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Concise Review: Perspectives and Clinical Implications of Bone Marrow and Circulating Stem Cell Defects in Diabetes

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Key Words. Diabetes • Regeneration • Hematopoiesis • Vascular disease

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Received April 20, 2016; accepted for publication May 28, 2016; first published online in STEM CELLS *EXPRESS* June 24, 2016.

1066-5099/2016/$30.00/0

http://dx.doi.org/10.1002/stem.2445

ABSTRACT

Diabetes mellitus is a complex systemic disease characterized by severe morbidity and excess mortality. The burden of its multiorgan complications relies on an imbalance between hyperglycemic cell damage and defective endogenous reparative mechanisms. Inflammation and abnormalities in several hematopoietic components are typically found in diabetes. The discovery that diabetes reduces circulating stem/progenitor cells and impairs their function has opened an entire new field of study where diabetology comes into contact with hematology and regenerative medicine. It is being progressively recognized that such rare circulating cell populations mirror finely regulated processes involved in hematopoiesis, immunosurveillance, and peripheral tissue homeostasis. From a clinical perspective, pauperization of circulating stem cells predicts adverse outcomes and death. Furthermore, studies in murine models and humans have identified the bone marrow (BM) as a previously neglected site of diabetic end-organ damage, characterized by microangiopathy, neuropathy, fat deposition, and inflammation. As a result, diabetes impairs the mobilization of BM stem/progenitor cells, a defect known as mobilopathy or myelokathexis, with negative consequences for physiologic hematopoiesis, immune regulation, and tissue regeneration. A better understanding of the molecular and cellular processes that govern the BM stem cell niche, cell mobilization, and kinetics in peripheral tissues may uncover new therapeutic strategies for patients with diabetes. This concise review summarizes the current knowledge on the interplay between the BM, circulating stem cells, and diabetes, and sets the stages for future developments in the field. STEM CELLS *2017;35:106–116*

SIGNIFICANCE STATEMENT

Diabetes leads to multiorgan complications that reduce life expectancy. Organ damage in diabetes results from glucose toxicity, defective tissue repair, inflammation, and disturbances in several hematopoietic components. Importantly, diabetes reduces circulating stem/progenitor cells involved in hematopoiesis, immunosurveillance, and peripheral tissue homeostasis. This alteration is attributable to microangiopathy, neuropathy, fat deposition, and inflammation in the bone marrow, which emerges as a previously neglected site of diabetic end-organ damage. As a result, diabetes impairs the mobilization and availability of stem/progenitor cells, which in turn predicts adverse outcomes and death.

AN OVERVIEW ON BONE MARROW-DERIVED CIRCULATING STEM/PROGENITOR CELLS

In healthy individuals, hematopoietic stem/progenitor cells (HSPCs), identified by the expression of CD34 in the $CD45^{dim}$ fraction, account for ~3 cells/μl of peripheral blood (PB), equal to ~0.05% of total white blood cells [1]. Once believed the result of a passive shedding from the bone marrow (BM), it is now clear that small numbers of HSPCs are actively and steadily released from BM to PB following a tightly regulated circadian rhythm [2]. HSPCs that have reached the peripheral circulation are not lost, as they will eventually home back to the BM in a few hours [3], thus preventing an otherwise ineluctable shrinking of their pool [4].

HSPCs may not need to circulate in PB, as their physiologic function is to generate blood cell progenies within the BM. In fact, the biological meaning of the in/out recirculation of HSPCs is incompletely understood, as is the interpretation of changes in PB HSPC levels [5]. The following hypotheses have been proposed: (i) The exit and re-entry

