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Forum Editorial

Emerging Roles of Reactive Oxygen and Nitrogen Species in Stem/Progenitor Cells

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INTRODUCTION

THE UBIQUITOUS PRESENCE OF REACTIVE OXYGEN SPECIES (ROS) within cells continues to defeat our simplistic thinking. At the crossroads of cell signaling with its response to injurious stimuli, changes in intracellular levels of ROS and/or redox equivalents are omnipresent, regulating biological effects such as differentiation, proliferation, and apoptosis. To add another level of complexity, nitric oxide (NO) and other reactive nitrogen species accompany ROS in their actions, besides the many functions specifically ascribed to them. The role of these highly reactive molecules in the biology of stem/progenitor cells is particularly challenging, raising important questions about signaling in these cells in general, with implications for their differentiation, as well as practical issues related to their dysfunctions in various pathological conditions.

We invited several researchers to summarize their work, to review their peers' activity, and to synthesize their opinions. The domains are representative of cardiovascular/hematopoietic, neuron, and tumor biology. Their contributions reveal a number of recently surfaced facts, indicating a deeply rooted role of ROS and NO metabolism in stem/progenitor cell functions and differentiation. The topics were not split up among stem and progenitor cells, because the distinction in itself (stem/progenitor) is still a matter of debate (18). For the purpose of this *Forum* issue of the *Journal,* the stem cells are defined by self-renewal (immortality) and pluripotency, and the progenitor cells are characterized by multipotency, while maintaining vigorous but limited proliferative capacity. The topics presented here encompass both embryonic and adult stem/progenitor cells.

Overall, the studies discussed here support a model proposed some time ago by Allen and Balin (1), accounting for the influence of oxidative stress on development and differentiation. This model was based on the fact that oxygen strongly influences the course of development, and that metabolic gradients exist in developing organisms. The authors suggested that the effects of these gradients on development might result from differential oxygen supply to tissues, because cells and tissues in various stages of differentiation exhibit discrete changes in their antioxidant defense and in the parameters of oxidation. Metabolically generated oxidants and ROS have been implicated as factors that direct the initiation of morphogenesis. Two recent studies confirmed that the antioxidant protective mechanism of endothelial progenitor cells (EPC) is highly amplified as compared with adult EC (5, 9). Moreover, it has been reported that low doses of ROS protect EC from apoptosis by increasing expression of thioredoxin-1 (8), indicating that, similar to adult cells, the effects of ROS in progenitors are concentration-dependent, and thus the background ROS formation is a prerequisite of normal cellular functioning. The Allen and Balin model also implicated the cellular distribution of ions and the cytoskeleton, both of which are influenced by oxidants. The impact of ROS on cytoskeleton organization was supported by other recent data in various experimental systems. For example, ROS were found to mediate actin polymerization in endothelial cells (EC) (16), and Rac1 to play a key role in this process (15).

ROS CONTROL DIFFERENTIATION OF STEM/PROGENITOR CELLS

In this issue, Heinrich Sauer and Maria Wartenberg discuss the participation of ROS in signaling in cardiac cell differentiation. They show that embryonic stem cell-derived embryoid bodies (EB) generate ROS through the activity of NAD(P)H oxidase (21). EB at early stages of differentiation exposed to ROS displayed an increase in the percentage of beating EB, demonstrating both the presence of cardiac cells in the EB and the redox control of differentiation. However, the role of ROS in cardiovascular differentiation of embryonic stem cells ap-

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pears to have antagonistic features, because continuous exposure to ROS results in inhibition of cardiomyogenesis and vasculogenesis, whereas pulsed exposure to low levels of ROS enhances differentiation toward cardiomyogenic, as well as vascular cell lineages. Furthermore, the "confrontation culture" method (where two tissues are let to grow side by side) for EB versus tumors showed that ROS were induced in both tissues. EB formed blood vessel-like structures, which invaded the tumor. Vitamin E treatment reduced this angiogenic activity of EB, further confirming the mechanistic role of ROS in the process.

