A novel treatment for anemia?

Check the kitchen pantry!

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This is to acknowledge that Joseph A. Garcia, MD,PhD does not have any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Garcia will be discussing off-label uses in his presentation.

Purpose and Overview:
Critically as well as chronically ill patients suffering from low red blood cell counts, or anemia, have decreased energy and worse clinical outcomes. Anemia normally stimulates production of erythropoietin, also known as Epo, a hormone that controls red blood cell production. The development of recombinant Epo several decades ago revolutionized treatment of anemia. Other treatments for anemia include periodic intravenous iron supplements or blood transfusions, which carry inherent risks and expense. Unfortunately, treatment with recombinant Epo or iron supplementation does not decrease mortality for critically or chronically ill anemia patients, including patients with congestive heart failure (CHF) or coronary heart disease (CHD). In this presentation, we will review the classification and epidemiology of chronic anemia patients with a focus on cardiac disease patients who frequently have anemia of chronic disease (ACD) with or without coexisting iron deficiency anemia (IDA). We will summarize results of previous attempts to correct anemia in cardiac patients. After outlining the molecular and cellular basis for control of erythropoiesis in mammals, we will discuss novel strategies to up-regulate endogenous Epo expression. In this manner, a more effective and inexpensive treatment may be developed that improves outcomes of convalescing and critically ill anemia patients, a substantial portion of veteran and community patients in the United States of America and elsewhere.

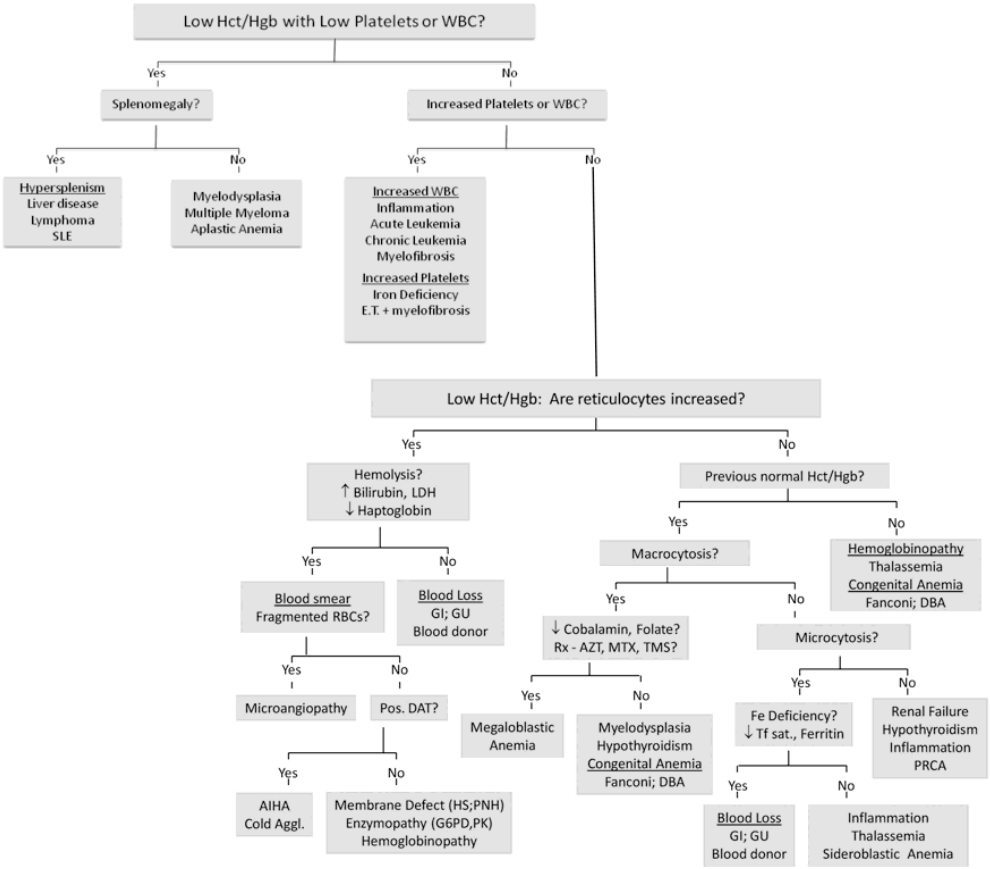
Objectives:
- To understand the basis and limitations of current anemia classification schemes
- To understand how clinical laboratory-based methods may be used to distinguish patients presenting with ACD, IDA, or combined ACD/IDA
- To review the physiological sites of erythropoiesis in mammals
- To review the molecular basis for erythropoietin regulation in mammals
- To understand how recent insights into erythropoietin regulation may allow for novel means of regulating erythropoietin production in anemia patients

About the author:
Dr. Garcia is a staff physician-scientist in the Department of Medicine, Cardiology Division and Director of the Cardiac Care Unit at the VA North Texas Health Care System. He is also an Associate Professor of Internal Medicine at the University of Texas Southwestern Medical Center. His overall scientific interests are in regulatory mechanisms involved in environmental stress response. Over the past fifteen years, his laboratory has focused on discerning the signaling mechanism and biological roles mediated by Hypoxia Inducible Factor 2 (HIF-2), a stress-responsive transcription factor. His laboratory demonstrated that HIF-2 regulates endocrine erythropoietin (Epo) expression in adult mammals. His most recent efforts have focused on the role of a novel mechanism of HIF-2 signaling in Epo regulation as well as in cancer growth and metastasis. One day, he may get around to figuring out what HIF-2 does in the heart.

Definition and classification of anemia, a multi-factorial disease

Anemia is a common medical condition that results from a reduced number or function of circulating red blood cells, and leads to the development of hypoxemia and tissue hypoxia. Presentations of anemia may be acute, for example in blood loss due to surgery or trauma, or may be chronic. The latter, which represents ~3/4 of all hospitalized patients diagnosed with anemia, is generally categorized as to one of three etiologies with roughly equal presentation in adults: anemia of nutrient disorder, anemia of chronic disease or inflammation, or anemia of unknown cause. Anemia of chronic disease is a particular problem in the elderly, in part because its effects may be attributed to or masked by symptoms associated with co-morbid conditions.

Although definitions of anemia vary according to organization, the threshold serum hemoglobin (Hb) levels for diagnosis of anemia generally are less than 13 to 13.5 g Hb/dL for men and less than 12 g Hb/dL for women or men older than 70 yr. A complete classification of anemia accounts for historical, physical exam, and laboratory findings[1]. The presence of splenomegaly in conjunction with parameters obtained from complete blood counts, reticulocyte counts, laboratory tests, and peripheral blood smears identify potential etiologies for chronic anemia. As our understanding has improved, immunological and biochemical parameters, including measurements of factors critical for iron uptake and transport, have further refined the classification scheme for anemia.