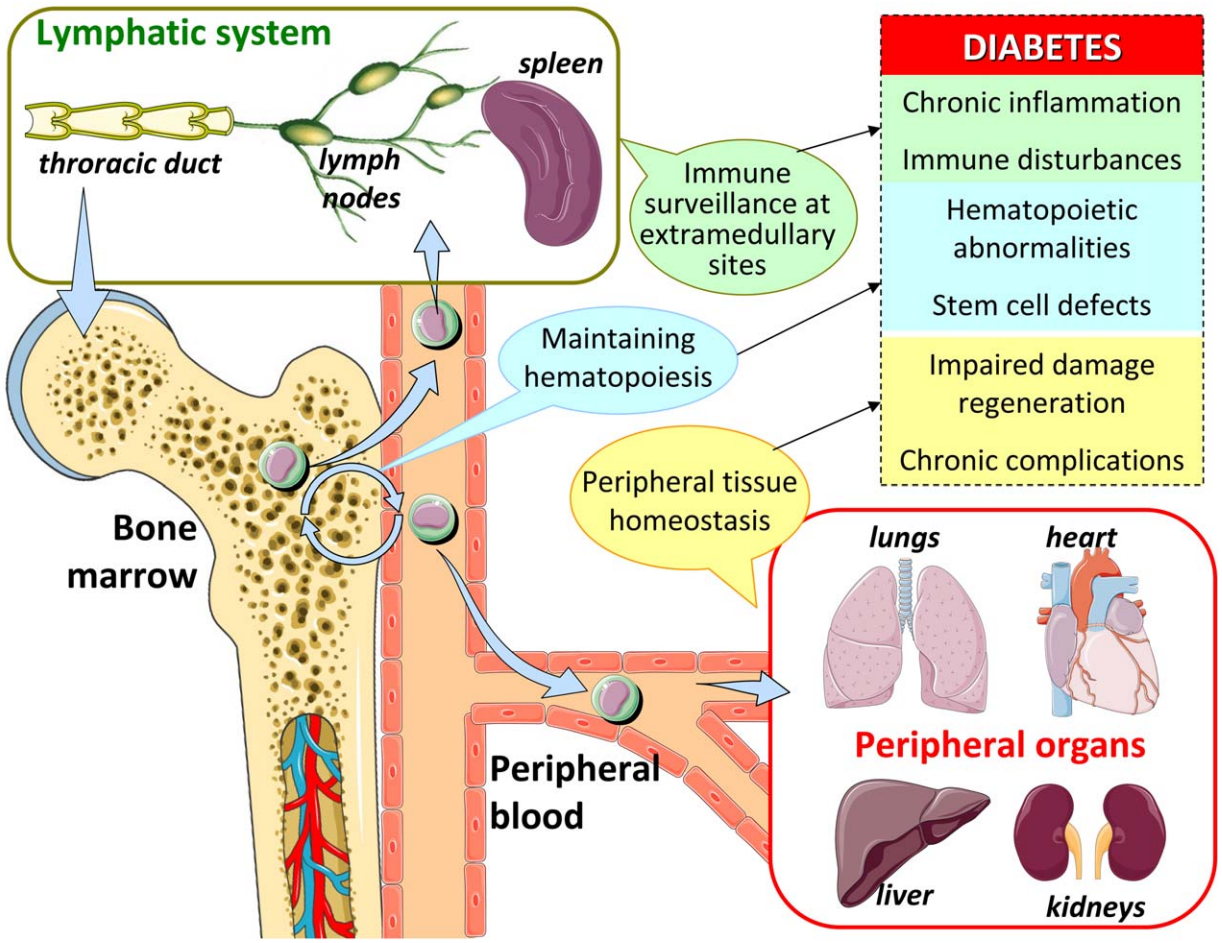

Figure 1. Schematic representation of the physiological functions of circulating stem/progenitor cells and the implication of their dysfunctions in diabetes. Stem/progenitor cells released from the bone marrow can either seed extramedullary sites to promote peripheral immunosurveillance, home to peripheral organs contributing to tissue homeostasis, or migrate back to the bone marrow to find preferential niche locations. Disruptions of the kinetics of bone marrow-derived stem/progenitor cells in diabetes leads to hematopoietic and immune abnormalities, chronic inflammation, and impair tissue regeneration, that collectively contribute to end-organ complications.

of HSPCs in the BM allows a better relocation of the cells in preferential niches, for example, where oxygen tension is lower, in order to optimize hematopoiesis. (ii) Circulating HSPCs patrol peripheral organs and give rise to mature cells in extramedullary sites, thereby contributing to local inflammation and immunosurveillance [6]. (iii) Subsets of BM-derived progenitor cells contribute to the homeostasis of peripheral tissues, for example, to maintain the endogenous repair capacity [7].

A combination of the aforementioned functions helps explaining the physiological role of circulating HSPCs. Tough they represent a small component of PB, a perturbation of the HSPC population is associated with adverse clinical sequelae, including excess mortality [8]. Indeed, BM failure and significant changes in the levels of circulating HSPCs are common findings in clinical conditions characterized by premature death [9], albeit the links between hematological disturbances and accelerated aging remain unclear.

The discovery that diabetes affects circulating stem/progenitor cells opened one of the most revolutionary fields of diabetology in the last 15 years. Diabetes-associated dysfunctions in circulating progenitor cells have mostly been interpreted in view of their putative role in cardiovascular repair. On the other side, the immunological properties of stem cells appear to be particularly relevant for the onset of type 1 diabetes, wherein autoimmunity destroys beta-cells. Several types of stem cells including, but not limited to, HSCs can promote beta-cell regeneration [10]. In addition, the immuno-modulatory function of mesenchymal stem cells are compromised in a murine model of type 1 diabetes [11, 12], whereas simply inducing HSC mobilization can delay the rejection of islet allograft [13]. This concise review takes a larger perspective on the interplay between the BM, circulating stem cells, and diabetes (Fig. 1), and sets the stages for future developments in the field. However, it should be noted that diabetes not only impacts on circulating stem cells, but also affects the function of tissue-resident committed stem cells. It has been recently reported that diabetic enteropathy, a relatively neglected but severe complication of long-standing diabetes, is attributable to disruption of human colonic stem cells [14]. These notions highlight how generalized disturbances in stem cell function underlie the entire diabetes spectrum, from pathogenesis to complications and therapy.