Interestingly, as a means for directly inducing ROS in EB, these authors chose to use pulsed electric fields. In this regard, it is worth noting that electromagnetic fields were independently shown to stimulate angiogenesis *in vitro* and *in vivo*, via the release of endothelial basic fibroblast growth factor (22).

Michel Pucéat highlights the roles of the small GTP-binding molecule Rac and of NAD(P)H oxidase complex in the inhibition of cardiomyogenesis and down-regulation of the cardiac-specific transcription factor MEF2C, via induction of excessive levels of ROS. He used a model of embryonic stem cells overexpressing a constitutively active form of Rac (19).

Paula van Hennik and Peter Hordijk review the roles of the major members of the Rho family (*i.e.,* Rac1, Rac2, RhoA, and Cdc42), in the organization of actin cytoskeleton and in the control of gene expression in hematopoietic cells, during processes such as chemotaxis and phagocytosis, both dependent on production of ROS (23). These functions are allocated to relatively small regions in the primary sequence of these GTPases. The identification of mutations in GTPases or in their regulators has provided novel insights in the structure–function relationship, as well as in their relevance for the development of hematological diseases.

Mark Noble and colleagues discuss the role of redox state in regulating the balance between proliferation and differentiation in neural progenitor cells (17). The authors developed a model to study how the redox state of a cell is involved in cell-fate decisions. The core evidence is provided by the authors' studies of oligodendrocyte progenitor cells isolated from rat optic nerve. They suggest that the intrinsic redox state of the cells affects their responsiveness to different stimuli, namely, cells in a more reduced state would respond more readily to inducers of survival and proliferation, whereas cells that are more oxidized would respond readily to inducers of differentiation or cell death. They further argue that this redox state can be altered by exogenous cues, in which case signaling molecules that induce a more reduced state stimulate self-renewal, and those agents that make a more oxidized state promote differentiation. The authors comment that, paradoxically, the results obtained through studies on the oligodendrocyte lineage are the opposite of what might be predicted from many other studies demonstrating the ability of ROS to enhance the effects of signaling through tyrosine kinase receptors and to induce cell proliferation. Comparing their own data with those obtained in other cell systems, the authors identified the existence of two distinct programs of cellular responses to changes in oxidative status. In one of these, slightly oxidized states are sufficient to inhibit proliferation and induce differentiation. In the second program, similar changes would enhance proliferation. To the question of how the cells can interpret similar signals in such opposite manners, we would suggest that a decisive factor in dictating their responses to ROS and to redox status might be their embryological origin. Oligodendrocytes are derived from the ectoderm, whereas others, such as the cardiovascular and hematopoietic cells, are of mesodermal origin. This hypothesis can be experimentally tested.

ABNORMAL ROS LEVELS PRODUCED IN DIABETES INTERFERE WITH STEM/PROGENITOR CELL FUNCTIONS

Another emerging field of investigation is the impact of externally imposed, chronic (pathological) oxidative stress on stem/progenitor cells. To illustrate the case, diabetic diseases were considered, which are notorious for induction of high concentrations of ROS in a variety of cells. Recently, the sensitivity of bone marrow-derived primitive EPC to diabetes-induced ROS was demonstrated by their increased response to oxidative stress and by reduced proliferative abilities, whereas the more differentiated monocytic EC precursors were less affected (2).

In this issue, Cindy Loomans and colleagues review the impact of type 1 diabetes on (mostly endothelial) progenitor cells (13), showing that the vascular regenerative potential of patients with diabetes is impaired, likely as a consequence of reduced number and deficient activity of circulating progenitor cells contributing to endothelial maintenance and ischemia-induced neovascularization. The authors also cite their own work supporting a role of oxidative stress in the diabetes-associated EPC dysfunction. Based on DNA microarray analysis, they suggest that EPC cultured from patients with type 1 diabetes display a proinflammatory phenotype, with implications for the proatherogenic mechanisms present in the diabetic patients. The authors also suggest that statins could reduce glucose-induced ROS production by the endothelium via inhibition of GTPase-mediated activation of the NADPH subunit $p22^{phox}$. The inhibitors of HMG-CoA reductase may also counteract the adverse phenotype observed in EPC from diabetic patients. The authors also propose that pharmacological intervention in angiotensin II signaling, known to be ROS-dependent, may also be beneficial to maintain EPC number and function in diabetes.

