HIF Pathway Mutations found in Tibetan Highlander Populations leads to Increased Cancer Risk: REVIEW

By: Cassidy Coghlan

Abstract:

In the Tibetan population, Non-Small Cell Lung Cancer (NSCLC) was reported to be the sixth deadliest cancer (X. Wang et al., 2020). Mutations in the *EGLN1* gene are present in 80% of people in Tibetan highlander populations. The *EGLN1* mutations D4E and C127S are known to confer a 2x increased risk for developing lung cancer in this population. The *PDH2* enzyme, coded for by the *EGLN1* gene is a crucial part of hypoxia sensing in the Hypoxia-Inducible-Factor pathway (HIF). Under normal conditions, the HIF pathway is among the primary pathways responsible for homeostatic processes such as increasing vascularization and proliferation of red blood cells (Graham & McCracken, 2019; Lukashev & Sitkovsky, 2008; Marxsen et al., 2004; Masoud & Li, 2015; van Vliet et al., 2021). Under hypoxic conditions, hydroxylase activity of *PHD2* is suppressed, resulting in accumulation of HIF1α proteins that activate downstream target genes, inducing physiological changes especially in red blood cell production and increased vascularization. The result of these mutations in hypoxia is decreased degradation of the HIF1α subunits (Andersen et al., 2011; Delamare et al., 2023; Lanikova et al., 2017a; Lorenzo et al., 2014). This acts to enhance oxygen delivery under hypoxic conditions. It is hypothesized that under normoxic conditions, $PDH2^{MUT}$ induces HIF1 protein accumulation that promotes angiogenesis which may contribute to cancer growth and persistence. In terms of a NSCLC therapy, this Tibetan population expressing $PDH2^{MUT}$ would respond most preferably to HIF1α targeted therapy, as hypoxia in tumors leads to resistance to most other cancer therapies like radiotherapy and chemotherapy. The anti-VEGF monoclonal antibody bevacizumab (Avastin), has been expanded to the treatment of NSCLC. I will look at current and upcoming therapies to identify if the *EGLN1* mutation associated with lung cancer can be treated with current technology and if the mutations would confer positive or negative response.

Background Info on the HIF Pathway:

The Hypoxia inducible factors (HIF) are crucial transcription factors that regulate oxygen homeostasis. These transcription factors are composed of an alpha and beta subunit. The alpha subunit is hypoxia-induced whereas the beta subunit is always on. Hypoxia is a condition in which there is a lack of oxygen entering the body, often present at high altitudes. The alpha subunit has three different isoforms, HIF-1α, HIF-2a and HIF-3α . The HIF-1α subunit is constantly expressed, whereas the HIF-2α and HIF-3α isoforms have tissue-specific differential expression (Keith et al. 2012, Carroll et al. 2006). When oxygen is present under normal conditions, the enzyme protein PHD, encoded by the gene *EGLN1,* catalyzes the process of hydroxylating HIF-1α triggering degradation of the HIFa subunit via the Von Hippel-Lindau (VHL) protein-ubiquitin-proteasome pathway. VHL serves as a ubiquitin ligase and attaches the ubiquitin to HIF proteins marking them for degradation. The ubiquitin-proteasome pathway then will recognize and break down proteins that have been marked for degradation by ubiquitin. (Andersen et al., 2011; Delamare et al., 2023; Lanikova et al., 2017; Lorenzo et al., 2014). Under hypoxia conditions, when little to no oxygen is present, the hydroxylation function of PHD

is repressed resulting in a build up and overexpression of HIF-1α (Figure 1). This response is beneficial because HIF-1α can then go on to trigger multiple downstream genes for transcription that involve oxygen homeostasis and metabolism It also allows for more HIF-2α (EPAS proteins) to be constantly produced, increasing the overall levels (and enabling more of these homeostatic processes to occur such as VEGF production, vascularization and erythropoiesis). HIF-2α is a subunit of HIF which controls genes in cell division, red blood cell production, vascularization and regulates the hormone erythropoietin. Accumulation of HIF-2α promotes the formation of new blood vessels and stimulates production of red blood cells. This promotes processes like angiogenesis (production of new blood vessels) and erythropoiesis (production of red blood cells) (Andersen et al., 2011; Carroll & Ashcroft, 2006; Jokilehto & Jaakkola, 2010; Onnis et al., 2009; Semenza, 2010).

