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histidine-rich glycoprotein have decreased ability to trap and kill bacteria.[9] The fact that histidine-rich glycoprotein is a negative acute phase reactant while fibrinogen, its major plasma ligand, is a positive acute phase reactant suggests that the ability of histidine-rich glycoprotein to put the brakes on factor XII will be diminished during acute phase responses, a condition that could therefore contribute to increased proinflammatory (and prothrombotic?) phenotypes.

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

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● ● ● PLATELETS & THROMBOPOIESIS

Comment on Liu et al, page 4106

Young maybe, but surely not immature

Nicolas Pineault HÉMA-QUÉBEC

Megakaryocytes derived from fetal or cord blood stem cells have long been viewed as immature compared with their adult counterparts. In this issue of *Blood*, Liu and colleagues dismiss once and for all this concept while providing new insights into the long sought molecular mechanisms responsible for the unique properties of neonatal megakaryocytes.[1]

While one mature erythroblast will give rise to a single red cell, one mature megakaryocyte will produce thousands of platelets. This production feat is possible because, in contrast to erythroblasts, megakaryocytes increase their size during maturation, in large part by becoming polyploid through endomitosis. Finally, after cytoplasmic maturation and proplatelet formation, platelets are released into the circulation through the marrow sinusoids.

Compared with their adult counterparts, fetal and neonatal megakaryocytes have a greater proliferative potential but remain mostly diploid and significantly smaller,[2-4] which has led many scientists including myself to refer to these as immature. In addition, the growth and polyploidization responses of neonatal and adult-derived megakaryocytes to thrombopoietin (TPO)[5,6] and to stromal-conditioned media[5] were previously shown to differ. Together, these developmental divergences are thought to be some of the many contributing factors responsible for the long delays in platelet recovery after cord blood transplantations. The mechanisms responsible for these differences remained poorly understood up to now, despite many studies aimed at identifying the predisposing factors. In their study, Liu et al further investigated this paradigm using megakaryocytes derived in TPO cultures from adult and neonatal stem cells.[1] They showed that in contrast to popular belief and to adult megakaryocytes, the majority of diploid (2N) cord blood megakaryocytes are in fact fully mature. In addition, the authors demonstrated that the increased proliferative response and reduced polyploidization of neonate megakaryocytes are, at least in part, because of increased TPO-mediated activation of JAK2 and mTOR signaling, and to reduced Cyclin-dependent kinase 4 (CDK4) expression, respectively.[1] Hence, these findings provide a rational molecular justification for some of the important developmental differences between megakaryocytes issue at distinct stages of ontogeny.

Without doubt, the past 15 years of clinical and fundamental research have provided a wealth of knowledge on the process of megakaryopoiesis. Several transcription factors (*GATA-1, FLI-1*) and other molecules (*JAK2, mTOR, Cyclin D1, Cdk4*) have had their role during the differentiation and maturation of megakaryocytes defined. The authors used this information smartly to reveal a wide spectrum of differences in the expression of several key regulators in neonatal megakaryocytes. Because GATA-1 is essential for the polyploidization and cytoplasmic maturation of megakaryocytes,[7] the authors investigated early on whether GATA-1 expression varied in megakaryocyte during ontogeny. Surprisingly, they discovered that GATA-1 levels were a near 2-fold greater in neonatal megakaryocytes. Consistent with this, the expression levels of the TPO receptor (C-MPL) and Cyclin D1, known targets of GATA-1, were also up-regulated. Perhaps because of the increased levels of C-MPL, cord blood megakaryocytes were shown to be hypersensitive to TPO and showed a more pronounced and sustained activation of JAK2 (based on phosphorylation) and mTOR signaling. Because both are known to promote proliferation, these results explain the increased proliferation of neonatal megakaryocytes in response to TPO.[8,9] However, the functional consequences of increased JAK2 phosphorylation in neonatal megakaryocytes on STAT proteins such as STAT-3 and -5 remain to be firmly addressed.

