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Concise Review: Challenges in Regenerating the Diabetic Heart: A Comprehensive Review

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Key Words. Stem cells • Diabetes • Cardiac regeneration • Epigenetic changes • MicroRNA • Long noncoding RNA • Stem cells engineering

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ABSTRACT

Stem cell therapy is one of the promising regenerative strategies developed to improve cardiac function in patients with ischemic heart diseases (IHD). However, this approach is limited in IHD patients with diabetes due to a progressive decline in the regenerative capacity of stem cells. This decline is mainly attributed to the metabolic memory incurred by diabetes on stem cell niche and their systemic cues. Understanding the molecular pathways involved in the diabetes-induced deterioration of stem cell function will be critical for developing new cardiac regeneration therapies. In this review, we first discuss the most common molecular alterations occurring in the diabetic stem cells/progenitor cells. Next, we highlight the key signaling pathways that can be dysregulated in a diabetic environment and impair the mobilization of stem/progenitor cells, which is essential for the transplanted/endogenous stem cells to reach the site of injury. We further discuss the possible methods of preconditioning the diabetic cardiac progenitor cell (CPC) with an aim to enrich the availability of efficient stem cells to regenerate the diseased diabetic heart. Finally, we propose new modalities for enriching the diabetic CPC through genetic or tissue engineering that would aid in developing autologous therapeutic strategies, improving the proliferative, angiogenic, and cardiogenic properties of diabetic stem/progenitor cells. STEM CELLS *2017; 00:000–000*

SIGNIFICANCE STATEMENT

Stem cell therapy is gaining global interest as the next generation of drug treatment in patients with ischemic heart disease. However, this approach is limited in patients with diabetes, due to the reduction in the available pool and functional deficit of the diabetic stem/progenitor cells. To our knowledge, this is the first review summarizing the molecular alterations in the diabetic cardiac progenitor/stem cells, which reduce its functional efficacy. We believe this review will provide a strong foundation for several future studies aiming to regenerate the diabetic heart by restoring the dysregulated molecular signaling cascade in the diabetic stem/progenitor cells.

INTRODUCTION

Heart failure, as a result of ischemic heart disease, is a worldwide epidemic caused by gradual loss of cardiomyocytes. The incidence of heart failure is more pronounced in patients with diabetes mellitus [1]. In 1974, Framingham study first reported diabetes as a discrete cause of congestive heart failure, with twofold higher risk of heart failure in men and fivefold higher risk in women, and two- to fourfold higher mortality than their nondiabetic peers [1]. Although recent studies show that adult human cardiomyocytes have a turnover rate [2, 3], it is limited and insufficient for restoration of contractile dysfunction during heart failure. Given the adverse structural and functional changes in diabetic heart [4], there remains a higher risk of developing heart failure in patients with diabetes [5].

Heart transplantation has so far been the only successful clinical approach to replace diseased myocardium that is unresponsive to other conventional therapies. However, lack of available donors and complications due to graft rejection limits its application for the large cohort of diabetic patients with heart failure. In the past decade, various preclinical [6–8] and clinical [9, 10] studies have developed different regenerative strategies to replace the lost cardiomyocytes in the diseased heart. As reviewed in detail by Lin and Pu [11], strategies for myocardial regeneration broadly fall under four categories: (a) stem/progenitor cell therapy, (b) activation of endogenous progenitor cells, (c) inducing proliferation of existing cardiomyocytes, and (d) reprogramming noncardiac cells to cardiomyocytes.

Regenerative therapies, which are clinically more relevant, are either through expansion of stem cells ex vivo and injecting them into the diseased heart (category 1), or by activation of endogenous progenitor cells to migrate and repair the diseased myocardium (category 2) [6–8]. Although efforts are under way to optimize the strategies of all the above categories, only the first strategy has evolved into clinical trials with encouraging results [9, 10]. Preclinical and clinical studies have transplanted plethora of multipotent stem/progenitor cells into ischemic hearts with variable, but mostly positive, results in terms of their survival, improved vascularization and differentiation into cardiac lineages (detailed description is reviewed elsewhere) [12].

The fate of a cell depends on the way it interacts with its cellular environment (niche) and indeed a mutual interaction between the cell and its niche exists. Therefore, any pathological disturbance in the niche could affect cellular function, resulting in homeostatic imbalance and ultimately cell death. Diabetes is one such condition, which has been demonstrated to affect the regenerative ability of stem/progenitor cells [13–18]. This is mainly attributed to hyperglycemia or sustained high blood glucose levels in diabetic individuals with diabetes affecting the cellular metabolism thereby impeding cellular homeostasis, which eventually induce senescence and apoptosis [19–21]. While the pathogenesis of most commonly occurring type 1 and type 2 diabetes are different, we along with others have shown that the pathological consequences of high glucose on stem cells is identical irrespective of the type of diabetes [22–24]. This review will focus on how diabetes has its impact on both stem/progenitor cell therapy, which is currently one of the most promising regenerative strategies. In this review, the term "stem cells" is used while referring to hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), whereas the term "progenitor cells" is used to refer endothelial progenitor cells (EPCs) and cardiac progenitor cells (CPCs).

Effect of Hyperglycemia on Stem/Progenitor Cell Senescence and Death

Senescence is a state of proliferative cell arrest induced by both external environment and endogenous cellular influences. Stem cell senescence is the prime reason for majority of the pathologies associated with aging due to its strong association with reduction in the ability of tissues to regenerate lost cells [25, 26]. The two major cell cycle regulating pathways that influence the cell proliferation and senescence are $p16^{INK4a}$/retinoblastoma (p16/pRb) and $p14^{ARF}$ (p14/p53) pathways. P16 and P14 are cyclin inhibitors that regulate the activity of the tumor suppressor pRb and p53, respectively. The state of senescence cumulatively activates p16/pRb [26] or p14/p53 pathways leading to cell cycle arrest [27, 28]. Diabetes has been widely demonstrated to have a dramatic impact on aging of stem cells. Generation of reactive oxygen species (ROS) leads to telomere shortening, expression of p53 and $p16^{INK4a}$, and apoptosis of CPC, impairing the cardiac turnover [29]. Stolzing et al. reported the negative impact of hyperglycemic treatment on proliferation and differentiation potential of MSC [18]. The proliferative and angiogenic capacities of adipose-derived stem cells were also reduced in response to hyperglycemic treatment [14, 17]. Furthermore, EPC [15] and CPC [16] showed marked diminution in secretion of paracrine growth factors and failed to promote angiogenesis when exposed to hyperglycemia. All these effects were attributed to increased senescence by high glucose treatment [18, 29]. This effect of hyperglycemia on stem cell senescence is not only restricted to the heart but also observed in other organs. For instance, in bone, hyperglycemia-induced inflammation through tumor necrosis factor-alpha (TNF-α) led to forkhead box O transcription factor 1 (FOXO-1) nuclear localization resulting in MSC apoptosis and reduction in bone fracture healing [30]. This FOXO-1 nuclear localization has been attributed to the post-translational modification process of O-GlyNAcylation, a process in which excessive glucose molecules attach to an oxygen atom on serine or threonine residues of proteins by O-linked-*N*-acetyl glucosamine (O-GlcNAc) transferase (OGT), which was accelerated under hyperglycemic conditions [31]. Thus, hyperglycemia can lead to accelerated cell cycle arrest, indicating the negative impact of the diabetic environment on stem cell senescence.

While hyperglycemia has been proposed to be the major cause for diabetes-induced stem cell dysfunction, results from the clinical trials action in diabetes and vascular disease: preterax and diamicron modified release controlled evaluation (ADVANCE) [32], action to control cardiovascular risk in diabetes (ACCORD) [33], diabetes control and complications trial (DCCT) [34], epidemiology of diabetes interventions and complications (EDIC) [35], and UK Prospective Diabetes Study [36] clearly showed that a very strict glycemic control does not reduce diabetes-induced macrovascular and cardiac complications. Hyperglycemia has a persistent adverse effect on the development and progression of these complications, leading to a phenomenon called metabolic memory [35, 37]. Among other factors, epigenetic changes have a crucial role in the process of metabolic memory.

Epigenetic Mechanisms Regulating Cardiac Stem/Progenitor Cells

Epigenetics is defined as a heritable phenotype that does not depend on the primary sequence of DNA [38]. Although the role of epigenetics was originally implicated in vascular complications, numerous reports have also now highlighted its key role in cellular senescence [15, 38–40]. We will discuss exclusively on two important epigenetic changes that affect stem cell integrity and induce senescence in diabetes: DNA and histone modifications and noncoding RNA (nonprotein coding) regulation through microRNA (miRNA) and long-noncoding RNA.

DNA and Histone Modifications

DNA or histone modifications have a major influence on chromatin structure and gene expression. DNA can be modified by methylation of cytosine residues in CpG dinucleotides, whereas the N-terminal tails of histone proteins are subjected to a wide range of different modifications, such as acetylation and methylation [41]. These modifications can have a substantial influence on chromatin structure and gene function, which depends on the type and location of the modification. Hypermethylation and deacetylation are known to cause gene silencing [42].