Adapted from Koury and Rhodes, Hematology Am Soc Hematol Educ Program, 2012:183-90 (2012)

Anemia adversely impacts cardiovascular disease

Anemia is associated with increased morbidity and mortality in patients with congestive heart failure (CHF) and coronary heart disease (CHD)[2]. With respect to prevalence of anemia in CHF patients, ~1/3 of patients with chronic CHF and ~1/6 of patients with new-onset CHF are anemic, with the latter likely including a significant fraction with dilutional anemia. The incidence of anemia in CHF patients is estimated at 10-16%/yr, although again it is not clear how many are a result of accompanying volume overload. For prevalence of anemia in CHD patients, ~1/10 to 1/5 of CHD patients are anemic. The etiologies of chronic anemia in CHF patients include iron deficiency, chronic disease/inflammation, decreased erythropoietin (Epo) production as a result of medication-induced renal effects, and reduced Epo production with chronic kidney disease[3]. The etiologies of anemia with CHD overlap those seen with anemia of CHF, but are less likely confounded by anemia due to volume overload.

As with any association, it is unclear whether anemia is a causal factor or, alternatively, is a marker for a different causal factor such as the cardiac disease itself. Nevertheless, a drop of Hb from 15 g/dL to 10 g/dL ("mild to moderate anemia") reduces the oxygen carrying capacity of blood from 20 mL O_2/100 mL blood to 13.3 mL O_2/100 mL blood. Compensation for this deficit can be met with an increase in cardiac output, enhanced release of oxygen from hemoglobin, or by an increase in red blood cell mass. Hence, it is perhaps not surprising that in both CHF and CHD patient populations, where an increase in cardiac output can exacerbate myocardial supply/demand mismatch, anemia is associated with poorer outcomes.

Anemia of heart failure or chronic disease with iron deficiency are similar

Anemia of chronic disease (ACD) is the second most common form of anemia world-wide after iron deficiency anemia (IDA) and is the most common cause of anemia in the elderly in the US. It is associated with a variety of conditions, most of which have significant inflammatory components to their presentation. Depending upon the nature of the underlying condition, ACD may be confounded by concomitant anemia of nutrient disorder including iron or vitamin deficiencies. Because of the dysregulated signaling induced by chronic inflammatory stimuli, iron availability is oftentimes limited because of iron sequestration by macrophages in the reticuloendothelial system, which subsequently leads to development of an apparent iron-deficient anemia. CHF and CHD have significant inflammatory components and therefore may present with ACD. However, iron deficiency may occur in CHF and CHD patients, even in the absence of overt anemia.

Besides interleukin-6, the hepatic factor hepcidin and its modulators are central to development of the reduced iron availability in ACD. Although traditional markers of iron deficiency can be used to distinguish ACD from IDA or mixed ACD/IDA, the use of hepcidin as a serum or urine biomarker may be of additional benefit for diagnosing mixed ACD/IDA. Hepcidin comprises three isoforms: hepcidin-20, hepcidin-22, and hepcidin-25 with hepcidin-25 being the biologically active isoform and its presence in urine or blood is assessed by liquid chromatography/tandem mass spectrometry.

Table 1. Use of laboratory investigations in the differential diagnosis of ACD.

Laboratory marker	ID without anaemia	IDA	ACD	ACD/IDA	AHF/IDA
Hb	Normal	Low	Low	Low	Low
MCV/MCH	Low	Low	Normal/low	Low	
Inflammatory markers	Negative	Negative	High	High	
Iron	Low	Low	Low	Low	Low
Ferritin	Low	Low	Normal/high	Normal/low	High
Transferrin		High	Normal/low	Low	
Transferrin saturation	Low	Low	Low	Low	Low
sTfR		High	Normal	Normal/high	High
sTfR/log ferritin ratio	High	High (<2)	Low (<1)	High (<2)	High
Serum hepcidin	Low	Low	Raised	Normal	

ACD = anaemic of chronic disease; Hb = haemoglobin; ID = iron deficiency; IDA = iron-deficiency anaemia; AHF = anaemic of heart failure
MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; sTfR = soluble transferrin receptor.

Adapted from Cullis, Clin Med, 13(2):193-6 (2013); Shah and Agarwal, Clin Inter Aging, 2013(8):111-22 (2013); Weiss and Goodnought, NEJM, 352:1011-23 (2005)

Current treatments of anemia in cardiac disease patients have unclear benefits

Clinical investigators have been interested in determining whether anemia is a causal factor for poorer outcomes associated with CHF or CHD[4]. To test this hypothesis, investigators have used blood transfusions, iron supplementation, or Epo injections as therapeutic agents, mostly performed or evaluated in small clinical trials[5]. Treatment of anemia in stable CHF patients has focused predominantly on iron supplementation, which improves quality of life (mostly class III NYHA patients) but not mortality. With respect to erythropoiesis stimulating agents (ESAs) including various Epo formulations, ESAs provide no clear benefit and may cause harm, likely because of venous thromboemboli. For CHD patients, data are mostly from observational studies where blood transfusions were used for anemic patients with acute coronary syndrome or those scheduled for non-cardiac surgery. These studies have not detected a strong signal in favor of either harm or benefit. Similar to CHF patients, anemic CHD patients treated with ESAs had no clear benefit and may have been at risk for increased harm, although the data from these trials was not examined in a subgroup-specific manner.

Table 2. **Evidence for the Effects of RBC Transfusions, ESAs and IV Iron for the Treatment of Anemia in Patients With Heart Disease**

