and characterized by ST elevation. The efficacy of the treatment was demonstrated by a significant improvement of the cardiac perfusion and a 50% reduction in scar formation after a 6-month follow-up (Houtgraaf et al., 2012). Similar results were obtained by the ADVANCE study. However, the MyStromalCell trial found an increase in exercise duration, a reduction of angina, and a general improvement of the quality of life after a 6-month follow-up post injection of autologous MSCs in patients affected by ischemic cardiomyopathy (source: www.clinicaltrials.gov) (Follin et al., 2013).

A series of clinical trials based on BM-derived MSCs, specifically TAC-HFT (NCT00768066_Phase 1), POSEIDON (NCT00587990_Phase 1), PROMETHEUS (NCT00587990_Phase 1), and Prochymal (NCT00877903_Phase 2), dealt with the effect of allogeneic and autologous MSCs isolated from BM as well as with the comparison of BM-derived MSCs *versus* BM-derived mononuclear cells in patients with acute MI. Overall, these clinical trials reported positively about the overall safety and tolerance of the treatment after 13-month follow-up and a decreased number of arrhythmic events or reduced chest pain, paralleled by an improved ventricular function (Hare et al., 2012; Karantalis et al., 2014).

Clinical trials related to resident cardiac MSCs were based on either the so-called "cardiosphere-derived" stem cells (CDCs) or the so-called cardiac stem cells (CPCs). At present, no method using cardiac fibroblasts (CFs) or another type of cardiac MSC (CMSCs) has been proposed. CDCs represent a mixed population of undifferentiated and highly clonogenic cells, expressing stem, mesenchymal, and endothelial progenitor markers, including c-kit, Sca-1, CD31, and CD34 (Messina et al., 2004). They can be easily isolated from a small cardiac biopsy and grown in vitro as self-adherent clusters. It is noteworthy that CDCs display spontaneous beating activity soon after their generation without loss of differentiation potential. In 2009, the CADUCEUS trial (NCT00893360_Phase 1) proposed the infusion of autologous CDCs in patients with ischemic cardiomyopathy. Once again, no safety concerns were identified. Instead, a regenerative potential was demonstrated by several parameters including a decreased scar size, an increased viable myocardium, and an improved although modest regional cardiac function 1 year after cell injection (Malliaras et al., 2014). More recently, the DYNAMIC trial (NCT02293603_Phase 1) proposed a study aimed at establishing the safety of CDCs in patients affected by dilated cardiomyopathy after 6- and 12-month follow-up (source: www.clinicaltrials.gov).

CSCs were proposed in two clinical trials designed for the treatment of ischemic cardiomyopathy: SCIPIO (NCT00474461_Phase 1) and ALCADIA (NCT00981006_Phase 1). The first trial is focused on the intracoronary injection of autologous CSCs isolated according to their positive expression of the cellular kit ($cKit^+$) lineage negative (lin^-) markers in patients with post-infarction left ventricular dysfunction (Bolli et al., 2011). Similar to other trials, no safety concerns have emerged to date from this study. Indeed, treated patients showed a significant increase in the left ventricular ejection fraction with a clear reduction in the infarct size after an 18-month follow-up (Bolli et al., 2011). Of note, in 2014, the Lancet published an expression of concern regarding the SCIPIO trial and, at the time of writing this review, the validity of its results is still under investigation (The Lancet, 2014). Finally, the ALCADIA trial proposed a hybrid cell therapy application, where autologous CSCs, with mesenchymal features, derived from endomyocardial biopsies were intramyocardially injected in patients with ischemic cardiomyopathy and heart failure, together with a biodegradable gelatin (hydrogel) sheet enriched with bFGF. However, no result has been published yet about this study (source: www.clinicaltrials.gov).

4.3. Summary

In conclusion, because of their intrinsic features, CMSCs may play pivotal roles in the regenerative processes occurring after a cardiovascular insult. The results reported in this review, including the overview of preclinical and clinical study outcomes, clearly demonstrate the substantial therapeutic potential of these cells, highlighting CMSCs derived from different tissues for future cell therapy applications. Nevertheless, more mechanistic studies are necessary to assess the feasibility of epigenetic intervention aimed at modulating the therapeutic properties of CMSCs and fostering their healing potential in the human heart.

Conflict of interest

The authors declare no conflict of interest.

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