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REVIEW

The SDF-1/CXCR4 axis in stem cell preconditioning

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Abstract We review the pivotal role of the stromal derived factor (SDF)-1 chemokine in tissue ischaemia and how it orchestrates the rapid revascularization of injured, ischaemic, and regenerating tissues via the CXC chemokine receptors CXCR4 and CXCR7. Furthermore, we discuss the effects of preconditioning (PC), which is a well-known protective phenomenon for tissue ischaemia. The positive effect of both hypoxic and acidic PC on progenitor cell therapeutic potential is reviewed, while stressing the role of the SDF-1/CXCR4 axis in this process.

Keywords SDF-1 • CXCR4 • Ischaemia • Preconditioning

1. SDF-1 and its receptors

SDF-1 (stromal derived factor-1)/CXCL12 is a small molecule, with alternatively spliced forms, belonging to the CXC chemokine family. Its N-terminus is necessary for receptor binding to and activation of chemokine receptors, which are characterized by seven transmembrane domains coupled to G proteins.[1–3] The main, and best studied, receptor for SDF-1 is CXCR4, which is a 352-amino acid molecule that, when triggered, transduces multiple signals leading to the control of biological functions such as cell chemotaxis, proliferation, apoptosis, survival, and differentiation.[4,5] The CXCR4 receptor is expressed on several cell types, including blood cells (i.e. lymphocytes, monocytes, etc.), haematopoietic stem cells (HSCs), and embryonic stem (ES) cells.[6–9] One of the main functions of the SDF-1/CXCR4 axis is the regulation of progenitor cell (PGC) trafficking during embryonic development, cell chemotaxis, and postnatal homing into injury sites.[10–14] SDF-1 also has a role in the quiescence for long-term HSC maintenance.[15] CXCR4 is expressed on endothelial cells (ECs), smooth muscle cells (SMCs), and endothelial progenitor cells (EPCs),[16–18] and promotes angiogenesis both *in vitro* and *in vivo*. In addition, this ligand–receptor pair promotes cardiomyocyte survival, neovascularization, and cardiac function after myocardial infarction (MI).[19] The SDF-1/CXCR4 axis plays a crucial role during embryonic development, which is demonstrated by the lethality of SDF-1$^{-/-}$ or CXCR4$^{-/-}$ mice, due to altered lymphopoiesis, myelopoiesis, cardiogenesis, angiogenesis, neurogenesis, and germ cell development.[20–22] SDF-1 induces neural PGC migration, axon development and guidance, and both dorsal root ganglia neuron and neural stem cell survival. Further proof of the SDF-1/CXCR4 role in neurogenesis is the abnormal development of both cerebellum and hippocampus found in these mice.[23]

SDF-1 is produced by many tumours and can promote primary tumour growth and metastases towards lung, liver, or lymph nodes, where SDF-1 levels are high. Tumour growth is regulated not only by the SDF-1 effect on cell proliferation, apoptosis, and survival, but also through its role in angiogenesis.[23]

More recently, the previously described orphan receptor RDC was renamed CXCR7 after it showed high-affinity binding to SDF-1 and to the chemokine ITAC.[24,25] The CXCR7 receptor has a more restricted surface expression, specifically in tumour cells, activated ECs, foetal liver cells, etc.[25] than CXCR4, and is yet poorly characterized. *In vivo* studies showed that CXCR7 signalling promoted tumour growth in animal models, favoured renal PGC engraftment in injured areas in an acute renal failure mouse model, and is involved in cardiac development[25,26] as defects in the ventricular septum and heart valve organogenesis were found in CXCR7$^{-/-}$ mice. CXCR7 is also involved in SDF-1-induced cell growth, survival, and adhesion,[25,26] whereas its role in calcium mobilization and chemotaxis is still controversial[25–27] and depends on the cell type. Liu *et al.*[28] showed that CXCR4 is required for mesenchymal stem cell (MSC) migration and adhesion, whereas CXCR7 is responsible for MSC adhesion and survival. It is interesting to note that, in response to SDF-1, CXCR7 signalling may have not only agonistic but also antagonistic activity;[25,26,29] CXCR7 has been shown to be an SDF-1 scavenger, inducing ligand internalization and degradation, which is thought to modulate the functioning of CXCR4, in specific cell types.[30,31]