Certain mutations allow for increased HIF degradation under hypoxic conditions. This increased degradation of the HIF pathway allows for more normal conditions for the Tibetan Highlanders who experience hypoxic conditions on a daily basis. The PDH2 D4E and C127S mutations ($PDH2^{MUT}$) are gain of function missense mutations. Under normal conditions, HIF 1α builds up and starts to degrade slower with less oxygen available to bind to PHD2. This mutation allows for more degradation in these low-oxygen areas, creating more normoxia-like conditions and similar rates of degradation that would be found at lower altitudes (Figure 2). (Bigham & Lee, 2014; Lanikova et al., 2017a; Lorenzo et al., 2014; Tashi et al., 2019; Yi et al., 2010a)

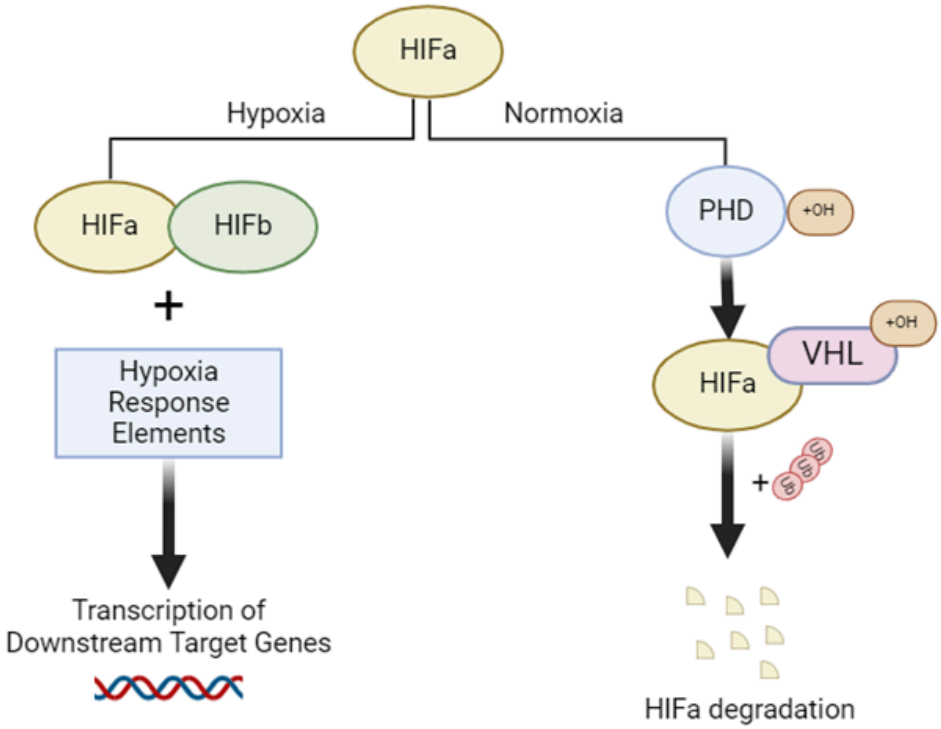

Figure 1: Schematic of HIFa pathways.

When oxygen is present under normoxic conditions, the enzyme protein PHD, encoded by the gene *EGLN1,* catalyzes the process of hydroxylating HIFa and thus triggering degradation of the HIFa subunit via the VHL protein-ubiquitin-proteasome pathway. Under hypoxic conditions, when little to no oxygen is present, the hydroxylation function of PHD is repressed resulting in a build up and overexpression of HIFa.

