Effects of altitude and duration of different hypoxia exposure on HIF-1α in the rat brain, lung and heart tissues

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Abstract

Objective: To explore the effects of hypoxic at different altitude and duration on the expression of HIF-1α in the rat tissues.

Methods: A total of 72 Wistar rats were randomly divided into 6 groups (n=12 in each group): normoxic group and 5 experimental groups. Normoxia group was placed in the normal circumstances of Lanzhou (1500 m altitude), rats in the experimental groups were exposed to hypoxia in the hypobaric hypoxic animal experiment chamber simulating the altitudes of 3000, 4500, 6000, 7500, and 8000 m for 12 h, respectively. HE staining was conducted to observe the pathological changes of hippocampus tissues and the expression of HIF-1α in the rat brain, lung and heart tissues under the condition of hypoxia was detected by RT-PCR and Western Blotting. A total of 72 Wistar rats were randomly divided into 6 groups (n =12 in each group): normoxic group and 5 experimental groups. Rats in the experimental groups were exposed to hypoxia in the hypobaric hypoxic animal experiment chamber simulating the altitudes of 7500 m for 6, 12, 24, 36 and 72 h, respectively. Detect the same indicator after dissection.

Results: We demonstrated that with the increased of hypoxia altitude and prolonged hypoxia, the expression of HIF-1α in the rat showed rising tendency ($P<0.05$) and the severe damage was revealed by pathological biopsy.

Conclusion: We conclude that the expression of HIF1α in the rat was enslaved to the different altitude and duration of altitude hypoxia exposure.

1. Introduction

Hypoxia, defined as a state of abated O_2 level below normal values (< 1% O_2) (1), occurs under various physiological (metabolism, development, adaptation to high altitudes by increased red blood cells, wound healing) as well as pathological (hereditary erythrocytosis, inflammation, cancer, traumatic shock, pulmonary arterial hypertension (PAH), acute mountain sickness (AMS), obstructive sleep apnea)conditions (2–4). Hypoxia with deficiency in oxygen supply can restrict their function of organs, tissues and cells and cause the various high altitude illnesses. As The Lake Louise AMS Score Consensus Committee stated “AMS is the most common form of acute altitude illness and typically occurs in unacclimatized persons ascending to altitudes > 2500 m” (5). Additionally, Hypoxia inducible factor (HIF) is recognized as a critical modulator of the transcriptional response to hypoxic stress. Besides its adaptive functions in physiological processes, recent studies have revealed important roles for HIF in pathological processes such as a series of high altitude illnesses including AMS, high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE) (6).

HIF is heterodimeric transcriptional factor, composed of the oxygen-regulated HIF-α subunits (including HIF-1α, HIF-2α and HIF-3α) and the constitutively expressed HIF-β subunits (also known as aryl hydrocarbon receptor nuclear translocator (ARNT)).These subunits belong to the family of the basic helix–loop–helix (bHLH) and PER-ARNT-SIM (PAS) domain-containing transcription factors. The bHLH-

PAS domains mediate DNA binding and dimerization; the other domains in the HIF-α subunits include a unique O2-dependent degradation domain (ODDD) and two transactivation domains: the N-terminal activation domain (NAD) and C terminal activation domain (CAD) (Fig. 1) (7–10). Although all the subunits are involved in regulation of the transcriptional response following exposure to hypoxia (11), the core contributing components are the HIF-1α and HIF-1β subunits.

Under normoxia conditions, proline residues within the oxygen-dependent degradation domain of HIF-1α subunits are hydroxylated by a family of 2-oxoglutarate-dependent enzyme prolyl hydroxylase domain-containing proteins 1, 2, and 3(PHD1-3), which consequently activates the ubiquitin ligase system and interacts and binds the von Hippel-Lindau tumor suppressor protein (pVHL), following by the ubiquitination and proteasomal degradation (12). Moreover, asparaginyl hydroxylation of HIF-1α by factor inhibiting HIF (FIH) disrupts its interaction with transcriptional coactivators, CREB-binding protein (CBP) and p300.Under hypoxia, the activity of PHD and FIH is inhibited, HIF-1α can translocate into nucleus where they heterodimerize with HIF-1β and bind to DNA at hypoxia-response element (HRE, 5'-(A/G) CGTG-3') (Fig. 2) (13–17), thereby transactivation of HIF target genes. HIF-1α confers sensitivity and specificity for hypoxic induction of HIF-1 transcriptional activity.

