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A niche for every cell, for every function

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Acute or chronic blood loss reduces the numbers of red blood cells (RBCs) in the circulation, reducing oxygen delivery and producing tissue hypoxia. One of the first cellular responses to hypoxia is increased production of reactive oxygen species (ROS) by mitochondria. With evolution, organisms have developed an efficient and rapid way to respond to erythroid challenges by using ROS as the 'ignition key' that activates the engine, defined stress erythropoiesis, which induces hematopoietic/stem progenitor cells to produce RBCs to compensate losses.^{1,2}

Much has been discovered in recent years on the mechanism(s) that regulate stress erythropoiesis. ROS stimulates kidney cells to produce greater levels of erythropoietin (Epo), the hormone that specifically stimulates RBC production.² In addition, ROS stimulates hematopoietic niches in the marrow to produce factors that induce hematopoietic stem cells to generate 'stress-specific erythroid progenitors' with enhanced ability to produce RBCs.³ In mice, 'stress-specific erythroid progenitors' egress from the marrow into the blood to home in 'stress-specific' niches in the spleen where they continue their maturation.^{4,5} The development of mechanisms that use 'ROS' as the sensor to increase RBC production in response to erythroid challenges is an evolutionary winner that has an important drawback. As described by Ulyanova *et al.* in this issue,⁶ maturation of erythroid cells is uniquely associated with accumulation of hemoglobin, the oxygen carrier molecule with the intrinsic property of increasing ROS production. Since above a 'crit-

ical' threshold, ROS activates the p53-dependent pathway of cell death, during their maturation, erythroid cells must maintain ROS constantly low by increasing production of enzymes, such as catalase, that are able to degrade ROS. The expression of these enzymes is controlled by the transcription factor forkhead-box protein O3 (FoxO3) activated by AKT⁷ (Figure 1). Therefore, in contrast to stem/progenitor cells, hypoxia-induced increases of ROS are deleterious to erythroblasts. Hence the need for these cells to have a safety circuitry up-regulating expression of enzymes that catabolize ROS under conditions of hypoxia. Without this circuitry, stress-specific erythroblasts would die in great numbers, eliminating the expected increases in RBC production mediated by ROS-induced proliferation of hematopoietic stem/progenitor cells.

Studies have been performed to clarify how increases in ROS production as a result of hypoxia induce hematopoietic stem cells to generate stress-specific progenitor cells.^{1,3} ROS induce the formation of 'stress-specific' niches that through specific adhesion receptors attract hematopoietic stem cells exposing them to growth factors that, in synergy with Epo, induce them to generate stress-specific progenitor cells. In contrast, little is known about how the control of ROS production becomes more stringent in erythroblasts maturing under conditions of hypoxia.

Integrins are heterodimers composed by an alpha(α) and a beta(β) subunit that interact with counter receptors present on niche cells and/or with fibronectin, a component of