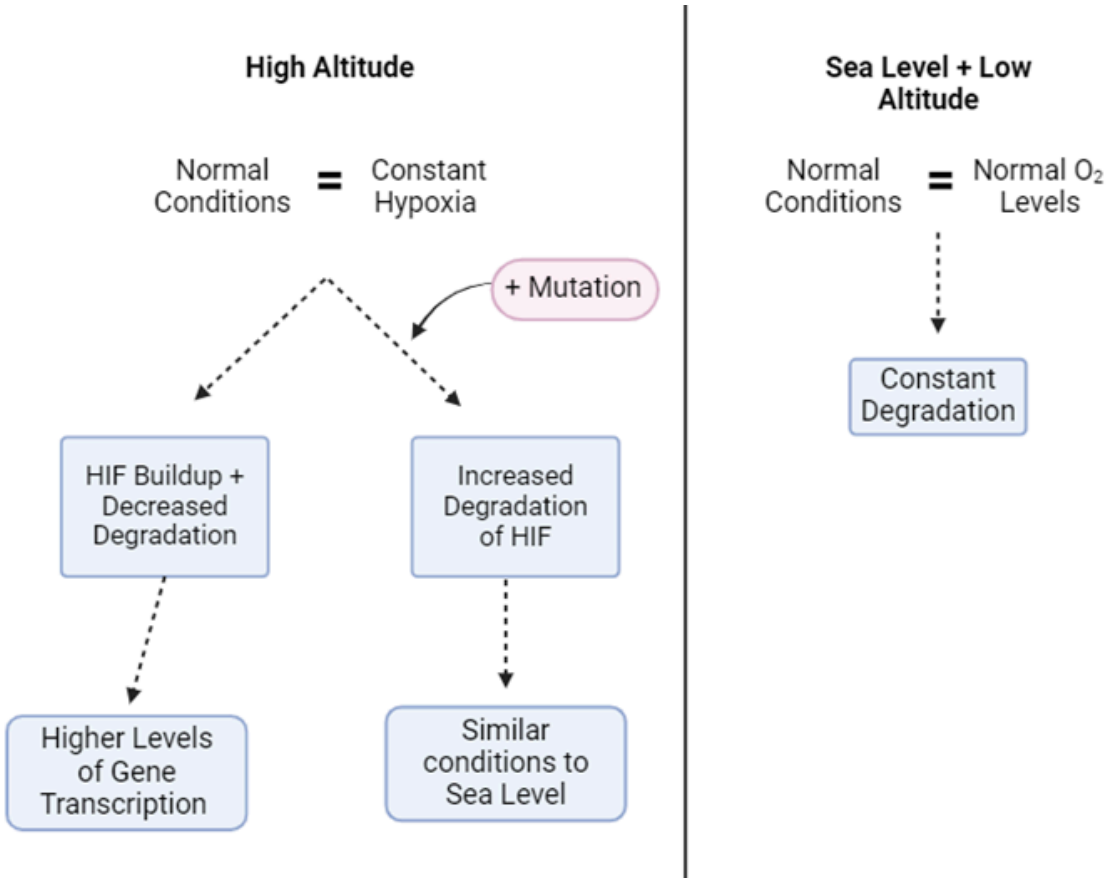

Figure 2: High Altitude vs. Sea Level Hypoxia response

High altitude populations live at a constant state of hypoxia, meaning they will have a much higher level of gene transcription regularly, activating many of the downstream pathways like angiogenesis, VEGF production, and Erythropoiesis. The mutation present in High Altitude Tibetan Populations has the ability to increase degradation to limit these effects and confer more sea-level normal conditions.

Natural Selection of HIF-1α mutation in Tibetan-Highlander Populations:

Due to the extreme conditions of their surroundings, many Tibetan populations are forced to live at unnaturally high altitudes. Many residents of this Tibetan Plateau live at elevations

exceeding 4000 meters, with oxygen concentrations about 40% lower than at sea level (Yi et al., 2010b). This constant pressure on their body has caused them to develop certain adaptations through natural selection over time in order to cope with the strains of high altitude. When lowlanders (people normally residing at sea level or near sea level altitudes) go up in altitude, their hemoglobin will increase along with their blood viscosity.(Amanat et al., 2020; Pérez-Gracia et al., 2010; Zhang et al., 2019) For people who have to constantly live at these altitudes, the effects are not sustainable for long term health and wellness and can lead to detrimental effects like Chronic Mountain Sickness (CMS) and certain cardiovascular complications. Some populations that live at high altitude elsewhere in the world, such as Andean and Ethiopian populations, have developed adaptations that allow increased levels of hemoglobin blood concentration, but the Tibetans are different. Tibetan highlanders show restrained average concentration of hemoglobin increase in comparison to Andean and Ethiopian populations due to their large plasma volume. Having a large plasma volume allows Tibetans to have higher than normal hemoglobin in total, but at a comparatively normal blood hemoglobin concentration. In addition to high plasma volume, (Beall, 2006; Lanikova et al., 2017b; Lorenzo et al., 2014; Pérez-Gracia et al., 2010; Simonson et al., 2010; Yi et al., 2010a).Tibetan populations also have another specific adaptation in their HIF pathway allowing for higher than normal degradation of HIF. Higher HIF degradation allows the body to regulate processes like angiogenesis and erythropoietin production by conferring more normal conditions with less access to oxygen.

Mutation background:

EGLN1 is the gene encoding the enzymatic protein HIF prolyl 4-hydroxylase 2 (PHD2). *EGLN1* is known as a hotspot gene meaning it is key in forming a central regulatory network between a series of upstream activators and downstream effector genes and is therefore more likely to be selected upon when high selective pressure is available. (*EGLN1 Gene: MedlinePlus Genetics*, n.d.; Tashi et al., 2019). Under normal conditions PHD is the protein that performs oxygen dependent hydroxylase functions to HIF1α proteins which triggers their degradation via additional enzymatic complexes. In Hypoxia the activity of this protein is repressed due to the lack of oxygen, allowing for an accumulation of HIF proteins which can go on to activate hundreds of downstream target genes, therefore inducing different physiological changes especially in red blood cell (RBC) production and increased vascularization.(Delamare et al., 2023; Tang et al., 2022) These mutations allow for increased degradation of HIF during hypoxia (Marxsen et al., 2004).This response provides protection against polyglycemia, preventing excess production of RBCs by mediating the upstream response and allowing less activation of downstream target genes (Graham & McCracken, 2019; van Vliet et al., 2021).