In diabetes, Vecellio et al. attributed the impaired proliferation, differentiation, and senescent behavior of diabetic CPC to changes in their chromatin conformation [40]. The major changes identified were hypermethylation of CpG islands, an increased trimethylation of Histone 3 lysine 9 (H3K9), H3K27,

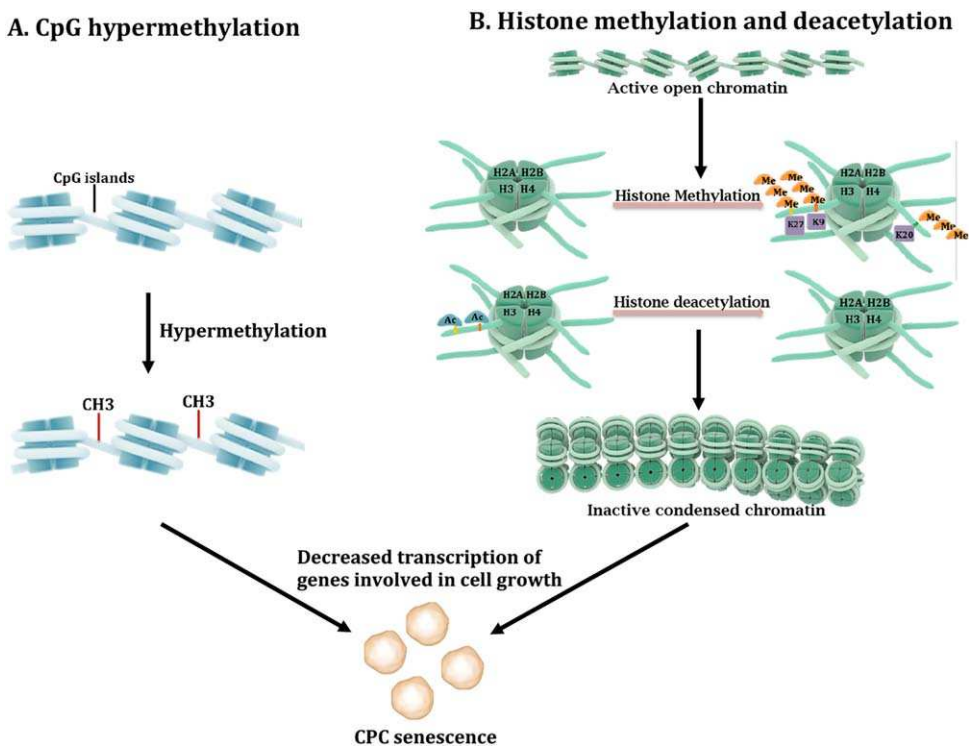

Figure 1. DNA and histone modifications in diabetic cardiac progenitor cell (CPC). **(A)**: Hypermethylation (excess methylation of cytosine) of CpG islands at the promoters of cell cycle regulators leads to their gene silencing and cell cycle arrest. **(B)**: Trimethylation of H3K9, H3K27, and H4K20 and deacetylation of H3K9 and K27 converts the active chromatin to inactive chromatin and inhibits the transcription of genes involved in cell growth and proliferation, which finally leads to CPC senescence in diabetes. Abbreviations: CPC, cardiac progenitor cell.

and H4K20, and a decreased monomethylation and acetylation of H3K9 (Fig. 1). All the above changes cause the chromatin to condense and initiate a repressive state to hinder the transcription of genes involved in cell growth and genomic stability. Interestingly, treatment of diabetic CPC with a proacetylation compound histone acetylase activator pentadecylidene-malonate 1b (SPV106) was able to reverse the chromatin conformation and partially rescue the proliferation and differentiation potential of diabetic CPC by increasing acetylation and decreasing CpG methylation [40]. Similarly, peripheral blood mononuclear cells (PBMC) derived from types 1 and 2 diabetic individuals showed a constitutive expression of H3K9 dimethylation pattern [43]. In addition, Pradhan et al. demonstrated that persistent activation of p65 subunit of nuclear factor-κB was associated with the recruitment of the methyl writer SET7 [44]. Using a knockdown approach, they demonstrated a key role for SET7 in inducing the expression of proteins involved in metabolic memory. Since CpG hypomethylation leads to gene activation and hypermethylation leads to gene repression, it would be interesting to determine whether SET7-mediated methylation has an indirect role in regulating genes involved in CPC proliferation during diabetes.

Noncoding RNAs

microRNA. miRNA are small regulatory noncoding sequences, which are involved in various cellular functions, including stem/progenitor cell proliferation [45]. They can be broadly classified into positive and negative regulators of stem cells (detailed description is reviewed elsewhere [45]). Here, we discuss the miRNAs that are involved in inducing senescence in stem/progenitor cells in diabetes.

miR-34a. Zhang et al. recently demonstrated that MSCs exposed to hypoxia/serum deprivation showed marked increase in miR-34a levels. Hypoxia/serum deprivation reduces cell survival of bone marrow-derived MSCs by increasing the rate of apoptosis through silent information regulator-1/FOXO-3a (SIRT-1/FOXO-3a)-dependent regulatory network [46]. SIRT-1, which deacetylates and represses FOXO-3a (a pro-apoptotic transcription factor), is a direct target of miR-34a (Fig. 2). Interestingly, treating the MSC with miR-34a mimic increased production of ROS, leading to increased expression of senescent markers p16, p53, and p21. Similarly, miR-34a induced senescence in EPC and impeded their angiogenesis by suppressing SIRT-1 [47]. The deleterious effects of miR-34a were partially abrogated by the addition of ROS scavenger *N*-acetylcysteine and completely abrogated by addition of miR-34a inhibitor through significant reduction in senescent markers [46]. Thus, miR-34a acts as a senescent inducer in both EPC and bone marrow-derived MSCs.

Diabetes is known to induce ROS and FOXO activities [48]; however, there are no studies on the role of miR-34a in CPC in diabetes. In contrast to its role in EPC and MSC, recent work from our laboratory (Gandhi. S et al., unpublished data) found that inhibition of miR-34a increased apoptosis in CPC isolated from diabetic individuals, suggesting a protective effect of miR-34a in diabetes. These data suggest that miR-34a exhibits differential roles in CPC, EPC, and MSC. Validating the specific role of miR-34a in different stem/progenitor cells could be a beneficial tool to improve the regenerative and therapeutic potential of diabetic stem/progenitor cells.

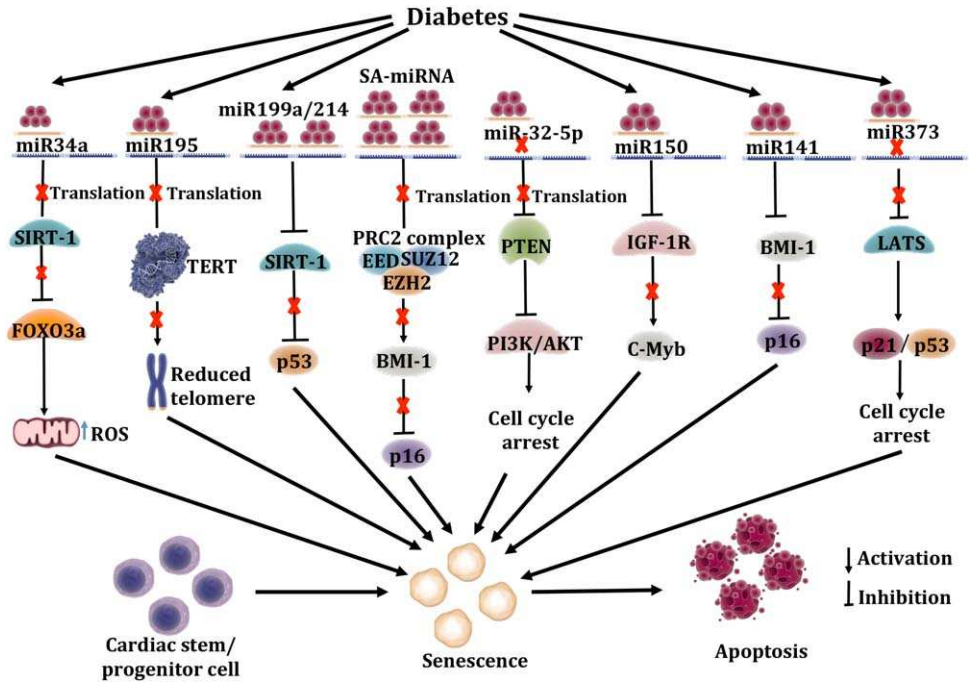

Figure 2. Proposed epigenetic regulation of cardiac progenitor cell senescence and apoptosis during diabetes through miRNA. Red cross indicates inhibition of the corresponding step. Abbreviations: BMI-1, PRC1 oncogene; EED, embryonic ectoderm development; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; FOXO3a, forkhead box O transcription factor 3a; IGF-1R, insulin-like growth factor 1 receptor; LATS, large tumor suppressor homolog; p16, cyclin-dependent kinase inhibitor 2A; P21, cyclin-dependent kinase inhibitor 1; p53, tumour suppressor protein; PRC2, polycomb repressive complex-2; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; SA-miRNA, senescence-associated miRNA; SIRT-1, silent information regulator-1 SUZ12, suppressor of zeste 12 homolog; ; TERT, telomerase reverse transcriptase.