It is well known that the brain, lung and heart are the most aerobic organs in the body, and therefore, both consume a great portion of respiratory oxygen. However, the changes of the nervous, pulmonary and cardiovascular system to high altitude hypoxia are variable, depending on individual predisposition, oxygen level and the exposed duration at high altitude. This study focused on simulating the effects of altitude and duration of different altitude hypoxia exposure on HIF-1α in rat brain, lung and heart tissues, and monitoring the changes of HIF-1α protein expression after different hypoxia exposures, so as to explore whether HIF-1α can be an attractive treatment target.

2. Materials And Methods

2.1. Animals

Adult male Wistar rats (200 ± 20) g of specific pathogen-free were purchased from the Laboratory Animal Center of the 940th Hospital of Joint Logistic Support Force of Chinese People's Liberation Army (License No. SYXK (Army) 2017-0046).

Animals were housed twelve per cage in plastic cages with sawdust bedding, and maintained on a 12 h light/dark cycle at a room temperature of (25 ± 1) °C. The rats were allowed ad libitum access to standardized pellet food and water. 10% chloral hydrate was used to anesthetize the rats before the collection of tissues and blood for the experiment. All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the 940th Hospital of Joint Logistic Support Force of Chinese People's Liberation Army (LZ20120907-35). All efforts were made to minimize animal sufferings and to use only the number of animals necessary to produce reliable scientific data.

2.2. Reagents

TaKaRa MiniBEST Universal RNA Extraction Kit, TB Green™ Premix Ex Taq™ II and PrimeScript™ RT Master Mix were provided by Takara Biotechnology (Dalian, China). 4% Paraformaldehyde was obtained from Biosharp (Hefei, China). BCA Protein Assay Kit, SDS-PAGE Gel Kit, SDS-PAGE loading buffer (4× (with DTT)), Non-Fat Powdered Milk, SDS, Tris, Glycine, 10 × TBST were purchased from Solarbio (Beijing, China). RIPA and PMSF were provided by Beyotime (Shanghai, China). Polyvinylidene fluoride (PVDF) microporous membrane was provided by Immobilon®-P Transfer Membranes (MA, USA). Medical X-ray film was provided by Carestream (Xiamen, China);PageRuler Prestained Protein Ladder was acquired from Thermo Scientific(Shanghai, China)Primary antibody against β-actin and secondary antibodies, horseradish-peroxidase-conjugated goat anti-mouse IgG (H + L) and horseradish-peroxidase-conjugated goat anti-rabbit IgG (H + L) were provided by Zhongshan Jinqiao Biotechnology (Beijing, China), while primary antibody against HIF-1α was obtained from Abcam (MA, USA). WesternBright ECL was acquired from Advansta (California, USA).Other reagents were of commercially available analytical grade and so on.

2.3. Experimental design

The total of 72 SPF male Wistar rats were randomly divided into normoxic groups and 3000 m, 4500 m, 6000 m, 7500 m, and 8000 m hypoxia group with 12 animals in each group. Except for the normoxia group placed in the normal circumstances of Lanzhou (1500 m above sea level), the other five groups were placed in the hypobaric hypoxic animal experiment chamber (FLYDWC50-C, Guizhou Fenglei Aviation Ordnance Co., Ltd)for hypoxic exposure at different altitudes. Anatomic samples were obtained after exposure for 12 h, Hematoxylin and eosin (HE) staining was conducted to observe the pathological changes of brain, lung and heart tissues and the expression of HIF-1α was detected by PCR and Western Blotting. According to the above, the tissues damage and the most obvious hypoxic exposure elevation of HIF-1α expression are fixed.

Then 72 SPF male Wistar rats were randomly divided into 6 groups (12 in each group): normoxic group, hypoxia 6 h, 12 h, 24 h, 36 h and 72 h group. Except for the normoxia group placed in the normal circumstances of Lanzhou (1500 m above sea level), the other five groups were placed in the hypobaric hypoxic animal experiment chamber at an altitude of 7500 m for hypoxic exposure at different durations, the samples were dissected and the pathological changes of brain, lung and heart were observed by HE staining and the expression of HIF-1α was detected by PCR and Western Blotting.