2. The role of SDF-1 and its receptors in response to ischaemia

Cerebrovascular, coronary artery, and peripheral artery disease all have a high incidence of morbidity and mortality. During the acute

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phase of ischaemia, hypoxia, trophic factor deprivation, tissue pH reduction, oxidative stress, and inflammation are among the factors inducing tissue damage.[32,33] After an ischaemic insult, rapid revascularization and tissue regeneration occur, which are essential to restore organ functions[34–37]; revascularization is achieved by the sprouting of small endothelial tubes from pre-existing capillary beds, by an enlargement of pre-existing collateral arterioles, and by the homing and differentiation of circulating, bone marrow (BM)-derived, or resident PGCs (*Figure 1*).[34–37] HSCs, EPCs,[2,11,17,38–40] MSCs,[41–43] cardiac progenitor cells (CPCs),[44] and human adipose tissue-derived stem cells[45] are among the PGCs leading to neovascularization after an ischaemic insult. Factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, platelet-derived growth factor (PDGF), angiopoietins (ANGs), semaphorin family members, Notch ligands, and a number of chemokines, play a pivotal role in angiogenesis and neovascularization[37,46] by affecting the function of mature and/or PGCs. VEGF and chemokines, such as SDF-1, IL-8, MCP-1, and MCP-3, promote homing to injured tissues, favour PGC differentiation, and activate mature ECs and/or SMCs. Furthermore, several other molecules act as chemoattractants

