COMMENTARY

Adult mesenchymal stem cells and the NO pathways

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Adult marrow-derived mesenchymal stem cells (MSCs) were named by me in the late 1980s, and data were published in the 1990s by our group and others (1, 2) to indicate that these cells were capable of differentiating into a number of mesenchymal phenotypes in culture. At that time, it was the dogma that only one adult stem cell could be found, the hematopoietic stem cell. We now know that there are a number of tissue- or organ-specific progenitors, including neural, cardiac, liver, epidermis, and gastrointestinal stem cells, to name the most obvious. Moreover, we now appreciate that MSCs originate in many tissues as perivascular or mural cells (pericytes) (3). Importantly, these pericytes, when detached from their blood vessel nest, become activated MSCs that secrete large amounts and a substantial array of bioactive molecules. These MSC injury site-secreted molecules have profound effects on the host's immune system and trophic effects that serve to establish a regenerative microenvironment (4). Indeed, in searching ClinicalTrials.gov, more than 285 MSC trials were listed for a vast array of clinical symptoms and diseases. For this reason, I have suggested that the stem cell activity of these cells was relatively minor, and that MSCs should be short for "medicinal signaling cells" (5).

In this context, the paper by Gomes et al. (6) documents that signaling by *S*-nitrosoglutathione reductase (GSNOR), an enzyme that metabolizes *S*-nitrosothiols (SNOs) to regulate protein nitrosylation (7), contributes to MSC-mediated vasculogenesis. Although the authors state that MSCs can differentiate into endothelial cells that form vessels, this differentiation capacity is far from certain (it is certainly not shown in this publication). To the authors' credit, the results of their experimentation on increasing or decreasing SNO bioactivity are explained by the MSC-mediated effects on vasculogenesis. Indeed, as MSCs contribute to vascular homeostasis, we and others have strongly argued that this is a result of their role as pericytes and by virtue of the spectrum of bioactive molecules the activated MSCs secrete, and that the MSCs can stabilize fragile new capillaries by becoming pericytes. Notably, NO is famous for such paracrine activity, and it is quite conceivable that NO or SNOs are secreted by MSCs to regulate vessel formation.

In this regard, GSNOR deficiency affects MSC-mediated postnatal vasculogenesis. The authors argue that the basis by which NO signaling through GSNOR directly affects MSC-mediated vasculogenesis is by regulating PDGF receptor abundance and thereby responding to VEGF-A (6). If one assumes that the source of endothelial cells is endothelial progenitor cells (EPCs) in the MSC preparation, the actual role of the MSCs would be to assume pericyte positions around the neovessels and thereby function to physically and chemically stabilize the newly formed vasculature. Thus, an extension of the interpretations discussed in this paper may be that the reduction in myocardial infarct size in GSNOR$^{-/-}$ mice (8) is a result of EPC activation and pericyte stabilization of neovasculature, as suggested by the authors (6).

Gomes et al. demonstrate that the central role of MSCs in developmental, repair, and regeneration processes uses SNO-regulated pathways.

There are several factors that contribute to the stabilization of neovasculature. Certainly, the ECM and its spectrum of bound bioactive molecules play a dominant role in the formation events. Both Matrigel and tissue-specific ECM—for example, the ECM of human papillary vs. reticular dermis (9)—support the formation of an intricate capillary vascular network. In the case of Matrigel, when this ECM–capillary network is implanted s.c., the capillaries fall apart unless stabilized by MSCs (10). Indeed, we would assert that an important test for MSCs isolated and expanded from a number of different tissues is whether they will stabilize a vascular network by taking up pericyte locations on the neovessels. The role played by NO in this regard is unstudied, although molecules such as PDGF-BB and VEFG-A seem to be obligatory. This is clearly documented in real-time in vivo sensing of PDGF secretion by sensor-engineered MSCs (11).

Although the authors focus on myocardial vasculature (rightfully so, as one of the authors is a cardiologist), it is clear that MSCs also play a crucial role in other organs and tissues, including in solid tumors and in metastasis. Recent studies by Correa et al. (12) based on data published by others (13) strongly indicate that MSCs as pericytes are obligatory and permissive agents for the transit of metastasizing melanoma cells into bone. In the absence of pericytes, melanoma cells do not enter into the bone stroma; in the presence of pericytes, the melanoma cells release and carry the detached pericytes, now MSCs, into the bone stroma, where the MSCs are corrupted to serve as a stimulus for tumor expansion and vascular support. Others have clearly shown that exogenously added MSCs locate in the established tumors and aid in the tumor expansion (14). How NO and NO mediators derived from MSCs might affect this system is unclear, especially as NO derived from inducible NO synthase (iNOS) attenuates tube formation in Matrigel in vitro, as shown by Gomes et al. (6), whereas NO derived from neuronal NO synthase is required for MSC function. The interactions between NOS1, NOS2, and GSNOR, which are constitutively expressed by MSCs (and many tumor cells), compared with NOS3 expression by endothelial cells (and tumor cells),

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suggest complex roles for NO in the formation and maintenance of vascularized tissues.

