Published in final edited form as:

Best Pract Res Clin Haematol. 2012 December ; 25(4): 493–497. doi:10.1016/j.beha.2012.10.014.

Molecular basis of polycythemic disorders due to aberrant hypoxia sensing and its relevance to acute leukemia

Josef T. Prchal, MD [Professor of Medicine, Pathology, and Genetics]

Division of Hematology, University of Utah School of Medicine, 30 North 1900 East, 5C402, Salt Lake City, Utah 8413, USA

Abstract

The author of this lecture has been especially honored to be selected to deliver the Ernest Beutler Memorial Lecture at the Acute Leukemia Forum 2012 and to write this overview. Ernest Beutler was the pivotal influence in my introduction to academic life, and his contribution to hematology in the last 5 decades was unsurpassed. Taking a cue from Ernie's example, I have elected in the keynote speech and this brief treatise, to start with an unconventional introduction and to expand on some discoveries made in my laboratory. Then I will extend these findings to the focus of the Acute Leukemia Forum to address potentially new approaches to therapies of acute leukemias. Somatic and germline mutations of acute leukemias are unfortunately caused by arrays of somatic and germline mutations. Simultaneous targeting of so many mutations makes it not possible to efficiently target all for cure. Albeit we should be aware that we should not in the near future ignore targeted therapy of those functionally important genetic and epigenetic events that are either initiating or contributing to aggressivity of acute leukemia, as these may be ameliorated by targeted intervention against one, or even a few together, of these defined molecular lesions. Yet, leukemic cells, like other cancer cells, have the unique metabolic feature to generate energy, referred as the Warburg effect, which can potentially be targeted to suppress or even eradicate cancer.

Keywords

Acute Leukemia Forum; Beutler; cancer cell metabolism; glycolysis; hypoxia-inducible factor; HIF; HIF-1; HIF-2; HIF-3; IDH1; IDH2; isocitrate dehydrogenase; leukemia; polycythemia; tumorigenesis; von Hippel-Lindau; Warburg

Introduction

Normal cells in the absence of oxygen derive energy from glycolysis, and in the presence of oxygen, by more efficient citric acid cycle. This is known as the *Pasteur effect*. The Otto Warburg Nobel Prize-winning experiments in the 1920s attacked this venerable postulate, demonstrating that cancer cells even in the absence of oxygen maintain active glycolysis. This is now known as the *Warburg effect*. This was surprising, and until recently,

© 2012 Elsevier Ltd. All rights reserved.

Tel: (801) 213-2075, Fax: (801) 585-3432, josef.prchal@hsc.utah.edu.

Conflict of Interest Statement:

No relevant financial relationships with any commercial interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

unexplained. Hypoxia-inducible factors (HIFs) are transcription factors that control a wide range of functions connected to the way cells respond to oxygen, including metabolism and the creation of red blood cells. Clearly, up-regulation of HIFs plays a central role in the Warburg effect.

Hypoxia, erythropoiesis, and polycythemia

How can the Warburg effect be linked to erythropoiesis, my principal interest? Erythroid production is largely controlled by responses of the body to hypoxia. Hypoxia leads to increased levels of master transcription factors, named hypoxia-inducible factors (HIFs). HIF-1 and HIF-2 are essential for production of the principal cytokine regulating erythropoiesis, ie, erythropoietin (EPO), but also have a direct erythropoietin-independent role in the augmentation of erythropoiesis. Seminal studies from Alan Erslev and others demonstrated the close relationship between hematocrit and EPO levels in humans and experimental animals, and also to hypoxia.

Studies of erythropoietin-producing cell lines resulted in the discovery of the oligonucleotide sequence, which is essential for hypoxic control of erythropoietin gene transcription named HRE, which subsequently led to the discovery of HIF-1 by several groups [1]. HIF-1 is a dimer subunit of alpha and beta subunits, and only the alpha subunit is hypoxia regulated. Subsequently, two other isoforms, HIF-1-alpha and HIF-2-alpha, and later HIF-3-alpha (also known as FIH) have been discovered. The seminal studies from Bill Kaelin and Peter Radcliffe's group [2] led to our concept of hypoxia sensing (Figure 1), indicating that in the presence of oxygen, HIF-alpha subunits are rapidly degraded by first being prolyl hydroxylated by one of the proline hydroxylase enzymes. This modified alpha subunit can then interact with von Hippel-Lindau proteins, which leads to ubiquitination and rapid degradation in proteasome. In the absence of oxygen, or with deficient or abnormal proline hydroxylase of von Hippel-Lindau proteins, the alpha subunits are stabilized. This leads to an increased level of HIF-1 and HIF-2, which regulate an array of genes essential for development, energy metabolism, iron metabolism, erythropoiesis, vasculogenesis, and many other essential body functions.

