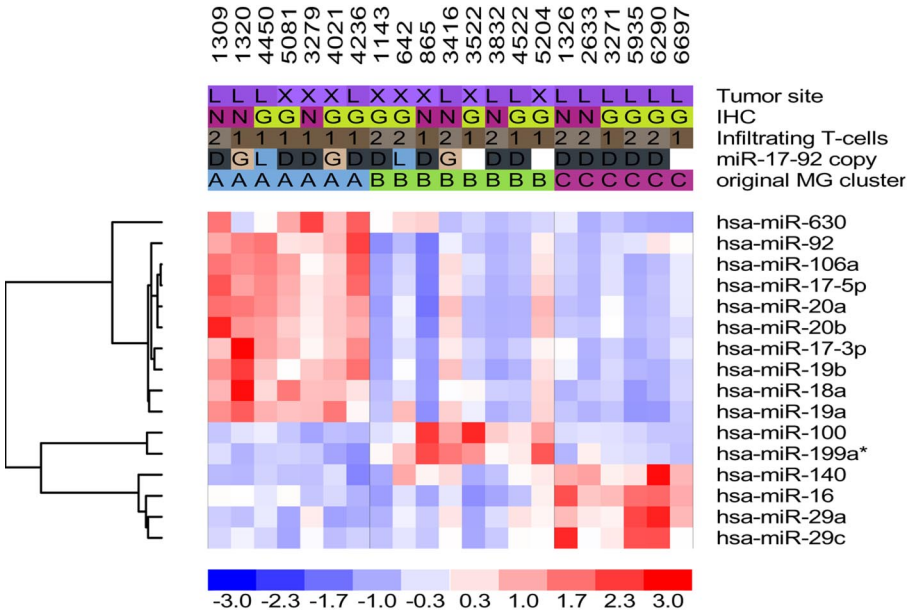

MicroRNA expression profiling in DLBCL: training and validation sets. Two rounds of one-way ANOVA testing identified subsets of 38 and 16 miRNAs whose expression could effectively discriminate DLBCLs into these 3 subsets. See the complete figure in the article beginning on page 6681.

gene amplification. Another example is miR-17-92, an oncogenic polycistronic cluster that is targeted by both gene amplifications and deregulated c-MYC, resulting in its overexpression in the "centroblast-like" subgroup of DLBCL. Normally, miR-17-92 is expressed at higher levels in centroblasts compared with naive and memory B cells.[4] c-MYC is not normally expressed in GCB cells, suggesting that the differential expression of miR-17-92 in centroblasts may be regulated by transcription factors other than c-MYC. Increased copy numbers and transcription induction by a deregulated c-MYC may result in persistent expression of miR-17-92, which would otherwise be down-regulated during transition from GCB cells to memory B cells. This study highlights the importance of c-MYC as well as miR-17-92 deregulation in the pathogenesis of DLBCL. Undoubtedly, investigations to dissect the influence of other oncogenes or tumor suppressors on the miRNA expression profiles in DLBCL are necessary to further understand the role of these complicated gene regulatory networks in lymphomagenesis.

Above all, the generation of these miRNA profiles has provided us with a framework to delineate the roles of specific miRNAs in DLBCL lymphomagenesis. Careful comparative studies of these miRNA profiles with the normal B-cell subset signatures should reveal miRNAs that are deregulated during specific stages of B-cell differentiation. In addition, comparison of mRNA profiles of DLBCL with differential expression of a particular miRNA should facilitate the identification of target genes coordinately regulated by the miRNA and the molecular mechanisms by which it contributes to lymphomagenesis.[8]

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● RED CELLS & IRON

Comment on Robach et al, page 6707

Muscle iron in stress erythropoiesis?

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Two related recent studies on erythropoiesis by Robach and colleagues describe surprising differential responses to erythropoietin with respect to skeletal muscle iron mobilization, apparently depending on normoxic versus hypoxic conditions.

Like other heavy metals, iron is toxic, albeit in bound form, essential for life. Thus elaborate mechanisms involving dozens of molecules balance systemic and cellular iron usage, detoxification, storage, and homeostasis, with the regulatory peptide hormone hepcidin a prominent recent addition to this repertoire.[1] As the main consumer (80%) of mobilizable iron, the erythroid compartment in response to its homeostatic regulator erythropoietin (Epo) is an indirect but significant player in this balance. First, erythroid iron intake and incorporation into heme represent a sort of temporary iron store. Second, the "outflow" when aged erythrocytes are eaten up by macrophages also has to be taken into account. Finally, lowered hepcidin levels, due to, for example, elevated Epo, enable increased transfer of iron between macrophages and liver as the major storage organ via up-regulated activity of the iron exporter ferroportin.[2] Although skeletal muscle cells contain up to 15% of total body iron in myoglobin and do express ferroportin, knowledge about the contribution of this potential iron storage depot to erythropoiesis has remained scarce so far.

