Hypoxia-induced pulmonary hypertension – utilising experiments of nature

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Abstract

An increase in pulmonary artery pressure is a common observation in adult mammals exposed to alveolar hypoxia. It is considered a maladaptive response that places an increased workload on the right ventricle. The mechanisms initiating and maintaining the elevated pressure are of considerable interest to understanding pulmonary vascular homeostasis and developing new treatments for pulmonary hypertension. In particular, it would be helpful to discover the key molecules in the integrated vascular response to hypoxia to inform potential drug targets. One strategy is to take advantage of experiments of nature; specifically, to understand the molecular basis for the inter-individual variation in the pulmonary vascular response to acute and chronic hypoxia. This is the motivation for genetic studies in populations and animals adapted to life at high altitudes. To date, these studies highlight the importance of hypoxia-inducible factor 2α (HIF-2α), encoded by EPAS1, and prolyl hydroxylase domain-containing protein 2 (PHD), encoded by EGLN1, and support efforts to pharmacologically manipulate HIF-2 activity as a treatment for pulmonary hypertension.

Introduction

Under physiological conditions, the adult pulmonary circulation is maintained as a high-flow, low-pressure and low-resistant system through which the entire cardiac output (CO) must pass. Exposure to hypoxia leads to the constriction of small resistant arteries in the lung, referred to as hypoxic pulmonary vasoconstriction (HPV) (Euler USV, 1946). It is a physiological mechanism to divert blood to better oxygenated lung segments and optimize gas exchange by adapting blood flow (perfusion) to alveolar ventilation. This is beneficial if*regional* obstruction of airflow occurs and between 30-70% of the lung is exposed to hypoxia (Sylvester, Shimoda, Aaronson & Ward, 2012); if the vasoconstricted area is less than 30%, the effect of lower PO_2 is negligible. In the case of *global* alveolar hypoxia, such as occurs at high altitude, HPV can be detrimental because it leads to a sustained overall vasoconstriction and a significant increase in pulmonary vascular resistance (PVR) and pulmonary artery pressure (PAP) – see Box 1 (Dunham-Snary et al., 2017; Penaloza & Arias-Stella, 2007b; Young, Williams & Thompson, 2019).

Sustained exposure to hypoxia leads to structural changes in pulmonary vessels that increase vascular stiffness and resistance to blood flow. HPV and vascular remodelling, together with a rise in haematocrit and thus blood viscosity, comprise the components of hypoxia-induced pulmonary hypertension (HPAH), which places an increased workload on the right ventricle (Dunham-Snary et al., 2017; Julian & Moore, 2019; Penaloza & Arias-Stella, 2007a; Soria, Egger, Scherrer, Bender & Rimoldi, 2016; Wilkins, Ghofrani, Weissmann, Aldashev & Zhao, 2015; Young, Williams & Thompson, 2019). Heterogeneity in HPV in the pulmonary circulation is credited with an important role in hypoxia-induced pulmonary oedema (HAPE); differential HPV may lead to over perfusion of some regions of the lung, causing oedema. Excessive erythrocytosis leading to hyper-viscosity characterises chronic mountain sickness (CMS) and emphasises the importance of correcting measurements of pulmonary vascular resistance for haematocrit when diagnosing HAPH. Here

we focus on HPAH and the contribution from studies in animals and humans exposed to chronic hypoxia to understanding underlying mechanisms and prioritising pharmacological targets.

Molecular basis of HPV.