How the HIF pathway impacts Cancer Risk

Recent data suggests HIF overexpression may correlate with a more aggressive tumor profile. As initial tumors form and expand, parts of them become hypoxic which in turn induces angiogenesis, engaging the HIF pathway that is associated with promoting aggressiveness and radiation resistance (Alì et al., 2023; Araghi et al., 2023a; Onnis et al., 2009). HIF1α also

primarily regulates the transcriptional activation of Vascular Endothelial Growth Factor (VEGF) response to hypoxia. VEGF is a signal protein produced by cells that stimulates the formation of new blood vessels. VEGF is one of the downstream target genes that is activated by HIF1α, especially when there is an accumulation due to overexpression in hypoxic conditions. (Lukashev & Sitkovsky, 2008; Ramakrishnan et al., 2014). This buildup and overexpression of HIF1α during hypoxia, such as when tumors become hypoxic, induces higher than normal expression of VEGF leading to more blood vessels being formed for that tumor, allowing it to continue to expand. Because of this relationship between HIF1α and VEGF, both of these pathways and signals have been targets of cancer therapies in recent years.(Masoud & Li, 2015). (Figure 3)

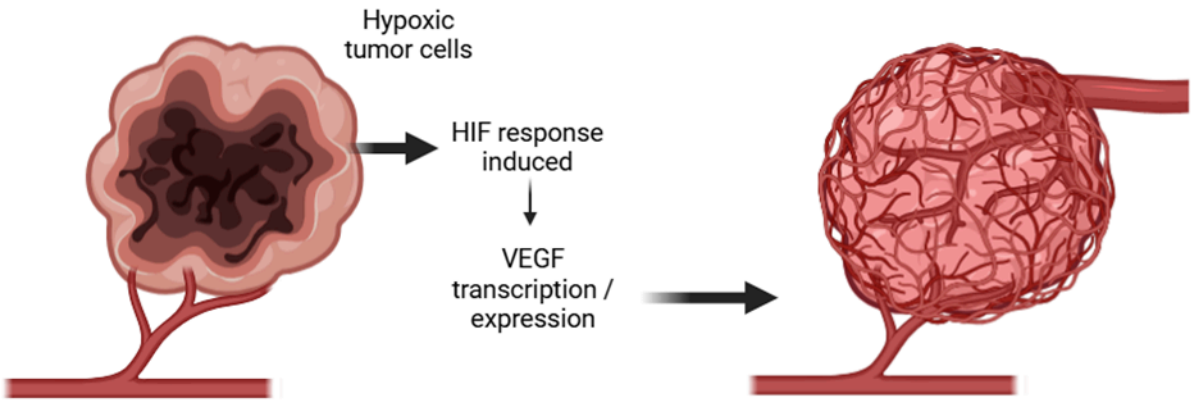

Figure 3: VEGF drives Vasculature in Hypoxic Tumor regions

As initial tumors form and expand, parts of them become hypoxic as they grow away from the initial source of blood. This in turn induces angiogenesis, engaging the HIF pathway and increasing VEGF transcription and expression as a result. VEGF induces more blood vessel growth towards the tumor, increasing its growth and aggressiveness.

How the Mutations affect Cancer Risk: (NSCLC)

While the mutations that have been selected for in the Tibetan-highlander populations are beneficial to the high altitude adaptation, they have also been found to confer a two times increased risk of lung cancer in these populations (Lanikova et al., 2017a).Much of the reason behind this increased risk is unknown, although it is known that the mutation somehow alters the

protein *PHD2* in the HIF pathway, altering the hydroxylase reaction that allows HIF to be broken down. When there is not enough oxygen to be used in this reaction, the *PHD2* protein slows and less HIF protein is degraded. $PDH2^{MUT}$ enhance the protein enzyme catalyst abilities, allowing the protein *PHD2* to increase the rate of reaction and bind more O_2 in a shorter amount of time than normal; the protein is therefore more efficient at hydroxylating HIFa subunits at lower oxygen than non-mutated *EGLN1.* (Andersen et al., 2011; Jokilehto & Jaakkola, 2010; Jun et al., 2017; Nisar et al., 2023; Price et al., 2019). This makes sense, for the results of this mutation have been observed to allow for increased degradation, which would result from increased rate of oxygen binding when a smaller amount of oxygen is available. $PDH2^{MUT}$confers higher rates of HIFa degradation with decreased inhibition of the hypoxia response element activity compared to $PDH2^{WT}$. $PDH2^{MUT}$ alters oxygen binding and perhaps also HIF-2α substrate binding. It is unclear whether this mutation is loss of function or gain of function as research points to evidence for both cases. (Delamare et al., 2023; Tang et al., 2022; Yang et al., 2016).