signaling pathways enhancing erythroid stress proliferative response in spleen

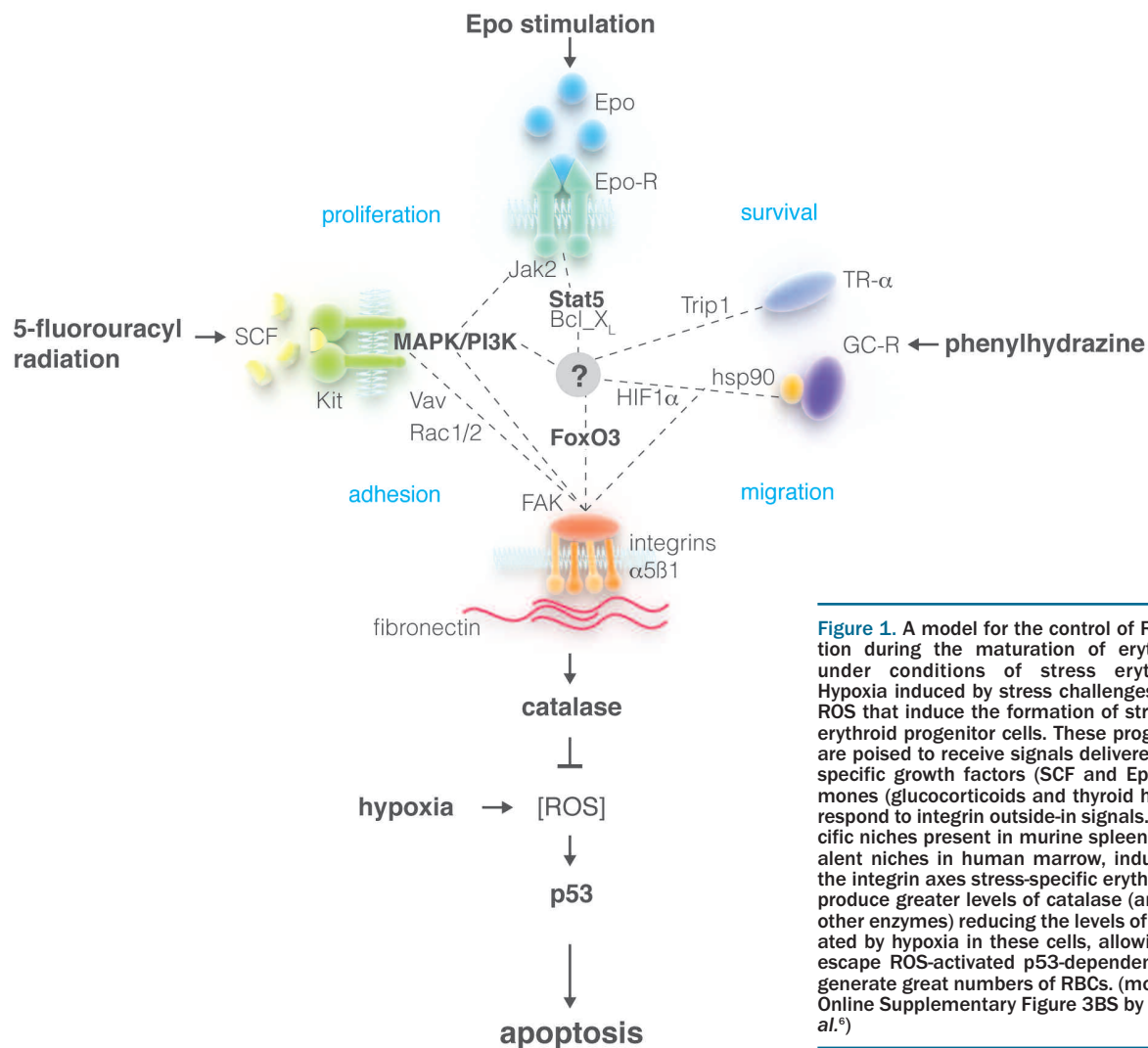


Figure 1. A model for the control of ROS production during the maturation of erythroid cells under conditions of stress erythropoiesis. Hypoxia induced by stress challenges generates ROS that induce the formation of stress-specific erythroid progenitor cells. These progenitor cells are poised to receive signals delivered by stress-specific growth factors (SCF and Epo) and hormones (glucocorticoids and thyroid hormone) to respond to integrin outside-in signals. Stress-specific niches present in murine spleen, and equivalent niches in human marrow, induce through the integrin axes stress-specific erythroid cells to produce greater levels of catalase (and probably other enzymes) reducing the levels of ROS generated by hypoxia in these cells, allowing them to escape ROS-activated p53-dependent death, to generate great numbers of RBCs. (modified from Online Supplementary Figure 3BS by Ulyanova *et al.*⁶)

the extracellular matrix used by cells to sense their position in the microenvironment.⁸ Integrins lack a kinase domain but upon activation deliver outside-in signals through association with the focal adhesion kinase (FAK).^{8,9} The combination of α and β subunits expressed is cell- and tissue-specific. Hematopoietic cells express mainly the $\beta 1$ subunit in combination with either $\alpha 4$ or $\alpha 5$.^{10,11} During erythroid maturation, expression of $\alpha 4$ increases between the proerythroblast and the basophilic stage and then decreases between the basophilic and polychromatic stage.¹²⁻¹⁴ Expression of $\beta 1$ parallels that of $\alpha 4$ with the exception that it is retained in terminally maturing cells. Pioneering work published, among others, by the Papayannopoulou laboratory has identified that the $\alpha 4\beta 1$ heterodimer prevents mobilization of stem/progenitor cells in response to a variety of stimuli, including those that elicit the erythroid stress response.^{1,5} In contrast, the pattern of $\alpha 5$ expression and its biological functions in erythropoiesis are less clear.

In this issue, Ulyanova *et al.*⁶ present a superb review of the effect of ROS on terminal erythroid maturation and the regulation of this process by the microenvironment through

the integrin axes (Figure 1). This information is used as a foundation for performing loss-of-function analysis of the role played by integrins in the regulation of the terminal phases of RBC production. The loss-of-function models utilized include mice lacking the $\alpha 4$ gene, mice lacking the $\beta 1$ gene (and therefore presumably lacking also $\alpha 5\beta 1$ heterodimers), mice lacking both the $\alpha 4$ and the $\beta 1$ gene, and wild-type mice treated with an inhibitor of FAK, the tyrosine kinase protein that mediates their intracellular signaling. The mutants were analyzed at baseline and during the recovery from stress challenges chosen to induce progressively more profound perturbations of the erythron (Figure 1).