Treatment	Patient Population	Outcome	Effect*	Data	Quality of Evidence
RBC transfusions	Stable CHD (all patients)	Mortality	(~)	RR, 0.86 (95% CI, 0.46 to 1.62)	Low
		Cardiovascular events	(~)	RR, 0.60 (CI, 0.34 to 1.03)	Low
	ACS	Mortality	(~)	RR, 0.23 (CI, 0.05 to 1.02)	Low
		Cardiovascular events	(~)	RR, 0.70 (CI, 0.24 to 2.07)	Low
	Noncardiac surgery	Mortality	(~)	RR, 1.44 (CI, 0.81 to 2.54)	Low
		Cardiovascular events	(~)	RR, 0.52 (CI, 0.27 to 1.01)	Low
ESAs	Stable CHF	Mortality	(~)	RR, 1.07 (CI, 0.98 to 1.16)	High
		Cardiovascular events	(~)	RR, 0.94 (CI, 0.82 to 1.08)	High
		Quality of life	(~)	No consistent differences	Moderate
		Exercise tolerance and duration	(~)	Mean difference in NYHA, −0.77 (CI, −1.21 to −0.32)	Moderate
		Hospitalizations	(~)	RR, 0.97 (CI, 0.87 to 1.10)	High
		Harms, including hypertension and cerebrovascular and thrombotic events	(−)	Hypertension: RR, 1.20 (CI, 0.90 to 1.59) Ischemic stroke: RR, 1.33 (CI, 0.93 to 1.89) VTE: RR, 1.36 (CI, 1.17 to 1.58)	Moderate
	Stable CHD†	Mortality	(−)	Increased mortality	Low
		Cardiovascular events	(~)	No effect	Low
		Quality of life	(~)	No effect	Low
		Venous thrombosis	(−)	Increased risk for venous thrombosis	Low
IV iron	Stable CHF	Mortality	(~)	NA	Insufficient
		Cardiovascular events	(+)	27.6% vs. 50.2% (P = 0.01)	Low
		Exercise tolerance and duration	(+)	Improvements in NYHA class and 6-min walk test	Moderate
		Quality of life	(+)	Improvement on various scales	Moderate
		Serious harms	(~)	No differences, although harms were sparsely reported	Moderate

ACS = acute coronary syndrome; CHD = coronary heart disease; CHF = congestive heart failure; ESA = erythropoiesis-stimulating agent; IV = intravenous; NA = not applicable; NYHA = New York Heart Association; RBC = red blood cell; RR = relative risk; VTE = venous thromboembolism.
*Effect: (+) indicates that treatment provided benefit; (−) indicates that treatment resulted in harm; (~) indicates mixed findings or no effect.
† Patients included in these studies had advanced kidney disease and/or end-stage renal disease, so the application to other patient populations is unclear.

Adapted from Qaseem et al., Ann Intern Med, 159:770-9 (2013)

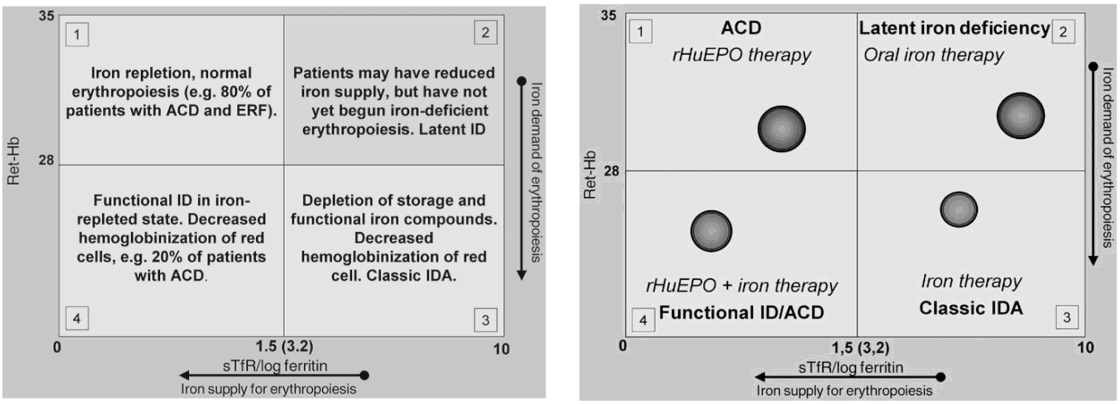
Advances in technology have facilitated more accurate assessments of anemia

The automation of complete blood cell assessments has provided the tools for more precise classification schemes for anemia[6]. Although used as a classification system for decades, mean cell volume (MCV) and red blood cell distribution (RDW) are only used in a confirmatory manner in current assessments of anemia. Because MCV measurements vary according to hematology analyzer platforms (impedance- versus flow cytometry-based instruments), reliance upon MCV for assessments of red blood cell mass may confound comparisons of hematocrit (Hct), which is calculated from the measured MCV. The use of mean cell hemoglobin content (MCH), which is derived from precise measurements of hemoglobin and red blood cell numbers, provides a very reliable platform-independent red cell index. However, mean cell hemoglobin concentration (MCHC) is subject to inaccuracy at the extremes of hemoglobin content and therefore may underestimate or overestimate hematocrits of anemia patients with hypochromic or hyperchromic red blood cells, respectively. Moreover, MCV and MCHC are subject to variation introduced by temperature and storage times. RDW values may also be subject to platform-dependent variations, in part because of how it is determined and also because of artifact effects at extreme hemoglobin values. Automated measurements of reticulocyte indices - including immature reticulocyte fraction, reticulocyte cell hemoglobin content reticulocyte volume and reticulocyte hemoglobin concentration - are of additional value for hematologists with reticulocyte hemoglobin content being of particular utility in the detection of IDA.

Distinguishing amongst chronic anemia subtypes facilitates treatment decisions

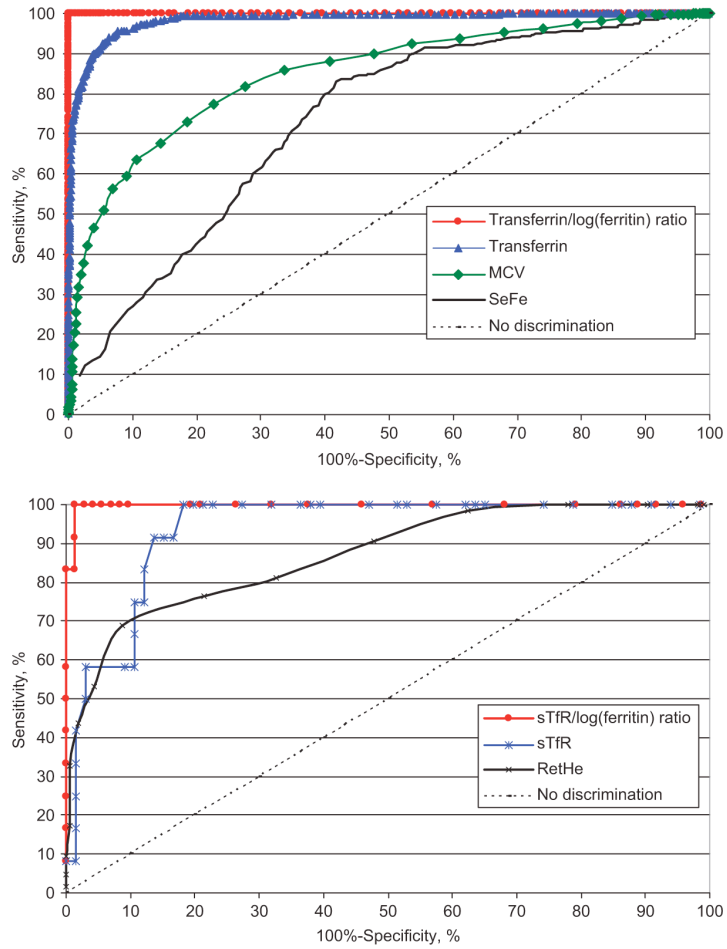
ACD or IDA may occur in isolation or together. Serum ferritin is the best single test for the diagnosis of IDA. Ferritin levels <20 µg/L are highly specific for IDA. However, because ferritin is an acute phase reactant, serum ferritin levels may be increased in inflammatory conditions, which decrease the sensitivity for ferritin in diagnosing IDA. Ferritin levels >100 µg/L usually exclude IDA. Thus, ferritin concentrations between 20 and 100 µg/L may be inconclusive. Unlike serum ferritin, sTfR levels are not affected by inflammation. The ratio between the soluble transferring receptor (sTfR) and serum ferritin concentrations may be a more reliable indicator of iron availability in patients being evaluated for iron deficiency who have equivocal ferritin levels.