miR-26b, -181a, -210, and -424. Recently, Overhoff et al. identified a group of four senescence-associated miRNAs (miR-26b, -181a, -210, and -424) that regulated the major cyclin inhibitor P16 (tumor suppressor) [49]. These senescence-associated miRNAs act together to inhibit polycomb repressive complex-2 (PRC2) which lead to the activation of P16 and thereby can mediate cellular senescence. PRC2 complex with its core subunits mediates the trimethylation of histone modification H3K27. H3K27 trimethylation helps to stable the expression of B cell-specific moloney murine leukemia virus integration site 1 (BMI-1), a PRC1 oncogene, which again represses P16-mediated cellular senescence (Fig. 2). The exact role of senescence-associated miRNAs in inducing CPC senescence remains unknown; however, they are differentially regulated during metabolic disorders. For example, activation of miR-181a downregulated the levels of SIRT-1, which decreased insulin sensitivity [50]. As SIRT-1 is also a deacetylator of the pro-apoptotic transcription factor FOXO-3a (as described above), it can be speculated that CPC senescence is under regulation of a miR-181a/insulin/SIRT-1 signaling pathway.

miR-195. miR-195 has been associated with cardiac hypertrophy and chronic heart failure by inducing cellular apoptosis through downregulation of SIRT-1 [51–53]. Zheng et al. demonstrated increased myocardial expression of miR-195 in a mouse model of both type 1 and type 2 diabetes, which was associated with reduced expression of its target protein SIRT-1 [52]. Okada et al. was the first to identify the pro-senescence activity of miR-195 in a population of bone marrow-derived MSC [54]. They reported an increased expression of miR-195 in MSC from aged mice, which induced senescence by targeting telomerase reverse transcriptase (*tert*) gene reducing telomere length (Fig. 2). Silencing miR-195 improved the regeneration capacity of aged MSC by reactivating *tert*, resulting in relengthening of telomere [54]. Thus, elevated levels of miR-195 in diabetes could potentially induce cellular senescence and apoptosis by reducing the key growth regulators SIRT-1 and *tert*. Silencing the expression of miR-195 may, therefore, revert the senescent phenotype of diabetic CPC regaining their proliferative, angiogenic, and cardiogenic properties.

miR-32-5p. miR-32-5p plays an agonistic role in the proliferation of bone marrow-derived MSC by inhibiting phosphatase and tensin homolog (PTEN), a negative regulator of PI3K/Akt signaling. Zhu et al. reported reduced miR-32-5p levels in bone marrow-derived MSC exposed to hyperglycemia, which in turn increased PTEN levels thereby reducing the prosurvival PI3K/Akt signaling cascade. This led to an inhibition of cell cycle progression from G0/G1 to G2/M and S phase, and resulted in cell cycle arrest (Fig. 2). Interestingly, overexpression of miR-32-5p rescued bone marrow-derived MSC from hyperglycemia-induced toxic effects and promoted cell cycle progression [55].

miR-199a/miR-214 Cluster. miR-199a/miR-214 are a cluster of miRNA situated in a long noncoding RNA (lncRNA), Dnm3os. Myocardial hypoxia following myocardial infarction activates Dnm3os, which leads to increased expression of miR-199a/miR-214 and subsequent downregulation of peroxisome proliferator-activated receptor-δ. These events cause a decrease in mitochondrial oxidative capacity of heart [56]. Costantino

et al. highlighted the differential involvement of miR-199a and miR-214 in cardiac fibrosis, hypertrophy, and heart failure under hyperglycemic conditions [57]. In the presence of insulin/insulin-like growth factor (INS/IGF) signaling cascade, Akt induces downregulation of miR-199a and prevents inhibition of SIRT-1 resulting in P53 inhibition [58, 59]. Similarly, Maes et al. demonstrated upregulation of miR-214 in aged liver tissue [60], which directly targets proteins involved in mitochondrial function and oxidative defense leading to cellular senescence. These evidences further support the speculation for the involvement of miR-199a/miR-214 cluster in regulating CPC senescence and proliferation (Fig. 2).

miR-133a. miR-133a belongs to the MyomiR group of muscle-specific miRNAs. It regulates heart and skeletal muscle biology and plays an important role in heart development [61]. miR-133a is progressively upregulated during in vitro cardiac differentiation of adult CPC, and miR-133a expression was enhanced during oxidative stress in vitro. miR-133a overexpression protected CPC against cell death by targeting the pro-apoptotic genes *Bim* and *Bmf* [62]. miR-133a overexpressed CPC clearly improved cardiac function in a rat myocardial infarction model by reducing fibrosis and hypertrophy and increasing vascularization and cardiomyocyte proliferation [62]. The levels of miR-133a are downregulated in the diabetic heart [63, 64]. Although Izarra et al. found no effect of miR-133a overexpression on CPC proliferation [62], the effect of miR-133a downregulation on CPC proliferation and senescence has not been reported yet. Downregulation of miR-133a-induced cardiomyocyte hypertrophy [64] has been associated with atherosclerosis due to decreased insulin growth factor 1 receptor (IGF-1R) expression, increased cardiac fibrosis and decreased contractility [63, 65]. Since miR-133a positively regulates IGF-1R expression, it could be speculated that downregulation of miR-133a in diabetic heart could result in decreased proliferation and increased senescence of CPC. This has to be further validated to develop a specific therapeutic strategy to regulate miR-133a expression.

miR-150. miR-150 is an important regulator of hematopoiesis. Its expression pattern varies during the differentiation of hematopoietic lineage, where it is highly expressed in mature B and T cells and show reduced expression in progenitor cells [66]. It has been reported to function as a tumor suppressor and its aberrant downregulation induced continuous activation of PI3-Akt pathway and subsequent telomerase activation [67]. Surprisingly, patients with type 2 diabetes and atherosclerosis were shown to have high plasma levels of miR-150 [68] and other studies have shown dysregulated miR-150 expression under hyperglycemic conditions to be associated with myocardial fibrosis, hypertrophy, and pathological angiogenesis [69–71]. Although a regulator of hematopoietic lineage, the effect of miR-150 upregulation in CPC proliferation, differentiation, and senescence has not yet been reported. However, Farhana et al. [70] demonstrated that upregulation of miR-150 induced apoptosis in pancreatic cancer cells by targeting IGF-1R and c-Myb (proto-oncogene). Both IGF-1R and c-Myb are important for maintaining cell proliferation, and their downregulation led to reduction in Bcl2 (anti-apoptotic) expression and hence apoptosis [70]. Considering the role of miR-150 in cell proliferation, differentiation, and angiogenesis, it is crucial to discover its importance as a therapeutic target to enhance the efficiency of stem/progenitor cell therapy for in diabetic conditions.

miR-141. miR-141 is another presenescent miRNA which has been shown to post-transcriptionally downregulated BMI-1 expression in human diploid fibroblasts [72]. BMI-1 is required for self-renewal of neural and HSCs [73, 74]. Dimri et al. showed that overexpression of miR-141 induces premature senescence in human diploid fibroblasts by directly targeting BMI-1 in normal but not in exogenous BMI-1-overexpressing HDF [72]. Induction of premature senescence was accompanied by upregulation of $p16^{INK4a}$, an important downstream target of BMI-1 and a major regulator of senescence. Interestingly, miR-141 is upregulated in the diabetic heart [75]. miRNA target prediction analyses identified miR-141 as a potential regulator of the inner mitochondrial membrane phosphate transporter, solute carrier family 25 member 3 (Slc25a3), which provides inorganic phosphate to the mitochondrial matrix and is essential for ATP production [75]. Overexpression of miR-141 in HL-1 cells elicited a decrease in Slc25a3 protein content, ATP production, and a decrease in ATP synthase activity, similar to the diabetic phenotype. This indicated that miR-141 could regulate Slc25a3 protein expression in the diabetic heart. While the effect of diabetes on BMI-1 in CPC is not known, Orlandi et al. showed marked reduction in BMI-1 in diabetic bone marrow cells [76] suggesting that restoration of BMI-1 through modulation of miR-141 could be another strategy to reduce premature senescence in diabetic CPC.

miR-373. miR-373 is an anti-senescent miRNA that can bypass Ras-induced senescence by directly targeting the 3′-UTR of p21 [77] and through the downregulation of large tumor suppressor kinase 2 (LATS2) [78]. miR-373 also prevented Ras-induced senescence in human fibroblasts [79] suggesting its universal immortalization mechanism. Although the status of miR-373 in diabetic CPC has not been explored, Shen et al. showed marked downregulation of miR-373 in diabetic heart [80]. They showed that activation of mitogen-activated protein kinase (MAPK) signaling pathway in the diabetic heart or high glucose treated cardiomyocytes downregulated miR-373 expression leading to cardiomyocyte hypertrophy. In addition, cardiomyocytes transfected with miR-373 mimic showed repression of MADS Box Transcription Enhancer Factor 2 - C (MEF2C), a transcription factor known to be involved in cardiac hypertrophy. Further studies are required to explore the role of miR-373 in diabetic CPC.

Long Noncoding RNA

LncRNAs are nonprotein coding transcripts larger than 200 nucleotides and with multiple exons that are subjected to alternative splicing to generate isoforms [81]. Accumulating animal and human studies highlight that lncRNAs plays a key role in pathogenesis of diabetes [82]. It is therefore crucial to improve our understanding of how lncRNAs regulate complex signaling networks to provide a therapeutic opportunity to modify glucose metabolism in stem/progenitor cells. This section will discuss four important lncRNA that may possibly be

involved in regulating the proliferation and differentiation of diabetic stem/progenitor cells.