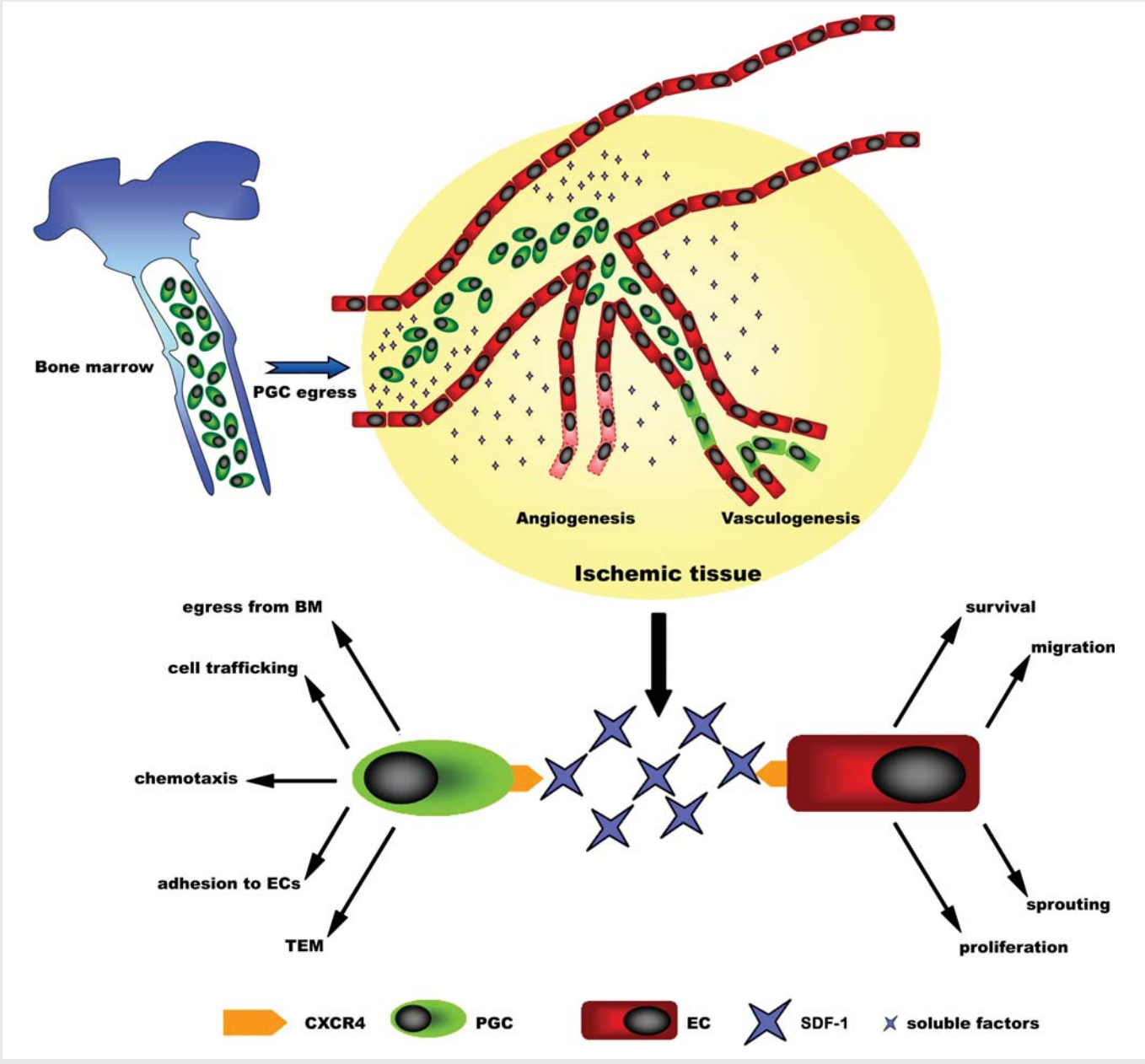

Figure 1 Revascularization after ischaemia. During the acute phase of ischaemia, neoangiogenesis and neovascularization are stimulated in order to restore organ perfusion. Regeneration is permitted by key soluble factors, such as VEGF, SDF-1, and G-CSF, released by ischaemic tissues, that promote the sprouting of small endothelial tubes from pre-existing capillaries, the egress of PGCs from BM, and their homing and differentiation into the injured tissue. The SDF-1/CXCR4 pair plays a pivotal role during neovascularization, regulating PGC and EC functions.

for BM PGCs following ischaemia; they include bioactive phospholipids, i.e. sphingosine-1-phosphate[47] and members of the kinin–kallikrein system, notably bradykinin, that chemoattract circulating EPCs via the kinin B2 receptor.[48] Other signalling pathways are involved in PGC homing and engraftment to ischaemic tissues, namely the stem cell factor—c-kit, leukaemia inhibitory factor (LIF)—LIFR, hepatocyte growth factor—c-met,[47] granulocyte colony-stimulating factor (G-CSF), and insulin-like growth factor 2 (IGF-2).[49] Integrins such as β1 and β2, and metalloproteases (MMP)s, specifically MMP-2 and MMP-9, are crucial not only in the homing process but also in vessel sprouting. Cell survival and proliferation of PGCs and mature cells involved in angiogenesis are tightly regulated processes achieved by the triggering of several signal transduction pathways including Raf/Mek/Erk and Akt.[50]