Diabetes, the Bone Marrow, and Circulating Stem/Progenitor Cells

Circulating Stem/Progenitor Cells

Interest in the rare populations of circulating hematopoietic and nonhematopoietic stem/progenitor cells was limited to hematology until the discovery of endothelial progenitor cells (EPCs) in 1997 [15]. Initially described as a population of cells expressing CD34 and KDR (type 2 VEGF receptor), endowed with vascular regenerative capacity, EPCs were soon adopted as an integrated component of the cardiovascular system [16]. Diabetes is now one of the clinical conditions wherein EPCs have been most extensively studied and EPC reduction/dysfunction most consistently shown [17]. EPCs, defined as $CD34^+$ KDR^+ with or without co-expression of $CD133^+$ in the CD45-positive, -diminished, or -negative gate are reportedly reduced in people with diabetes, especially in the presence of chronic complications affecting the macrovasculature and microvasculature [18–21]. Though the mechanisms accounting for such reduction remained long obscure, EPC alterations were claimed as a pathway of end-organ damage in diabetes, and a target of therapy [17, 22]. In addition to blunting the generation of EPCs and their ability to promote vascular healing, diabetes skews the differentiation capacity of circulating progenitor cells toward an inflammatory phenotype [23, 24], thereby turning this cell type from protective to harmful.

Our knowledge on the biology of EPCs, in terms of definition, identity, origin, and function, has much evolved over time [25–27]. Most studies still argue that BM-derived immature cells can repopulate nonhematopoietic tissues and the vasculature. However, the frequency and the physiological meaning of rare integration of BM-derived cells into the peripheral endothelium have been questioned [28, 29]. It is now recognized that most EPC phenotypes overlap with hematopoietic cells [30, 31], and that they promote vascular healing and angiogenesis indirectly by means of paracrine signals [32–35]. Advances in the understanding of how blood cells develop from the hemogenic endothelium helps to interpret the hemato-endothelial overlap of EPCs [36]. Sophisticated culture protocols have clarified that the PB harbors even smaller populations of endothelial colony forming cells (ECFC), distinct from traditional EPCs [37, 38]. However, whether culture-defined cell types truly exist in vivo or they represent in vitro artifacts is unknown.

From Circulating Cells to the Bone Marrow

Notwithstanding and partly owing to the critical appraisal on EPCs, investigators have moved from the analysis of definite stem/progenitor cell populations to study how diabetes decreases the availability of circulating regenerative cells.

A reduction of progenitor cell levels in PB may be the result of (i) insufficient release from the BM, (ii) shortened survival in the bloodstream, and (iii) homing outside the vasculature, or a combination thereof [39]. It should be emphasized that diabetes has been shown to affect several different hematopoietic and putatively nonhematopoietic stem/progenitor cells, mostly derived from the $CD34^+$ cell pool [19]. In fact, the total PB $CD34^+$ HSPC population is itself diminished in diabetic patients [18, 21]. Early in vitro studies on monocytic EPCs found an increased apoptotic rate when cells were isolated from patients with diabetes or exposed to high glucose [40, 41]. However, such findings were not replicated by ex vivo studies evaluating the apoptotic rate of $CD34^+$ cells [18]. The well-established notion that diabetes induces endothelial damage may suggest that a larger than normal amount of endothelial-reparative cells is wasted in the target tissues. However, the idea that extravascular homing accounts for a reduced number of progenitor cells in PB is not supported by data, as studies show that homing of BM-derived immature cells to the site of hyperglycemic tissue damage is actually impaired in diabetic mice [42, 43].

Rather, since 2006 data have suggested that diabetes may affect the ability of the BM to release stem/progenitor cells upon remote stimulation by tissue ischemia or by direct stimulation with growth factors and mobilizing agents [44]. The reasons for this BM hyporesponsiveness were initially unknown. It took some years before investigators realized that hyperglycemia damages the BM microenvironment to the same extent it negatively affects the structure and function of the heart, kidneys, nerves and retina [28, 45]. Microangiopathy is a widespread result of chronic hyperglycemia in most tissues and organs of people affected by diabetes; while myocardial, vasa nervorum, retinal, and renal microangiopathy are responsible for traditional diabetic complications [46], diabetes also causes microangiopathy in the endocrine islets [47], brain [48], lungs [49], liver [50], muscles [51], corpora cavernosa [52], placenta [53], and BM [54]. All these microangiopathies have different clinical implications, which, altogether, contribute to the severe morbidity and mortality of diabetic patients, which goes well beyond cardiovascular disease [55].