H19. H19 is a maternally imprinted gene that is abundantly expressed during the development of embryo [83] and is one of the highly studied lncRNA on stem cells. Its exon 1 acts as a template for prosurvival and antisenescent miR675 [84]. Deng et al. showed that activation of H19 on resident MSCs improved the regenerative ability of these cells [85]. In another study, Cai et al. demonstrated that inhibition of oxidative stress-induced p53 and p21 proteins by H19 derived miR-675 in CPC [86]. While the role of H19 on diabetic CPCs is not known, a study by Liu et al. showed that H19 acts a negative regulator of cardiomyocyte hypertrophy by regulating levels of miR675 and targeting Ca^{2+}/calmodulin-dependent protein kinase II delta (CaMKIIδ) [87]. In a successive study, Li et al. overexpressed H19 in diabetic rats which attenuated oxidative stress, inflammation, and apoptosis and consequently improved left ventricular function through upregulation of miR-675 that targets proapoptotic voltage-dependent anion channel 1 [88].

Braveheart. The lncRNA Braveheart (Bvht) has been demonstrated to be crucial for the commitment of embryonic stem cells to the cardiovascular lineage commitment [89]. In an elegant study, Klattenhoff et al. showed that Bvht functions upstream of MesP1 to direct embryonic stem cells toward cardiac commitment [89]. MesP1 acts as a master regulatory switch during the cardiovascular specification of stem cells [90]. Indeed, the expression of MesP1 was sufficient to transdifferentiate dermal fibroblasts into CPC. In addition to MesP1, Bvht interacts with SUZ12, a core component of PRC2 [91], suggesting the Bvht is important for epigenetic regulation of cardiac genes. Recently, Hou et al. showed the importance of Bvht in promoting the cardiogenic differentiation of MSC. Overexpression of Bvht in MSC isolated from mouse bone marrow showed a better cardiogenic differentiation and importantly inhibition of MesP1 significantly attenuated the transdifferentiation of MSC overexpressed with Bvht [92]. Being a relatively newcomer to the family of lncRNA, the role of Bvht on CPCs and diabetes is still not known, although the evidence suggests Bvht as a strong candidate requiring further studies.

ANRIL. Antisense noncoding RNA in the INK4 locus (ANRIL) is a scaffold lncRNA that forms a complex with multiple molecular components that act as transcriptional activators or repressors. It recruits and interacts with PRC1 and PRC2 leading to silencing of the INK4b-ARF-INK4a locus which plays a critical role in cell cycle inhibition and senescence [93]. Genome-wide analysis reported decreased levels of ANRIL in senescent WI-38 lung fibroblasts, and its silencing increased the abundance of cell cycle inhibitors and induced senescence [94]. Downregulation of ANRIL significantly enhanced the levels of the cyclin-dependent kinase (cdk) inhibitor p15 and moderately increased the levels of p16 [95]. ANRIL has been described as a genetic risk factor for coronary artery disease [96, 97] and type 2 diabetes [98, 99], and its expression level is associated with left ventricular dysfunction after myocardial infarction [100]. To date, the role of ANRIL on stem cells is unknown although, based on the published evidence, it is logical that downregulation in ANRIL could be one of the major risk factors in CPC senescence.

Kcnq1ot1. The *Kcnq1ot1* is a 91-kb long lncRNA that causes transcriptional gene silencing of the *Kcnq1* gene locus by influencing histone modifications [101]. It recruits the histone methyl-transferases G9a and PRC2 leading to H3K9 and H3K27 trimethylation that repress transcription of *Kcnq1* gene locus. One of the genes within this locus is the cdk inhibitor and tumor suppressor *CDKN1C*, encoding $P57^{KIP2}$ [102]. Suppression of *Kcnq1ot1* expression allows expression of genes on the *Kcnq* locus relevant to age-associated diseases like type 2 diabetes. Thus, *Kcnq1ot1* can affect aging and senescence through its impact upon the cell cycle. Therefore, it would be a significant effort to profile *Kcnq1ot1* lncRNA levels in diabetic stem/progenitor cells, which might possibly be involved in inducing senescence and hence viability.

Thus, miRNA and lncRNA could possibly regulate the self-renewal and proliferation of CPC by crosstalking with cell cycle regulators through various signaling pathways and transcription factors. However, the major question that remains to be answered is: What is the mechanism by which noncoding RNA synchronize their activity with signaling pathways to regulate stem/progenitor cell senescence? Another important question is: Do miRNAs/lncRNAs regulate stem/progenitor cells individually or as a group? Identifying the differential effects of noncoding RNA could therefore become a pioneering discovery in the field of cardiac regeneration. Although noncoding RNA act as important regulatory elements, recent studies show that extracellular vesicles such as exosomes could be other key players, which are tightly regulated and secreted to illicit physiological response.

Exosomes

Exosomes (30–100 nm) are the population of extracellular vesicles released from cells when a vesicle fuse with the plasma membrane in a highly regulated manner and release their contents [103]. Exosomes carry a composite cargo of molecules, including miRNAs, proteins, and lipids, whose quality and quantity vary with cell types and the environmental conditions. Moreover, the exosome cargo could be transferrable to other cell types, where the recipient cells respond to exosome uptake with expressional and functional changes [104, 105].

Cardiac cell communication via exosomes in both healthy and pathological conditions is an emerging area of research. Gray et al. observed that exosomes derived from CPC cultured in hypoxic conditions had more angiogenic potential than exosomes released by CPC cultured under normoxic conditions [106]. Compared with normoxic exosomes, hypoxic exosomes contained higher amounts of proangiogenic miRNAs, including miR-132 and miR-146a, and could efficiently induce tube formation in cultured endothelial cells [106]. Thus, hypoxia triggered exosome release may serve as a positive feedback mechanism to counter ischemic environment. However, it is important to evaluate whether hyperglycemia induced hypoxia could trigger similar proangiogenic effect on exosomes.

The exosome status of CPC from a diabetic heart is yet to be studied, although a few elegant studies highlight the role

of exosomes in diabetes-induced heart disease. In a recent study, Wang et al. demonstrated the release of detrimental exosomes from diabetic cardiomyocytes, which contain lower levels of heat shock protein (Hsp) 20 than the exosomes released by healthy cardiomyocytes [107]. In a subsequent study, using a transgenic mouse model with overexpression of Hsp20, they were able to engineer the detrimental exosomes to induce protective effect as evidenced by significant attenuation of cardiac dysfunction, hypertrophy, apoptosis, fibrosis, and microvascular rarefaction in the diabetic heart through activation of miR-320 [108]. Importantly, blocking the exosome generation by GW4869 remarkably reduced Hsp20-mediated cardioprotection in diabetic mice [108]. Another study by Chaturvedi et al. observed exercise-induced secretion of cardiac exosomes containing anti-fibrotic miRNAs (miR-29b, miR-323-5p, miR-455, and miR-466) in diabetic rats [109]. These miRNAs downregulate abnormally elevated proinflammatory matrix metalloproteinase-9 in the diabetic heart. Therefore, while the role of exosomes on diabetic CPC is yet to be determined, it is plausible that exosomes released from diabetic CPC could be adversely modulated.

In addition to CPC, $CD34^{+ve}$ bone marrow cells [110] and MSCs [104] have been used as a source for cardioprotective exosomes. Interestingly, cardiomyocytes and cardiac endothelial cells, but not fibroblasts, selectively took up exosomes from $CD34^{+ve}$ bone marrow cells, suggesting the presence of cell-specific receptors on the exosome membrane [110]. This provides an option for targeted exosome-mediated delivery of cardioprotective drugs.

Another major cause for stem cell senescence and impaired cardiac repair is the production of ROS. The prime source of ROS generation is the mitochondria, and hyperglycemia can induce mitochondrial aberrations and damage leading to further increase in ROS.

Mitochondrial Aberrations in Diabetic Stem/Progenitor Cells

Mitochondria regulate several key processes including metabolism, respiration, and cell death. They are responsible for producing energy via oxidative phosphorylation for cellular development, differentiation, and growth [111–113]. Defects in mitochondria and aberrant uncoupling of mitochondrial complexes can lead to excessive ROS production causing cellular damage by inducing senescence and apoptosis [111, 113]. This section will discuss two major mechanisms that could affect the efficiency of mitochondria of stem/progenitor cells in diabetes.

Advanced Glycation End-products

Under hyperglycemic conditions, by-products are formed by nonenzymatic glycosylation of proteins called advanced glycation end-products (AGEs) that can result in metabolic memory [37]. Especially, levels of methyl glyoxal, a by-product of glycolysis, increase during diabetes. Methyl glyoxal induced modifications of mitochondrial proteins have been shown to inhibit mitochondrial respiration [37]. The mitochondrial respiratory proteins, which undergo glycation, are prone to produce superoxide anion independent of glycemic levels. This AGE-induced process would therefore increase ROS levels that could trigger various pathological events to induce senescence of CPC similar to bone marrow stem cells [114].