The precise mechanism of HPV remains unclear (Sylvester, Shimoda, Aaronson & Ward, 2012). Published studies indicate that both the sensor and effector mechanisms required for acute HPV are located in pulmonary arterial smooth muscle cells (PASMC) (Bigham & Lee, 2014; Wang, Weigand, Lu, Sylvester, Semenza & Shimoda, 2006; Young, Williams & Thompson, 2019), while endothelial cells (EC) and extracellular matrix cells play an important role in modulating HPV (Dimmeler, Fleming, Fisslthaler, Hermann, Busse & Zeiher, 1999; Fukuroda, Ozaki, Ihara, Ishikawa, Yano & Nishikibe, 1994; Sakao, Tatsumi & Voelkel, 2009; Tian, McKnight & Russell, 1997; Xu, Tan, Tampe, Sanchez, Zeisberg & Zeisberg, 2015). The initial event for HPV is probably a mitochondrial redox signal in response to low PO_2 (Dunham-Snary et al., 2017; Peng et al., 2011), then the "O_2 sensor" signals to the "effectors" leading to smooth muscle contraction. The canonical mechanism of HPV includes PASMC membrane depolarization due to acute hypoxia-induced reduction of K^+ channel activity. This subsequently opens voltage-dependent L-type of Ca^{2+} channels (VDCC) and increases cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) via Ca^{2+} influx through VDCC. Meanwhile, hypoxia can also directly open receptor-operated Ca^{2+} channels (ROC), and store-operated Ca^{2+} channels (SOC), causing an increase in $[Ca^{2+}]_{cyt}$ in PASMC (Dunham-Snary et al., 2017; Luks & Swenson, 2015; Mauban, Remillard & Yuan, 2005; Sylvester, Shimoda, Aaronson & Ward, 2012). Elevated $[Ca^{2+}]_{cyt}$ is a major trigger for PASMC contraction and proliferation (He et al., 2018; Kuhr, Smith, Song, Levitan & Yuan, 2012; Song et al., 2018). Therefore, if mitochondria or the mitochondrial respiratory chain is the O_2 sensor in PASMC, the membrane receptors and ion channels and cytosolic Ca^{2+} are the effectors for HPV.

Hypoxia-induced vascular remodelling.

The initial structural changes include endothelial blebbing and disruption of the endothelial barrier, allowing influx of plasma proteins, including growth factors. The hallmark of remodelling is the extension of vascular smooth muscle to previously unmuscularised arterioles. Medial and adventitial thickening are also observed, the former from smooth muscle cell hypertrophy as well as accumulation of smooth muscle cells, the latter from an increase in fibroblasts and myofibroblasts and in extracellular matrix. An influx of inflammatory cells is also evident but less pronounced than in, for example, pulmonary arterial hypertension (PAH).

Indeed, the remodelling witnessed with hypoxia differs from that seen with PAH in that with most species, including man, it is less severe than in PAH and there is no occlusion of vessels; one exception is the neonatal hypoxic calf model which can develop marked intimal thickening associated with a very high PAP (Stenmark et al., 1987). Moreover, the concept that hypoxia leads to an increase in PVR from remodelling that narrows the vessel lumen has been challenged, as has the idea that hypoxia leads to vascular rarefaction or "pruning". Hypoxia stimulates angiogenesis. Studies in rats have reported that chronic hypoxia increases total pulmonary vessel length, volume, endothelial surface area and the number of endothelial cells. Coupled with experimental studies in rodents that show that inhibition of the anti-angiogenic factor, angiostatin, aggravates and that overexpression of vascular endothelial growth factor protects rats from hypoxia-induced pulmonary hypertension, the suggestion is that, at least in rodents, angiogenesis plays a significant role in the response of the pulmonary circulation to chronic hypoxia, perhaps acting to reduce the effects of HPV and structural changes elsewhere on the right ventricle.

An increase in haemodynamic stress with hypoxia is a factor in initiating and perhaps sustaining pulmonary vascular remodelling; banding of the pulmonary artery has been shown to prevent and reverse occlusive lesions in a hypoxia-dependent rodent model of pulmonary hypertension (Abe et al., 2016). Unlike the immediate vasoconstrictor response to hypoxia, vascular remodelling requires new protein synthesis. A panoply of factors (reviewed elsewhere)(Wilkins, Ghofrani, Weissmann, Aldashev & Zhao, 2015) have been implicated in mediating the structural changes, from vasoactive molecules (such as endothelin) to growth factors (e.g. platelet-derived growth factor) and cytokines (e.g. interlukine-6 and tumour necrosis factor-α). Changes in the underlying response of vascular cells to these factors are also involved. Not surprisingly, the

role of hypoxia-inducible factors (HIFs) and their target genes are of primary interest.

HIF signalling pathway

HIFs belong to a group of basic-loop-helix-PER-ARNT-SIM (bHLH-PAS) proteins that function as transcription factors responding to oxygen and other stresses (Semenza, 2012; Wang, Jiang, Rue & Semenza, 1995). HIFs function as heterodimeric transcription factors comprising a constitutively expressed beta unit, HIF1β (also known as aryl hydrocarbon receptor nuclear translocator, ARNT), and an alpha subunit that is oxygen liable (Figure 1). Currently, three alpha subunits have been identified and include HIF1α, HIF2α, HIF3α. The α and β subunits share a high percentage of both amino acid and structural homology, the N-terminal region (bHLH) which enables DNA binding, two PAS domains (PAS-A and PAS-B) that contribute to heterodimer stability and an oxygen dependent degradation domain.