Determining whether ACD patients require iron repletion is not made from examination of iron stores, which may be normal or elevated, because there may be a functional discrepancy between what is needed for erythropoiesis and the actual iron supply. To facilitate decisions regarding iron and/or Epo needs in ACD patients, clinicians may use the Thomas plot[7], which relates indicators of functional iron deficiency (iron demand assessed by reticulocyte hemoglobin content) to indicators of functional iron availability (iron supply assessed by the ferritin index, expressed as the soluble transferrin receptor/log ferritin ratio). Hepcidin-25 used in place of the ferritin index in the Thomas plot may potentially distinguish in real-time patients with anemia and reduced iron supply[8]. Although subject to limitations in special cases, the Thomas plot may identify IDA, ACD, and ACD/IDA patients who will benefit from iron supplementation alone, Epo injections alone, or combined iron supplementation/Epo injections, respectively.



Adapted from Thomas et al., Medical Oncology, 23(1):23-36 (2006)

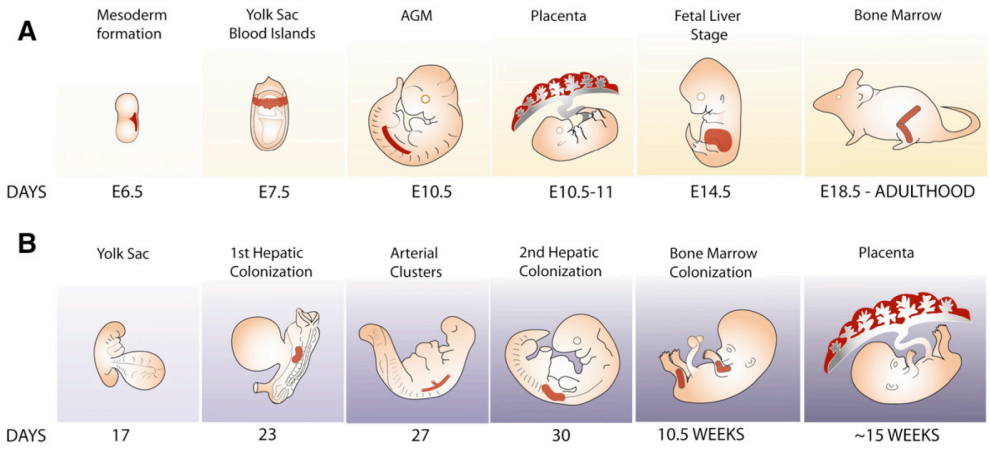
A more facile laboratory comparison, which may provide slighter better discriminatory power for patients with indeterminant ferritin levels, is the transferrin/log ferritin ratio[9].



Adapted from Castel et al., Clin Chem Lab Med, 50(8):1343-9 (2012)

Hematopoietic development is a complex, highly regulated process

Erythropoiesis is one lineage of hematopoiesis, a complex and elegant developmental process[10-13] that occurs in overlapping phases[13,14]. Primitive hematopoiesis in mice occurs as a single wave that originates in the extra-embryonic yolk sac around embryonic day 7.5 (E7.5) and results in development of primitive erythroid cells. The yolk sac is also the origin of the second wave of hematopoiesis that produces definitive erythroid and other cells. The third wave of hematopoiesis also produces definitive erythroid cells and arises from mobilized hematopoietic stem cells that localize first to the aorta-gonad-mesonephros (AGM) region at E10.5, transit to the placenta around E10.5, move to the fetal liver by E14.5, and finally settle in the bone marrow and spleen by E18.5. Hematopoiesis in humans shares some features with hematopoiesis in mice, but there are some differences in primary hematopoiesis sites in developing embryos.



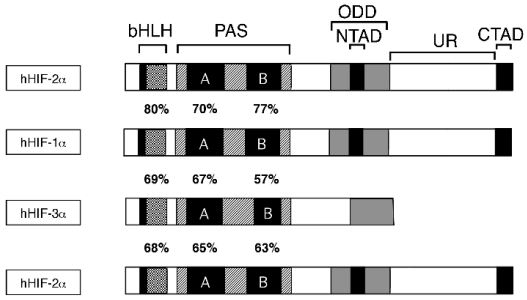
Adapted from Baron et al., Blood Cells, Molecules, and Diseases, 51:213-9 (2013)

Erythropoietin is a key regulator of red blood cell production

Erythropoiesis is regulated by the endocrine hormone erythropoietin (Epo), which augments erythroid development in medullary and extra-medullary sites. Epo is a prototypical hypoxic gene expressed in the fetal liver, adult kidneys and adult spleen[15-19], anatomic regions of developing and adult mammals that overlap sites of hematopoiesis[15-20]. Epo protein is notoriously difficult to localize and hence its sites of production have been gleaned from sites of Epo mRNA gene expression. Although some questions remain[19], sites of Epo production largely inferred from Epo mRNA localization in mouse studies have established the primary sites of synthesis[21-27]. Epo mRNA is first detected at E6.5 in the mesoderm and yolk sac just prior to primitive erythropoiesis, then transitions to the AGM and fetal liver by E14[25,26], which precedes the onset of definitive erythropoiesis and time of death for Epo null mice[28]. Epo mRNA thereafter declines and is undetectable in fetal liver or kidney by E19, reappears in neonatal kidney by post-natal day 9 (P9), peaks by P30, and then declines again to basal levels by P60. In anemic adult mice, Epo mRNA levels increase in kidney and liver with recruitment of renal interstitial cells[23,29] or increased production in hepatocytes and possibly other interstitial cells in the liver[24,30].