Effect of Mitochondrial Mutations on CPC Differentiation

Mitochondrial DNA replicates independent of the nuclear DNA [115]. Accumulation of mtDNA mutations in the heart is associated with increased ROS and apoptosis, which result in the development of cardiomyopathy [116]. Mitochondrial replication requires several proteins, among which mitochondrial DNA polymerase-γ-1 (PolgA) is a catalytic subunit with proof reading ability [117–119]. Differentiation of stem cells and CPC is associated with increased demand in supply of energy [120–122], and therefore an increased expression of proteins involved in oxidative phosphorylation and concurrent decrease in glycolytic proteins is required. Orogo et al. demonstrated the effect of PolgA mutations on CPC differentiation. They showed that CPC with a mutation in PolgA are deficient in mitochondrial respiration and hence do not depend on oxidative phosphorylation for the production of ATP, but rely on glycolysis [123]. An interesting finding from their study was that CPC with the PolgA mutation turn off their glycolytic pathway during differentiation despite having damaged mitochondria [123]. Interestingly, Tewari et al. showed PolgA mutation in retinal cells in a mouse model of diabetic retinopathy [124]. Although not reported, diabetic CPC with a defective PolgA might result in malfunctioned mitochondrial as well as glycolytic pathways and being unable to meet the increased energy demand during differentiation [123]. These findings demonstrate the consequences of accumulating mitochondrial DNA mutations and importance of mitochondrial DNA integrity in CPC homeostasis and their potential in regenerating the diabetic heart. The mitochondrial DNA mutations cause mitochondrial proteins to misfold, aggregate, and form nonspecific aqueous pores whose opening leads to mitochondrial permeability transition [125]. Under minimal stress conditions, the mitochondrial permeability transition pore promotes mitophagy, a form of autophagy to remove damaged mitochondria, thereby preventing the necrosis of host cell [126].

Apart from mitophagy, autophagy also plays an important role in promoting cardiac differentiation that is differentially regulated during stress conditions, as discussed in the Autophagy section.

Autophagy

Autophagy is an evolutionarily conserved catabolic process, which regulates cellular homeostasis by degrading and recycling damaged organelles [127]. Understanding and regulating autophagy would help to gain important insights on how CPC respond to the proliferation and differentiation signals. The two important regulatory pathways that are involved in autophagy are mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) pathways. The mTOR acts by directly inhibiting phosphorylation and inactivation of ULK/Atg1 (uncoordinated-51-like kinase/autophagy related-1) and Atg13, which are essential for the induction of autophagy. AMPK, an energy sensor, directly activates autophagy by

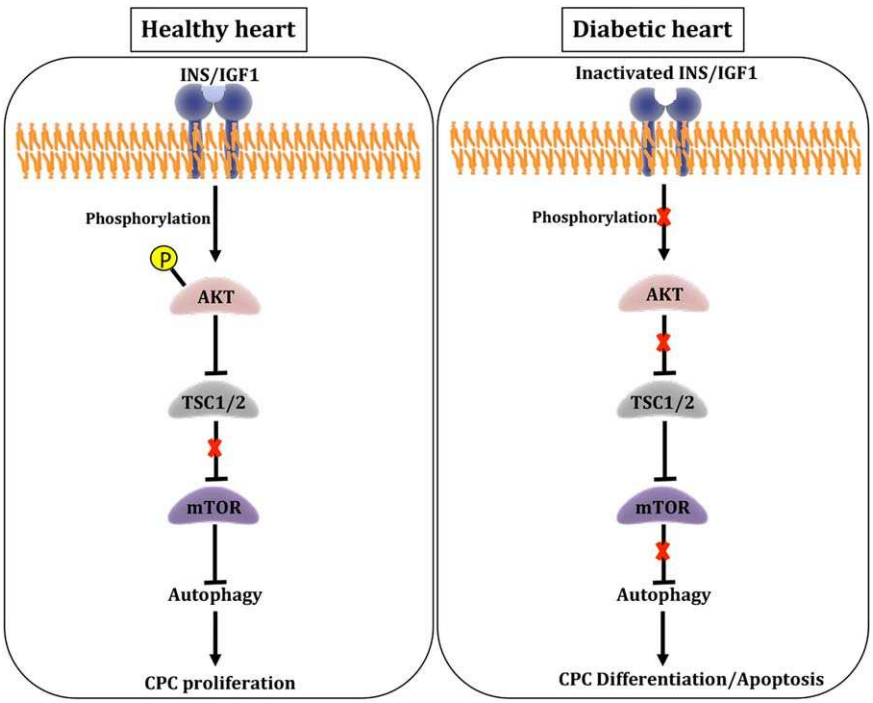

Figure 3. Proposed mechanism of autophagy regulation by insulin/insulin-like growth factor 1 (INS/IGF-1) signaling pathway. **Left**: In the healthy heart, positive signals from INS/IGF-1 inhibits autophagy by activating Akt resulting in cardiac progenitor cell (CPC) proliferation. **Right**: In the diabetic heart, inactivated INS/IGF-1 signaling pathway leads to activation of autophagy, which may lead to either premature CPC differentiation or apoptosis. Abbreviations: CPC, cardiac progenitor cell; INS/IGF-1, insulin/insulin-like growth factor 1; mTOR, mammalian target of rapamycin; TSC1/2, Tuberous sclerosis complex 1/2.

initiating ULK/Atg1 initiation complex. AMPK can inhibit mTOR by phosphorylating mTORC1 and promotes autophagy [128].

Autophagy in CPC Proliferation and Differentiation

Zhang et al. was the first to demonstrate the role of autophagy in regulating cardiac differentiation of CPC [129]. Through cardiac-specific ablation of fibroblast growth factor (FGF) receptor and its substrate FGF receptor substrate 2α, they demonstrated the positive role of FGF signaling in suppressing the premature differentiation of CPC and embryonic stem cells through inhibition of autophagy by activation of Akt (protein kinase B) and MAPK pathways. They also confirmed that activation of autophagy induced the differentiation of CPC [129]. In addition to its role in CPC and embryonic stem cells, recent studies have shown autophagy as a driving factor in differentiation of MSC to various lineages including osteocyte, adipocyte, and chondrocyte [127]. While FGF signaling is beneficial in the early stages, in a different study, Zhang et al. showed that persistent increase in FGF could prevent differentiation of cardiomyocytes at a later stage, suggesting that FGF could act as a double-edged sword in cardiac differentiation [130].

The exact role of autophagy in the diabetic heart is still not clear with contradictory results. Kubli and Gustafsson showed reduced autophagy activity in type 1 diabetic hearts due to increased expression of mTOR [128]. In contrast, a recent study from our laboratory demonstrated a marked increase in autophagy proteins in human and mouse type 2 diabetic hearts, which was associated with increased apoptotic cell death [131]. One possibility for the difference between both the studies could be the difference in the type of diabetes. Positive signals from growth factors such as INS/IGF-1 induce phosphorylation of Akt. Phosphorylated Akt in turn inactivate tuberous sclerosis complex-1/2 (TSC1/2), releasing the inhibitory effect of TSC1/2 on mTOR activity, which eventually inhibits autophagy. In addition, INS/IGF-1 signaling directly regulates CPC proliferation [132]. Its role in inhibiting autophagy and inducing CPC proliferation leads to the speculation that INS/IGF-1 signaling could act as an upstream regulator of autophagy in regulating cardiac homeostasis. However, diabetes dramatically reduces the secretion of growth factors such as INS/IGF-1 or expression of their respective receptors in the heart. Therefore, impaired INS/IGF-1 signaling might result in activation of autophagy and premature CPC differentiation or apoptosis (Fig. 3). However, the exact role of autophagy in regulating CPC proliferation or differentiation is yet to be deciphered.

Role of Autophagy during Stress

Nutrient (glucose) deprivation and hypoxia are two of the most important factors of cellular stress during diabetes. In nutrient-deprived conditions, cardiac homeostasis depends on autophagy. Hariharan et al. showed that interaction of pro-apoptotic transcription factor FOXO-1 with the metabolic sensor SIRT-1 is required for induction of autophagy [133]. In the presence of insulin, Akt phosphorylates FOXO-1 to an inactive state and sequesters it from nucleus to cytosol, thereby inhibiting the transcription of autophagy related proteins [134]. However, in glucose-deprived conditions, FOXO-1 remains in the active dephosphorylated state to interact with SIRT-1 leading to activation of autophagy [133]. In addition, Warr et al. demonstrated that HSC retain an intact FOXO-3a driven pro-autophagy program to survive in a nutrient-deprived bone marrow niche [135]. Therefore, nutrient-deprived conditions may induce premature differentiation or apoptosis of CPC.

Table 1. Summary of dysregulated molecular pathway in diabetic stem/progenitor cells

Cell type	Dysregulation	Outcome	Subject	References
Bone marrow stromal cells	↑SDF-1, ↑M1-macrophages	↓Migration	Human, mouse	[140]
Hematopoietic stem cells	↑SDF-1	↓Migration	Human	[142]
CPC	↓Ephrin	↓Migration	Human	[143]
CPC	↓SCF, ↓ERK1/2, ↓p38 MAPK	↓Migration and homing	Rat	[144–146]
CPC	↓IGF-1R	↓Proliferation, ↑senescence, ↑apoptosis	Human	[147]
CPC	↑$P66^{Shc}$	↑Senescence, ↑apoptosis	Mouse	[28]
CPC	↑Methylglyoxal, ↑AGE	↓Angiogenesis	Human, mouse	[148]
c-kit^{+ve} CPC, Cardiomyocytes	↓Pim-1	Apoptosis	Human, mouse	[21, 149–151]
c-kit^{+ve} CPC, Endothelial cells	↓ Pin-1/↑ Pin-1	↓Proliferation, ↑Senescence, ↑Differentiation		[152–155]

Abbreviations: AGE, advanced glycation end products; CPC, cardiac progenitor cells; ERK, extracellular signal-regulated kinase; IGF-1R, insulin-like growth factor 1 receptor; MAPK, mitogen-activated protein kinase; Pin-1, peptidylprolyl *cis/trans* isomerase, NIMA-interacting 1; Pim-1, proviral integration site for moloney murine leukemia virus-1; SDF, stromal-derived factor; SCF, stem cell factor.

