High Altitude Illness and Adaptation: Hints from Proteomics

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Abstract

Proteomics has been successfully applied to many biomedical fields. Among these, high altitude (HA) proteomics has attracted researchers in past few years. Adaptation to hypobaric hypoxia (HH) is necessary for humans under several environmental, physiological and pathological situations. High altitude environmental challenges are major concern for sojourners, soldiers and mountaineers not only during their initial days of induction to the hypoxic environment but during long term residency. Hypoxia triggers oxidative stress and damage to proteins, lipids and DNA. This condition can occur due to environmental factors (altitude), toxicity or diseases. Sometimes body overcome the stress caused by hypoxia through unique process of self recovery and adaptation. Identifying the molecular variables playing key roles in this process are of prime importance in understanding the mechanisms for counteracting the negative effects oxygen deprivation that may lead to several diseases. Proteins play vital role in these physiological conditions and can be considered as molecular signature for altitude adaptation. Several proteins have been identified and categorised on the basis of their role in hypoxia and HA adaptation. Recent advances in proteomics have opened new vistas to understand the functional aspect of differential expression of proteins. Linking existing proteins together, finding newer proteins and their functional analysis can provide better understanding of the physiological mechanism underlying adaptation to hypoxia. New information coming from the analysis of the novel proteins offers opportunity to further analyse the cellular responses to HH.

Keywords: Hypobaric hypoxia; Gene regulation; Proteomics; Hypoxia associated proteins; Two-dimensional electrophoresis; Mass spectrometry

Introduction

The human habitation includes diverse range of environments; some of the most extreme of these environments are found at high altitudes. The three major HA regions with long-term resident populations are the Himalayan region/Tibetan Plateau (Tibetans, Ladakhis, and Sherpas), the Andes (Quechua and Ayamara), and Northern Africa (Ethiopians) [1-3]. Residents of these regions descend from a long line of highland ancestors who have lived there for generations despite the physiological challenges associated with chronic oxygen deprivation. The study of indigenous HA residents therefore, provide the opportunity to identify molecular markers that might have played a role in hypoxic adaptation. Hypoxia is a pathophysiological condition which occurs due to lower of circulating oxygen levels in body [4,5]. Hypoxia can either be due to environment (HA), diseases or any kind of cellular toxicity. Despite having different underlying causes, all types of hypoxia have similar responses and consequences (Figure 1). Therefore in the present article we have discussed all those proteins whose expression has been found altered in either of the hypoxic conditions. More extensive studies are required for validating many of these proteins as HH markers. Hypobaric hypoxia can be divided into four different phases depending upon the altitude and corresponding reduced oxygen pressure (Table 1) [6-8]. Along with the altitude, the pressure of oxygen in atmosphere and level of saturation of haemoglobin in the blood of the residents play important role in determining the HH stage and degree of hypoxia [9]. Different degree of impaired functions of brain and other organs are noticed at various stages of HH [10]. These stages of hypoxia are important in understanding the major behavioural and physiological changes taking place at any particulars stage [11].

Adaptation to altitude has two different aspects. The first refers to residents living at high altitude (high landers) and second refers to people who ascend to HA regions for relatively shorter period of time. The high landers do not show many complications because they have adapted to these environments as the result of residing there for generations. People, who ascend to HA show variable degree of complications due to sudden lowering of oxygen content in atmosphere. The second aspect is of importance as it has clinical implications. Altitude sickness, some times leading to even fatal conditions, can develop in these people and therefore identification of molecular markers is required for development of either therapeutic or predictive medicines. High altitude pulmonary edema (HAPE), high altitude cerebral edema (HACE) is common diseases associated with HA.

Genomic changes during hypoxia have been extensively investigated; hypoxia-induced changes in the proteome have gained the interest of researchers in past few years. In past few years proteome-wide alterations during hypoxia have been reported through many proteome studies [12-15] discussed in further sections.

The cellular responses to hypoxia are rather complex and characterized by alterations in expression of a number of stress related proteins, proteins associated with energy metabolism and angiogenic pathway proteins such as HSPs, NOS, GAPDH, VEGF, Erythropoietin [16-18]. Above all, the most protein which first comes when hypoxia is discussed is HIF (hypoxia inducible factor) which is a transcriptional regulator for most of the hypoxia related genes. It is well established that HIF-1, controls cellular oxygen homeostasis and plays a key role

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in energy metabolism [19]. HIFs represent the link between oxygen sensors and effectors at the cellular, local and systemic level [20]. An account of many altered proteins which can be perspective biomarkers has been presented in this article.