Hypoxia Inducible Factors (HIF) are stress-responsive genetic regulators

Stress signaling plays a unique role in Epo regulation and in erythropoiesis. Adult animals subjected to systemic hypoxia or severe anemia exhibit marked increases in serum Epo protein, a result of increased *Epo* mRNA synthesis in the kidneys and liver[31]. Identification of hepatoma cell lines that increase *Epo* mRNA in response to hypoxia[32,33] facilitated delineation of a minimal enhancer in the Epo gene[34], which was used as a molecular handle for the biochemical purification of a seminal stress-responsive transcription factor, Hypoxia Inducible Factor 1 alpha (HIF-1α)[35]. Subsequent perusal of the Human Genome Project revealed a structurally similar protein, HIF-2α[36-38], which raised questions about which HIF might regulate *Epo* gene expression[39].

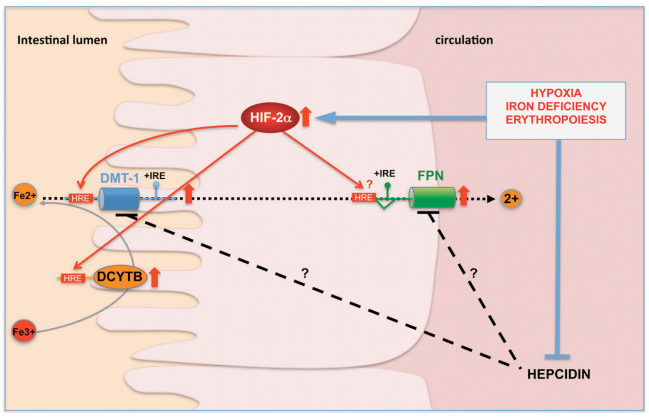


HIF-2 regulates *Epo* gene expression in late embryonic and adult mammals

Genetic ablation studies identified distinct roles for HIF-1α and HIF-2α in erythropoiesis and Epo regulation[39-44]. HIF-1α null embryos have reduced yolk sac hematopoiesis and Epo gene expression at E9.5[45] and expire by E10.5[46-48]. Most HIF-2α null mice expire around E12[49], preceding the transition from primitive to definitive erythropoiesis and a complex change in Epo regulation[50-54]. We generated HIF-2α null mice that survived embryogenesis[42,55] and noted multi-lineage hematopoietic defects[42] as well as dysregulated Epo expression in embryonic liver[40] and adult kidney[41]. Other investigators reported that HIF-2α also regulates Epo expression in adult liver[43]. Activating mutations in human HIF-2α result in increased Epo production and erythrocytosis[56-58].

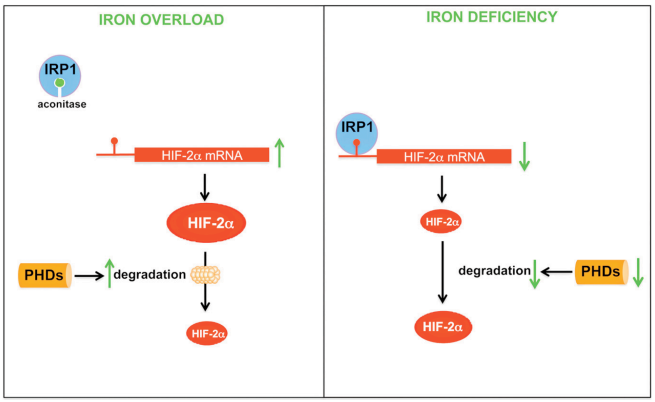
HIF-2 regulates expression of genes involved in iron metabolism

Stress signaling mediated by HIF-2α is also important for iron metabolism. Several HIF-2 target genes include an iron transporter (divalent metal-ion transport 1, DMT1), a diheme-containing and ascorbate-dependent ferric reductase (duodenal cytochrome b561, DCYTB), and possibly an iron exporter (ferroportin, FPN)[59-61]. Expression of DMT1 and FPN is also regulated at the translational level; the mRNAs encoding DMT1 and FPN contain iron-responsive elements (IRE), which are regulated by iron responsive element binding proteins (IRP) that bind to the IRE when iron levels are low. For DMT1, binding of IRP1 to an IRE in the 3' untranslated region of the mRNA enhances stabilization of the message and thereby increases translation. For FPN, binding of IRP1 to an IRE in the 5' untranslated region of the mRNA inhibits translation.



Adapted from Mastrogiannaki et al., Blood, 122: 885-92(2013)

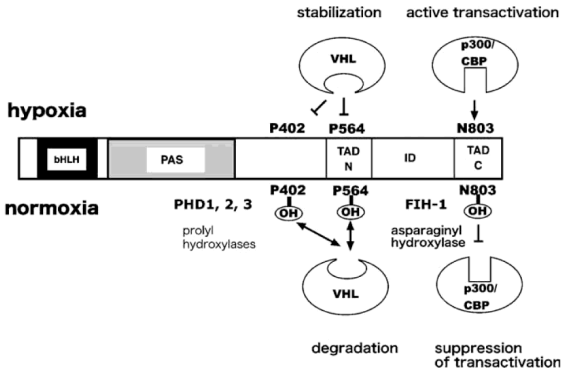
The mRNA for HIF-2α itself contains an IRE in the 5' untranslated region and may be subject to translational control in duodenal enterocytes by IRPs as a result[62-64]. However, the inhibitory effects of IRP1 on HIF-2α translational efficiency, and hence HIF-2α steady-state protein levels, during iron deficiency may be counteracted by the effects of iron deficiency on the iron-requiring PHDs, which are inhibited with iron restriction and thereby lead to increased HIF-2α protein stability or activity.



Adapted from Mastrogiannaki et al., Blood, 122: 885-92(2013)

Oxygen-dependent modifications impair HIF-1 activity

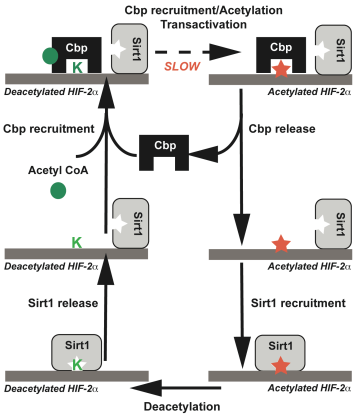
HIF-1 activity is prominently regulated by post-translational modifications controlled by two classes of oxygen-dependent enzymes[65]: the prolyl hydroxylases (PHDs)[66,67], which during normoxia modify two proline residues in HIF-α members[68] and thus target HIF-α members to the proteosome for degradation[69-71], and the asparaginyl hydroxylase Factor Inhibiting HIF-1 (FIH-1)[72], which during normoxia hydroxylates a single asparagine residue in the carboxy termini of HIF-α members[73], thereby reducing their ability to interact with the coactivator protein p300. During hypoxia, the oxygen-requiring PHDs are impaired and HIF-α members are stabilized[74], allowing formation of heterodimeric HIF complexes composed of oxygen-sensitive HIF-α and oxygen-insensitive HIF-β subunits, and the oxygen-requiring FIH-1 is inhibited, allowing HIF dimers to efficiently recruit p300 and increase expression of target genes.