Similarly, when EPCs were exposed to a different stressor, hypoxia, they showed increased expression of pro-autophagy markers Beclin-1 and microtubule- associated protein 1 light chain 3 - II (LC3-II) along with increased proliferation. Interestingly, inhibition of autophagosome formation by treating the cells with 3-methyl adenine (inhibitor of lysosomal degradation) reduced the proliferation of EPC while increasing their cell death [136].

Finally, in the genetic Otsuka Long-Evans Tokushima fatty rats (OLETF) model of type 2 diabetes, Murase et al. demonstrated the protective effects of dipeptidyl peptidase-4 (DPP4) inhibitor vildagliptin in reducing the acute mortality after myocardial infarction through activation of autophagy response. Vildagliptin suppressed Beclin-1-Bcl-2 interaction and increased both LC3-II protein level and autophagosomes in the nonischemic region. The serum glucagon-like peptide-1 (GLP-1) levels also increased after treatment with vildagliptin suggesting that the action of vildagliptin is via GLP-1-mediated mechanism [137]. However, the role of DPP4 inhibitors in regulating autophagy in CPC in diabetes is yet to be studied and future studies could help in developing novel therapeutic strategy. Thus, in the diabetic heart, molecular alterations in autophagy signaling could play a major role in determining the functional efficacy of CPC.

Migration and Homing of Stem/Progenitor Cells in Diabetic Heart

Survival of transplanted cells in the host tissue is a major challenge for successful regeneration therapy. The metabolic maladaptations in a failing heart reduce migration, homing, and survival of transplanted cells, the first step in the regenerative process [138]. This process is exacerbated in the diabetic heart, resulting in reduced therapeutic efficacy of the transplanted cells [139–141]. This section will discuss the underlying molecular signaling cascades that regulate the disturbed process of migration and homing of stem cells in diabetes. A detailed list of aberrant molecular pathways is in Table 1.

Role of Inflammatory Macrophages

Diabetic mobilopathy is characterized by the failure of either endogenous or transplanted stem cells to mobilize to the area of injury [139]. Recently, Albiero et al. highlighted the involvement of macrophages in diabetic mobilopathy. They showed an increased polarization of proinflammatory M1 macrophages in the bone marrow of type 1 diabetic individuals, which inhibit mobilization of bone marrow stromal cells by activation of stromal cell-derived factor-1-α (SDF-1α) [142], a potent chemoattractant that has the ability to orchestrate the mobilization of stem cells [156]. They further showed an increase in $CD169^{+}$ pro-inflammatory M1 macrophages in the bone marrow (with stem cells retaining activity) of streptozotocin-induced type 1 diabetic mice compared with the nondiabetic controls. Interestingly, treating these animals with clodronate liposomes (a liposome mediated macrophage "suicide" approach) improved stem cell mobilization [142]. Using in silico analysis, they identified that oncostatin-M, a soluble factor secreted by the inflammatory macrophages, activates SDF-1α in diabetic bone marrow stromal cells. By neutralizing oncostatin-M, they were able to inhibit the induction of SDF-1α, thereby restoring the mobilization of diabetic bone marrow stromal cells (Fig. 4A) [142].

SDF-1α/CXCR4 Signaling Axis

Granulocyte-colony stimulating factor (G-CSF) is a glycoprotein that has been shown to stimulate the bone marrow to produce granulocytes and stem cells and release them into the bloodstream [157]. G-CSF is involved in reducing the levels of the chemokine and HSC retention factor SDF-1α, which is responsible for mediating HSC trafficking in and out of the bone marrow through its receptor C-X-C chemokine receptor type 4 (CXCR4) [158] (Fig. 4A). Fadini et al. investigated the effect of G-CSF in mobilization of HSC from diabetic individuals and reported it to be ineffective compared with nondiabetic individuals. However, a combination of plerixafor, a CXCR4 antagonist, and G-CSF was more effective in mobilizing HSC than G-CSF alone [159]. In a contrasting study, it was reported that the activation of CXCR-4/SDF-1α expression through adenoviral mediated overexpression of Ang-1 into ischemic heart of a diabetic mice attenuated cardiac apoptosis and improved cardiac repair by promoting the homing of $CD133^{+}$/$C\text{-}Kit^{+}$ stem cells [143]. While the exact mechanism is unknown, the differential regulation of migration and homing of stem cells by CXCR-4/SDF-1α could be attributed to the differential origin of stem cells.

Role of Ephr-A2 Signaling

In an age-associated study on human CPC migration, Goichberg et al. demonstrated a key role for ephrin (Ephr) in regulating the mobilization of progenitor cells [160]. Activation of Ephr-A2 by Ephr-A1 ligand promoted the migration of human

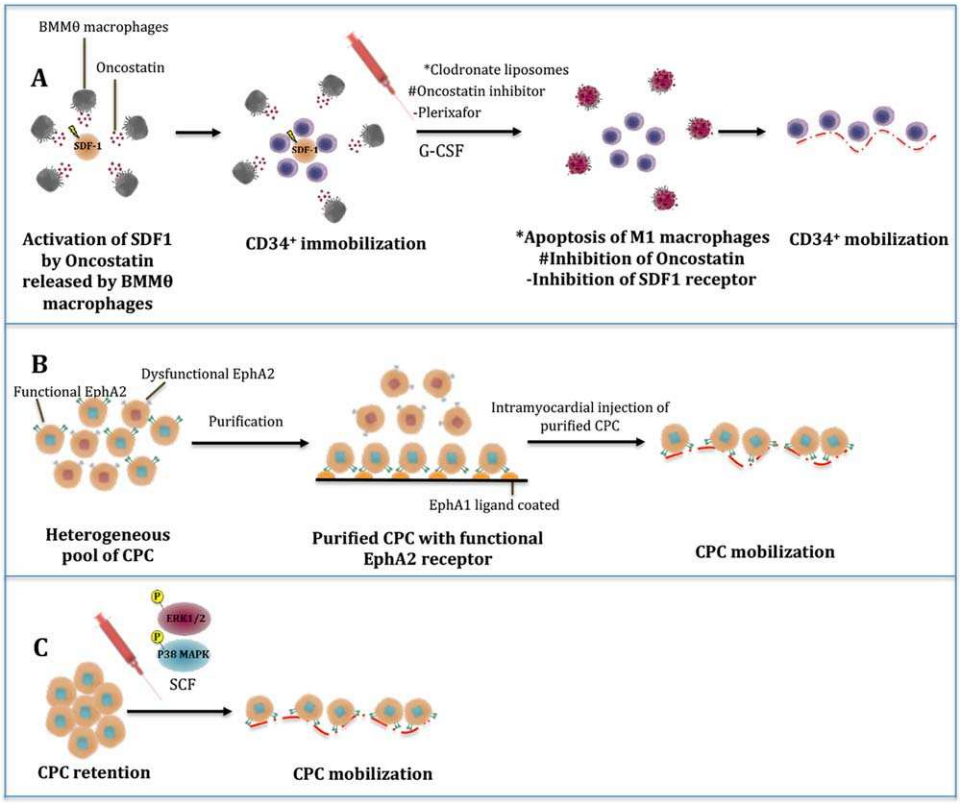

Figure 4. Strategies to mobilize diabetic stem/progenitor cells. **(A)**: Combinatorial treatment to induce mobilization of hematopoietic stem cells from bone marrow. **(B)**: Purification of cardiac progenitor cell (CPC) with functional Ephrin-A2 signaling to enhance mobilization. **(C)**: CPC mobilization by administering stem cell factor or by activating ERK1/2-P38 MAPK by phosphorylation. Abbreviations: BMMθ, bone marrow M1 macrophages; CPC, cardiac progenitor cell; G-CSF, granulocyte-colony stimulating factor; SCF, stem cell factor; SDF-1, stromal derived factor-1.

CPC to the injured myocardium. They showed that inadequate activation of the Ephr-A2 receptor in aged human CPC could be a reason for impaired migratory capacity compared with young human CPC. Furthermore, selective induction of Ephr-A2 signaling resulted in enhanced migration of CPC [160] (Fig. 4B). Diabetes hastens the aging process [29] and reduced the mobilization of stem cells [139, 141]. Although there are no reports yet on regulation of Ephr-A2 in diabetic hearts, Ephr-B2 treatment enhanced the wound healing property (a characteristic of cellular migration) of PBMC in diabetes [161]. These studies suggest that activation of Ephr signaling could serve as an effective tool in selecting therapeutically efficient CPC in both diabetic and nondiabetic scenario.