In this issue of *Blood*, Robach et al describe hematologic and molecular responses in muscle samples upon Epo injections.[3] Eight healthy volunteers received "clinical" doses of recombinant human Epo (65 IU per kg body weight), for the first 2 weeks every other day

	normoxia	hypoxia
erythropoietin (a.u.)	1.0 → 3.3 injected; 2nd week	1.0 → 1.4 endogenous; day 8
hemoglobin (a.u.)	1,00 → 1.07	1.00 → 1.16
ferroportin (a.u.)	1.0 → 1.7	1 → 7.0
myoglobin (major iso-forms; a.u.)	1.0 → ~ 0.9	1.0 → ~ 0.6

Changes in selected molecular components directly or indirectly involved in iron metabolism upon exposure of human subjects to either normoxic or hypoxic conditions. Data are shown in arbitrary units to facilitate comparison between factors of response and are average values deduced from the authors' original figures and tables.

and then once per week. Besides measuring classical blood parameters as well as urinary hepcidin levels, the authors performed small muscle biopsies, a commendable effort by both patients and investigators. As expected, erythropoiesis was enhanced while hepcidin levels decreased. In muscle, there was a concomitant increase in ferroportin expression by about 70%, which, however, was accompanied by only a very minor reduction in myoglobin levels and thus more or less futile in terms of iron mobilization. To make the results even baffling, expression of the iron uptake protein transferrin receptor (TfR) went up in muscle, together with an elevated abundance of the iron storage protein ferritin (see figure). This indicated that there was an increased iron influx into muscle cells upon Epo administration, but this iron was not ending up in myoglobin. Measuring total cellular iron levels further corroborated this interpretation. At this stage, the overall picture might have been interpreted as expected hematologic results and a "complicated" situation in muscle.

The story, however, becomes really interesting when seen in conjunction with an earlier paper from the same group.[4] There, similar parameters were quantitated, again with human volunteers but at high altitude, to be precise in the Monte Rosa region at 4559m. In this really hypoxic environment (101 kPa → 59 kPa), the ensuing rise in hematocrit was accompanied by a significant loss of muscle-iron and myoglobin (see figure), lowered TfR and ferritin levels, and a massively elevated level of ferroportin. In this case, muscle cells indeed appeared to become a source of mobilizable iron.

How can these seemingly disparate data be reconciled? One could always argue that experimental protocols were not identical. While probands tested under normoxic conditions received Epo injections boosting their total levels by 350% throughout the second week, the mountain volunteers had to rely on an observed 40% increase of endogenous Epo (day 8). This does not offer an adequate explanation since the lower Epo concentrations had a much larger impact on ferroportin levels. The equation, more Epo/less hepcidin/more ferroportin activity/more iron release that beautifully explains the role of macrophages in iron homeostasis, appears to fail for muscle.

In their present paper, Robach et al discuss a variety of possible scenarios these differential responses could take on. One physiologically meaningful hypothesis sticks out, despite involving a notorious "factor X." Such a factor might sense an emergency need for enhanced erythropoiesis, especially under hypoxia or eventually also upon blood loss. Thereby it would open a route to utilize the otherwise protected source of iron in muscle. In contrast, elevated Epo under normoxia might be sensed as a more "normal" situation, leading to the conventional route of increasing red blood cell production by involving the reticuloendothelial system. It will be interesting to see whether and under which specific conditions myoglobin iron might also become mobilized under normal oxygen supply.

Biologic systems persist to be more complex than any possible preconception. Despite the decades that have passed since the first applications of Epo[5] for example, to treat anemia in kidney diseases, cancer or—less beneficially—for blood doping, it looks as if there are still facets of this molecule and its role in iron homeostasis that need further study.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● VASCULAR BIOLOGY

Comment on Horst et al, page 6726

Myeloid cell–induced angiogenesis: a sticky business

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In this issue of *Blood*, Horst and colleagues demonstrate that myeloid cells expressing the adhesion molecule CEACAM1 play a role in promoting angiogenesis and resolving inflammation.

In the adult, angiogenesis is essential to wound repair and inflammation and for highly specialized functions, such as the regeneration of the endometrium. Angiogenesis is also involved in tumor growth, chronic inflammatory diseases and atherosclerosis. Vast literature has emerged that investigates the cellular and molecular mechanisms underlying the formation of new vessels. One of the important findings over the past few years has been the identification of the role of different cell types in angiogenesis.

As with endothelial cells (ECs) and their bone marrow (BM) precursors, heterogeneous