Adapted from Hirota and Semenza, Biochem Biophys Res Com, 338:610-6 (2005)

Cyclical acetylation/deacetylation modifications augment HIF-2 signaling

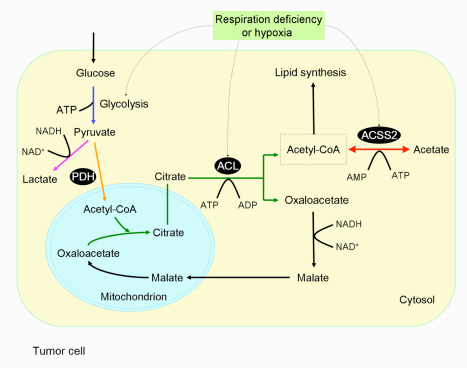
Although modifications induced by oxygen-dependent requiring enzymes are critically important for regulating HIF-1, their effects on HIF-2 protein stability and signaling are less important, which suggests that other PTMs are important for HIF-2 signaling during hypoxia. Indeed, HIF-2α is acetylated by and complexes with the acetyltransferase/coactivator Cbp[75], then is deacetylated by Sirtuin 1 (Sirt1)[40], a deacetylase and genetic regulator of diverse biological processes[76-79].



Adapted from Chen et al., J Biol Chem, 287:30800-11(2012)

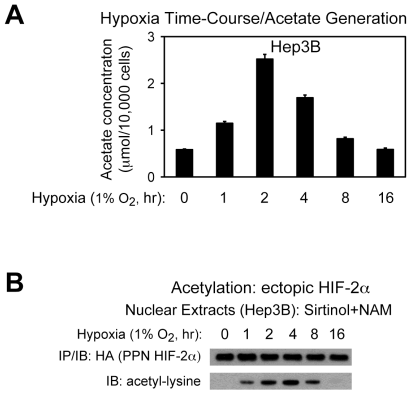
The acetate switch links intermediary metabolism with stress signaling

In contrast to HIF-1, HIF-2 activity during hypoxia is prominently controlled by two cyclical acetylation and deacetylation reactions. HIF-2α acetylation, the rate-limiting step, requires an enzyme, Cbp, and two substrates, HIF-2α lysine residues and acetyl CoA. The three potential *de novo* sources of acetyl CoA include ATP citrate lyase (Acly), which generates most cytosolic acetyl CoA used for intermediary metabolism; pyruvate dehydrogenase (PDH), which is inactivated by PDH kinase during hypoxia[80,81]; and acetate-dependent acetyl CoA synthetases (Acss), which are located in mitochondria and cytosol[82-84], but like Acly also may be present in the nucleus[85].



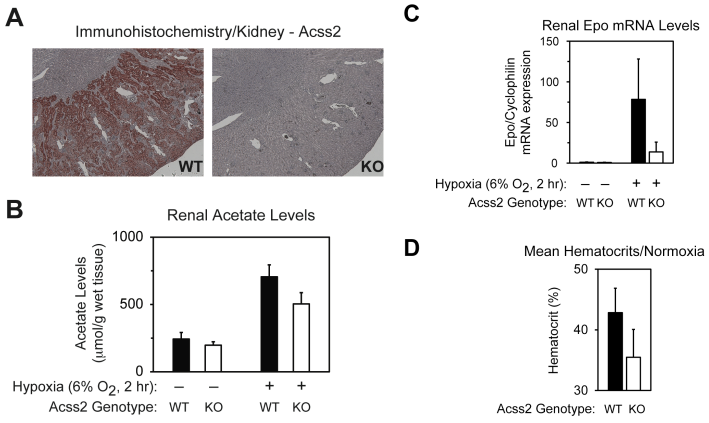
Adapted from Yoshii et al., Cancer Sci, 100(5):821-7 (2009)

During hypoxia, acetate secretion[82-84] and Acss2 expression[82,83] increase in tumor cells, which provide a potential inducible biochemical trigger and source for acetyl CoA. Indeed, Acss2 controls HIF-2α acetylation, Cbp/HIF-2α complex formation, Cbp/HIF-2α recruitment to the Epo enhancer, and HIF-2 signaling in hypoxic cells and mice[86].



Adapted from Xu et al., Nature Medicine, 20(9):1018-26 (2014)

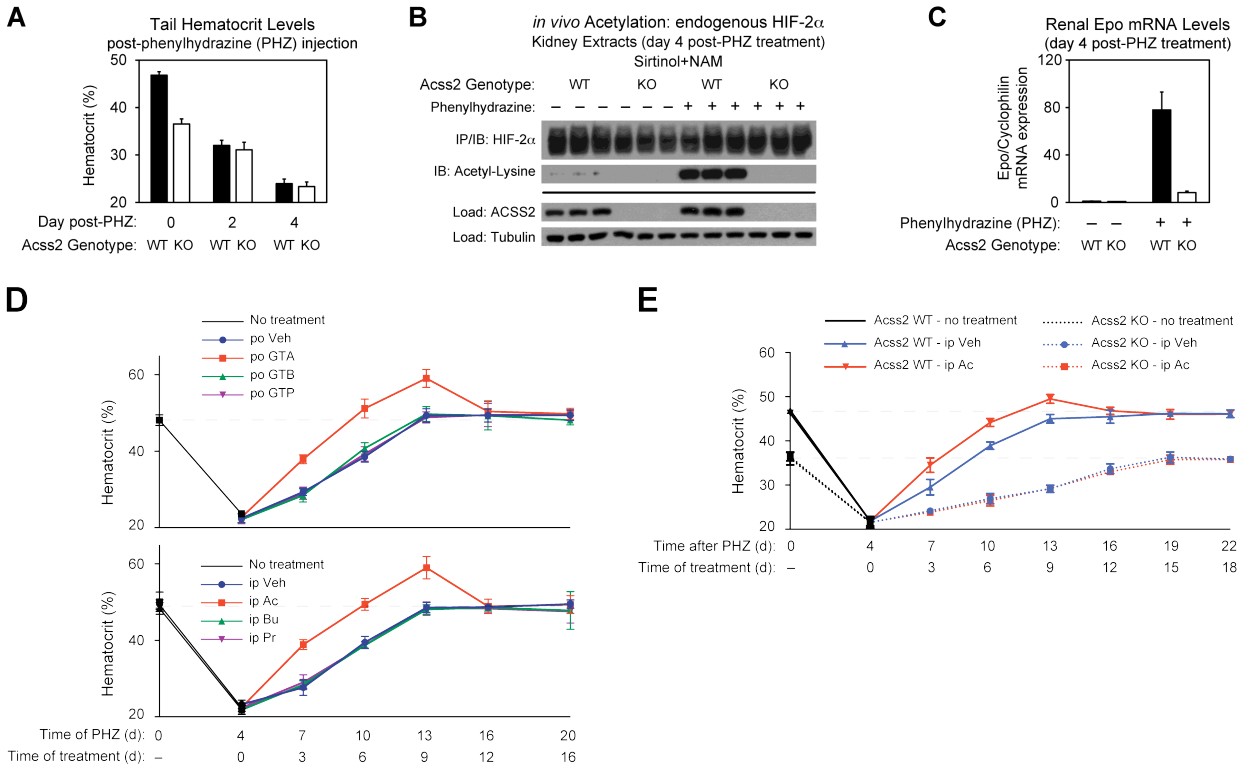
Recently, we evaluated Acss2 knockout mice and found that these mice have blunted induction of Epo as a result of impaired HIF-2 signaling induced upon hypoxia exposure.