The BM microcirculation, composed by arterioles, capillaries, and sinusoids, is instrumental to BM function. For instance, quiescent HSPCs associate specifically with small arterioles that are preferentially found in endosteal bone marrow [56], whereas sinusoids are associated with activated and migratory stem cells [57]. Histopathological analyses of BM specimens from diabetic mice and humans show rarefaction of all microvascular components and relocation of stem cells according to deranged perfusion gradients [54, 58]. Together with excess vascular permeability of the diabetic BM microvasculature [59], these features closely resemble pathological changes typically ongoing in the diabetic retina. Other typical characteristics of BM pathology in murine models and human diabetes include fatty infiltration [54, 58, 60]. As BM adipocytes regulate the stem cell niche [61], this apparently passive filling may turn out to be a critical element affecting BM function in metabolic diseases.

Besides its complex microvasculature, the BM is also dense of nerve terminals [62]. The sympathetic nervous system (SNS) is critically involved in the circadian release of leukocytes and stem cells from the BM to PB [63] and their recruitment to tissues [64]. This is relevant to diabetes as autonomic neuropathy is a common finding in patients with diabetes and it can hit any organ in the body [65]. In the BM, experimental and human diabetes leads to a depletion of SNS terminals, which in turn prevents stem cell mobilization in response to ischemia or G-CSF [66–68]. Similarly, a pauperization of sensory nerve fibers within the BM of diabetic mice and patients is responsible for impaired

Table 1. A summary of the histopathological alterations in the diabetic BM in animal models and in humans

Component	Observation	Model (reference)
Stem cells	Depletion	Mouse [54, 78], human [39, 58]
	Kathexis	Mouse [70], rat [68]
	Relocation in the niche	Mouse [54, 70, 77],
	Increased apoptosis	Mouse [54], human [58]
Microcirculation	Rarefaction	Mouse [54], human [58]
	Reduction in blood flow	Mouse [54]
	Increased permeability	Mouse [59]
Innervation	Reduced sympathetic nerve fibers	Mouse [67, 70], rat [68], human [66]
	Reduced nociceptive fibers	Mouse [66], human [66]
Fat	Increase adipocyte numbers	Mouse [54], rat [68], human [58, 66]
Inflammation	Increased inflammatory cells	Mouse [79]
	Increased inflammatory cytokines	Mouse [77, 78]

nociception-mediated stem cell mobilization and recruitment [66].

It is not surprising that the molecular mechanisms of BM microangiopathy and neuropathy so far identified are those typically induced by high glucose in other organs, including oxidative stress evoked by the pentose phosphate pathway and p66Shc, proapoptotic signals, DNA damage and epigenetic dysregulation [54, 58, 59, 67].

In diabetes patients and animals, pathological features of the BM do not result in steady-state hematological abnormalities, but cause an impaired release of stem/progenitor cell from the BM to PB upon specific triggers, including tissue damage and G-CSF stimulation [44, 66, 67, 69–72]. The functional defect in stem cell mobilization seems to be attributable at least in part to a dysregulation in the CXCL12/CXCR4 axis [70, 73]. During physiologic mobilization and mobilization induced by G-CSF, the chemokine and retention signal CXCL12, normally produced by BM stromal cells, is shed into the circulation and its expression dramatically downregulated to allow the cells migrating out of the BM niche [74]. In diabetes, the switch in CXCL12 concentrations is defective [75], accompanied by a maladaptive response of the CXCL12-degrading enzyme DPP-4 [39, 72, 76].

Inflammation is another pathologic feature of the diabetic BM. In the BM of mice with long-term type 1 diabetes, stem cells alterations, enhanced myelopoiesis, and impaired repopulation were found to be associated with increased concentrations of inflammatory signals, such as gp130 ligands [77, 78]. The excess generation of inflammatory macrophages contributes to blunt stem/progenitor cell mobilization because macrophages sustain CXCL12 expression by stromal cells through the production of Oncostatin M (OSM) [79]. Table 1 resumes histopathological alterations observed in the diabetic BM in different murine models and in humans. Figure 2 shows representative immunofluorescence imaging of the BM vasculature, sympathetic innervation and fat infiltration in nondiabetic and diabetic mice.