**Proteomic analysis**

The field of proteomics has grown many folds in post genomic era. It is now possible to identify and quantify large number of proteins using proteomic technologies. Proteomics is generally accepted as complementary to genetic profiling and comparative proteomic studies have been critical in identifying systems/ networks of proteins that contribute to disease progression. A significant aspect of proteomics lies in its ability to be quantitative and provide high-throughput strategy for global profiling of the changes in protein expression. An account of proteome coverage.

The protein expression levels are measured directly, rather than being inferred from abundance of the corresponding mRNAs that are imperfectly correlated to protein concentration due to variable rates of post-transcriptional modifications, differences in mRNA stability and translational efficiency [21,22]. The techniques commonly used for global analysis of protein expression include two-dimensional electrophoresis (2DE) in association with mass spectrometry (MS) [23], multidimensional chromatography coupled with tandem MS [24,25] and chip technologies coupled with either antigens or antibodies [26].

Two-dimensional gel electrophoresis coupled with matrix-assisted laser desorption/ ionization (MALDI) was the most extensively used approach for identifying and quantifying the changes in protein expression [27] during early proteomic era and will continue to be an integral part of proteomic research for the foreseeable future [28]. However, electro spray ionization spectroscopy coupled with MS [29]; multidimensional chromatography coupled to MS provides better separation power and extended concentration range to analyse complex samples than the one dimensional liquid chromatography coupled with mass spectrometry [30]. Surface-enhanced laser desorption-ionization (SELDI) mass spectrometry is another technique that has been used and has solved the biomarker conundrum [31]. Different labelling methods such as iTRAQ (isobaric tags for relative and absolute quantitation), mTRAQ (mass differential tags for relative and absolute quantitation), TMT (tandem mass tags) and metabolic labeling/SILAC (stable isotope labeling of amino acids in cell culture) are being used currently for quantitative proteomics [32].

Exosomal proteome profiling (considered as potential multi-marker cellular phenotyping tool) is a recent advancement in proteomics [33]. Biofluids contain cell-secreted proteins in membrane-bound vesicles, termed exosomes. These small vesicles (50-100 nm in diameter), so far under-represented in body fluid proteome studies, originate from multivesicular bodies/endosomes and are being exploited in secretome proteomic research. The low abundant and membrane-bound proteins play important role in cell-to-cell communication. The protocols to isolate and purify exosomes and advances in mass spectrometry-based proteomics have helped in analysis of exosome content. Malignancy leads to hypoxia, resulting in release of exosomes from cancer cells. This may be responsible for malignant transformation of normal recipient cells and may result in malignant cell proliferation and migration [34-36].

The quantative proteomics has been coupled with bioinformatics tools to develop the shotgun proteomics. Bioinformatics part in shotgun proteomics comprises different databases, search engines, softwares such as msPecT, Mascot, OMSSA, SEQUEST, MyriMatch, XTandem, Trans-Proteomic Pipeline (TPP), MSBlender and PepArML [37]. The advanced proteomic tools, databases and bioinformatics software and their applicability [32] together has driven the field of proteomics. This approach is being applied for proteomic analysis of many of the known diseases to correlate proteins profile with pathogenesis and identify potential biomarkers. Proteome coverage for these biomarker candidates has been depicted in Figure 2. In addition to these serum/plasma proteins have been extensively utilized in biomarker studies and are equally important. A very recent plasma proteome study of HH induced rat model using 2 DE coupled with MALDI-TOF with peptide mass fingerprinting (PMF) and MS/MS analysis followed by database searching describes a number altered proteins like transthyretin, peroxiredoxin-2, Glutathione peroxidase3, Apolipoprotein A-1 during HH [38].
Overall steps involved in proteomic analysis of protein from different source like tissue, serum/plasma is depicted in Figure 3 (flow diagram).