- *Phenylhydrazine-induced hemolytic anemia*: this drastically depletes the number of circulating RBCs. Mice recover from this challenge by rapidly amplifying the compartment of erythroid precursors which generates RBCs within several days. Recovery requires activation of the glucocorticoid receptor that induces proerythroblasts into a transient self-replication state.¹⁵

- *Epo treatment*: this induces polycythemia by increasing

proliferation of cells ranging from commitment progenitor cells to proerythroblasts.¹⁶

- *5-fluorouracyl treatment and radiation*: these challenges induce death of cells in proliferation such as hematopoietic progenitor cells of multiple lineages but spare quiescent hematopoietic stem cells and RBCs.¹⁷ Onset of anemia is delayed and is due to the fact that reductions in progenitor cell numbers hamper the ability of the marrow to produce new RBCs in sufficient numbers to compensate their physiological turnover due to aging. Recovery involves induction of proliferation of quiescent hematopoietic stem cells and is mediated by the interaction of cKIT, a receptor present on hematopoietic stem cells, with stem cell factor (SCF), a growth factor produced by the microenvironment.¹⁸

Both glucocorticoid receptor and cKIT activation up-regulate expression of FAK^{19,20} poising the cells for outside-in signaling by integrins (Figure 1).

The data presented indicate that $\alpha 4\beta 1$ and $\alpha 5\beta 1$ are not essential for normal erythropoiesis because the hematocrit of mice lacking either one of these integrin complexes is normal. However, careful analysis of the phenotype of $\alpha 5\beta 1$ deficient mice demonstrated that they express a modest uncompensated anemia because of impaired ability to compensate for the physiological increases in ROS production occurring during the late stages of maturation. This result suggests that signaling from $\alpha 5\beta 1$ integrin may be specifically responsible for regulating ROS catabolism (Figure 1).

This hypothesis was confirmed by demonstrating differences in phenotype of $\alpha 4\beta 1$ and $\alpha 5\beta 1$ deficient mice under conditions of stress erythropoiesis. Mice lacking the integrin $\alpha 4$ had delayed but quantitatively normal responses to all the challenges used. In contrast, mice lacking $\alpha 5\beta 1$ were unable to respond to any of these challenges because their maturing erythroid cells expressed great levels of ROS and died of apoptosis. Further investigations indicated that erythroid cells from $\alpha 5\beta 1$ -deficient mice were unable to activate FAK and FOXO3 and to increase antioxidant response. These results indicate that although stress-specific erythroid cells are poised to activate the machinery that reduces ROS, an outside-in signal delivered by $\alpha 5\beta 1$ integrin is necessary for activation of the machinery and ROS (and consequent p53-mediated death) reduction under hypoxic conditions.

The analyses of the phenotype of double knock-out mice provided additional information on the role exerted by the two integrins during stress erythropoiesis. Mice lacking both $\alpha 4\beta 1$ and $\alpha 5\beta 1$ responded to challenges perturbing only the terminal erythroid compartments (phenylhydrazine and EPO) as single $\alpha 5\beta 1$ mutant mice, confirming that $\alpha 5\beta 1$ is necessary and sufficient to control ROS production in maturing erythroid cells. By contrast, double knock-out mice responded to challenges perturbing the initial phases of the hematopoietic process, (5-fluorouracyl and radiation) more severely than single $\alpha 5\beta 1$ mutants dying in great numbers following these challenges. This result confirms that $\alpha 4\beta 1$ is necessary to induce hematopoietic stem cells to generate stress-specific erythroid progenitors.

The results described by Ulyanova *et al.* are important for three reasons.

1) They demonstrate for the first time that $\alpha 4\beta 1$ and $\alpha 5\beta 1$ control different levels of the response to erythroid

stress. This specialization may represent an evolutionary mechanism developed by the hematopoietic system to decrease ROS in response to hypoxia only in those cells, i.e. mature erythroblasts, which, having already high ROS levels, would be induced into apoptosis by further increases.

2) They identify the first outside-in signaling activated by integrins represented by the FAK, AKT, FoxO3 and catalase axis. Although much is known about the activity of integrins in hematopoietic stem/progenitor cells, the outside-in signaling they activate in these cells still has to be characterized.