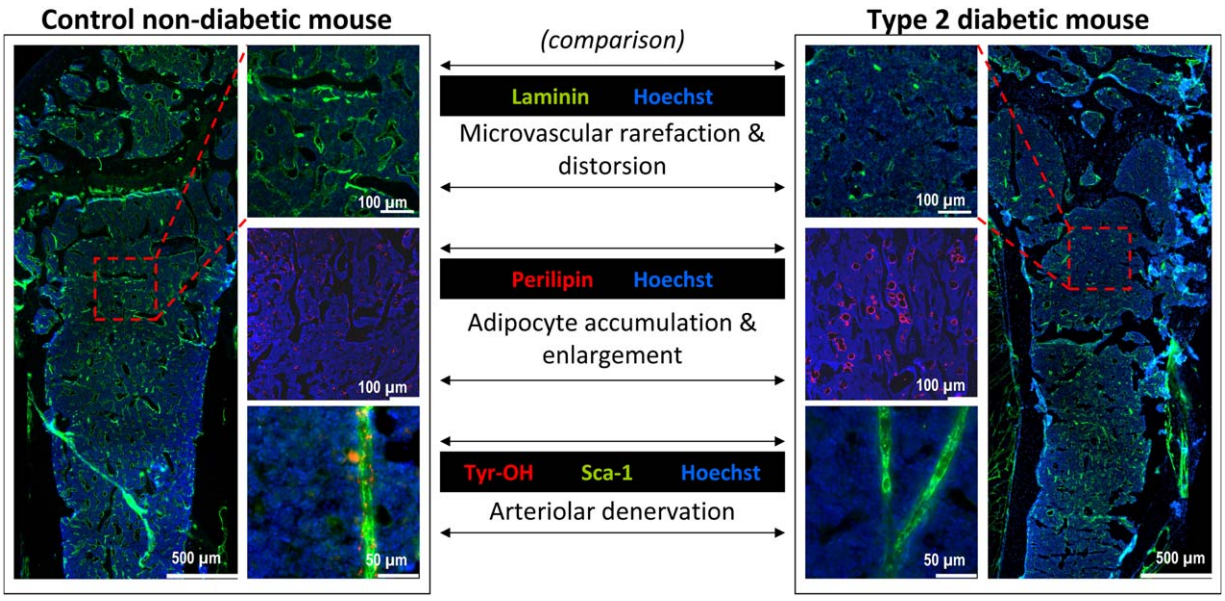

Figure 2. Representative immune-fluorescence imaging of bone marrow abnormalities in experimental diabetes. The figure features some of the typical alterations seen in a murine model of the 2 diabetes (induced by high fat diet) versus control. A low magnification picture of the diaphiseal and epiphiseal BM stained with Hoechst and laminin shows the microvasculature and its rarefaction and distortion in diabetes. Staining for perilipin identifies adipocytes, which are increased and enlarged in type 2 diabetes. The staining for tyrosine hydroxylase (Tyr-OH) on Sca-1^{+} arterioles shows how diabetes induces arteriolar denervation.

The notion that stem cells are retain within the diabetic BM via CXCL12/CXCR4 signaling makes this peculiar complication of diabetes similar to myelokathexis observed in the WHIM syndrome, wherein a CXCR4 point mutation sustains its signal by impeding downregulation via vesicular trafficking [80]. Restoring stem cell mobilization by targeting the CXCL12/CXCR4 axis has several benefits in diabetes, such as improvement in survival of transplanted islets, acceleration of wound healing, and amelioration of endothelial health [13, 75, 81, 82]. However, this approach may have significant downsides, as CXCL12/CXCR4 signaling is itself important for islet survival [83] and for stem cell homing to the damaged vasculature [84].

Implications for the Development and Progression of Diabetic Complications

Preclinical Studies

A wealth of experimental studies in animal models have demonstrated that BM-derived cells contribute to the homeostasis of peripheral nonhematopoietic tissues, via lineage specific progenitors, like EPCs [85]. Different lines of evidence support this concept and its relevance for diabetic end-organ damage. Indeed, diabetes has been defined as a condition of "impaired damage control" [86], wherein tissue damage is not adequately counterbalanced by endogenous repair.

The use of chimeric mice harboring lineage-specific expression of GFP or other genetic reporting systems in BM cells allowed the demonstration that cells derived from the BM home to peripheral tissues, integrate with the vasculature or parenchyma and contribute to organ function [87]. The extent to which this occurs is still a matter of debate. In mice with diabetes, homing to peripheral tissues, differentiation and integration is defective, and such defect associates with impaired recovery from damage, as shown in the setting of delayed wound healing [42, 43].

In parallel, experimental cell therapy approaches based on injection/infusion of BM-derived stem/progenitor cells have elucidated that cells isolated from diabetic animals show an impaired ability to repair the myocardium [88], the vasculature [89, 90], and the wounded skin [91, 92]. These observations, together with the well-known dysfunction in endothelial and vascular progenitor cells of diabetic mice and humans [93], supports the idea that a restrained availability of functional BM-derived progenitor cells contributes to end-organ diabetic complications. Other pathologic changes of BM cells can directly contribute to the onset of complications. Hyperglycemia has been shown to promote an inflammatory expression of the proinsulin (PI) gene in BM cells. In turn PI^+ cells have fusogenic properties, traffic to peripheral organs, fuse with neurons and nephons, giving rise to typical features of diabetic neuropathy and nephropathy [94, 95]. That pathogenic changes in BM cells under diabetic conditions is supported by a study showing adoptive transfer of diabetic nephropathy features by transplantation of BM cells from diabetic donors to nondiabetic recipient mice [96].