Proteomics of hypoxia

By using the different proteomic approaches (above mentioned), it has been possible to analyze differentially expressed proteins and assess the changes in metabolic pathways in response to hypoxia at the tissue level. Comparative analysis of hypoxia related proteins suggests that hypoxia up-regulates or down-regulates a number of proteins in cell type or tissue dependent manner. Major consequences and responses of hypoxia depicted in Figure 1 are result of the alterations in expression of these proteins.

Hypoxia regulators

Activation of transcription factors is one of the rapid cellular events that take place in response to cellular stress caused by hypoxia. Heat shock factor-1 (HSF-1) and HIF-1 represent two separate classes of transcription factors that are specifically and rapidly activated in response to cellular stress [39,40].

HIF-1: HIF-1 is the key molecule involved in hypoxia. It is a heterodimeric complex composed of HIF-1α and HIF-1β subunits. The dimer binds to specific DNA enhancer sequences and regulates the expression of target genes. Both HIF-1α and HIF-1β are constitutively expressed under normal oxygen conditions (normoxia), however, HIF-1α gets quickly degraded before its dimerization with HIF-1β [41]. Normoxic HIF-1α degradation is mediated by a series of hydroxylations and ubiquitinations that tag HIF-1α for disposal through proteosomes [42,43]. Following a shift to low oxygen environment, the α-subunit gets stabilized and translocates to nucleus. HIF-1α has an oxygen-dependent domain (ODD) that is important for regulation of its stability. Under normoxic conditions the ODD is recognized by the product of Von Hippel Lindau (pVHL) suppressor gene, a component of a multisubunit ubiquitin-protein ligase complex that tags the subunit with polyubiquitin to promote HIF-degradation.

Heat shock factor-1 (HSF-1): Heat shock factor-1 (HSF-1) is important for the induction of heat shock proteins (HSPs) such as HSP-27, and HSP-70 which in turn act as chaperones preventing the aggregation and inactivation of essential cellular proteins [39].

HIF-1α is transcriptionally inactive and is rapidly degraded by the ubiquitin (Ub) proteasome pathway. In hypoxia, HIF-α undergoes protein stabilization and translocation from the cytoplasm to the nucleus, where it dimerizes with HIF-β to induce the transcription of related genes.

HIF-2: HIF-2 is closely related to HIF-1 and both are related key transcriptional regulators of hypoxic response [48]. Preferential binding of HIF-2α to HRE (hypoxia responsive elements) at the Epo (erythropoietin) locus has been proposed by Ratcliffe [49]. While HIF-1α binds to isolated HRE, HIF-2α binds preferentially at the HRE within the native Epo3' enhancer. Similar to HIF-1α, HIF-2α also escapes proteolysis during low oxygen condition and dimerizes with HIF-1β, recruits coactivators, and activates transcription via HREs [49]. Recently it has been reported that HIF-2 requires another regulatory factor, the upstream stimulatory factor 2 (USF2), for hypoxic transcriptional response, for activation of HIF-2 target genes [50]. This suggests that HIF-2 also has also regulatory effect on hypoxia responsive gene. However, the role of HIF-1 has been more explored as key regulator of hypoxia than HIF-2. It’s another isoform, HIF-3 is also reported which is a distantly related isoform generated by alternate splicing and encodes for a polypeptide that antagonizes HRE-dependent gene expression [49].

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Figure 3: Overview of steps involved in proteomic analysis.
are vital in maintaining cellular homeostasis and are up-regulated during hypoxic condition [12]. In unstressed cells, HSF-1 binds to HSP-90 and remains in inactive form. During cellular stress, HSF-1 rapidly dissociates from HSP-90, gets trimerized and is translocated to the nucleus, where it activates the transcription of genes that contain the characteristic heat shock response element such as HSP-27 and HSP-70 thereby regulating hypoxia [51-52]. Induction of the heat shock pathway during hypoxia is also dependent on HIF-1 [53]. Recently it has been reported that HSF-1 is involved in hypoxic tumour progression via regulation of HIF-1 [54].