2.4. Hematoxylin and eosin staining

Three different tissues which were expected to be involved in pathogenesis were collected and fixed in 4% paraformaldehyde at room temperature for 72 h. Tissues were brain, lung and heart. Then tissues were embedded in paraffin wax. Sections of 5 μm were cut for hematoxylin and eosin staining. These sections were examined using light microscopy. Pathological evaluation was conducted by experienced pathologists in a single-blind way.

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCR was used to determine the mRNA expression of HIF-1α. Total RNA of brain, lung and heart samples were extracted using TaKaRa MiniBEST Universal RNA Extraction Kit following the manufacturer's instructions. The concentration and purity of the total RNA of different samples were measured using the Nanodrop 2000 spectrophotometer. Meanwhile, TB Green™ Premix Ex Taq™ II and PrimeScript™ RT Master Mix were utilized for reverse transcription of cDNA and qRT-PCR of HIF-1α and GAPDH mRNA. Relative expression was calculated on the basis of the $2^{-\Delta\Delta Ct}$ method.

The following primers were used: HIF-1α (5'-AAACCTAATGTTCTGCCTAC-3', 5'-GGATGTTAATAGCGACAAAGT-3'), GAPDH (5'-ATGACATCAAGAAGGTGGTG-3', 5'-CATACCAGGAAATGAGCTTG – 3').

2.6. Western blotting analysis

The brain, lung and heart tissues were homogenized in RIPA lysis buffer (1% Triton X-100, 1% deoxycholate.0.1% SDS) containing a protease inhibitor cocktail (RIPA: PMSF = 100:1). After determining the protein concentration by the BCA method, the proteins were separated by using a Bio-Rad Tris-Glycine Gel system according to the manufacturer's instructions. Then, proteins in the gels were blotted to polyvinylidene difluoride (PVDF) membranes, followed by blocking with 5% non-fat milk. After rinsing, membranes were incubated at 4°C overnight with primary antibodies against HIF-1α and β-actin (dilution, 1:1000). After rinsing, secondary antibody conjugated to horseradish peroxidase (dilution, 1:5000) at room temperature for 2 h. After rinsing, immunoblots were detected with an ECL Plus chemiluminescence reagent kit and the bands were scanned with the ChemiDoc-It2 610 chemiluminescence imaging system and optical densities were analyzed by Image J software and β-actin was used as the quality control.

2.7. Statistical analysis

The results of multiple experiments are presented as the mean ± standard deviation. Statistical analysis was performed using SPSS 24.0 statistical software by one-way analysis of variance (ANOVA) followed by the LSD, Student-Newman-Keuls (S-N-K) and Duncan's post hoc test. Image-Pro Plus software was used for processing images. $P < 0.05$ was considered to indicate a statistically significant result.

3. Results

3.1. Acute hypobaric hypoxia leads to pathological damage of brain, lung, and heart tissue

Whenever the defense mechanisms become exhausted for too prolonged hypoxia tissue injury ensues with consequent cellular damage. Due to their low regenerative potential, apoptosis surges as a reliable marker of brain, lung, and heart tissue injury, with associated changes in HIF-1α.

Under light microscopy, it could be seen that the brain tissue structure of normoxia group was normal which showing that the size of neurocyte was uniform with intact shape and neat arrangement, and the large ratio of nucleoplasm was observed, even the light blue-violet and round or elliptical nucleus on the center and the nucleolus was clearly visible. The degree of damage was mild in the hypoxia 3000 m and the hypoxia 4500 m group, mainly manifested as cellular edema. Swelling and nerve cells death occurred after hypoxia with irregularly stained cytoplasm and swollen or disappeared nucleus. In the hypoxia 6000 m, 7500 m and 8000 m groups, the nucleus of neurocyte which soma shrink could be constricted into a triangle or a polygon, even chromosomes disappeared and hyperchromatic cytoplasm. The normal cells were decreased in number and the injury of cell aggravated when the altitude of hypoxia increased (Fig. 3 − 1). In the 6 h and 12 h hypoxia groups, the neurocyte damage was minor. In the 24 h, 36 h and 72 h hypoxia groups, the damage was mainly neuronal cell necrotic and apoptosis with nuclear shrinkage deformation and nuclear condensation even disintegration as well as interstitial cells are filled with cellular debris and apoptotic bodies (Fig. 3 − 2). With the prolongation of hypobaric hypoxia, the number of normal cells in brain tissue decreased, and the damage gradually increased.