It is important to recognize that there is no direct demonstration that BM pathology and circulating stem cell defects can per se promote vascular damage, tissue or organ dysfunction, as long as the direct effects of hyperglycemia cannot be dissected. The demonstration that supply of nondiabetic stem/progenitor cells exerts protection against typical features of diabetic complications in animal models supports a therapeutic, but not necessarily physiologic, relevance of putative regenerative cells.

Clinical Studies

The most apparent consequence of BM microangiopathy, neuropathy, and mobilopathy in diabetes is reduction in the steady-state levels of circulating EPCs, HSPCs, and other immature cell types. The quantitative and qualitative defects in putative regenerative cells have long been claimed as a pathophysiological mechanism of cardiovascular disease induction and progression in diabetes [97]. Relevance of BM-derived cells in human physiology is sustained by studies conducted on BM transplanted individuals, showing that donor cells repopulate to some extent several nonhematopoietic tissues, including the myocardium [98], endothelium [99], lungs [100], liver [101], adipose tissue [102], and kidneys [103]. This is important because diabetes not only increases cardiovascular mortality, but also the risk of death from any cause, including respiratory, digestive, renal, infectious disease, and cancer [55]. Speculatively, the widespread pathological involvement of the BM and the shortage circulating stem cells may be a more general feature associated with accelerated aging and excess mortality seen in diabetes. This concept is supported by the observation that low levels of HSPC phenotypes (e.g., $CD34^+$ and $CD34^+$ $CD133^+$) are independent determinants of all-cause mortality in different population of subjects, such as patients with metabolic syndrome [104], cardiovascular disease, [105, 106] or renal disease [107]. A meta-analysis of prospective observational studies shows that a reduction in the levels of circulating cells putatively provided with regenerative properties represents a risk factor for adverse cardiovascular outcomes and death [108]. Furthermore, the observation of preserved levels of stem/progenitor cells in patients with extreme-duration type 1 diabetes without CVD, supports the idea that endogenous factors exist to neutralize the adverse effects of metabolic abnormalities of diabetes on vascular tissues [109]. It is surprising that a reduction in the rare population of HSPCs (or even the rarer EPCs), which has no clinical relevance in hematologic terms, causes premature death [8]. Indeed, such epidemiological data does not provide any clue as to whether stem cell reduction is a mechanistic factor or a bystander biomarker of aging, disease burden, and risk. If the mechanistic role has to be questioned, low stem cell levels should be considered as an epiphenomenon of BM dysfunction, thereby pointing to a prominent role of the BM as a central housekeeper of global organismal health.

A common observation in patients with diabetes is that the presence of microangiopathy (e.g., retinopathy) precedes and predicts the development of macroangiopathy (e.g., coronary artery disease), and death [110, 111]. Despite severity of hyperglycemia and disease duration may be the common ground for all complications, such epidemiological associations persist after statistically adjusting for known confounders. Therefore, the pathophysiologic link between distant and disparate end-organ diabetic complications remains elusive. Importantly, a recent study shows that low levels of $CD34^+$

Table 2. Hot topics and unanswered questions in the study of circulating stem/progenitor cells in diabetes and its complications

Basic	Bone marrow	Links between BM pathologies and other diabetic complications in animal models
		Therapies to prevent BM remodeling induced by experimental diabetes
		The interplay between different niche cell types in the diabetic BM
	Circulating BM-derived stem/progenitor cells	Moleculr mechanisms underlying diabetic stem cell mobilopathy
		Lineage-tracking of peripheral trafficking of BM-derived stem/progenitor cells
		Relevance of BM-derived stem/progenitor cell homing to sites of diabetic complications
Clinical	Circulating stem/progenitor cells as biomarkers	Which subpopulation of $CD34^+$ cells are mostly affected by diabetes?
		Which phenotype of circulating stem/progenitor cells has the strongest prognostic power in diabetic patients?
		The prognostic capacity of circulating stem/progenitor cells beyond cardiovascular diseases
		To what extent circulating stem/progenitor cell measures can improve clinical practice?
	Therapeutic applications	Efficacy of BM-cell therapies in diabetic versus nondiabetic patients
		Conditioning regimens to improve autologous BM-cell therapy in diabetes
		Smart therapies directed to re-educate endogenous stem/progenitor cells to tissue repair

HSPCs predict development and progression of microangiopathy in the retina, kidney and nervous system of diabetic patients [112]. Speculatively based on such data and taking into account that microangiopathy occurs within the BM itself, it is possible that BM impairment represents the common soil for the development of both microvascular and macrovascular diabetic complications, that collectively shorten life expectancy [45].