Erythropoietin (EPO): Erythropoietin is required for the proliferation and differentiation of erythroid progenitor cells to yield RBCs. Its response is elicited in a number of tissues, depending on level of expression of its receptor (EPOR). During low oxygen tension HIF-1 binds to hypoxia response elements and activates the transcription of EPO and other hypoxia-responsive genes [55-56]. The EPOR expression and the biological response to EPO have been observed in endothelial, neuronal, muscle, cardiac and certain other cell types [57]. In neuronal cells, EPORs are induced by hypoxia resulting in increased EPO response due to its binding to EPORs [58]. EPO maintains several activities in cardiovascular system; the induction of NO production by EPO mediated cascade under hypoxic conditions provides one of the explanations for the protection conferred by EPO in myocardial infarction as it may rapidly increase vasodilatation and facilitate effective collateral circulation [59]. Hypoxic-stimulated enhanced erythropoietin secretion is also reported in mice [56]. The regulation of EPO through HIFs has been recently reviewed [60].

Vascular endothelial growth factor (VEGF) is an important growth and permeability factor for endothelial cells and is expressed in lungs at high level. Hypoxia is a potent inducer of VEGF gene expression. The regulation takes place at transcriptional level through activation of HIF-1a and HIF-2a that bind to response element in VEGF gene. In alveolar epithelial cells, hypoxia induces an up-regulation of VEGF mRNA transcripts due to transcriptional activation of VEGF gene without any change in mRNA stability [61]. There are strong evidences to suggest that HIF plays a major role in hypoxia-induced VEGF regulation of VEGF gene expression modulates epithelial cell proliferation and surfactant protein expression [62]. However, the precise role of increased VEGF secretion in overall adaptation process of alveolar epithelial cells in response to hypoxia remains to be fully elucidated. Rehn et al. [63] have reported the hypoxia induced VEGF regulation of murine hematopoietic stem cell function.

### Mitochondrial autophagy

Reactive oxygen species serve as signalling molecules at low level but at high level these damage cell organelles [64]. The most severe response and mechanism during hypoxic adaptation is mitochondrial autophagy due to ROS formation. Mitochondrial autophagy is necessary to protect cell against oxidative stress [65-66]. This process requires HIF-1 dependent expression of BNIP3 (BNIP3 is a pro-apoptotic BH3-only protein which is associated with mitochondrial dysfunction and cell death) and the constitute expression of Beclin-1 and Atg5 (both are regulators of autophagy) [67-68]. In cells subjected to prolonged hypoxia, mitochondrial autophagy has been found to be an adaptive metabolic response which is necessary to prevent increased ROS levels and cell death [69].

The roles of all these proteins in hypoxia have been well established. These are regarded as classical regulators of hypoxia associated genes.

### HIF-independent hypoxia regulation

As discussed HIF-1 and Pur-a are the identified regulators of hypoxia. Hypoxia leads to inflammation and binding of circulating leukocytes to endothelium with resultant induction of hypoxia-responsive genes. Such binding is mediated by monocytes that directly sense hypoxic conditions and respond by inducing expression of the β-2 integrin family of adhesion molecules. Earlier it was thought that induction of β-2 integrin is dependent on HIF-1 [70], however, later studies reported that induction of β-2 integrin can also take place in HIF-1 independent manner through Pur-a. Pur-a binds to HIF-1 independent β-2 integrin promoters under hypoxic condition. Mutagenesis in Pur-a binding site abolishes the ability of the promoters to respond to hypoxia [71]. The role of Pur-a need to be studied further. Recently a hypoxic study on Drosophila melanogaster provided evidences that HIF and non-HIF dependent pathways are complemented by the action of estrogen-related receptor [72]. Surprisingly, the results of this study emphasized more prominent role of HIF-independent pathways than previously thought.