Hypoxia is one of the main factors stimulating neoangiogenesis and neovascularization[32,51–53]; key effector molecules consist of hypoxia-inducible factor (HIF) family members[32,51] that directly regulate the transcription of growth factors (i.e. VEGF, PDGF, HGF, SCF),[40,54–57] chemokines (i.e. SDF-1, IL-8),[52,58,59] and cytokines (i.e. TNF-α, IL-6)[57,60] that concur to induce angiogenesis. SDF-1 expression is upregulated by HIF-1 in ECs, and its expression, in ischaemic tissue, is proportional to reduced oxygen tension.[52,61] Furthermore, hypoxia induces HIF-1-mediated CXCR4 expression and activation[62] in different cell types, leading to enhanced SDF-1-driven chemotaxis.[59,61] Physiologically, SDF-1 is constitutively expressed in the the BM endothelium and in endosteal bone osteoblasts, where the oxygen tension is low.[63] As a consequence, most PGCs remain in BM because of high-SDF-1 expression. The inhibition of the CXCR4 receptor activity, through desensitization, down-regulation, or treatment with the AMD3100 selective antagonist, or the SDF-1 degradation, by proteases such as CD26, induces PGC mobilization from BM to the peripheral blood, thus indicating that SDF-1/CXCR4 signalling promotes PGC retention in BM.[61] Nevertheless, low numbers of PGCs continuously circulate between BM and peripheral blood. The regulation of BM PGC egress and retention is a complex process; in fact, the over-expression of SDF-1 in the plasma induces stem cell mobilization in peripheral blood, indicating the importance of SDF-1 gradient formation for cell mobilization. Thereafter, SDF-1 regulates PGC adhesion to ECs, by activating integrin molecules,[64–66] and favours transendothelial migration (TEM); PGCs then seed into the ischaemic tissue after extravasation.[11,12,17,56] PGCs, en route to the injured tissue, begin their differentiation into ECs through cytokine expression/activity, shear stress, and mechanical forces.[10,13,17,19,36,38,67,68]

SDF-1 expression is transiently induced by ischaemia[11,12] and can strongly up-regulate the expression of EC markers in PGCs[13,17,38,68]; in particular, it plays a role in the EC differentiation of BM-derived PGCs, CPCs,[38,69] MSCs,[70] cord blood EPCs,[71] and of human ES cells.[68] These cells, once recruited into the ischaemic tissue, support adult ECs during repair and re-endothelization via paracrine factor secretion and differentiation into developing vessels. In fact, PGCs, injected soon after an ischaemic insult, induce an enhancement of functional recovery, as demonstrated by several clinical trials.[72–75]

CXCR4, when triggered, transduces several signalling cascades, leading to angiogenesis. In particular, it induces calcium mobilization and activation of PKC, PI3K, proline-rich tyrosine kinase 2 (Pyk-2), Crk, p130 Cas, and NF-κB, which regulate cell migration, cytoskeleton reorganization, and extracellular matrix cell adhesion, and activates several pathways, including ERK-1/2 and Akt, that contribute to the control of cell growth, apoptosis, and survival.[4,61]

Despite the experimental evidence showing the positive role of the SDF-1/CXCR4 axis on revascularization and functional recovery post-ischaemia, some studies have challenged these findings. Recent works showed that SDF1/CXCR4 signalling could be detrimental for infarct size and left ventricular function in an ischaemia–reperfusion injury model,[76] and that CXCR4 signalling leads to an increase in scar size probably due to the recruitment of inflammatory cells and fibrocytes. Moreover, a profibrotic effect of SDF-1 has been demonstrated in a dilated cardiomyopathy mouse model and in pulmonary fibrosis. Further, it has been shown the negative effect of SDF-1 on left ventricular function in a model of MI[77] and the negative inotropic effect of SDF-1 on adult cardiomyocytes.[14] Therefore, further studies are necessary to clarify and definitively assess the therapeutic potential for the SDF-1/CXCR4 axis in ischaemia.

Unlike CXCR4, the role of CXCR7 in BM cell mobilization and homing in the revascularization of ischaemic/damaged tissues is poorly characterized. Yan *et al.*[78] recently published data showing that CXCR7 had no role in SDF-1-induced human EPC migration and proliferation. On the contrary, CXCR7 mediated EPC tube formation, thus promoting angiogenesis, and MMP-2 production. Furthermore, it was found to have a positive role in SDF-1/CXCR4-induced EPC adhesion and TEM. Noteworthy, SDF-1 mediated EPC survival, predominantly via CXCR7. It is interesting to note that CXCR7 signalling up-regulated the expression of pro-angiogenic factors such as IL-8 and VEGF in tumour cells.[79] It is therefore possible that this would be one of the mechanisms through which CXCR7 promotes revascularization following ischaemia, along with the effects on EPC survival, adhesion, TEM, and angiogenesis.