In an accompanying article, Matthew Callaghan and colleagues review the current knowledge regarding type II diabetes and EPC function (3). They note that hyperglycemia alone, through mitochondrial overproduction of ROS, can induce changes in gene expression and cellular behavior in diabetes. Experimentally, the impairment of EPC functions would prevent new blood vessel growth and is potentially reversible by manipulations to decrease ROS. They underline that novel strategies aimed at reducing hyperglycemia-induced ROS may again be a useful adjuvant to antihyperglycemic

therapies in the restoration of vasculogenesis and prevention of diabetic complications.

NO REGULATES STEM/PROGENITOR FUNCTIONS

NO constantly accompanies O_2 and/or ROS in most of their physiological activities. It became customary to see that in most of the studies addressing one mechanism, the other was considered as well. There are multiple reasons for this. One is the cross-talk between the signaling pathways, in production and/or consummation of these molecular species (11). It was hypothesized that in states where superoxide anion is increased, especially in the mitochondria, whole-body O_2 consumption will be increased, because of the inactivation of NO. Reaction of NO with superoxide leads to formation of peroxynitrites, which have many important consequences on cellular physiology (12). Remarkably, the "overlap" between NO and ROS metabolism goes as deep as their alternate synthesis by the same enzyme, NOS (4). When reviewing the role of ROS in cardiac cell differentiation, Sauer and Wartenberg did not omit the role of NO (21), underscoring that the roles of NO and ROS in certain stages of cardiovascular differentiation are similar. Recently, a wealth of data on the key role of NO in EPC mobilization and homing was obtained using eNOS knockout mice (for a review, see 6).

As an application of the concepts discussed before, the original contribution to this *Forum* by Ellis *et al.* (7) highlights the interplay between NO and ROS in EPC-based vasculogenesis in diabetic eyes. The authors used animals with targeted mutations to either inducible NOS (iNOS$^{-/-}$) or the endothelial form of NOS (eNOS$^{-/-}$) to characterize the isoform-specific role of NOS in retinal pathology. The absence of iNOS in transgenic animals protected against development of retinal pathology following long-term galactose feeding, minimized neovascularization after laser injury, and prevented the increase in nitrotyrosine, as opposed to galactosemic eNOS$^{-/-}$ and wild-type mice. Following laser injury to the retina, eNOS$^{-/-}$ mice produced in the eyes many unbranched, nonperfused tubes (indicating poor functionality), whereas the iNOS$^{-/-}$ animals had only few areas of perfused new vessels, similar to the wild-type controls. The authors concluded that not only the levels of NO, but also their sources (underscoring the role of inflammatory cells), modulate hematopoietic stem cell behavior and vascular phenotype in the retina. These findings of the increased presence of bone marrow-derived progenitors (although of limited functionality) in the retinal injury areas of eNOS$^{-/-}$ mice are at odds with the reduced bone marrow homing of EPC in eNOS$^{-/-}$ mice (6), which suggests a nuanced interpretation of the role of NO in EPC physiology. We anticipate that this paradox, as well as the previous one regarding the opposite responses of various classes of stem cells to redox imbalances, will be a rich source of future discoveries.

It is said that good science is the one that produces more questions than answers. This is definitely the case with the articles collected in this issue, and with the field at large. There are of course other instances, not discussed here, where ROS, NO, and their pharmacological modulators regulate signaling mechanisms in stem/progenitor cells. For sure, the coming years will unveil much more exciting facts and interpretations with particular application of ROS and NO metabolism to developmental biology and tissue regeneration.

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ABBREVIATIONS

EB, embryoid bodies; EC, endothelial cells; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cells; iNOS, inducible nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; ROS, reactive oxygen species.

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