### Categorization of hypoxia evoked proteins

<table>
<thead>
<tr>
<th>Classification</th>
<th>Proteins</th>
<th>Physiological function</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Proteins of energy metabolism including</td>
<td>Glycolytic enzymes</td>
<td>Often referred as hypoxia-associated proteins (HAPs), these proteins include the glycolytic enzyme GAPDH and non-neuronal enolase unique to endothelial cells. Expression of these proteins helps in up regulated tolerance and adaptation to lack of oxygen. This may be one of the mechanisms to overcome the ischemic injury during hypoxia. Increased expression of heat shock proteins have been found in hypoxic condition.</td>
<td>[27,73,74]</td>
</tr>
<tr>
<td>Stress related proteins</td>
<td>Heat shock Proteins (HSPs)</td>
<td>The enhanced synthesis and accumulation of several functionally and compartmentally distinct families of HSPs such as HSP70, HSP90, HSP60, and HSP27 is one of the cellular mechanisms for protection against the stress induced by environmental cues. Inflammatory responses during hypoxia are due to over expression of these proteins.</td>
<td>[75,12,76]</td>
</tr>
<tr>
<td>Membrane-bound proteins that include transporters and receptors</td>
<td>Acid-base transporters, glucose transporter</td>
<td>The influence of intermittent hypoxia exposure on the expression of acid-base transporters in the mouse central nervous system, especially in the cerebellum is the decreased expression of sodium/hydrogen exchanger (NHE) isoform 1 and sodium-bicarbonate co-transporter. Edema of CNS due to hypoxia is related to alteration in these proteins.</td>
<td>[77-79]</td>
</tr>
<tr>
<td>Cytosolic proteins</td>
<td>Antioxidants, signalling cascade proteins</td>
<td>The expression of antioxidants and the proteins associated with signalling cascade is altered in hypoxia. The expression of nitric oxide synthase (NOS) endothelial (eNOS) and inducible (iNOS) forms is increased during hypoxic condition. Overexpression of NOS leads to vasodilatation to minimise the effect of hypoxia.</td>
<td>[80-82]</td>
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Table 2: Categorization of hypoxia evoked proteins.
oxygen metabolism or transport. This includes skeletal muscles where energy metabolism is activity dependent and enhanced by a magnitude with onset of contraction. A study carried out on comparative profile of skeletal muscle proteome of human volunteer natives of Tibet (HA) and low landers reported seven differentially expressed proteins [83]. It was found that glutathione-S-transferase P1-1, Δ2-enoyl-CoA hydratase, phosphoglucone mutase, and myoglobin were significantly overexpressed while NADH-ubiquinone oxidoreductase was only slightly overexpressed in highlanders. On the other hand, glycolaldehyde-3-phosphate dehydrogenase and lactate dehydrogenase were slightly down-regulated in highlanders. From this study it became clear that hypoxia affects energy metabolism pathways. The result of a similar study state that hypoxic induction and reoxygenation of primary human hepatocytes induce protein changes in protein associated with glutamine metabolism, oxidative protection and peroxisomal function [84]. It has been reported that exposure to hypoxia results in reduced skeletal muscle mass [85,86]. At the cellular level the alterations include reduction in number as well as size of mitochondrial volume density and accumulation of lipofuscin, a product of lipid peroxidation. Differential analysis has indicated that proteins involved in iron transport, TCA cycle, oxidative phosphorylation, and oxidative stress responses are significantly decreased in hypoxia. Hypoxia markers such as HIF-1α and pyruvate dehydrogenase kinase 1 (PDK1) remained unaltered [87]. Another acute vs. chronic hypoxia study reports that repeated acute hypoxic exposure have the potential to slow down muscle atrophy and even to stimulate muscle mass accretion when coupled to resistance exercise as is the case with occlusion training [88]. Recently it has been found that chronic hypobaric hypoxia in rats increases fast-twitch and slow-twitch limb muscle force and fatigue in rats [89]. The analysis of lung proteome during pulmonary arterial hypertension (PAH, which is a common manifestation of sudden HA exposure) in male Sprague-Dawley rats showed differential expression of HSP27, septin 2, tropomyosin β-chain, annexin 3, HSP70, F-actin capping protein, biliverdin reductase and ERp29 (an endoplasmic reticulum protein) in PAH as compared to controls (without PAH) [89]. In another proteomic study conducted on patients with idiopathic PAH it was found that 25 proteins were differentially expressed in lung [12]. These include chloride intracellular channel protein 1, annexin A3, phospholipase C-like protein 5, and serum deprivation-response protein. These proteins could be associated with cell growth, proliferation, intracellular trafficking and signalling pathways. The changes in some of these proteins have been validated by Western blotting. The validated proteins include pericentrin, haptoglobin and chloride intracellular channel protein 1 (CLIC-1) and chloride intracellular channel protein 4 (CLIC-4) [13]. Acute hypoxia also induces apoptosis of pancreatic β-cells by activation of the unfolded protein response and upregulation of CHOP (C/EBP homologous protein) which is a pro-apoptotic transcription factor, indicating insulin metabolism impairment that ultimately results in altered energy metabolism [69]. In another report it was found that chronic intermittent hypoxia (CIH) and chronic sustained hypoxia (CSH) differentially regulate left ventricular inflammatory and extracellular matrix responses. The levels of matrix metalloprotein (MMP-9) and fibronectin are lowered in CIH, suggesting decreased inflammatory status whereas in case of CSH extracellular matrix and adhesion molecule were found to be up regulated indicating an increased inflammation during CSH [90]. Another study reports an increase in cysteine oxidation in endothelial cells in response to acute hypoxia which could be reversed by reoxygenation [91]. Surprisingly, humoral immune system modulation was also observed following single and repeated exposure to hypoxia in Eurasian perch [91]. In a recent study on comparison of human plasma proteome between sea level and high altitude natives it was reported that there is alteration in number of anti inflammatory proteins in highlanders [14]. The up-regulated proteins are vitamin D-binding protein, hemopexin, alpha-1-antitrypsin, haptoglobin b-chain; apolipoprotein A1, thymosin-β4 and hemoglobin b-chain, the down-regulated proteins are transferrin, complement C3, serum amyloid, complement component 4A and plasma retinol binding protein. These results show that the adaptive mechanism sustains the inflammation balance in HA natives exposed to HH. Another exploratory proteomic analysis of human volunteers under hypoboric hypoxia and acute mountain sickness showed the abundance of antioxidant proteins such as peroxiredoxin 6, glutathione peroxidase and sulphhydril oxidase [15]. Hypoxia is present in most solid tumors and is clinically correlated with increased metastasis and poor patient survival. A very recent, in situ hypoxia proteome study on tumor samples presents idea of more than 100 proteins that are found to differentially expressed [92].