Therapeutic Applications of the Study of Bone Marrow and Stem Cells in Diabetes

Cell Therapies

Since the discovery of EPCs, BM-derived stem/progenitor cells have been considered suitable for autologous cell therapy approaches for diseases wherein vascular regeneration is desirable [113]. Indeed, in the last 15 years, several clinical trials have been performed using BM cells for the treatment of cardiovascular disease, including acute myocardial infarction, chronic ischemic heart disease, heart failure, peripheral vascular disease, and critical limb ischemia [114]. Only a few studies have been performed specifically in diabetic patients, and, therefore, results need to be inferred from trials including both diabetic and nondiabetic patients. A detailed description of such therapies goes beyond the scope of this concise review. It is worth underlining that most studies employed mixed populations of BM mononuclear cells, containing small percentages of stem/progenitors, while a few studies used specific cell populations, such as $CD34^+$ or $CD133^+$. Overall, the most recent meta-analyses of randomized clinical trials indicate that BM cell therapy for acute myocardial infarction provides only transient benefits on surrogate endpoints without affecting hard outcomes [115]. Rather, cell therapy appears to be consistently beneficial in patients with chronic ischemic heart disease or heart failure, being associated with improved symptoms, functional outcomes, and hard endpoints [116, 117]. In the setting of peripheral arterial disease and critical limb ischemia, meta-analyses consistently indicate positive effects of BM cell therapy on symptoms, exercise tolerance, perfusion indexes, wound healing, and risk of amputation [118, 119]. This is particularly pertinent to diabetes, as the ischemic foot and delayed wound healing is a devastating complication with limited treatment options and high mortality [120]. In light of the small amount of truly regenerative cells present in most cell products, there is general agreement that the beneficial effects of cell therapy depend on humoral factors, that is, the paracrine secretion of growth factors and cytokines [114].

Based on the notion that diabetes affects the BM niche, decreases the availability of circulating stem/progenitor cells, impairs the function of vascular progenitors, and prevents mobilization, it has been hypothesized that autologous cell therapies may be less beneficial in diabetic than in nondiabetic patients [121], as seen in animal models [89]. For instance, diabetes is the strongest negative clinical determinant of the yield in $CD34^+$ cells when patients receive G-CSF before apheresis for cell therapy of cardiovascular diseases [122]. As the amount of $CD34^+$ cells in the cell product has been shown to be a determinant of therapeutic effects, the diabetic stem cell mobilopathy is likely to have a negative impact on this approach. Similarly, in patients with myeloma or lymphoma undergoing HSPC mobilization with G-CSF for autotransplantation, diabetes negatively affects the yield of $CD34^+$ cells, which is a critical determinant of engraftment and hematopoietic recovery [123]. However, in a small randomized controlled trial on diabetic patients with critical limb ischemia, intramuscular injection of mobilized $CD34^+$ cells improved symptoms, perfusion indexes, and wound healing, compared to standard therapy [124]. This would suggest that impaired homing is the most important defect of stem cell kinetic in the pathophysiology of diabetic vascular disease and that simply taking cells from the BM to the target tissue exerts therapeutic effects notwithstanding mobilopathy and impaired cellular function. More recently, a study reported that diabetes prevents the increase in ejection fraction after $CD34^+$ cell therapy for nonischemic dilated cardiomyopathy [125], thereby lending support to the relevance of intrinsic cell dysfunction. A detailed analysis of whether diabetes limits the benefits of cell therapies for cardiovascular diseases would clarify how much the above-described alterations in BM and stem cells impact on their therapeutic efficacy. Unfortunately, most clinical trials do not report differential efficacy measures in subgroups of patients, such as diabetic versus

nondiabetic, and meta-analyses also provide no useful meta-regression data. This issue should be matter of intense future investigation. In fact, cells isolated from diabetic patients may need conditioning regimens before being used for cell therapy, in order to improve their function. Several ex vivo experimental treatments have been proposed, ranging from physical to chemical, pharmacologic, and genetic intervention [92, 126–128], most of which introduce different degrees of manipulation, having various regulatory implications [129].

Beyond the treatment of diabetic complications, various types of stem cells are being used for beta-cell replacement and/or immunomodulation in type 1 diabetes [10, 11]. For instance, a multicentric meta-analysis reported that autologous nonmyeloablative HSC therapy associated with immunosuppression can induce long term remission of new-onset type 1 diabetes [130]. An approach using autologous cord blood transfusion in children with type 1 diabetes has been tempted, but failed to preserve insulin secretion [131, 132]. Paradoxically, it had been reported that cord blood- [133], but not bone marrow-derived [134], stem cells can differentiate into pancreatic insulin-producing cells in humans though at very low frequency. This suggests again that paracrine modulation of endogenous repair mechanisms is more important than transdifferentiation.