ERK1/2-P38MAPK-SCF Axis

MAPK-like extracellular signal regulated kinase 1/2 (ERK1/2) and P38 MAPK are a family of serine/threonine kinases that regulate the diversity of cellular activities by multiple cascade of phosphorylation [144]. Various studies have indicated the involvement of stem cell factor (SCF) and its receptor (c-kit) signaling in the migration of CPC to peri-infarct region of the myocardium through activation of ERK1/2 and p38 MAPK pathways [145, 146, 162, 163]. She et al. demonstrated reduced expression of SCF in diabetic rat hearts through reduction in ERK1/2 and p38 MAPK phosphorylation. In support of this, diabetic hearts showed a significant reduction in the accumulation of injected CPC following experimental myocardial infarction, which was possibly due to the impaired migration of CPC to the infarct zone due to inactivation of ERK1/2 and p38MAPK pathway [164]. Therefore, combinatorial activation of ERK1/2 and P38 MAPK and administration of SCF could help in boosting the migration potential of CPC in a diabetic scenario (Fig. 4C).

Preconditioning and Enrichment of Diabetic CPC

Having discussed the pathology and defects in the diabetic stem/progenitor cells, the next section of this review will discuss how preconditioning and enrichment of diabetic CPC could potentially overcome these drawbacks to exhibit the beneficial regenerative effects. Preconditioning and enrichment of diabetic CPC could enhance their therapeutic efficacy before transplantation, which can be implemented either by treating CPC with pharmacological analogues and growth factors or by regulating the expression of a gene of interest of the CPC.

Pharmacological Preconditioning of Diabetic CPC

Antioxidants. The pentose shunt pathway is an anabolic alternative of glycolysis, which plays a key role in maintaining the redox state and contractility of cardiomyocytes by generating pyridine nucleotide nicotinamide adenine dinucleotide phosphate [165]. However, this pathway is dysregulated in a diabetic heart [166]. Using the vitamin B1 derivative benfotiamine to

boost the pentose shunt pathway in a mouse model of type 1 diabetes, we demonstrated an improved survival. Similar results were observed in human CPC exposed to hyperglycemia [22]. Similarly, Laviola et al. showed the protective effect of GLP-1, a neuropeptide released by gut, on human CPC through inhibition of oxidative stress-mediated apoptosis by inactivating the phosphorylation and nuclear localization of stress kinase c-Jun N terminal protein kinase [167]. Other studies have also demonstrated the cardioprotective effects of GLP-1 in improving cardiac contractile function in chronic heart failure patients, with or without diabetes [168, 169]. Since generation of ROS represent the major event leading to dysfunction and death of diabetic CPC [22, 114], it is highly likely, but not yet shown, that anti-oxidants could be used as one of the major pharmacological substances to improve the regenerative potential of diabetic CPC.

Paracrine and Growth Factors. Recent studies suggest that the major mediator of the therapeutic potential of stem cells is through secretion of paracrine and/or growth factors, which either activate the endogenous resident progenitor cells or attract them to the site of injury [170, 171]. In line with this, dose-dependent administration of insulin-like growth factor-1/hepatocyte growth factor (IGF/HGF), through coronary circulation, activated C-Kit^{+ve}CD45^{-ve} CPC in a porcine model of myocardial infarction, improving the cardiomyogenic differentiation and vascularization [172]. Eren et al. demonstrated the paracrine factor-mediated enhancement of progenitor cell efficacy by treating the bone marrow mononuclear cells and EPC with conditioned medium from adipocytes [173]. They demonstrated marked improvement in the survival and proliferation of bone marrow mononuclear cells and increased differentiation of EPC following treatment with the conditioned medium. They further identified that adiponectin, a circulating hormone secreted by adipocytes, play a key role in this cytoprotective effect [173]. While the above studies were conducted in nondiabetic models, it is well known that diabetes dysregulates IGF, HGF, and adiponectin levels [174–176]. Hence, treatment of diabetic CPC with these paracrine and growth factors is likely to improve their regenerative potential.

O-GlcNAc Modification. O-GlcNAcylation is a post-translational modification of nuclear and cellular proteins. A study by Zafir et al. demonstrated protective effect of protein O-GlcNAcylation on proliferation of mouse CPC and cell cycle [177]. In subsequent studies, they showed activation of prosurvival signaling cascade by protein O-GlcNAcylation in both c-kit^{+} [178] and Sca-1^{+} mouse CPC [179] following hypoxia-reoxygenation injury. They observed significant exacerbation of post-hypoxic cell death following reduction in protein O-GlcNAcylation, while augmenting O-GlcNAc levels enhanced cell survival. In contrast, sustained elevation of protein O-GlcNAc levels have been associated with diabetes induced reduced Ca^{2+} sensitivity in myofilaments [180] impaired cardiomyocytes mitochondrial function [181, 182], impaired vasodilation [183] and increased cardiac injury [184]. Earlier studies reported that O-GlcNAc reduce the levels of centromere protein-A (CENP-A) [185]. CENP-A is a critical component of cell cycle process and recently McGregor et al. showed that reduction in the levels of CENP-A induce senescence in CPC [186, 187]. While physiological O-GlcNAcylation of proteins may serve as an important cardioprotective feedback mechanism, sustained high levels of OGT by hyperglycemia in diabetes could induce senescence of CPC in an O-GlcNAc dependent manner. Therefore, restoration of protein O-GlcNAc levels in diabetic CPC could be beneficial in improving its therapeutic efficacy.

In Vitro Stem Cell Engineering

IGF-1R Expressing CPC. As described above, diabetic hearts dramatically reduce the expression of IGF-1R, which plays a major role in cell growth, survival, and delaying cellular aging through activation of nuclear phospho Akt [147]. D'amario et al. demonstrated that C-Kit^{+} human CPC expressing high level of IGF-1R activity exhibited high proliferation, high telomerase activity, and attenuated apoptosis [188]. Interestingly, cells without IGF-1R but with IGF-2R and angiotensin 1 receptor exhibited senescent phenotype and increased apoptosis [188]. Furthermore, human CPC expressing only IGF-1R synthesize both IGF-1 and IGF-2, which are potent modulators of stem cell replication and their commitment to the myocyte lineage and differentiation. Therefore, purification and enrichment of IGF-1R expressing C-Kit^{+ve} cells or genetically engineering CPC with IGF-1R could improve the therapeutic efficiency of diabetic CPC.

Deletion of p66^{Shc} in CPC. p66^{shc} is a member of Shc family of proteins that affects the cell growth negatively by increasing ROS formation and shortening of the telomere eventually increasing the cellular senescence [149]. Using a mouse model of insulin-dependent diabetes mellitus, Rota et al. showed that hyperglycemia-induced ROS leads to telomere shortening, increased expression of the senescence proteins p53 and p16^{INK4a} and apoptosis of CPC. Altogether, they impair the growth reserve of the heart. However, ablation of the p66^{shc}gene prevented this negative impact of diabetes on CPC senescence and consequently reduced the development of cardiac dysfunction in diabetic mice [29]. Therefore, CPC specific ablation or repression of p66^{shc}gene serves as a promising target in ameliorating CPC senescence and improving their therapeutic efficiency.

Overexpression of Glo-1. A hyperglycemic environment would enhance the glucose flux and increase glycolytic intermediates such as dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Breakdown of these glycolytic intermediates leads to accumulation of methylglyoxal resulting in the formation of AGE and oxidative stress [16]. Molgat et al. demonstrated the effect of glyoxalase-1 (Glo-1), a methylglyoxal detoxifier, on regenerative potential of CPC, where overexpression of Glo-1 restored the angiogenic ability of human and mouse CPC exposed to hyperglycemia. In addition, they demonstrated that Glo-1 transgenic mice were resistant to diabetes-induced deterioration of CPC efficacy [16]. Therefore, methylglyoxal-mediated damage could be countered by overexpression of Glo-1 or by development of Glo-1 inducers to improve the regenerative efficacy of CPC in patients with diabetes.

Overexpression of Pim-1. Pim-1 is a member of serine-threonine protein kinase family that increases the proliferative activity of CPC by inducing c-myc, nucleostemin, and cyclin E expression and p21 phosphorylation [150, 189]. We showed marked downregulation of myocardial proviral integration site for moloney murine leukemia virus-1 (Pim-1) expression from

the early stage in mouse model of type 1 [151] and type 2 diabetes [190], which was also confirmed in human diabetic hearts [151]. Using murine cardiomyocytes [151] and C-kit^{+ve} [22] cells we further confirmed that the hyperglycemia-induced downregulation of Pim-1 is one of the major molecular causes for enhanced apoptotic cell death in the diabetic heart. In support of this, overexpression of Pim-1 significantly improved the regenerative potential of endogenous CPC in vivo and proliferative capacity of CPC in vitro respectively [152]. Given the above evidences, activation of Pim-1 expression has a major effect in improving regenerative capacity of CPC both in vitro and in vivo.