Kavanagh and Kalia[80] showed that CXCR4/CXCR7 heterodimers exert an increased calcium mobilization in response to SDF-1 when compared with CXCR4 monomers, and CXCR7 blockade with specific antagonists inhibits an SDF-1-mediated arrest of PGCs on immobilized VCAM-1. Although these data strongly suggest that CXCR7 plays a role in tissue revascularization following ischaemia, further studies are however necessary for a better characterization.

SDF-1 induces not only postnatal revascularization but also tissue regeneration.[61] Indeed, soon after an ischaemic insult, SDF-1, CXCR4, and CXCR7 are modulated during myogenic differentiation[81–83] and they promote muscle regeneration, by inducing muscle PG/satellite cell differentiation *in vitro* and *in vivo*,[83,84] thereby contributing to organ function restoration. Moreover, as mentioned above, CXCR7 promoted tissue regeneration in a mouse model of acute renal failure.[26]

SDF-1 is a neuro-protective factor in BM multipotent stromal cells. Interestingly, CXCR7, but not CXCR4, induces neural PGC survival during hypoxia exposure[85] and its expression increased in the brain after stroke.[86]

3. Preconditioning of PGCs might enhance their therapeutic potential: a role for the SDF-1/CXCR4 axis

Cell therapy is a promising approach in the treatment of ischaemic diseases[7,12,13,17,34,42,43,72–74,87,88] but much work still needs to be done in order to define the optimal donor cell type, cell transfer timing, cell delivery method,[89] and cell pre-treatment.[41,70,71,90–92] It

has previously been shown that age and cardiovascular risk factors, including diabetes,[38,93] hypercholesterolaemia,[94] hypertension,[94] and smoking,[95] reduce the availability and function of PGCs, thus limiting their therapeutic potential. Furthermore, the unfavourable microenvironment present in the ischaemic tissue may impair the effectiveness of cell transplantation. The improvement obtained in injured tissue function, following stem cell transplantation, is directly related to the number of cells injected and retained at the cell graft site. Since a considerable number of PGCs undergo cell death during and after cell transplantation, many strategies have been applied to optimize and enhance stem cell survival, activation, and homing to injury sites.

Preconditioning (PC) is an endogenous protective mechanism, activated by ischaemic stress, where tissues can sense and adapt to the environment by changing their cellular phenotype and function[96–98] and developing tolerance to an ischaemic environment. PC has therefore been adopted as a strategy to improve PGC therapeutic potential through brief exposure to a particular stress for a specific period of time. Exposure to hypoxia, anoxia or acidosis,[41,90–92] heat shock,[99,100] cytokines,[70,71] and pharmacological treatments,[67,70,71,101–104] prior to cell injection into the damaged tissue, render PGCs more resistant to ischaemia. Different strategies are used to precondition stem cells *in vitro*; exposure to the selected stress can be brief (a few minutes to 1 h),[70,71] long-single (from 1 to 24 h),[90,92,105–107] or short-multiple (two or more cycles of brief exposure).[108] Here, we discuss the PC achieved with brief or long-single exposure to exogenous SDF-1, hypoxia, or acidosis. All of these strategies can influence/potentiate SDF-1/CXCR4 functions both *in vitro* and in animal models (*Table 1*). We are not aware, otherwise, of any clinical evidence of PC strengthening of the SDF-1/CXCR4 system. One of the benefits of PC is the increase of transplanted PGC recruitment, retention, and survival and the induction of a more supportive environment, within the host tissue, following the secretion of angiogenic factors.[41,90–92,109]

Peripheral or cardiac ischaemia often occur in patients with atherosclerosis. As already mentioned, atherosclerosis may negatively regulate PGC engraftment and regenerative potential; this may, in part, occur via the SDF-1/CXCR4 axis impairment, as shown in ApoE null aged mice[110] that have defects in CXCR4 surface expression of BM-derived cells, and an SDF-1 reduction in serum and BM. Interestingly, clusterin, a stress-inducible apoliprotein, up-regulates CXCR4 and SDF-1 expression and thus enhances CPC migration.[111] As PC plays a protective role in hypercholesterolaemic rabbits with atherosclerosis,[112] during ischaemia, it is possible that PGC function impairment in atherosclerotic aged patients could be positively affected by CXCR4 expression enhancement and PC-induced functions.