The increased levels of GATA-1 are also consistent with the normal cytoplasmic maturation of neonatal megakaryocytes. However, because increased GATA-1 expression in murine megakaryocytes leads to increase polyploidization, why do the majority of neonatal megakaryocytes remain diploid? The answer can be extrapolated from the elegant work of Muntean et al[10] that demonstrated that GATA-1–mediated polyploidization is dependent on the coexpression of Cyclin D1 and Cdk4, and that overexpression of Cyclin D1 alone resulted in the formation of slightly smaller and less polyploid cells (sounds like neonatal megakaryocytes!). Hence, it would

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appear that the failure of neonatal megakaryocytes to sustain elevated Cdk4 levels (50% reduction) is likely to be one of the factors precluding neonate megakaryocytes initiating and undergoing multiple rounds of endomitosis. However, this remains an assumption because no functional proof of this has been shown in neonatal megakaryocytes, although Bornstein et al did demonstrate a significant reduction in CDK2 activity in cord blood–derived megakaryocytes.[4] Thus, future studies aimed at better characterizing the activity, and transcriptional and posttranslational regulation of Cdks and related genes (eg, $p16^{ink4a}$) in neonatal versus adult-derived megakaryocytes would be useful to determine whether these are effectively responsible for the low propensity of neonatal megakaryocytes to become polyploid.

Another concept already well accepted in this field is that polyploidization and cytoplasmic maturation are 2 independent processes, and that only the latter is essential for platelet production. Liu and colleagues now provide an interesting new twist to this concept by showing that inhibition of mTOR with rapamycin results in a partial reduction of polyploidization in adult-derived megakaryocytes, while having no significant effects in neonatal megakaryocytes. The different impact of rapamycin on the process of polyploidization at distinct stages of ontogeny further highlights the important molecular differences between them.

The results presented here by Liu et al might just be the tip of the iceberg. Nonetheless, they have already revealed that numerous proteins of diverse functions (transcription and translation factors, cell-cycle regulators, and signaling molecules) normally implicated in the regulation of megakaryocyte maturation are tuned differently in neonatal megakaryocytes. The precise functional reasons for these developmental divergences remain unclear. However, such properties could enable a more efficient expansion of the megakaryocyte lineage to populate the rapidly expanding bone marrow during development, while maintaining normal platelet counts. In addition, they could be the molecular foundation allowing the development of megakaryocytes in different microenvironments during fetal development. Certainly, these are exciting times and future functional studies will be required to complement these findings and to further our understanding of megakaryopoiesis.

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

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● ● ● VASCULAR BIOLOGY

Comment on Brunner et al, page 4154

uPAR in angiogenesis regulation

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In this issue of *Blood*, Brunner and colleagues demonstrate that uPAR, the urokinase plasminogen activator (uPA) receptor that drives endothelial invasion and proliferation in the initial phases of angiogenesis, undergoes down-regulation by density-enhanced phosphatase-1 (DEP-1) in confluent endothelial cells.[1] Together, uPAR and DEP-1 provide a mechanism that regulates the response of endothelial cells to angiogenic stimuli.

The investigators who first identified uPAR also found that uPAR expression was inversely related to cell density. This find lead to some dismay as it was possible that the uPA, the uPAR ligand used for binding studies, could stick to culture-dish plastic in the absence of a cell monolayer. However, mud sticking to the sole of a shoe does not mean that shoe soles have mud receptors. The bulk of evidence regarding uPAR functions led to acceptance of uPAR down-regulation as having significant biologic function.[2]

uPAR is becoming more and more important as a target molecule for cancer therapy due to its role in regulating cell proliferation and invasion. Recent studies have shown that besides the classic extracellular matrix-degrading protease cascade triggered by uPA binding, uPAR also triggers robust proliferative cell-signaling responses and accounts for gripping properties of the cells to extracellular matrix via its interaction with vitronectin. Such features make the uPAR/uPA system an inviting target for control of malignant cell growth and spread.[3] As endothelial cells exploit this cell-associated growth/invasion machinery, it is also an intriguing target for antiangiogenesis molecules.[4]

Twenty-five years after uPAR was first described, the scientific community finally has a satisfactory answer to the problem of the inverse relationship between uPAR and cell density. Using a battery of in vitro assays, Brunner and colleagues demonstrated that confluent endothelial cells not only show decreased responses toward VEGF stimulation, but also express less uPAR. The factor responsible for down-regulating ERK1/2 activation and subsequent decreased uPAR expression has been identified with the density-enhanced

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