Regulation of Pin-1 Expression. Prolyl isomerase peptidyl-prolyl *cis/trans* isomerase, NIMA-interacting 1(Pin-1) is a molecule that regulates multiple signaling cascades by modulating protein folding and thereby the activity and stability of phosphoproteins. Proliferating cells express Pin-1, which influences multiple regulatory molecules of the mitotic processes including cyclins and cell cycle inhibitors [153, 191]. Toko et al. demonstrated the key role of Pin-1 in C-Kit^{+ve} CPC proliferation, differentiation, and senescence through regulation of the cell cycle [154]. They showed that reduced Pin-1 expression causes cell cycle arrest of CPC in G1 phase and thus leads to decrease in number of CPC both in vitro and in vivo. Moreover, loss of Pin-1 also directly affects the levels of cyclin D and cyclin B expression, and activates prosenescent p53 and Rb pathways inhibiting cell cycle progression. In contrast, overexpression of Pin-1 impaired the proliferation of CPC through G2/M phase cell cycle arrest by initiating differentiation [154]. These reports lead to the speculation that the level of Pin-1 expression may act as a deciding factor for regulation of CPC proliferation, differentiation, and senescence. Although the role of Pin-1 in diabetic CPC is still not studied, Paneni et al. recently reported marked increase in the expression of Pin-1 in diabetic human endothelial cells that drives the development of vascular dysfunction through increased oxidative stress [155]. Gene silencing of Pin-1 suppressed the ROS production and improved the nitric oxide release from diabetic endothelial cells [192]. The above evidence indicate that Pin-1 has a differential role in regulating CPC senescence and apoptosis depending upon the level of its expression and the mechanism of its action at different phases of cell cycle. Therefore, understanding the role of Pin-1 in proliferation or differentiation of diabetic CPC would help us decipher the on/off switch for proliferation and differentiation and thus restoring their functional efficacy.

While it is encouraging that the functionality of diabetic CPC can be improved in number of proposed ways, the most important limitation of these approach is the lack of long-term study demonstrating the sustained potential benefits. This eventually leads to another unanswered question on the side effects of these methods. Therefore, future long-term studies are warranted to determine the clinical feasibility of these approaches.

iPSC-Derived Cardiovascular Cells

Generation of induced pluripotent stem cells (iPSCs) has provided a new avenue for cardiovascular therapies [193, 194]. Somatic cells were isolated and reprogrammed to iPSC that could subsequently give rise to any kind of cell lineage [195, 196]. However, to provide an autologous therapy for a diabetic patient, it may be essential to generate iPSC specific to diabetic individuals (diPSC). Generating diPSC-derived cardiovascular cells would not only help in understanding their efficiency in differentiating into cardiovascular cells but also aid in developing patient specific autologous therapy. In addition, diPSC would provide a versatile platform for disease modeling and regenerative applications. Hence, it is important to understand the effect of diabetes on the reprogramming and differentiation efficiency of diPSC. Maehr et al. reprogrammed iPSC from type 1 diabetic individuals and demonstrated that they have the ability to be differentiated into insulin-producing cells [197]. Similarly, Ohmine et al. demonstrated the feasibility and reproducibility of reprogramming keratinocytes from elderly individuals with type 2 diabetes to iPSCs, which expressed pluripotent genes, exhibited telomere elongation, and downregulation of senescence- and apoptosis-related genes [198]. Furthermore, Kikuchi et al. have generated functional cardiomyocytes from iPSC derived from type 1 diabetes individuals using the monolayer-based cardiomyocytes differentiation protocol [199]. They demonstrated that diabetic and nondiabetic iPSC-derived cardiomyocytes exhibited similar characteristics in cardiac-specific protein expression, spontaneous contractions, action potentials, and response to β-adrenergic stimulant isoproterenol.

Although the above reports provide insights into the use of iPSC in regeneration of diabetic cardiovascular cells, Stepniewski et al. demonstrated impaired differentiation of iPSC derived from fibroblasts of type 2 diabetic mouse (db/db) to EPCs and thus impaired angiogenesis function due to leptin deficiency. In support of this, a recent study by Bhatt et al., demonstrated impaired growth, reprogramming and self-renewal of iPSC derived from type 1 diabetic individuals associated with diabetes-induced complications [200]. While still in infancy, future studies specifically focusing on deriving disease specific iPSC to overcome the impairment due to diabetes could help in understanding their diseased memory status and develop efficient protocols to develop functional cardiovascular cells [148].

Conclusion and Future Directions

Diabetes is a metabolic disorder that causes a series of pathological changes in the myocardial environment of which some of the molecular events show resemblance with ageing. Regenerative therapy, by stem/progenitor cells, aims to reverse the loss of cardiomyocytes and vasculogenesis in patients with heart failure and is currently under clinical trial; however, this strategy could be ineffective in individuals with diabetes due to increased senescence, decreased migration, and homing of transplanted cells. This ineffectiveness is mainly attributed to the retained unfavorable metabolic memory in stem/progenitor cells isolated from diabetic individuals. Cellular senescence is an attribute of ageing developed due to hyperglycemia-induced metabolic memory caused by epigenetic changes in terms of DNA methylation, post-translational histone modification, and regulation by noncoding RNA-like miRNA and lncRNA, which could have deleterious effect on

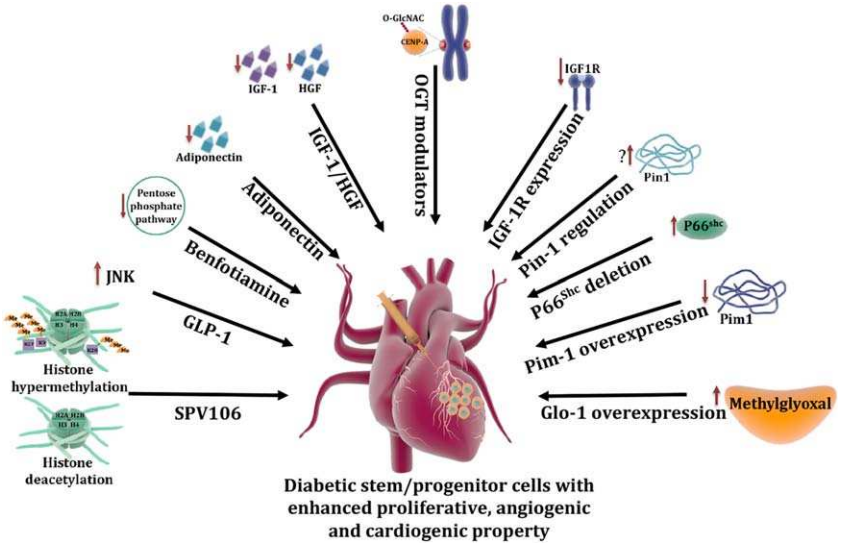

Figure 5. A pictorial summary of plethora of molecular dysregulation in diabetic stem/progenitor cells and their corresponding methods of preconditioning to enhance their therapeutic efficacy in regenerating diabetic heart. Abbreviations: Glo-1, glyoxalase-1; GLP-1, glucagon-like peptide-1; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; JNK, c-Jun N terminal protein kinase; Pim-1, proviral integration site for moloney murine leukemia virus-1; Pin-1, peptidylprolyl *cis/trans* isomerase, NIMA-interacting 1; SPV106, swinepox virus 106.

the regenerative ability of stem/progenitor cells. In addition, the efficacy of endogenous/transplanted cells is further regulated by exosomes, which carry the molecular cargo and influence the angiogenic and cardiogenic properties of the diabetic heart. Aberrant release of diseased exosomes or reduced secretion of therapeutic exosomes would affect the regenerative ability of diabetic stem/progenitor cells by reducing their proliferation and inducing senescence. The metabolic stress exerted through hyperglycemia also leads to AGE production and thus ROS generation, which could stimulate mutations in mitochondrial DNA polymerase-γ and in turn exacerbate ROS production. These pathological maladies of diabetes would contribute to senescence and apoptosis of stem/progenitor cell.

In addition to cellular senescence, migration and homing of stem/progenitor cells are affected in a diabetic heart. An increase in proinflammatory macrophages and SDF-1 levels, inactivation of Ephr, and reduction in ERK1/2 and P38 MAPK signaling are all the possible pathological molecular contributors to the severely impaired migration and homing capacity of stem cells in diabetes.

The proliferation and differentiation of CPC must be well regulated to maintain the homeostatic balance of the heart. Although the role of autophagy in regulating the proliferation and differentiation of CPC in diabetes has not been determined, understanding the interaction of INS/IGF-1 signaling in regulating autophagy in diabetic heart will open new opportunities to determine the efficiency of CPC proliferation and differentiation in diabetes.

The limited intrinsic regenerative capacity of diabetic CPC indicates that extrinsic modulation of stem/progenitor cells through genetic or pharmacological engineering is essential to enhance their proliferation, homing, and migration of the transplanted cells. Alternatively, given the adverse microenvironment in the diabetic heart, preconditioning the stem/progenitor cells by pharmacological, genetic, or synergistic preconditioning could help stem/progenitor cells to withstand the disturbed diabetic microenvironment and enhance their regenerative capacity (Fig. 5). Therefore, it is crucial to understand the key molecular events that regulate stem cell proliferation, differentiation, and senescence under diabetic conditions to optimize the existing therapies or to develop novel regenerative strategies.

Finally, diPSC-derived cardiovascular cells might make an ideal source of disease model. Recent studies have successfully reprogrammed somatic cells from both type 1 and type 2 diabetic individuals implicating the possibility to erase the parent cell memory. However, deriving healthy cardiovascular cells from diabetic iPSC by overcoming the diseased memory is still debatable. Therefore, profiling the genetic and epigenetic alterations in diabetic iPSC and diabetic iPSC-derived cardiovascular cells would make a significant contribution in evaluating the functional and therapeutic efficacy of this population in regenerating a diabetic heart and pave the way for efficient autologous therapy.

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Author Contributions

V.R.S.: designed the idea, drafted the manuscript; R.R.L.: drafted the manuscript, made critical revision of the manuscript; R.G.K.: designed the idea, drafted and made critical

revision of the manuscript. *RRL and RK contributed equally to this article.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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