In an oxygen rich environment, HIFs are instantaneously inactivated (Jewell, Kvietikova, Scheid, Bauer, Wenger & Gassmann, 2001) by posttranscriptional hydroxylation of conserved proline amino-acid residues within the α subunit. Hydroxylation generates a high affinity binding site for pVHL, leading to polyubiquitination and degradation by the proteosome. HIF prolyl hydroxylation is mediated by prolyl hydroxylases (PHDs). PHD activity requires several co-factors including molecular oxygen; a K_M greater than 250 μM is above the oxygen concentration typically found in arterial blood (185 μM), and so the intracellular PO_2 will always fall below the K_M for oxygen, allowing enzyme activity to be modulated by oxygen availability over the entire physiological range (Hirsila, Koivunen, Gunzler, Kivirikko & Myllyharju, 2003). Obviously, evolution has come up with a mechanism that constitutively synthesizes molecules that allow a very fast response to hypoxia. This seems to be a crucial response. The prize for this is that if low oxygen conditions do not occur, all alpha subnits of HIFs are degraded without any further use.

Under hypoxic conditions, the loss of hydroxylation leads to the accumulation of HIFα isoforms and the formation of heterodimers with the constitutively expressed HIF1β (Kaelin & Ratcliffe, 2008). This HIF complex, together with a histone-acetyltransferase (p300), binds to hypoxia-response elements (HRE) in DNA, initiating or enhancing transcription of target genes. There is, however, a second tier of HIF regulation through the action of an asparagine hydroxylase, known as factor inhibiting HIF (FIH). Originally found to be a negative regulator of HIF1α, it was later shown to be an asparaginyl hydroxylase capable of hydroxylating N^{803} in the C-terminal domain of HIF1α (Schofield & Ratcliffe, 2004). FIH has a K_M for molecular oxygen between 90 to 200μM. FIH hydroxylates target proteins with a higher efficiency at lower oxygen tensions than the PHDs, consequently continuing to hydroxylate target proteins in oxygen tensions below 5% (68μM). The efficiency of FIH hydroxylation appears to be substrate dependent; hydroxylation of HIF1α occurs with a greater efficiency (90%) than HIF2α (<30%) in normal physiological oxygen (Bracken et al., 2006; Yan, Bartz, Mao, Li & Kaelin, 2007). However, unlike the PHDs, FIH has a greater number of HIF-independent targets; for example, the efficacy for NOTCH hydroxylation appears to be far greater than HIFα (Coleman et al., 2007).

Both HIF1α and HIF2α isoforms have been extensively studied in pulmonary hypertension. The first direct evidence came from mice hemizygous for either HIF1α or HIF2α (Brusselmans et al., 2003; Shimoda, Manalo, Sham, Semenza & Sylvester, 2001). Pulmonary disease progression following chronic hypoxia exposure was substantially delayed in these models. The aberrant stability of both HIF1α and HIF2α was initially reported in whole lung tissue from PAH patients, then subsequently in pulmonary endothelial and smooth muscle cells from this patient group (Ball et al., 2014; Barnes, Chen, Sedan & Cornfield, 2017; Bonnet et al., 2006). Murine studies identified a definitive tissue-specific HIFα expression profile in the pulmonary vasculature, where HIF2α was found to be highly expressed in the endothelium. Genetic ablation of pulmonary endothelial HIF2α prevents the initiation and development of pulmonary vascular remodelling associated with pulmonary hypertension (Cowburn et al., 2016). Several groups have now reported that endothelial-loss of PHD2 or*EGLN1* in mice leads to the aberrant stability of HIF2α with the development of occlusive vascular lesions and severe pulmonary hypertension (Dai, Li, Wharton, Zhu & Zhao, 2016; Tang et al., 2018). The concomitant genetic ablation of endothelial PHD2 and HIF2α in this model also inhibited the phenotype, offering near complete protection from pulmonary hypertension (Dai, Li, Wharton, Zhu & Zhao, 2016;

Kapitsinou et al., 2016).