Which Available Cancer Therapies would be best suited to treat mutation specific NSCLC cases ?

Type	**Name**	**Target**	**Function**	**Status**
Monoclonal Antibodies	Bevacizumab (Avastasin)	VEGF	Inhibits Angiogenesis	FDA approved for treatment of NSCLC
Chemotherapeutic	Topotecan	Topoisomerase I	Topotecan is a strong inhibitor of HIF-1α and HIF-2α subunits in hypoxic neuroblastoma cells, leading to decreased VEGF expression and	Still in trials for NSCLC (3)

			angiogenic activity. Topotecan is also an FDA approved chemotherapeutic agent used as a second line therapy for patients with small cell lung cancer. It also has demonstrated positive results with NSCLC. (Puppo et al., 2008)	
Small Molecule Inhibition	Trials using 2 independent shRNA sequences	PHD2	Knocks down PHD2 function reliant on HIF1α, successfully dramatically slows tumor growth and progression (Kondo et al., 2002)	Has only been tested on immunocompetent mice; not an actual drug yet

Small Molecule Inhibition	Aminoflavone	HIF-1α mRNA expression	Amino flavone is a ligand of the aryl-hydrocarbon receptor inhibits HIF-1α accumulation, in an aryl-hydrocarbon-independent fashion (Klotzsche-Von Ameln et al., 2011)	Currently only in phase I clinical trials in patients with metastatic cancer; not tested specifically with NSCLC
Small Molecule Inhibition	PX-478	HIF-1 inhibitor	Inhibition at multiple levels demonstrating anti-tumor activity with reduced levels of HIF-1 (Luo et al., 2022)	Currently still in clinical trials

In order for a cancer to grow, it needs to be able to continue to provide itself with oxygen and blood, and activated VEGF stimulates the process of growing and providing blood through new blood vessels, often directly to the cancer. Because of this direct relationship between VEGF expression and cancer growth, VEGF has been a main target for cancer therapies in recent years (Meadows & Hurwitz, 2012). There are a few anti-VEGF drugs that currently exist for use and there are also more that are currently being investigated or are in trials. Because the HIF pathway plays a direct role in the activation of VEGF, HIF inhibition drugs would be able to block VEGF expression from a more upstream standpoint. Current anti-VEGF pathway inhibition drugs use small-molecule inhibitors to inhibit specific parts of the VEGF pathway.(Araghi et al., 2023a, 2023b; Klotzsche-Von Ameln et al., 2011; Onnis et al., 2009; L. M. Wang et al., 2019) . Bevacizumab was the first VEGF inhibitor approved for the treatment of cancer and is approved for the treatment of colorectal, non-small-cell lung, breast, and renal cell cancers, and glioblastoma. Bevaciumab is a immunotherapy that needs to be used in conjunction with various methods of chemotherapy (Bevacizumab - NCI, n.d.).Numerous other drugs are currently being studied in clinical trials.

Discussion:

Identifying and understanding the risks and causes of cancer is an important first step in helping the most people in the world. This specific mutation in the Tibetan Highlander Populations helps us understand how the HIF pathway plays into cancer risk and cancer growth. Understanding how this specific mutation works and affects cancer in this population and especially with NSCLC we can expand the field of knowledge that will help not only the people currently affected by the mutation but also further the understanding of cancerous mutations in general to help more cases and recognize more cancerous risks as they arise. Similar mutations in the HIF pathway will have a baseline of knowledge and research to go off of to more effectively help patients. There are a few drugs currently available to treat this specific mutation in cancerous bodies, but HIF-1α inhibition is on the rise in drug development. In future years there will be many more drugs coming into and out of clinical trials. In either case, a few studies have been published as to the reasoning behind the increased cancer risk for tibetan patients with either gain of function or loss of function mutations. One further speculation is that because this protein already has a more normal adaptation to hypoxia, when patients with this mutation develop cancer, and the cancer develops hypoxic regions these cells already have the machinery to survive much more hypoxic conditions than the cells of lowlander populations.

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