Adapted from Xu et al., Nature Medicine, 20(9):1018-26 (2014)

The acetate switch can be exploited to treat acute and chronic anemia in mice

Acetate is generated in liver and kidney of mice with acute anemia. Exogenous acetate, delivered either by oral or intra-peritoneal means, augments Epo gene expression and increases resting hematocrits in mice with acute anemia[86].



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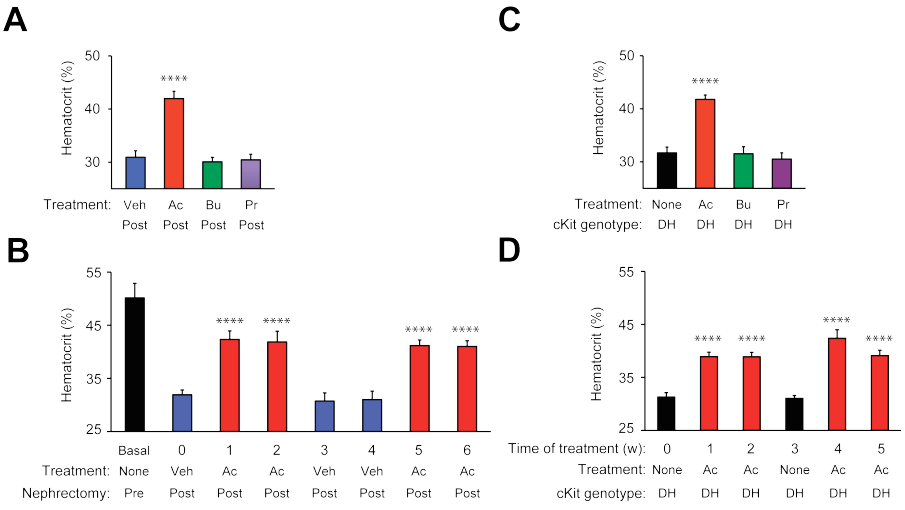
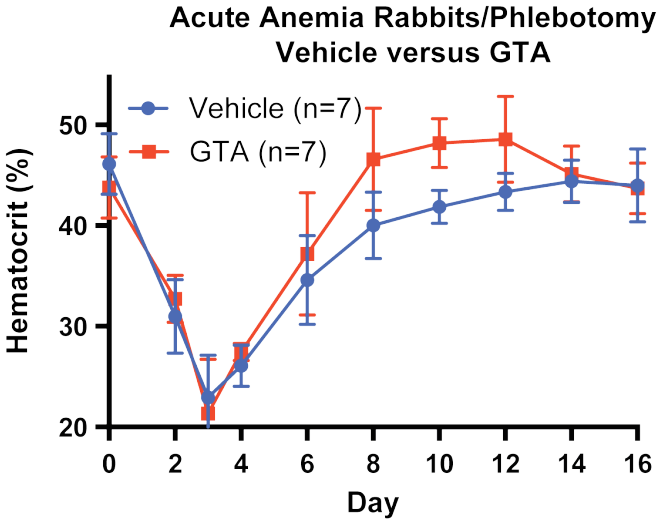
Exogenous acetate also augments Epo gene expression and increases resting hematocrits in mice with chronic anemia due to congenital or acquired reasons[86].



Adapted from Xu et al., Nature Medicine, 20(9):1018-26 (2014)

The acetate switch can be exploited to treat acute anemia in rabbits

A significant number of findings from mouse studies have not translated to humans. To increase potential relevance, some in the biomedical research and regulatory community advocate that non-rodents should be an essential, intermediate bridge from rodent studies to human clinical trials. Exogenous acetate delivered daily by oral gavage to rabbits rendered anemic by phlebotomy restores hematocrits to normal levels in approximately half the time otherwise required.



In conjunction with data from a rat model of hemorrhagic shock that suggests volume restoration with acetated Ringer's solution is superior to use of lactated Ringer's solution or normal saline, an argument could be made that acetated Ringer's solution may have immediate as well as delayed effects on recovery from acute blood loss[87].

Current anemia treatments carry inherent risks and/or substantial costs

Depending upon their clinical status, anemic patients may be treated by red blood cell transfusions, Epo injections, or intra-venous iron infusions. Transfusions are costly, have a limited lifespan, and carry a risk of transfusion reactions. Epo may result in hypertension and thrombosis. Intra-venous iron may be safe, but peripheral veins are oftentimes sclerosed, which complicates placement of arterial-venous shunts in renal failure patients who become hemodialysis-dependent. If a suitable alternative or adjunct to red blood cell transfusions, Epo injections, or intra-venous iron infusions is found, then the reduced economic costs and faster recovery times will result in millions of dollars saved per year, given the prevalence and cost of care for anemic patients.