While murine genetic manipulation studies established HIF2α as the predominant HIFα isoform driving pulmonary hypertension, additional support has been found in gain-of-function gene mutations. Patients or mice with a HIF2α-gain-of-function mutation develop pulmonary vascular disease (Formenti et al., 2011; Tan et al., 2013), while mutations in VHL leads to mice with "Chuvash polycythemia" and pulmonary hypertension, which is rescued by heterozygous deletion of HIF-2α (but not HIF-1α) (Hickey et al., 2010).

Populations living at high altitude

Accurate up-to-date data are difficult to come by but a commonly used statistic reports that roughly 7% of the world's population live above 1500m with some 140 million highlanders above 2,500m (Moore, Niermeyer & Zamudio, 1998) (Figure 1). Barometric pressure and the fraction of inspired oxygen (FiO_2) fall with increasing altitude, leading to hypobaric hypoxia (Imray et al). At 2,500 m above the sea level, barometric pressure falls from 101 Pa at sea level to 75 Pa and FiO_2 from 21% to 15%. For reference, on Mount Everest (summit 8,848 m), barometric pressure is 36 Pa and $FiO_2$7% (Hopfl, Ogunshola & Gassmann, 2003). Of note, the air's oxygen concentration remains at 21% even at that high altitude.

The extent to which HAPH is a health problem for residents at altitude is a relevant question. Most non-natives that ascend to these altitudes experience health problems in the early days, from breathlessness and headaches, to HAPE and high altitude cerebral oedema (HACE) in more severe cases. CMS is recognised among high altitude dwellers. A recent meta-analysis has provided a more complete picture of the prevalence of HAPH among highlanders than obtained from single reports. The analysis identified 12 studies that had collected echocardiographic estimations of PAP from a total of 834 high-altitude residents; all but one study was performed between 3,600m and 4,350m (Soria, Egger, Scherrer, Bender & Rimoldi, 2016). Mean systolic PAP was approximately 7 mmHg higher than recorded at low altitudes. It is noted, however, that HAPH was rare, with <1% of those studied recording a mean systolic PAP above 27.1 mmHg.

Nonetheless, PAP does increase with ascent and high-altitude regions offer a natural laboratory for investigating hypoxic response mechanisms in humans. Of particular interest is relating genotype to phenotype. Given that environmental hypoxia is a potent selection pressure, particularly at birth, individuals that exhibit the lowest PAPs at altitude might be expected to host genetic variants associated with adaptive molecular pathways. As genetic variants linked to phenotype offer a powerful strategy for defining critical molecular pathways, studying the genetic basis of adaptive responses to hypoxia provide an important approach to elucidating major drivers of HAPH.

The high-altitude populations for which most data are available are Tibetans, Andeans, Ethiopians and, somewhat less, the Kyrgyz (Figure 1). Humans have occupied the Tibetan plateau for over 25,000yrs, and those we refer to as Tibetans today split from Han Chinese, the usual control group in comparator studies, around 2750 ~ 5500 years ago (Yang et al., 2017). Human inhabitation of the Andean Altiplano began around 11,000yrs ago, the Semian Plateau in Ethiopia around 5,000yrs ago and the Tien-Shen mountains in Kyrgyzstan only in the last 1,000yrs. Given their longer history at altitude, Tibetans have had more time to adapt.

The most robustly quantitative traits studied at altitude are haemoglobin (Gassmann et al., 2019) and O_2-carrying capacity, and here there is agreement. Tibetans have a higher resting ventilation but lower arterial O_2 content than Andean or Han Chinese migrants and are arguably the most hypoxic of the high-altitude populations commonly studied. They also run lower haemoglobin concentrations, by around 1g/dl compared to Han Chinese at the same elevation. Han Chinese at the same altitude.

Accurate cardiopulmonary phenotyping in the field is more challenging. A widely held view is that Tibetans are more resistant to HAPH and there is a small but persuasive body of data in support of this. An early study of 5 Tibetans who underwent direct cardiac catherization at [?]3600 m reported PAP measurements in the same range as those measured in populations at sea level and minimal change in PVR when those subjects exposed to greater hypoxia (Groves et al., 1993). Related to this, histology of lung specimens from deceased