My personal interest in this field was initiated by my work in polycythemic disorders, including not only polycythemia vera, but also congenital polycythemic disorders that included the study of autosomal-dominant disease due to gain-of-function of the erythropoietin receptor [3]. This has led to the referral of patients with unusual phenotypes, and one of these was a young girl with an aberrant congenital polycythemic disorder whose hematocrit was over 70% and whose erythropoietin level was normal. Our attempt to treat her marginal symptoms, which we attributed to possible hyperviscosity, led to a series of phlebotomies that did not improve her symptoms. But her erythropoietin level markedly increased, however, and then, with discontinuation of phlebotomies, returned to the same level. We hypothesized that this could indeed be a congenital disorder of hypoxia sensing, resulting in augmented responses of erythropoietin to oxygen level changes (Figure 2). When, some 20 years ago, I attempted to present this hypothesis at the Hematology Forum, it was not well received. But that just persuaded me to pursue this idea with even greater vigor. The possibility of the existence of augmented hypoxia sensing by a genetic inherited cause was eventually confirmed because of a serendipitous phone call I received from Dr Victor Gordeuk, who graciously introduced me as the Beutler lecturer at the Acute Leukemia Forum. Dr Gordeuk visited Russia and learned about an endemic polycythemic disorder in the Chuvash Autonomous Region of Russia. When he called me from Russia about this disorder, not only did I have no knowledge about it, but could not find anything about it on Google. Fortunately, Dr Gordeuk persisted and made arrangements for me to be invited to the Erythropoiesis Conference in Moscow, partially sponsored by the Gorbachev

Foundation. The organizer of this meeting, Professor Tokarev, brought to the symposium two families affected by this polycythemia unknown in the Western. We performed some additional clinical studies, demonstrating that these people had increased EPO levels, which was not linked to EPO and the EPO receptor genes.

With the collaboration of the Chuvash physicians, we became acquainted with the seminal, unpublished work of Lydia Polyakova, who had written a doctoral thesis on this disorder. The thesis was not published and not accepted by the academic hierarchy in the Soviet Union because Dr Polyakova used genetic methods not tolerated by the Communist oligarchy that had ruled Soviet academic research. Dr Polyakova clearly established in her thesis that this is an inherited and autosomal recessive disorder. In collaboration with Dr Gordeuk and our Chuvash colleagues [4, 5], we conducted genome-wide association studies, which indicated that the polycythemic phenotype is localized to chromosomes where only a few genes are found, one of which is the von Hippel-Lindau gene, ie, a principal negative regulator of HIF. Mutations of the von Hippel-Lindau gene were well-known to cause von Hippel-Lindau syndrome, ie, a cancer predisposition syndrome associated with high penetrance for the development of renal cell cancer, pheochromocytoma, and cerebral angiosarcoma, which we did not see in this population. Therefore, we had to perform more rigorous studies to demonstrate that homozygosity for the von Hippel-Lindau gene mutation was indeed causative of increased levels of HIF-1-alpha in other abnormalities, including low blood pressure, propensity to thrombosis, high veg-F, varicose veins, and others [6, 7].

Cancer cell metabolism

How does this relate to acute leukemia and the subject of the Acute Leukemia Forum 2012? Dr Otto Warburg's seminal Nobel Prize-winning study leading to the description of the unique metabolism of cancer cells, including that of acute leukemia cells, is still not fully explained, but the molecular basis of this observation relates to upregulation of HIFs in cancer cell metabolism. This area is of intense interest to cancer researchers [8, 9]. In essence, it is clear that HIFs lead to down-regulation of mitochondria and oxygen metabolism that can be viewed as cancer cells protecting themselves from the reactive oxygen species generated by mitochondria.

Only in the last decade, our understanding of the unique metabolism of cancer cells has exploded, and it has become clear that cancer metabolism is largely mediated by hypoxia-inducible factors (HIF-1, HIF-2 and HIF-3). Indeed, the existence of many tumor-predisposing syndromes beautifully fulfills the Warburg hypothesis, wherein upregulation of glycolysis occurs from overexpression of HIF-1 and HIF-2, resulting from mutations of von Hippel-Lindau (VHL) in the VHL cancer predisposition syndrome. This establishes the HIFs' key role in tumorigenesis, cancer progression, and invasion when these transcription factors are stabilized/overexpressed [8, 9]. The peculiar features of the metabolism of cancers are characterized not only by increased glycolysis (Warburg effect), but also by the heretofore unappreciated metabolic features of tumor mitochondria, such as their utilization of glutamate and their connection to glutathione and lipid metabolism.