It is important to stress that paradoxical differences of human vs. murine MSCs exist relative to microbial growth, antimicrobial T-cell responses, and the roles played by NO and NO-generating or NO-related enzymes. For example, the tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) is stimulated with inflammatory cytokine exposure of human MSCs and is responsible for the broad-spectrum antimicrobial effector function directed against a range of clinically relevant bacteria, protozoal parasites, and viruses. In contrast, murine MSCs fail to express IDO when expressing iNOS (15). In addition, human MSCs upregulate the synthesis and secretion of a powerful protein antibiotic called LL37, whereas this is not the case with murine MSCs. Thus, the synthesis of NO and other noncytokine mediators have profound activities on MSC-mediated immune-modulatory and antimicrobial activities, with substantial differences observed between marrow-derived human and murine MSCs.

Data recently published (16) indicate that chemokines secreted by inflammatory cytokine-stimulated murine MSCs are responsible for the immune enhancement effects of MSCs and that immune function is regulated by iNOS. In this context, the relationship between SNO signaling as controlled by GSNOR and IDO has not been explored. Further, the differences between human and murine EPCs compared with MSCs would, likewise, be of considerable interest with regard to vasculogenesis and immunomodulation. As an aside, it is also of considerable interest to explore the relationship of MSC-mediated vasculogenesis and MSC-antimicrobial activity.

The NO, NO-related enzymes, and nitrosylated proteins that subserve metabolic and regulatory pathways are extremely complex, with thousands of moving parts. What is clear is that NO levels, per se, are not the key components to the control and regulatory mechanisms. During all developmental processes and those associated with repair and regeneration, there is a huge flux in the identity and activity of the NOS isoforms and the proteins being nitrosylated. The control of stem cells and their lineage descendants clearly is related to the sum of all of these components. For example, the GSNOR$^{-/-}$ mouse may be protected against severe effects of myocardial damage because the whole animal has an increased level of angiogenesis, and this can be controlled at a number of points and could be different in different tissues (7). Although the discussion by Gomes et al. (6) is in terms of NO levels, the key here is which enzymes are active and which proteins are being nitrosylated. This translates into the identity of the molecules being locally nitrosylated and how this results in the changing biologic activity that is being assayed. Again, it is the nitrosylation target, the various enzymes that are churning and how this affects stem cells and their individual lineage descendants. There is ample literature to support this thesis not only for MSCs, but for hematopoietic stem cells, neural stem cells, and others (15, 17). The new data in the study of Gomes et al. (6) demonstrate that the central role of MSCs in developmental, repair, and regeneration processes uses SNO-regulated pathways, which reflect the churning and changing of multiple NO enzymes and target molecules being nitrosylated. The key issues are not only where, when, and how these cells influence various biologic phenomena, but the metabolic and cellular landscape that contributes to the resulting events. The challenge is to provide a 3D, dynamic picture as it relates to each biologic process and organ system. Clearly, GSNOR, IDO, and NOSs all are coordinately interacting.

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1 Caplan AI (1991) Mesenchymal stem cells. *J Orthop Res* 9(5): 641–650.
2 Pittenger MF, et al. (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284(5411):143–147.
3 Crisan M, et al. (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 3(3):301–313.
4 Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. *J Cell Biochem* 98(5):1076–1084.
5 Sorrell JM, Caplan AI (2010) Topical delivery of mesenchymal stem cells and their function in wounds. *Stem Cell Res Ther* 1(4):30.
6 Gomes SA, et al. (2013) *S*-nitrosoglutathione reductase (GSNOR) enhances vasculogenesis by mesenchymal stem cells. *Proc Natl Acad Sci USA* 110:2834–2839.
7 Haldar SM, Stamler JS (2013) S-nitrosylation: integrator of cardiovascular performance and oxygen delivery. *J Clin Invest* 123(1): 101–110.
8 Lima B, et al. (2009) Endogenous S-nitrosothiols protect against myocardial injury. *Proc Natl Acad Sci USA* 106(15):6297–6302.
9 Sorrell JM, Baber MA, Caplan AI (2009) Influence of adult mesenchymal stem cells on in vitro vascular formation. *Tiss Eng Part A* 15(7):1751–1761.
10 Sanz L, et al. (2008) Long-term in vivo imaging of human angiogenesis: critical role of bone marrow-derived mesenchymal stem cells for the generation of durable blood vessels. *Microvasc Res* 75(3):308–314.
11 Zhao W, et al. (2011) Cell-surface sensors for real-time probing of cellular environments. *Nat Nanotechnol* 6(8):524–531.
12 Correa D, Lin P, Caplan AI (2011) Bone marrow pericytes/MSCs: gatekeepers controlling skeletal metastasis from melanoma. MSC 2011, Cleveland, OH. Available at http://www.msc2011.net/abstract_listing2011.htm. Accessed January 24, 2013.
13 Gerhardt H, Semb H (2008) Pericytes: Gatekeepers in tumour cell metastasis? *J Mol Med (Berl)* 86(2):135–144.
14 Klopp AH, et al. (2007) Tumor irradiation increases the recruitment of circulating mesenchymal stem cells into the tumor microenvironment. *Cancer Res* 67(24):11687–11695.
15 Krasnov P, et al. (2008) Neuronal nitric oxide synthase contributes to the regulation of hematopoiesis. *Mol Med* 14(3-4): 141–149.
16 Li W, et al. (2012) Mesenchymal stem cells: a double-edged sword in regulating immune responses. *Cell Death Diff* 19(9): 1505–1513.
17 Peunova N, Enikolopov G (1995) Nitric oxide triggers a switch to growth arrest during differentiation of neuronal cells. *Nature* 375 (6526):68–73.