All these findings suggest that different cellular responses are evoked in different organs under hypoxic condition (either due to disease or altitude) that lower its ill effect, failure of which leads to serious complications. The hypoxic adaptation involves simultaneous modulation of a number of different pathways. More studies are needed to understand the precise role of these responses and their coordination during hypoxic adaptation.

**Prospective biomarkers for high altitude adaptation**

A biomarker can be defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [93]. Biomarkers are key indicator for molecular or cellular events that link the exposure to specific environmental condition with health. These play important role in understanding the relationship between exposure to environmental chemicals, the development of chronic human diseases, and the identification of subgroups that are at increased risk for disease. Much progress has been made in identifying and validating new biomarkers that can be used in diagnosis and prognosis of certain life threatening diseases. Some of the proteins which can be prospective biomarkers for high altitude adaptation are discussed below. These proteins show marked altered expression during hypoxic exposure.

**Pur-α:** Pur-α is a transcriptional activator protein that has been implicated as a novel hypoxia response factor. It is responsible for coordinated induction of β-2 integrin family of adhesion molecules [71]. Increased abundance of Pur-α has also been found in lungs of the patient with idiopathic PAH [13]. Pur-α expression leads to increased angiogenesis.

**Chloride intracellular channel protein-4:** CLIC-4 has been reported to have role in angiogenesis by supporting the acidification of vacuoles along the intracellular tubulogenic pathway [94]. Increased CLIC-4 expressions was found in human pulmonary artery endothelial cells in PAH that may play a role in pathogenesis of PAH patients and provide novel insights into disease pathogenesis and treatment strategies [13].

**Periostin:** Periostin is a TGF-β inducible, 90-kDa protein that advances the atherosclerotic and rheumatic cardiac valve degeneration by inducing angiogenesis. Up-regulation of this protein has also been reported in patients with idiopathic PAH [13]. Periostin mediates the increased pro-angiogenic activity of gastric cancer cells under hypoxic conditions [95].
Macrophage migration inhibitory factor (MIF): It has been reported that hypoxia stimulates the expression of MIF in human vascular smooth muscles via HIF-1α dependent pathway [96]. In this study the expression of both, the MIF mRNA and protein both found to be up-regulated as early as 2 hours after cultured human VSMCs were exposed to moderate hypoxic condition (3-10% O2). MIF has also been found to be one of the mediators of hypoxia-induced pulmonary hypertension [97].