It has previously been shown that *ex vivo* stimulation of human PGCs with VEGF,[43] bFGF, β2-integrins activators,[67] statins,[102,113] or eNOS-enhancing drugs[101,103,104] may improve cell survival, proliferation, adhesion, migration and differentiation,[13] and/or stem cell effectiveness in tissue repair.[73] Since SDF-1 is critically involved in PGC recruitment and tissue retention, *ex vivo* strategies have been adopted to enhance the SDF-1/CXCR4 axis.[70,71,114] Pasha *et al.*[70] showed that PC with recombinant SDF-1, of BM-derived MSCs, is cytoprotective and stimulated cell proliferation and VEGF secretion *in vitro*. Moreover, SDF-1 PC is beneficial in an infarcted myocardium rat model, since it suppresses MSC apoptosis, enhances their proliferation, reduces the infarct size and fibrosis, and improves cardiac function (*Table 1*).

In addition, Zemani *et al.*[71] showed that SDF-1 PC of EPCs could enhance their angiogenic potential through increased cell migration, adhesion to activated endothelium, differentiation into vascular tubes, and increased cell therapeutic potential in a mouse model of hind limb ischaemia.[71]

The cytoprotective effects of ischaemic PC can also be mimicked by genetic modification; in fact, the forced overexpression of angiogenic growth factors, i.e. VEGF,[43] or anti-apoptotic factors, i.e. Bcl-XL, or pro-survival kinases, i.e. Akt,[42] in PGCs, has proved to be effective. Kahn *et al.*[115] showed an increase in cell proliferation, SDF-1-induced migration, and NOD/SCID repopulation of the spleen by overexpressing CXCR4 on human $CD34^+$ cells by a lentiviral gene transfer.

The pre-activation of damaged target tissue represents an alternative choice to augment cell homing and integration. A direct injection of chemotactic factors, such as VEGF and SDF-1, may be a strategy to attract infused PGCs into injured tissues.[17,114,116] For example, Hiasa

Table 1 The SDF-1/CXCR4 axis in stem cell PC

First author	Cell type	PC	Protocol	Effect (increase)	Reference
Zemani *et al.*	EPC	SDF-1	100 ng/mL SDF-1 for 30 min	Angiogenic capacity Regenerative potential in ischaemic hind limb (CXCR4-mediated)	71
Pasha *et al.*	MSC	SDF-1	50 ng/mL SDF-1 for 60 min	CXCR4-mediated survival Regenerative potential in ischaemic myocardium (CXCR4-mediated)	70
Hung *et al.*	MSC	HP	1% O_2 for 24 h	CXCR4 expression SDF-1-induced migration Engraftment into developing embryos	105
Liu *et al.*	MSC	HP	3% O_2 for 24 h	CXCR4 and CXCR7 expression SDF-1-induced migration, adhesion, survival	28
Kubo *et al.*	PBMNC	HP	2% O_2 for 24 h	CXCR4 expression Adhesion Retention in ischaemic limb	106
Tang *et al.*	CLK	HP	0.1% O_2 for 6 h	CXCR4 expression Regenerative potential in ischaemic myocardium (CXCR4-mediated)	92
Cencioni *et al.*	BM-$ckit^+$ cells	AP	pH 7.0 for 24 h	CXCR4 expression SDF-1-induced migration, differentiation (CXCR4-mediated) Regenerative potential in ischaemic hind limb	90

et al.[116] showed that SDF-1 gene transfer to an ischaemic limb enhances vasculogenesis and angiogenesis *in vivo* through VEGF/eNOS signalling. Similarly, SDF-1-overexpressing myoblasts injected into an infarcted myocardium promoted stem cell homing and induced angiomyogenesis.[117]

Hypoxic or acidic PC has been used as an approach to enhance PGC survival, adhesion, and homing *in vitro*/*ex vivo*[41,70,71,90–92,118,119] but we shall discuss this in more detail below.