Acetate supplementation is safe, inexpensive, and facile

Acetate has been studied in a variety of human clinical research settings, predominantly for measurements of substrate oxidation. Sodium acetate - administered intra-venously to young, healthy subjects at 20 μmol/kg bodyweight/min for 3 hr - raised serum acetate levels nearly 10-fold after 30 min and approached steady-state levels at nearly 20-fold elevations after 3 hr[88]. Similarly, sodium acetate administered to young, healthy or type II diabetic subjects at a concentration of 20-40 μmol/kg bodymass/min (administered at a fixed rate of 2.5 mmol/min from a 150 mmol/L solution, which was made from six 10 mL ampules of 2.5 mmol/mL acetate stock diluted with sterile water to 1 L total volume) raised serum acetate levels nearly 5-fold after 20 min and was at steady-state levels nearly 10-fold above baseline by 40-60 min[89]. There was no appreciable change in blood pH, pCO2, or pO2 while the base excess increased just after the infusion in both normal and diabetic subjects, consistent with a compensated metabolic acidosis. Infusions at a similar rate (33 μmol/kg bodymass/min) for 60 min in young, healthy volunteers also resulted in ~5-fold increase in serum acetate levels and had no detrimental impact on cardiac function[90]. Indeed, acetate administered in this manner is a weak arterial vasodilator as well as diuretic and may slightly improve cardiac as a result. Infusions for longer periods of time (120 min at a fixed rate at 2.5 mmoles/min) decreases ventilation and increases oxygen consumption, but does not affect oxygen delivery to peripheral tissues likely as a consequence of the increase in cardiac output.

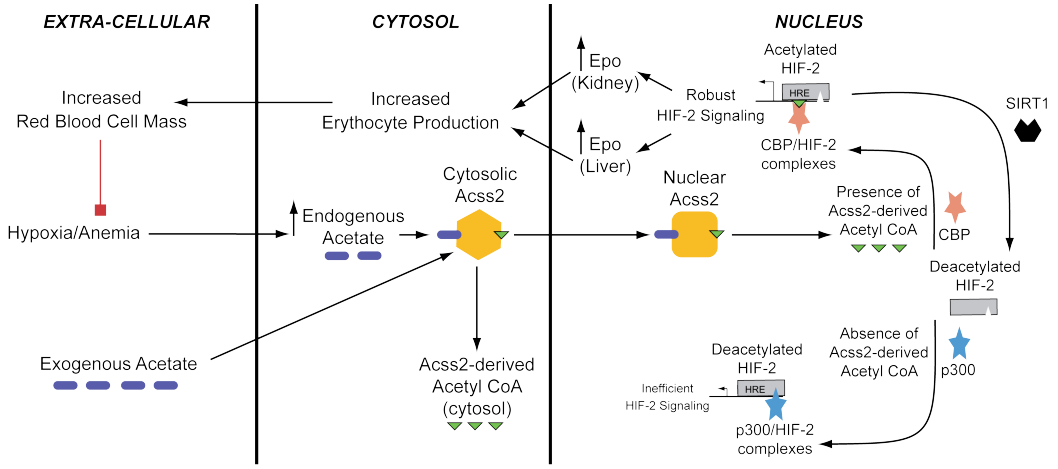
Consumption of acetate as vinegar in capsules or drinks (750 mg acetic acid, ~1.5 tablespoons household vinegar, in 100 mL) by young healthy volunteers raised serum acetate levels just under 2-fold in 30 min and ~3-fold in 15 min for the vinegar capsules or drinks, respectively; serum acetate levels returned to normal by 60 min or by 120 min for the vinegar drink versus capsule groups, respectively[91]. In young, healthy volunteers provided with acetate in an energy drink following exercise (2.5 mmoles/kg bodymass consumed in a 500 mL drink within 5 min), serum acetate levels increased substantially (~100-fold) by 30 min and returned to baseline levels by 120 min after ingestion; a control energy drink containing sodium bicarbonate had no significant effect on serum acetate levels[92]. Blood acid-base status was similar between the two groups. In another study, repetitive doses of oral acetate (15 mmol every 15 min over a 2 hr period for a total of 135 mol; administered with either intra-venous saline or glucose solution) resulted in ~10-fold increase in serum acetate levels ~1.5 hr into the infusion protocol and returned close to baseline by 60 min following completion of the oral load[93].

Has the lay public discovered this treatment already?

An internet search using “anemia vinegar” identifies several websites that report alleviation of anemia induced by menorrhagia or chronic illnesses. These sites often suggest consumption of a homemade cocktail comprised of apple cider vinegar and a foodsource high in iron such as blackstrap molasses, figs, or beetroot juice. Although a rigorous scientific assessment of the efficacy of such treatments is lacking at this time, the scientific basis to support the efficacy of vinegar-based oral drinks in treatment of anemia now is evident. Clinical studies to test acetate as a primary or adjuvant treatment for anemia in select patient populations appears warranted.

Summary, clinical relevance, and future studies

A substantial fraction of chronic cardiac disease patients have anemia, which is associated with worse clinical outcomes. Current treatments of anemia in these patient populations, which includes bolus Epo injections or intra-venous iron supplementation, have not been effective at changing clinical outcomes. HIF-2 signaling regulates physiological gene expression of Epo as well as iron metabolism factors. The same biochemical pathway that controls HIF-2 during hypoxia in cells, termed the acetate switch, can be stimulated in mice with anemia by treating them with exogenous acetate, which increases production of the HIF-2 target gene Epo and thereby restores hematocrit levels. Thus, the acetate switch is a potential therapeutic target for treating anemia, one that may restore Epo and possibly functional iron levels in a more physiological manner compared with bolus Epo therapy or HIF-stabilizing factors.



Translation of these bench findings to the bedside will require assessing safety as well as efficacy of acetate supplementation in target human anemia patient populations. In collaboration with clinical research investigators, preclinical studies will be needed to determine pharmacokinetics and optimal dosing of oral acetate supplementation in healthy and select anemia human patient populations. With this knowledge in hand, safety and efficacy trials in acute and chronic anemia human patient populations may be considered. If effective, acetate supplementation may positively impact lives of, while reducing health care costs for, convalescing and critically ill anemia patients, a prevalent patient population in the US veteran and general patient population.

It is important to know about co-morbid conditions that may be exasperated if HIF-2 signaling is augmented, particularly for chronic acetate supplementation. For example, several cancers are a relative contraindication for bolus Epo therapy and are a theoretical risk for any therapy that augments HIF signaling, which may exacerbate tumor growth. Indeed, chronic acetate supplementation augments Acss2/HIF-2 signaling and tumor growth in a flank tumor cancer mouse model[94]. Nevertheless, acetate has a selective action on HIF-2 signaling and does not augment HIF-1 signaling, which may have important advantages over non-selective HIF activators (e.g. PHD inhibitors) that are being evaluated for use as Epo-stimulating agents. As for any treatment, careful consideration of risks and benefits is always warranted and best appreciated when knowledge is greatest.

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