HSP-70: Preinduction of HSP-70 promotes hypoxic tolerance and facilitates acclimatization to acute hypobaric hypoxia in mouse brain up-regulation of HSP-70 has also been reported in lung tissues of male Sprague-Dawley rats after induction of PAH [12]. HSP-70 protects intestinal epithelial cells from hypoxia/reoxygenation injury via a mechanism involving mitochondrial pathways [98].

Rho-A: Hypoxia activates Rho-A and subsequently Rho-A kinase activity [99]. It has been reported that prolonged hypoxia increases Rho-A and ROS signaling and activation in pulmonary artery smooth muscles and endothelial cells [100]. Under hypoxic condition, RhoA activation promotes VEGF secretion which has a prominent role in hypoxia [101]. However, the mechanism by which Rho-A is activated during hypoxia is not well established.

p53 and hypoxia

The tumor suppressor activity of p53 is well documented. Its expression is induced by hypoxia. The expression and activity of p53 under hypoxia bears relationship to tumor inducing action of mutated p53, as the microenvironment in tumor tissue is hypoxic [102,103]. Earlier studies have demonstrated an indirect interaction between p53 and HIF-1α, probably through MDM2 [104]. However, in a later study evidences were presented for direct interaction between p53 and HIF-1α. Two p53-binding sites have been identified within the oxygen dependent degradation domain of HIF-1α, suggesting that one molecule of HIF-1α interacts with one p53 dimer [105]. Recent studies have shown that hypoxia-mediated p53 activation is through the ATR-Chk1-MDMX-14-3-3y signaling pathway [106]. Hypoxia activates ATR and then Chk1, which in turn phosphorylates MDMX at S367, suppressing its activity and activating p53 [106]. It has been found that wild type p53 rescues mutated p53 and leads to hypoxic tumor regression [68]. This novel role of wild type p53 as molecular chaperone raises the possibility that p53 self-rescue may be involved in reversing the oncogenic function of mutated p53 in cancer cells. Further, it has been shown that there is a novel chaperone-like activity that resides in p53 N-terminal domain. This study might have significance in understanding the role of p53 NTD in its self stabilization, conformational activation and apoptosis under heat-stress conditions [107]. Although the chaperone and apoptotic potential of wild type p53 are comparable with that of NTD, the tumor regression potential of NTD is about one half of wild type p53 which suggests that additional in vivo p53-bound modulating factors may also be involved in tumor regression [106]. The properties of p53 and further understanding of the relationship between p53 and HIF may provide new insight into both hypoxic adaptation and cancer therapy.

Conclusion and Future Prospects

Hypoxia is a generalised term referring to low oxygen condition. The high altitude hypoxia can not be completely separated as a pathological condition either from hypoxia due to cellular toxicity or a disease. Studies have shown that there are marked differences between genetics, proteomics and physiology among high land residents and low land residents. People who fall in between the i.e. those who ascend to HA regions necessarily face sickness ranging from altitude sickness to either normalization or turning into more serious conditions like HAPE, HACE etc. Proteomics has been helpful in understanding the role of differentially expressed proteins. Hypoxia affects a number of cellular processes and has been recognized as the major contributing factor in a variety of pathological and physiological processes. Single or whole proteome analysis of expression and posttranslational modifications of proteins in response to hypoxia have revealed that complex interplay of molecules takes place in multiple cellular compartments that helps the cell in coping with reduced oxygen availability for cellular functions. During hypoxia, the genes involved in various physiological processes such as metabolism, signal transduction, transcription, apoptosis, cell cycle and ubiquitination pathways are differentially expressed. Several proteins have been identified and categorised, however, the precise role of many these proteins is not fully understood. Proteomics provides new directions for better understanding of cellular mechanisms associated with the response and adaptation to hypoxia. Further, some of the proteins which are only indirectly related to hypoxia may also prove to be important candidate biomarker for HH. Hypoxic condition, whether due to some pathological condition (diseases) or physiological changes (altitude), cannot be distinguished completely from each other and therefore the proteins found to be altered under one condition will have role under other condition also. At present genomic profiling related to high altitude adaptation and sickness is much ahead of proteomic findings. It is therefore relevant to focus on hypoxia proteomics to find newer proteins and relate them with existing one to get better understanding of unique mechanism of adaptation. Such studies will also have implications in better management of hypoxia related health problems.

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