cell movement? Are there inherited or acquired abnormalities of megakaryocyte production that could be due to progenitor trapping in the osteoblastic niche?

There are elegant techniques available for in vivo microscopy of the marrow space in living animals that could be used to study these questions and I am confident that these experiments will be or are being done. I look forward to more details regarding the movement of megakaryocyte and other progenitor cells from their respective niches so they can get on with the important business of producing blood cells.

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

REFERENCES

Comment on van Rooijen et al, page 6449

Dissecting VHL-associated pathologies

Eric Metzen and Ulf Brockmeier

In this issue of Blood, van Rooijen and colleagues demonstrate that zebrafish carrying a mutation in the von Hippel Lindau gene (VHL) develop Chuvash polycythemia, a hereditary human disease characterized by the dysregulation of hypoxia-inducible proteins including erythropoietin.

Chuvash polycythemia (CP), a disorder associated with inappropriately high erythropoietin secretion and increased proliferation of erythrocyte precursors, has been linked to a specific mutation of the von Hippel Lindau (VHL) gene. The C598T mutation leads to replacement of arginine by tryptophan ([Lindau 18 JUNE 2009 I VOLUME 113, NUMBER 25 6273](www.bloodjournal.org). In addition, patients with CP do not have an increased risk of tumor development. This is important because inactivating mutations of the VHL gene are observed in VHL disease, which is a hereditary cancer syndrome. VHL disease predisposes affected individuals to visceral cysts and hemangiomata of the retina, of the central nervous system (described by Eugen von Hippel in 1904 and by Arvid Lindau in 1926, respectively), to pheochromocytomas, and to clear cell renal cell carcinomas (CCRCC). In addition, in the majority of cases of sporadic CCRCC, VHL mutations are detectable. Many different VHL mutations can cause VHL disease. Notably, none of the patients carrying a VHL mutation displays all these pathologies. This raises the question: How can defects of a single gene lead to so many diverse and apparently unrelated pathologies?

To answer this, it is important to consider the cell physiologic functions of pVHL. It is well established that pVHL is the substrate-recognizing subunit of an E3 ubiquitin ligase, which is critical for the oxygen-dependent regulation of the c-subunit of hypoxia-inducible transcription factors (HIFs). The canonical pathway of HIF-activation by hypoxia occurs as follows: when oxygen concentration is normal, HIF-α is hydroxylated on specific proline residues in a strictly oxygen-dependent enzymatic reaction by prolyl hydroxylase domain proteins (PHDs), pVHL specifically binds the hydroxylated transcription factor. As a consequence of pVHL binding, HIF-α rapidly undergoes poly-ubiquitination and proteasomal degradation. Therefore, in pVHL competent cells, HIF is active only when the oxygen concentration becomes low and thus limiting for hydroxylation. In cells expressing nonfunctional or no pVHL, however, this regulation is lost and HIF drives target gene expression even at high oxygen concentration. In this scenario, tissue specific HIF-subunit distribution and target gene expression enable different pathologies to arise from VHL inactivation. Importantly, however, pVHL also takes part in HIF independent processes, for example, extracellular matrix formation and the regulation of microtubule stability. 4 How the loss of HIF-independent pVHL functions contributes to pathologic conditions in VHL-related diseases remains largely unresolved.

Van Rooijen et al have generated 2 zebrafish lines carrying inactivating VHL mutations. The mutant embryos developed polycythemia (see figure) and showed up-regulation of hypoxia-inducible genes as well as hyperventilation, thus recapitulating key features of CP. Moreover, chemical activation of HIF by application of a prolyl hydroxylase inhibitor largely reproduced the Vhl-deficient phenotype. Injection of human wild-type VHL mRNA rescued the phenotype while the Arg200Trp mutant was almost ineffective. Of note, in cells from individuals affected with CP, the Arg200Trp mutation reduces but does not abrogate HIF binding. Therefore, it is thought that intermediate HIF activation causes human CP but is not sufficient to produce tumors. On the other hand, VHL patients carry a germline mutation of 1 VHL allele while the second allele is inactivated by somatic mutation later in life, which leads to

![BroD U incorporation assay in wild-type and Vhl/zebrafish. Increased cell proliferation is detectable in all hematopoietic tissues of Vhl/embryos 7.5 days after fertilization, as shown in whole mount and cross-sections (original magnification ×20). CHT indicates caudal hematopoietic tissue; and PHT, pronephric hematopoietic tissue. See the complete figure in the article beginning on page 6449.](image)
tumor development in specific tissues. To date, the molecular event between biallelic VHL inactivation and tumor development is not entirely understood. Erythropoietin-producing cells, however, are not affected and therefore patients with VHL disease are usually not polycythemic.