The morphology and structure of lung tissue cells were normal in the normoxic group, and the alveolar cavity structure was regular and complete with a vacuole-like thin-walled structure showing well defined boundaries. Compared with the normoxic group, the lung tissue structure of rats in hypoxia had pathological changes. With the increase of hypoxia altitude and duration, the hypoxia lung pathological damage was mainly featured with destroyed alveoli, thickened alveoli septum and included alveolar space shrinks, dropsy in different degree and inflammatory cell infiltrate (Fig. <link rid="fig13">3</link>–3; Fig. 3–4).

The myocardial tissue of the normoxic group was smooth and flat, the myocardial cells and muscle fibers were arranged neatly, continuously and without interruption. Some cardiomyocytes in the hypoxia 3000 m, 4500 m and 6000 m groups were lysed to different extents, indeed arranged disorderly, discontinuous, and muscle fibers which had different degrees of fracture and destruction. The myocardial cells in the 7500 m hypoxia group were severely disordered, and the degree of muscle fiber fracture was severe. Cardiomyocytes and muscle fibers were close to normal at 6 h, 12 h, 24 h and 36 h after hypoxia, while in the 72 h hypoxia group, the myocardial cells were slightly disordered, with few muscle fiber breaks and inflammatory cell infiltration (Fig. 3–5) (Fig. 3–6).

3.2. Effect of hypoxia on expression level of HIF-1α mRNA in tissues

3.2.1 The expression of HIF-1α mRNA in brain tissue after hypoxic exposure

HIF-lα mRNA in the normoxic group have a certain amount of expression, hypoxia group expression was more obvious ($P < 0.05$) in brain.

The expression of HIF-1α mRNA was increased significantly in brain tissue after hypoxic exposure at different altitudes (duration 12 h) as presented in Fig. 4A when compared with the normoxic group ($P < 0.05$), especially when the altitude at 7500 m. At different point-in-time, HIF-1α mRNA of brain showed an analogous trending enhancement with significant differences with normoxic group ($P < 0.05$) (Fig. 4B).

3.2.2 The expression of HIF-1α mRNA in lung tissue after hypoxic exposure

After exposure to hypoxia, the HIF-1α mRNA expression level in the lung of the hypoxia groups was significantly higher than that in normoxic group ($P < 0.05$). Compared with the normoxic group, the expression of HIF-1α in the 6000 m and 8000 m groups was significantly increased ($P < 0.01$) (Fig. 4A).

As shown in Fig. 4B, there was a significant increase in the HIF-1α mRNA in the lung in the hypoxia groups, suggesting a marked rise in HIF-1α generation in different anoxic duration, particularly exposure to hypoxia after12, 24 and 72 h($P < 0.01$).

3.2.3 The expression of HIF-1α mRNA in heart tissue after hypoxic exposure

PCR revealed differences in the expression of HIF-1α mRNA in the heart of normoxic and hypoxia rats. Compared with the normoxic group, the expression level of HIF-1α in the hypoxia 3000 m, 4500 m, 6000 m and 7500 m was significantly different from that in the normoxic group ($P < 0.05$), and the expression in the hypoxia 8000 m group was the most obvious one ($P < 0.01$).

After exposure to hypoxia at 7500 m in different anoxic duration, the expression of HIF-1α in each hypoxic group was significantly different from that in the normoxic control group ($P < 0.05$), and the expression in the hypoxia 72 h group was the most.

3.3. Effect of hypoxia on expression level of HIF-1α mRNA in tissues

3.3.1 The expression of HIF-1α protein in brain tissue after hypoxic exposure

The expression of HIF-lα protein in the normoxic group have few expression, but hypoxia group expression was more obvious ($P < 0.01$) in brain except 3000 m.