However, it has recently been proposed that inappropriate HIF activation can paradoxically suppress tumor growth in other contexts, such as in the presence of common and acute leukemia mutations of isocitrate dehydrogenases (IDH1 and IDH2). The paper published in *Nature* by Koivunen and colleagues in collaboration with William Kaelin's group in Boston and others [10] clarifies the basis of this exception to the principal role of increased HIFs in tumorigenesis and provides an explanation for the aberrant metabolism associated with leukemic isocitrate dehydrogenase (IDH1 and IDH2) mutations. The proline hydroxylases (PHDs; EGLNs) utilize 2-oxoglutarate as a substrate and are principal negative regulators of

the alpha subunits forming the HIF-1 and HIF-2 complex. Metabolism of 2-oxoglutarate by proline hydroxylase leads to degradation of HIF's alpha subunit by polyubiquitination and proteasomal degradation and ultimately decreased activity of HIF-1 and HIF-2. Koivunen and colleagues demonstrated that the mutant IDH1 and IDH2 proteins convert 2-oxoglutarate to 2-hydroxyglutarate (2HG), primarily to the (*R*)-enantiomer and to a lesser degree to its (*S*)-enantiomer. Many substances are present in *R*- or *L*-enantiomers, which are mirror images of each other, and often have unique metabolic properties. The (*R*)-enantiomer of 2-hydroxyglutarate ((*R*)-2HG) has been shown to increase proline hydroxylase activity (EGLN3), resulting in decreased HIF levels that promote the proliferation of IDH mutant tumor cells. Further, (*R*)-2HG and its *S*-enantiomer can promote tumorigenesis by another mechanism; they inhibit TET2. TET2 loss-of-function mutations are mutated in many hematologic neoplasms. TET2 catalyzes the conversion of 5-methylcytosine (mC) to 5-hydroxymethyl-cytosine (hmC), which is strongly implicated in epigenetic mechanisms of tumorigenesis [11], thus augmenting the tumor promoting abilities of IDH mutations.

These observations provide another novel therapeutic target that can be exploited in aberrant metabolism of tumor-promoting mutations, as in IDH1 and IDH2.

References

1. Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell*. 2012; 148(3):399–408. [PubMed: 22304911]
2. Kaelin WG Jr, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell*. 2008; 30(4):393–402. [PubMed: 18498744]
3. Prchal JT, Crist WM, Goldwasser E, Perrine G, Prchal JF. Autosomal dominant polycythemia. *Blood*. 1985; 66(5):1208–1214. [PubMed: 4052634]
4. Sergeyeva A, Gordeuk VR, Tokarev YN, Sokol L, Prchal JF, Prchal JT. Congenital polycythemia in Chuvashia. *Blood*. 1997; 89(6):2148–2154. [PubMed: 9058738]
5. Ang SO, Chen H, Gordeuk VR, et al. Endemic polycythemia in Russia: mutation in the VHL gene. *Blood Cells Mol Dis*. 2002; 28(1):57–62. [PubMed: 11987242]
6. Gordeuk VR, Sergueeva AI, Miasnikova GY, et al. Congenital disorder of oxygen sensing: association of the homozygous Chuvash polycythemia VHL mutation with thrombosis and vascular abnormalities but not tumors. *Blood*. 2004; 103(10):3924–3932. [PubMed: 14726398]
7. Bushuev VI, Miasnikova GY, Sergueeva AI, et al. Endothelin-1, vascular endothelial growth factor and systolic pulmonary artery pressure in patients with Chuvash polycythemia. *Haematologica*. 2006; 91(6):744–749. [PubMed: 16769575]
8. Cheong H, Lu C, Lindsten T, Thompson CB. Therapeutic targets in cancer cell metabolism and autophagy. *Nat Biotechnol*. 2012; 30(7):671–678. [PubMed: 22781696]
9. Dang CV. Cancer cell metabolism: there is no ROS for the weary. *Cancer Discov*. 2012; 2(4):304–307. [PubMed: 22576206]
10. Koivunen P, Lee S, Duncan CG, et al. Transformation by the (*R*)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. *Nature*. 2012; 483(7390):484–488. [PubMed: 22343896]
11. Chowdhury R, Yeoh KK, Tian YM, et al. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep*. 2011; 12(5):463–469. [PubMed: 21460794]

Hypoxia Sensing

Figure 1. Hypoxia sensing

Hypoxia sensing is the mechanism by which cells sense a decrease in oxygen and initiate an appropriate response, allowing the organism to adapt to new conditions. Hypoxia inducible factor-1 (HIF-1) plays a crucial role in this process. The cellular level of the alpha subunit is controlled by oxygen level. Oxygen activates prolyl hydroxylase, which will hydroxylate HIF-1-alpha. This leads to the binding of a tumor suppressor, von Hippel-Lindau protein, and subsequent ubiquitination of HIF-1 α . In contrast, in hypoxia, HIF-1 α can associate with the beta subunit. The complex then binds to hypoxia-responsive elements within the genome, activates associated genes, and initiates or increases production of related genes that comprise EPO, VEGF, etc. VHL has been widely studied as a tumor suppressor gene and for its role in hypoxia sensing.

16 year old girl with polycythemia Defect of hypoxia sensing?

Figure 2. Defect of hypoxia sensing in polycythemia

The inverse relationship between hematocrit and serum immunoreactive erythropoietin suggests that polycythemia could indeed be a congenital disorder of hypoxia sensing.