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The regulation of normal and neoplastic hematopoiesis is dependent on microenvironmental cells

Kenneth Kaushansky*, Huichun Zhan

Stony Brook University School of Medicine, Stony Brook, NY, USA

A B S T R A C T

Each day the adult human produces 4×10^{11} red blood cells, 1×10^{11} white blood cells and 1×10^{11} platelets, levels of production which can increase 10–20 fold in times of heightened demand. Hematopoiesis, or the formation of the ten different types of blood and marrow cells, is a complex process involving hematopoietic stem cells (HSCs), cytokine growth factors and cell surface adhesion molecules, and both specific and ubiquitous transcription factors. The marrow micro-environmental niche is defined as the site at which HSCs reside and are nurtured, receiving the signals that lead to their survival, replication and/or differentiation. Using microscopic, biochemical and molecular methods many different cells and the signals responsible for niche function have been identified. Early studies suggested two distinct anatomical sites for the niche, perivascular and periosteal, but the preponderance of evidence now favors the former. Within the "vascular niche" much evidence exists for important contributions by vascular endothelial cells (ECs), CXCL12-abundant reticular (CAR) cells and mesenchymal stromal cells, through their elaboration of chemokines, cytokines and cell surface adhesion molecules. In a series of studies we have found, and will present the evidence that megakaryocytes (MKs), the precursors of blood platelets, must be added to this list.

In addition to normal blood cell development, numerous studies have implicated the perivascular niche as contributing to the pathogenesis of a variety of hematological malignancies. Our laboratory focuses on the Ph (Crane et al., 2017)-negative myeloproliferative neoplasms (MPNs), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). These diseases are characterized by clonal expansion of HSCs and one or more mature blood cell types, hypermetabolism, a propensity to disorders of hemostasis (thrombosis > bleeding) and in some, evolution to acute leukemia. While a variety of therapies can control the abnormal expansion of the progeny of the malignant HSC, the only curative therapy is myeloablation with conditioning therapy or immunological means, followed by allogeneic stem cell transplantation (SCT), a procedure that is often inadequate due to relapse of the malignant clone. While the three disorders were postulated by Dameshek in the 1950s to be related to one another, proof came in 2005 when an acquired mutation in the signaling kinase Janus kinase 2 ($Jak2V_{617}F$) was identified in virtually all patients with PV, and ~50% of patients with ET and PMF. Since that time a number of other mutations have been identified that account for the "$Jak2V_{617}F$ negative" MPNs, including the thrombopoietin receptor, c-MPL, other mutations of Jak2, calreticulin and a variety of epigenetic modifier genes (e.g. TET2). Using a cell-specific Cre recombinase and SCT techniques we can introduce $Jak2V_{617}F$ into murine megakaryocytes and platelets, hematopoietic stem cells, and endothelial cells, alone or in combination, in order to probe the role of the mutant kinase in various cells on several aspects of the MPNs. Using these tools we have found that the expression of $Jak2V_{617}F$ in HSCs and ECs drives a MPN

* Corresponding author.
E-mail address: kenneth.kaushansky@stonybrookmedicine.edu (K. Kaushansky).

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characterized by neutrophilia, thrombocytosis and splenomegaly, eventually evolving into myelosclerosis. Somewhat surprisingly, we found that Jak2V$_{617}$F-bearing ECs were required for many features of the MPN, such as enhancing the growth of Jak2V$_{617}$F-bearing HSCs over that of wild type HSCs, its characteristic radioresistance, and a hemostatic defect. Altogether, our studies suggest that the malignant vascular niche is a critical element in the pathogenesis of MPNs, and a more thorough understanding of the molecular basis for these findings could lead to improved treatment for patients with these disorders.

1. Introduction

The marrow micro-environment, or "niche" is defined as the site at which hematopoietic stem cells (HSCs) reside and are nurtured, receiving the signals that lead to their survival, replication and/or differentiation into all the mature cells of the blood. Using microscopic, biochemical and molecular methods many different cells and the signals responsible for niche function have been identified. Early studies suggested two distinct anatomical sites for the niche, perivascular and periosteal, but the preponderance of evidence now favors the former (Crane et al., 2017). Within the "vascular niche" much evidence exists for important contributions by vascular endothelial cells (ECs), CXCL12-abundant reticular (CAR) cells and mesenchymal stromal cells, through their elaboration of chemokines, cytokines and cell surface adhesion molecules. In a series of studies we have found, and will present the evidence that megakaryocytes (MKs), the precursors of blood platelets, must be added to this list.