The expression of HIF-1α protein was increased significantly in brain tissue after hypoxic exposure at different altitudes (duration 12 h) as presented in Fig. 5A when compared with the normoxic group ($P < 0.01$), especially when the altitude at 8000 m. At different time points, HIF-1α of brain showed an analogous trending enhancement with significant differences with normoxic group ($P < 0.01$) (Fig. 5B).

3.3.2 The expression of HIF-1α protein in lung tissue after hypoxic exposure

After exposure to hypoxia, the HIF-1α protein expression level in the lung of the hypoxia groups was significantly higher than that in normoxic group ($P < 0.05$), particularly exposure to hypoxia in the 6000 m,7500 m and 8000 m($P < 0.01$) (Fig. 5A).

As shown in Fig. 5B, there was a significant increase in the HIF-1α protein in the lung in the hypoxia groups, suggesting a marked rise in HIF-1α generation in different anoxic duration ($P < 0.01$).

3.3.3 The expression of HIF-1α protein in heart tissue after hypoxic exposure

Compared with the normoxic group, the expression level of HIF-1α in the hypoxia 6000 m, 7500 m and 8000 m was significantly different from that in the normoxic group ($P < 0.05$), and the expression in the hypoxia 8000 m group was the most obvious one ($P < 0.01$).

After exposure to hypoxia at 7500 m in different anoxic duration, the expression of HIF-1α in each hypoxic group was significantly different from that in the normoxic control group ($P < 0.01$), and the expression in the hypoxia 6 h group was the slightly remarkable compare with other hypoxia groups.

4. Discussion

Degree of brain, lung and heart tissues pathology injury is strongly related to the hypoxia altitude and duration. Morphologically, the degree of the brain, lung and heart tissues damage was aggravated after the difference hypoxia. Positive correlations were found between the degree of the brain, lung and heart damage and the hypoxia altitude and duration, meanwhile, HIF-1α showed an increasing trend as the altitude of hypoxia increased and the duration of hypoxia increased.

In the state of hypoxia, the pathological damage of brain and lung tissue is more serious than that of myocardial tissue. It may be because the sensitivity of myocardial tissue is not higher than in brain and lung tissue when the individual acute exposed to plateau. Whereas a recent report by Li showed that myocardial tissue damage was aggravated with the prolongation of exposure to hypoxia after 3 d, 7 d, 15 d, and 30 d. It was observed that the arrangement of myocardial cells was disordered, the boundary of muscle fibers was blurred, and inflammatory cells infiltrated (18). In Li's study, the hypoxia duration of the rats was at least 72 h but the hypoxia duration of cardiomyocytes in our experiment was at most 72 h, suggesting that the heart should be considered for the hypoxia tolerance in the late-stage study.

This process is currently considered to be produced at the cellular level endogenous protective mechanism. Between different organs of the mechanism of hypoxic acclimatization have not the same. Elevation of HIF-1α at the early terms after hypoxic exposure established in rats seems to promote rapid and more effective adaptation of these rats to hypoxia.

This is a “productivity system” when the rapid degradation of HIF-1α and the stability regulation of its transcriptional activity for rapid perceptive response to hypoxic. In our results, a complex HIF “switch” mechanism to regulate the cellular or whatever responded to hypoxia with specific altitudinal and temporal roles. Different hypoxia altitude and duration tests showed that the expression of HIF-1α emerged an upward trend along with altitude and duration. When stand in the middle and low altitude region for a short time, the organism has an adaption to the hypoxic via mobilizing HIF-1α to activate many hundreds of target genes in the signal pathway (19), meanwhile, HIF-1α is in dynamic equilibrium on the account of continuously entering the nuclear transcription and translation on the one hand, on the other hand continuously degrading by PHD or others recognition simultaneously, now HIF-1α is a beneficial signal factor for the body. Nevertheless, when stay at high altitude for long-term, the dynamic balance of HIF-1α is destroyed assuming that the synthesize rate is greater than the degradation rate. Excrescent HIF-1α causes hyperirritable target genes reaching the peak of the physical performance, thereby multiple negative impacts damaging the tissue cells, destroying the cell structure and growth. At this moment, HIF-1α has become a harmful signal factor to the body.