Himalayan residents show no remodelling of small pulmonary arteries (Gupta, Rao, Anand, Banerjee & Boparai, 1992). More recent studies of ethnic Tibetans in a UK laboratory using Doppler echocardiography found a blunted pulmonary vascular response to both acute (minutes) and sustained (8 h) hypoxia compared to Han Chinese (Petousi et al., 2014). Also supportive is the low prevalence of chronic mountain sickness in Tibetans compared to Han Chinese or Peruvian Quechuas. At odds with these observations is the previously mentioned meta-analysis where the echocardiography derived PAP pressures in Tibetans living between 3,600 and 4,350 m were similar to those of Andeans and Caucasians at the same elevation (Soria, Egger, Scherrer, Bender & Rimoldi, 2016). That Tibetans maintain a similar resting PAP to, say, Andeans despite lower arterial oxygen levels supports the concept that they are more resistant, but the marked overlap in distribution of measurements in the different populations puts that concept in context. The relative resistance of the Tibetan pulmonary vascular bed to a hypoxia-induced rise in PAP may be more pronounced at higher altitudes (e.g. >5,000 m), but here there are few data. It is worth noting that the measurements of PAP at altitude show a wider distribution than those taken at lower altitude and comparing extremes of response within a population can be informative (Wilkins et al., 2014).

Genetic studies in humans

Excellent overviews of the genes identified in population studies as selection targets for high-altitude adaptation is provided by Witt and Huerta-Sanchez (Witt & Huerta-Sanchez, 2019) and Bigham and Lee (Bigham & Lee, 2014; Brutsaert et al., 2019). The nominated genes for Tibetans and for Andeans are distinct but show some overlap. One might anticipate that each population would exhibit a different adaptive response reflecting differences in the genetic backgrounds of the original settler populations; any convergence on a common adaptation would serve to highlight its importance. In that context, genes encoding proteins in the hypoxia-inducible factor (HIF) signalling cascade dominate both lists and speak to the possibility that selection for adaptation to chronic hypoxia as opposed to some other environmental pressure has been most important in permitting survival at altitude (Bigham & Lee, 2014; Brutsaert et al., 2019).

The two genes most consistently identified in studies of Tibetans are*EPAS1* and *EGLN1* (Beall et al., 2010; Bigham et al., 2010; Peng et al., 2011; Simonson et al., 2010; Wang et al., 2011; Xu et al., 2011; Yi et al., 2010) (Figure 1). *EPAS1* encodes HIF2α while*EGLN1* encodes PDH2. The strongest genotype-phenotype association has been the linkage of *EPAS1* to lower haemoglobin concentrations (Beall et al., 2010; Yi et al., 2010), which given the contribution of blood vicosity to PVR can be seen as to contribute to cardio-pulmonary adaptation to hypoxia. An association between two *EPAS1* variants (rs149594770 and rs73926265) and lower PAP measurements in Tibetans living at 4,700 m has been reported but the extent to which the lower haemoglobin levels account for this is not clear (Peng et al., 2017). The variants reported in *EPAS1* are in non-coding space but, in support of reduced function, *EPAS1* expression is reduced in endothelial cells derived from umbilical cords (Peng et al., 2017) and lymphocytes (Petousi et al., 2014) from Tibetan subjects.

EGLN1 is a candidate gene for selection in both Tibetans and Andeans, but only linked to haemoglobin levels in Tibetans (Bigham et al., 2013; Simonson et al., 2010; Xiang et al., 2013). Two non-synonymous coding variants are more common in Tibetans than Han Chinese, who are less well adapted to high altitude. The assumption is that these variants are associated with an increase in PHD2 activity. While *EGLN1* transcript levels are not altered in lymphocytes of Tibetans at low altitude (Petousi et al., 2014), the PHD2 [Asp4Glu; Cys127Ser] variant enriched in Tibetans exhibits a lower Km for oxygen than wild-type PHD2 (Lorenzo et al., 2014).

Interestingly, another candidate gene for selection common to both Tibetans and Andeans is *EDNRA* , which encodes endothelin receptor type A, a validated drug target for the treatment of pulmonary hypertension. Neither have been phenotypically linked with pulmonary vascular phenotype in these indigenous populations. Other biologically plausible candidates for a role in the pulmonary vascular response to hypoxia common to both Tibetan and Andean studies include *NOS1*and *NOS2* , *IGFBP1* and *IGFBP 2* , *VEGFA* and*IL6* . That is not to say that other candidates identified, such as*PPARA* , encoding the nuclear peroxisome proliferator-activated receptor α (PPARα), *PRKAA1* and *BRINP3* are not relevant to pulmonary vascular homeostasis.

But distinguishing their direct contribution, as opposed to adaptation through energy metabolism and muscle function (Horscroft et al., 2017), is important if any are to be exploited as pharmacological targets for pulmonary hypertension.