3.1 Hypoxic PC

In the ischaemic tissue, cells encounter severe hypoxic conditions, ranging from 0.4 to 2.3% O_2, which often result in cell apoptosis.[91] These lethal effects might be prevented or attenuated by exposing cells to mild hypoxic conditions, i.e. 2–3% O_2, for a short period of time before transplantation into ischaemic tissues (hypoxic PC). The PC strategy leads to the production of paracrine factors, which are essential for tissue regeneration. It is well established that hypoxia up-regulates both SDF-1 and CXCR4 expression, in cells such as ECs, monocytes, and tumour cells, through HIF-1α activation and transcript stabilization, and increases the chemotactic response to SDF-1, which regulates trafficking in and out of the ischaemic tissue.[52,53]

Several reports have shown a protective role for hypoxic PC in ischaemia in several stem cell types and have highlighted its pro-survival and pro-regenerative effects.[28,41,91,92,105,106] Moreover, a number of PGC types, preconditioned with hypoxia, improved their function/retention into ischaemic myocardium.[41,92] Human MSCs derived from the BM or adipose tissue have a high regenerative potential and can differentiate into osteoblasts, adipocytes, and chondrocytes.[120,121] Rosanova *et al.*[91] demonstrated that MSCs exposed to hypoxia activate the Akt signalling pathway, preserving cell viability and cell cycle rates; further, the injection of preconditioned MSCs into the ischaemic limb accelerated blood flow restoration and revascularization. Hu *et al.*[41] showed that hypoxic PC increased the expression of pro-survival and pro-angiogenic factors (HIF-1, ANGs, VEGF, Flk-1, erythropoietin, Bcl-2, and Bcl-xL) in MSCs. Also, hypoxic preconditioned MSCs transplanted into an MI mouse model resulted in an increase of angiogenesis and enhanced functional effects.

Evidence of the SDF-1/CXCR4 involvement in response to hypoxic PC has been reported in several studies. Hung *et al.*[105] showed that hypoxic PC up-regulated expression of both CX3CR1 and CXCR4, thus promoting enhanced fractalkine and SDF-1-directed cell migration, respectively, and increased MSC engraftment *in vivo* in chick embryo developing organs. Similarly, Liu *et al.*[28] found an up-regulation of CXCR4 and CXCR7 expression in MSCs, with hypoxic PC, via the PI3K/Akt signalling and HIF-1α stabilization; they also found that hypoxic PC promoted SDF-1-directed cell migration, adhesion, and survival of MSCs *in vitro*. Another study showed that hypoxic PC of mouse peripheral blood mononuclear cells (PBMNCs) increased cell adhesion and both integrin alpha M and CXCR4 expression. The retention of such cells in ischaemic hind limbs increased at day 3 after intramuscular injection. Kubo *et al.*[106] showed that CXCR4 signalling had a role in increasing cell retention and angiogenic activity of hypoxic PC-PBMNCs in ischaemic hind limbs (*Table 1*).

Resident CPCs are endogenous components of the adult heart and appear to be responsible for the physiological and pathological turnover of cardiac myocytes and other cardiac cells.[122] After an ischaemic insult, the SDF-1/CXCR4 axis promotes c-kit$^+$ CPC migration to the infarct zone and their differentiation into ECs.[69,123] Moreover, SDF-1 plays a pivotal role during the hypoxic PC of c-kit$^+$ CPCs; indeed, Tang *et al.*[92] found that hypoxic PC increased CXCR4 expression in an HIF-1α-dependent manner in c-kit$^+$ CPCs, named CLK (cardiosphere-derived, Lin$^-$ c-kit$^+$ PG) cells, and augmented their migration to SDF-1 *in vitro*. In addition, hypoxic PC increased their recruitment *in vivo* in murine ischaemic myocardium, thereby reducing the infarct size and improving heart function. The beneficial effects of hypoxic PC were mostly mediated by the SDF-1/CXCR4 axis (*Table 1*), thus suggesting that therapies targeting this ligand–receptor pair may enhance CPC-based regenerative therapy.[92]