Obviously, with respect to VHL functions and the consequences of VHL loss, many important issues have to be addressed. Therefore, van Rooijen et al have created a valuable tool to study VHL-associated human pathologies.

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

TRANSPLANTATION

Comment on Clave et al, page 6477

Is a little GVHD a good thing?

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In this issue of Blood, Clave and colleagues explore the impact of acute GVHD on thymic recovery following hematopoietic stem cell transplantation and begin to address the mechanism by which alloreactivity may impair thymic function in humans.

Although recovery of the innate immune system occurs rapidly following allogeneic hematopoietic stem cell transplantation (HSCT), full reconstitution of adaptive immunity is protracted, resulting in risk of infection and disease relapse. Reconstitution of T cells occurs via 2 major pathways. Mature T cells contained in the graft, remaining in the host following conditioning, or given as a separate infusion have tremendous potential to expand and can regenerate substantial numbers of progeny. However, this process results in an oligoclonal T-cell repertoire that is limited by the specificities contained in the starting population of T cells and further constrained during the expansion process. Full immunologic recovery, repertoire diversification, and immune competence coincides with recovery of thymic function but can be quite delayed. It is the delay in thymic recovery that is at the crux of the protracted course of full T-cell recovery following HSCT. Ultimately, a better understanding of the mechanisms limiting thymic function will be critical if we are to improve outcomes following HSCT.

A number of factors contribute to delayed thymic recovery including age, regimen-related toxicity, and chronic GVHD. Less well-defined in humans is the impact of acute GVHD. Mouse models have demonstrated loss of thymic architecture, impaired thymic selection, and decreased numbers of double positive thymocytes with GVHD (reviewed in Krenger and Hollander). Loss of thymic epithelial cells with diminished levels of proliferative factors, such as IL-7 and flt3L, and aberrant T-cell selection contribute to reduced thymic output and the generation of “auto”-reactive T cells. Importantly, these defects occur with the infusion of alloreactive T cells without conditioning, demonstrating that alloreactivity is sufficient to mediate GVHD effects on the thymus and indicate that the thymus is a target organ. Although useful, mouse models of GVHD are imperfect and not always predictive of the situation in humans, where the ability to measure what is truly happening in the thymus is difficult since most data comes from peripheral T cells. T cell–receptor excision circles (TRECs), which are generated during T cell–receptor rearrangement, have been instrumental in providing a picture of thymic function in humans and have confirmed the detrimental impact of age, regimen-related toxicity, and chronic GVHD. However, the data for acute GVHD have been limited.

Clave et al contribute to our understanding of thymic function following HSCT by performing longitudinal analyses in a cohort of patients of varying ages treated at a single institution. Their results confirm the profound impact of chronic GVHD and age on thymic recovery. In addition, they demonstrate that patients with acute GVHD also have decreased signal joint (sj) TREC levels and an oligoclonal T-cell repertoire. As long as the acute GVHD successfully resolved, recovery of TREC was seen. Interestingly, the authors indicate in the discussion that the decline in TREC was seen even in patients with grade 1 GVHD. However, their analysis goes one step further by measuring TRECs generated during β chain rearrangement in a subset of patients. Since this rearrangement occurs earlier than sj TREC, the ratio of β TREC to sj TREC has been proposed to indicate the amount of proliferation occurring between the 2 rearrangement steps in the thymus (see figure).

REFERENCES


A

B

TCR rearrangement involves sequential deletions of intervening DNA sequences in germline DNA resulting in episomal DNA circles (TREC). DJ-β excision occurs earlier than sj TREC generation, with thymocyte proliferation occurring between the 2 steps. In panel A, greater intrathymic expansion results in higher thymic T-cell output and a higher ratio of sj/DJ-β TREC when compared to panel B where less intrathymic expansion results in lower thymic output and a lower ratio of sj TREC to DJ-β TREC. With the same expansion seen in panel A (and the same TREC ratio), decreased thymic output would indicate changes either earlier (at the T-cell progenitor stage) or later (such as apoptosis of postrearrangement thymocytes) than the 2 rearrangement steps.
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