Genetic studies in animals

Studies of animals living at high-altitude (Witt & Huerta-Sanchez, 2019) support the role of *EPAS1* in adaptation to hypoxia and have suggested a number of other candidates but apart from studies in the yak and cow, little has been reported that links genetic variants to pulmonary vascular homeostasis.

An advantage of animal studies is the potential to cross-breed strains to isolate the gene of interest. This strategy was used successfully to understand the relative resistance of the rat F344 strain to pulmonary hypertension from chronic hypoxia when compared with the WKY strain (Zhao et al., 2015). Successive backcrossing of offsping from a F344xWKY cross onto the more sensitive WKY parental strain resulted in the introgression of DNA from the F344 strain (carrying the resistance gene) onto the WKY background. Sequencing the introgressed DNA demonstrated a stop-codon in *SLC39A12* , which encodes the zinc transporter, ZIP12. Mutation of this gene in the WKY strain rendered the WKY strain more resistant to hypoxia-induced pulmonary hypertension. ZIP12 is over expressed in lungs from patients with PAH and it remains to be seen whether pharmological inhibition of ZIP12 is a therapeutic strategy for these patients. Studies are ongoing to develop pharmacological tools for further experimental studies.

Genetic studies in patients with PAH

Insight into the genetic basis of PAH was first provided through studies of families and identified mutations in *BMPR2* , bone morphogenetic protein type 2 (Deng et al., 2000; International et al., 2000). More recent studies of carefully curated cohorts of patients with idiopathic PAH have confirmed that *BMPR2* is the most commonly affected gene associated with PAH but identified rare variants in a number of other genes that appear to be pathogenic (Southgate, Machado, Graf & Morrell, 2020; Zhu et al., 2019). Several converge on the TGF-B signalling pathway but other novel pathways are also implicated, including deleterious changes in genes encoding potassium channels (*KCNK3*and *ABCC8*) and transcription factors (*TBX4* and*SOX17*) (Southgate, Machado, Graf & Morrell, 2020). These genetic insights are informing drug development, and studies to evaluate restoring BMPR2 signalling or the imbalance in BMP-TGF-b signalling are in progress.

Aside from Chuvash polycythaemia, where arguably the main impact is on haematocrit, the rare variants identified in PAH families and cohorts do not affect genes that have emerged from genetic studies in high-altitude subjects and animals. This might reflect the different underlying pathology; remodelling is severe in PAH than HAPH. It might reflect the heterogeneity of clinical pulmonary hypertension; that it is the end stage of a number of pathological processes that converge on this phenotype. Nonetheless, comparison of the genetically driven pathways that emerge from studies in PAH and high-altitude subjects remains a potentially fruitful strategy, as one may inform the other, as work to exploit pharmacologically the HIF signalling pathway exemplifies.

Pharmacological exploitation of the HIF signalling pathway

Genetic mutations that operate from birth may invoke developmental influences that contribute to a protective phenotype. Nonetheless the enrichment of genes for *EPAS1* and *EGLN1* in adapted highlanders has given added weight to evaluating HIF signalling as a target for pulmonary hypertension. Bigham and Lee (Bigham & Lee, 2014) discuss the possible combinations of loss-of-function and gain-of-function *EPAS1* and *EGLN1* alleles to explain Tibetan adaptation and suggest it may be necessary to target both HIF2α and PHD2 to reproduce the Tibetan phenotype (Figure 1). To date, most progress has been made with exploring inhibition of HIF2α as a drug target for pulmonary hypertension.

As a transcription factor that activates gene expression through protein-protein interactions, HIF2α was generally regarded as intractable for small molecule inhibition. But biophysical studies have revealed that the inner core of the PAS-B domain of HIF2α possesses a hydrophobic cavity that can bind small molecules that allosterically disrupt its dimerization to ARNT and thereby block transcriptional activity (Scheuermann,

Tomchick, Machius, Guo, Bruick & Gardner, 2009). A cellular screening approach for HIF2α inhibitors has identified Compound 76 (C76) as a useful tool compound (Zimmer et al., 2008). C76 inhibits HIF2α translation by binding to iron regulatory protein-1 that enables association with the IRE in the 5'UTR, even during hypoxia exposure. This association continuously represses HIF2α translation. C76 has shown promising results in both the prevention and treatment of pulmonary hypertension in rodent models (Dai et al., 2018) but demonstrates only micromolar potency *in vitro* , and is not orally bioavailable.