In addition to normal blood cell development, numerous studies have implicated the perivascular niche as contributing to the pathogenesis of a variety of hematological malignancies (Medyouf, 2017). Our laboratory focuses on the Ph (Crane et al., 2017)-negative myeloproliferative neoplasms (MPNs), which includes polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). These disorders are not rare, together affecting approximately 1 in 1000 individuals in the United States, are characterized by clonal expansion of HSCs and one or more mature blood cell types, hypermetabolism, a propensity to disorders of hemostasis (thrombosis > bleeding) and in some, especially if treated with genotoxic agents to control blood cell counts, evolution to acute leukemia. While a variety of therapies can control the abnormal expansion of the progeny of the malignant HSC, the only curative therapy is stem cell transplantation (SCT), a procedure that is rather toxic and often inadequate due to relapse of the malignant clone.

While the three disorders were postulated by Dameshek in the 1950s to be related to one another, proof came in 2005 when an acquired, mutation in the signaling enzyme Janus kinase 2 (Jak2V$_{617}$F) was identified in virtually all patients with PV, and ~50% of patients with ET and PMF (Kaushansky, 2005). The mutation was found to lead to constitutive activation of the kinase (actually, stabilization of the active form of the kinase), and to result in erythrocytosis and the pathognomonic finding in patients with MPNs, "spontaneous colony formation", in which marrow progenitor cells form blood cell colonies independent of hematopoietic growth factors. Since then a number of other mutations have been identified that account for the "Jak2V$_{617}$F negative" MPNs, including the thrombopoietin receptor, c-MPL, other mutations of Jak2, calreticulin and a variety of epigenetic modifier genes (e.g. TET2). Using a cell-specific Cre recombinase and SCT techniques we can introduce Jak2V$_{617}$F into murine megakaryocytes and platelets, hematopoietic stem cells, and endothelial cells, alone or in combination, in order to probe the role of the mutant kinase in various cells on several aspects of the MPNs. Using these tools we have found that the expression of Jak2V$_{617}$F in HSCs and ECs drives a MPN characterized by neutrophilia, thrombocytosis and splenomegaly, eventually evolving into myelosclerosis. Somewhat surprisingly, we found that Jak2V$_{617}$F-bearing ECs were required for many features of the MPN, such as enhancing the growth of Jak2V$_{617}$F-bearing HSCs over that of wild type HSCs, the radioresistance that characterizes MPN stem cells (as evidenced by relapse following SCT), and a hemostatic defect. Altogether, our studies suggest that the malignant vascular niche is a critical element in the pathogenesis of MPNs, and a more thorough understanding of the molecular basis for these findings could lead to improved treatment for patients with these disorders.

2. Results and discussion

2.1. Megakaryocytes (MKs) are an important component of the perivascular stem cell niche

As part of a study to generate a murine model of MPNs, we used an inducible human JAK2V$_{617}$F gene (termed FF1) (Tiedt et al., 2007) and drove its expression in HSCs and ECs using a Cre recombinase under the control of the Tie2 promoter ("Tie2/FF1 mice"). This model was chosen as much evidence indicates that mutant kinase is expressed in these cell types in patients with MPNs. As expected, these mice developed a robust MPN characterized by thrombocytosis, neutrophilic leukocytosis, splenomegaly and hematopoietic stem and progenitor cell expansion within 2 months of birth. As a control for these experiments, and to gauge the effects of JAK2V$_{617}$F-bearing MKs on MPN development, we also crossed FF1 mice with Pf4-Cre mice (which bear a Cre recombinase driven by the megakaryocyte-specific platelet factor 4 promoter) (Tiedt et al., 2007) to express JAK2V$_{617}$F exclusively in the MK lineage (Pf4$^+$FF1$^+$). Our expectation was that the Pf4$^+$FF1$^+$ mice would develop thrombocytosis, splenomegaly, and greatly increased marrow megakaryopoiesis, but not a MPN. Somewhat surprisingly, the mice also developed significant increases in CD45$^+$EPCR$^+$CD48$^-$CD150$^+$ (E-SLAM) cell numbers, a highly purified long-term repopulating HSPC population (Kiel et al., 2005; Kent et al., 2009). Rigorous efforts were undertaken to be certain that the mutant kinase was not also expressed in the HSC