3.2 Acidic PC

Tissue acidification is a consequence of oxygen depletion which occurs in association with blood vessel occlusion and ischaemia.[124–126] Hypoxia, in fact, induces a switch from aerobic metabolism to anaerobic glycolysis, leading to the generation of lactic acid as well as protons. Similar to hypoxia, acidosis might represent a stress factor able to precondition stem cells.[127]

It has previously been demonstrated that acidification enhances cytosolic $[Ca^{2+}]_i$ and induces intercellular adhesion molecule-1 (ICAM-1) expression on the EC surface[128]; further, acidosis modulates the expression of molecules including VEGF, MMP-1, and IL-8,[129,130] inhibits EC proliferation, chemotaxis, and differentiation, and protects ECs from cellular apoptosis.[131,132] Although acidosis is an important stress factor under ischaemic conditions, a short acidic pre-treatment may protect cells against ischaemic stress. PC with brief episodes of acidosis is known to limit ischaemia/reperfusion injury in the heart,[87,133] lung,[134,135] and endothelium.[118,119] The possible mechanism(s) underlying this response include activation of pro-survival kinases, such as Akt and ERK, and the overexpression of anti-apoptotic protein Bcl-XL.[118,119] Flacke *et al.*[118] showed the effect of acidic PC on rat coronary EC, with a transient activation of p38 and Akt kinases, and a significant reduction of apoptotic cells via the inhibition of caspase-12/-3 cleavage and over-expression of the anti-apoptotic protein Bcl-xL.[118] The pro-survival effect of acidic PC was also reported by Kumar *et al.*[119] in coronary ECs exposed to simulated ischaemia. In their study, they found results similar to Flacke *et al.*[118] and a reduction of cytochrome *c* release from mitochondria.

As already mentioned, the SDF-1/CXCR4 axis is a major player in response to ischaemia.[11,12,17] Interestingly, acidosis regulates CXCR4 expression in a cell-specific manner. In fact, it induces a marked decrease of CXCR4 expression at pH 7.0 in ECs[136]; an acidosis-dependent CXCR4 mRNA up-regulation is instead observed in NT2-N neurons,[137] whereas in other cell types CXCR4 levels are unchanged.[136]

In our recent work,[90] acidic PC increased CXCR4 expression and phosphorylation levels through a nitric oxide (NO)-dependent mechanism both under baseline conditions and upon exposure to SDF-1 on mouse BM-derived ckit$^+$ cells. Acidic PC also induced increased chemotaxis and TEM towards SDF-1. It was shown that the SDF-1/CXCR4 axis mediated the acidic PC-driven differentiation of BM-derived ckit$^+$ cells into ECs. In a mouse model of hind limb ischaemia, acidic PC promoted a NO-dependent increase of angiogenesis and blood flow and enhanced the number of regenerating muscle fibres (*Table 1*).[90] Thus, the SDF-1/CXCR4 axis is an important effector not only of hypoxic but also of acidic PC.

4. Conclusions

The SDF-1 chemokine, via its receptors, plays a pivotal role during the response to ischaemia. It orchestrates the rapid revascularization of injured, ischaemic, and regenerating tissue that is essential to restore organ function. Hypoxic or acidic PC represents an approach to enhance PGC functions *in vitro* and their therapeutic potential in animal models, in part accomplished via the SDF-1/CXCR4 axis. PC could potentially be used, in the near future, as part of cell preparation protocols for clinical use. Nevertheless, more studies are needed to assess the feasibility of PC in increasing the therapeutic potential of PGCs in the treatment of human ischaemic diseases.

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