Finally, similarly to what observed for cardiac and vascular cell therapy, potential downsides also apply to immunomodulatory stem cell therapy, as autologous BM cells may display immunological incompetence in diabetes [12], whereas sensitization may occur with allogeneic cell therapies [11].

Smart Therapies

Owing to the aforementioned limitations of autologous cell therapy in patients with diabetes, together with the costs and regulatory constrains applied to advanced medicinal products, researchers have developed the idea of smart therapies to re-educate endogenous stem/progenitor cells by acting on disease-specific molecular pathways.

Various medications routinely used for the treatment of diabetes have shown favorable effects on EPC levels and function [22]. DPP-4 inhibitors are used to lower glucose levels because they protect incretin hormones (especially GLP-1) from enzymatic degradation, thereby enhancing glucose-induced insulin secretion. As a pleiotropic effect, DPP-4 inhibitors prevent CXCL12 degradation in the circulation, thereby raising EPCs levels [76, 135]. Likely through modulation of membrane-bound DPP-4/CD26, DPP-4 inhibitors also improve the function of myeloid EPCs in vitro [136]. Despite these evidences, DPP-4 inhibitors show no direct vascular protective effects in clinical trials [137], and provide no clinical advantage when associated to G-CSF stimulation in patients with myocardial infarction [138].

As noted above, neuropathy and dysregulation of the CXCL12/CXCR4 axis are major determinants of stem cell myelokathexis in diabetes. This can be targeted by the direct CXCR4 antagonist plerixafor (AMD3100), that acts independently of the SNS and, contrary to G-CSF, efficiently mobilizes HSPCs also in patients with diabetes [123]. Preclinical studies suggest that plerixafor may improve peripheral ischemia and promote wound healing [75, 139], though this needs to be confirmed in clinical trials.

As CXCL12-sustained stem cell retention in the diabetic BM is at least in part mediated by OSM production by macrophages, OSM inhibition is an attractive strategy to restore stem cell mobilization, as shown in diabetic mice [79]. One may argue that raising circulating EPCs or stem cells is insufficient to stimulate tissue regeneration, as long as cell function and homing are not simultaneously improved. Interestingly, OSM neutralization appears to reverse the BM retaining signal, yet letting the mobilized cells reach target tissues, such as ischemic muscles of diabetic mice [79].

Conclusions and Future Directions

The discovery that circulating stem/progenitor cell defects need to be traced back to BM involvement by diabetes has opened an entirely new avenue of research in the field of diabetology, which closely comes into contact with hematology and regenerative medicine. At the same time, data on the impact of circulating stem/progenitor cells as prognostic biomarkers improve our understanding of how BM alterations are interconnected with other diabetic complications over time. Table 2 shows a nonexhaustive list of hot-topics that are likely subject of future investigation in this field. The potential for circulating stem/progenitor cells to aid tissue repair and naturally contrast the development and progression of diabetic complications can be exploited therapeutically. However, in view of the global burden of diabetes and its complications, it is unlikely that costly cell therapies can be applied on a large scale. Rather, smart therapies, developed through pharmacologic modulation of endogenous stem cells, represent an attractive and cost-effective strategy. Much still needs to be clarified about the BM niche and how it regulates stem/progenitor cell mobilization and trafficking in tissues. Meanwhile, a complex set of hematological abnormalities are being recognized in diabetes, not limited to stem cells, but also affecting monocyte-macrophages [140] and neutrophils [141]. Notably, such myeloid cells are primarily involved in regulating the BM niche and stem cell mobilization [3, 79], as well as in propagating inflammation to the adipose tissue and atherosclerotic plaques [142, 143]. For decades, research on the pathobiology of diabetic complications focused on hyperglycemia and associated metabolic abnormalities. While it is impossible to deny the importance of such well-established damage pathways, the notion that diabetes is an accelerated aging condition with impaired damage control gives foreground importance to regenerative approaches. A better understanding of the cellular and molecular network that regulates BM stem/progenitor cells is likely to boost the development of therapies potentially suitable for diabetic patients.

Acknowledgments

Supported by a grant from the Italian Ministry of Health (RSF GR-2011-02347600, "*Stem cell dysfunction and its reversal in diabetes associated multiorgan pathology*").

Author Contributions

G.P.F., S.C., and M.A.: designed the outline of the manuscript, collected data, wrote the manuscript, and approved the final version.

Disclosures

The author(s) indicates no potential conflicts of interest.

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