A series of highly selective, orally bioavailable HIF2α inhibitors with a favourable safety and tolerability profile have been developed, with reported efficacy in rodent cancer models and metastatic clear cell renal cell carcinoma (ccRCC) patients (Chen et al., 2016; Cho et al., 2016; Courtney et al., 2018; Wallace et al., 2016; Wehn et al., 2018). PT2567 has shown efficacy in rodent models of pulmonary hypertension (Hu et al., 2019).

Conclusion

Exposure of the adult mammalian pulmonary circulation to alevolar hypoxia triggers rapid onset vasoconstriction and subsequent structural remodelling, co-ordinated through an integrated series of molecular events. Genetic studies in humans and animals coupled with genetically manipulated animal models have the potential to elucidate the conductors of this "orchestra". HIF-2α (encoded by *EPAS1*), PHD2 (encoded by*EGLN1*) and ZIP12 provide proof-of-concept for this strategy, although translating this into a new drug therapy for pulmonary hypertension remains to be demonstrated. Moreover, these studies provide more insight into the vascular remodelling than the vasoconstrictor component of the hypoxic response. Perhaps not surprising, phenotype studies of residents at high altitude have focused more on accessible physiological characteristics, such as haemoglobin and oxygen saturation, than more challenging measurements, such as pulmonary physiology. Such data remain few. The potential rewards give fresh impetus to the need for further detailed genotype-phenotype studies addressing pulmonary vascular adaptation of high altitude residents.

Competing Interests: None

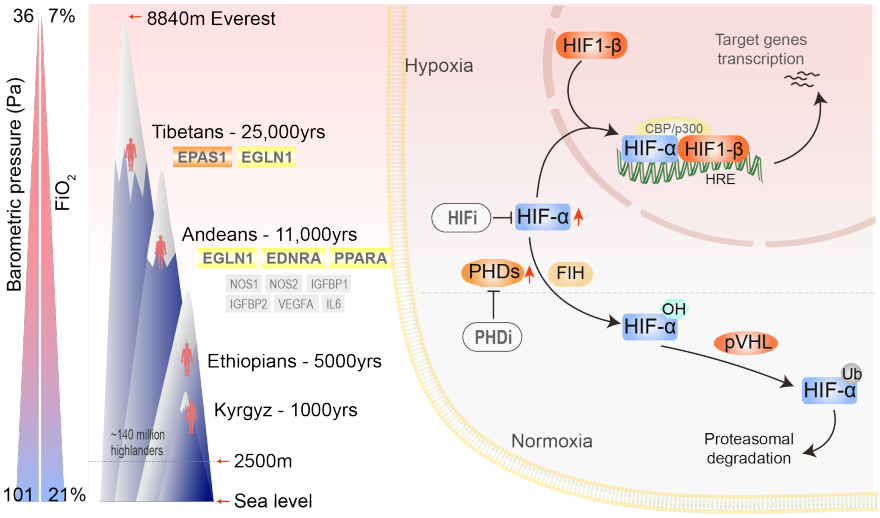

Figure 1. There are 140 million highlanders living above 2,500m. On the very left the barometric pressure and the FiO2 are shown at increasing altitudes. Note that the air's oxygen concentration always stays at 21%, even at high altitude. The populations are depicted at their average residence altitude and the time since they have occupied that region is given in years. Genetic studies in populations adapted to life at high altitudes, e.g. Tibetans, Andeans, Ethiopians and Kyrgyz, revealed the importance of EPAS1 gene (encodes hypoxia-inducible factor-2α, HIF2α) and EGLN1 gene (encodes hypoxia-inducible factor prolyl

hydroxylase 2, PHD2), supporting efforts to pharmacologically manipulate HIF pathway as a treatment for pulmonary hypertension. A schematic on the right shows degradation of hydroxylated HIFα subunits in normoxia via the von-Hippel Lindau (VHL) pathway. In hypoxia, HIFα stabilization and dimerization with HIFβ occurs, together with a histone-acetyltransferase (p300), binds to hypoxia-response elements (HRE) in DNA, initiating or enhancing transcription of target genes. HIF inhibitors (HIFi) with specific activity against HIF-2α are investigated as potential treatment for pulmonary hypertension. PHDi:prolyl hydroxylase inhibitors; FIH: factor inhibiting HIF.

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