3) Downstream to the hematopoietic stem/progenitor cells there is a plethora of hematopoietic precursors whose appropriate maturation is essential for the generation of adequate numbers of blood cells. These precursors are embedded in a mesh of microenvironment elements. A movie documenting the complex physical contacts established by maturing erythroblasts with macrophages may be downloaded here (Movie 1). By providing evidence that the microenvironment provides not only 'niches' that promote specific hematopoietic stem cell functions but also 'niches' that control differentiation/maturation of erythroid precursors, these data provide proof-of-principle for the existence of 'microenvironmental niches' that may control every function of every cell type.

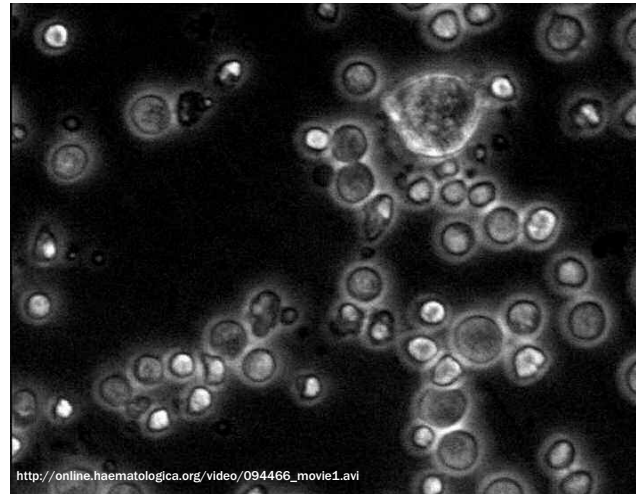
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Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

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Movie 1. Interaction between macrophages and human erythroblasts *in vitro*. Time lapse videomicroscopy of cells obtained after 14 days of culture under Human Erythroid Massive Amplification (HEMA) conditions from peripheral blood of one healthy donor. Samples were observed by an Olympus phase-contrast microscope equipped with incubator chamber and high speed, high-resolution camera (Hamamatsu), objective lens x20. Two hours of video recording are shown. Frames were collected every 30 seconds and movie was accelerated at 10 frames per second. The movie presents erythroblasts at various stages of maturation surrounding a macrophage (top right) recognizable for being larger than the other cells, for its motility, and for its ability to protrude its cytoplasm. During the movie, the macrophage appears to explore the space in its surroundings protruding filaments of different sizes. These filaments establish contacts with numerous erythroblasts. Some of the contacts are temporary while others persist in time. The variegation of these interactions suggests that the macrophage is engaging in multiple types of biological conversations possibly in response to specific signals emitted by each erythroblast. Kindly provided by Mario Falchi PhD, National Aids Center, Istituto Superiore di Sanità, Rome, Italy.

Autonomic regulation of hematopoiesis and cancer

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The functions of the autonomic nervous system have expanded in the last years from ‘fight-or-flight’ responses to fine-tuned control of multiple homeostatic and pathological processes. Recent evidence has demonstrated that hematopoiesis, cancer progression and metastasis do not escape the control of this rheostat.

The autonomic nervous system comprises two branches that frequently exert opposite biological actions: the sympathetic and the parasympathetic nervous systems. Some of the first evidence of sympathetic regulation of hematopoiesis came from studies on circadian rhythms, the daily oscillations that govern most biological processes. It has been shown that the postsynaptic neurotransmitter of the sympathetic nervous system (SNS) noradrenaline exhibits a circadian rhythm in the murine bone marrow (BM), peaking at night. This peak coincides with an increase in the number of cells in the G2/M and S phases of the cell cycle.¹ Also, admin-

istration of noradrenaline stimulated BM cell proliferation and rescued mice from chemotherapy,² and this has recently been confirmed.^{3,4} Similarly, noradrenaline was shown to directly stimulate the proliferation and migration of human hematopoietic stem cells (HSC).⁵ The role of the SNS in regulating HSC traffic and enforced mobilization was only recently uncovered by serendipitous observations. Different groups found that the selectin inhibitor fucoidan increased circulating hematopoietic progenitors (HSPCs) independently of selectin function.^{6,7} The hypothesis was advanced that sulfated glycans expressed in the BM microenvironment, like sulfatide, might regulate HSC trafficking. Sulfatide, a major component of myelin, is synthesized by the UDP-galactose:ceramide galactosyltransferase (Cgt) enzyme. Cgt^{-/-} mice did not only show reduced myelin and activity of myelinated nerve fibers,⁸ but also compromised G-CSF-induced HSC mobilization.⁹ Although these mice exhibited a