Now that HIF-1α is a production at the cellular level against hypoxic exists in the animal brain, heart, liver and other tissues, organs and cells, which can increase the tolerance to hypoxia. Going higher, oxygen needs to be transported preferably to the life-sustaining organs: brain, heart, and lungs. An increase of HIF-1α, with the increase of altitude and duration, is a double-edged sword for organizations such as the brain.

Brain is a type of high oxygen consumption organs. Despite it constitutes only 2% of total body weight, it accounts for about 20% of whole O2 consumption. Furthermore, brain is characterized by high energy consumption and low energy reserve, particularly susceptible to hypoxic conditions (20). As a consequence, the brain is extremely sensitive to hypoxia. So when people during the rapid ascent to high altitudes, likely lead to the High-altitude cerebral edema (HACE) which is a life-threatening illness (21). HIF-1α expression enhanced the hypoxia adaptation capability of the rat through the regulation of expression of multiple genes. According to our findings, these experiments showed an increased expression of HIF-1α by hypoxic of altitude and duration increased.

As well, the lung is also responsible for oxygen uptake and the pulmonary vascular responses that ensure adequate blood flow to the alveoli in response to low oxygen tensions but likely results in pulmonary hypertension (PH) under chronic hypoxic (22). HAPE is induced by the high altitude hypoxia, a serious life-threatening acute mountain sickness, their common characteristics as pulmonary hypertension and vascular permeability changes, and prolonged exposure to alveolar hypoxia (due to chronic lung disease or residence at high altitude) is a significant cause of pulmonary hypertension (23).The results suggest that in lung tissue with low expression under normoxia HIF-1α, along with raising the level of hypoxic of altitude and duration, HIF-1α expression level gradually increased, indicating that HIF-lα in the regulation of hypoxic acclimatization process. To understand the precise role of the HIF system in lung development and in lung diseases such as pulmonary hypertension and HAPE, and the identification and creation of

tools to manipulate HIF levels in vivo, hold promise for better therapeutic options to treat lung diseases caused by high altitude.

With exposure to hypoxia, the cardiac output and heart rate rise acutely (24), however, acute altitude exposure may trigger serious cardiovascular adverse events in subjects at risk (25). In our study, analysis of HIF-1α protein level in the rat heart, the results showed a slight difference between the normoxic group and hypoxic rats. When compared with normoxic group, HIF-1α protein level slightly increases in hypoxia group. The highest level of HIF-1α protein is seen in the hypoxia group at 8000 m and hypoxia for 72 hours group. Hypoxia induces gradual upregulation of HIF-1α, which in turn induces the expression of adaptive genes erythropoiesis, vascular endothelial growth factor, glucose transporter-1, nitric oxide synthase (26, 27). This means that there is an increase in HIF-1α protein level in hypoxic conditions and systemic adaptation occurs gradually at the cellular level in hypoxic conditions (27). The increase is thought to occur because tissues begin to adapt to hypoxic conditions.

5. Conclusion

A reduction in oxygen delivery to the brain, lung and heart, hypoxia, is sensed and responded to by the HIF and its family of proteins, by regulating the oxygen-dependent signaling cascade and subsequent response. Hypoxia is one of the main drivers of metabolic change in HACE, HAPE, ischaemic disease and myocardial infarction, and we therefore suggest that HIF may be an attractive therapeutic target.

Declarations

Ethics approval and consent to participate

All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the 940th Hospital of Joint Logistic Support Force of Chinese People's Liberation Army (LZ20120907-35). All efforts were made to minimize animal sufferings and to use only the number of animals necessary to produce reliable scientific data.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or used during the study appear in the submitted article, if you want to more, please contact authors.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Competing interests

The authors declare that they do not have any conflict of interests related to the contents of this article.

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Authors' contributions

Xiao-lin Li designed, analyzed, wrote, processed and reviewed the manuscript.. Mao-xing Li is guarantor of integrity of entire study and study design. Xiu-yu Tian and Lan Wu helped in resources, materials, data collection and literature search Li-Ping Chen, Wen-di Tao and Zhi-qiang Yang designed, supervised and analyzed the manuscript. All authors read and approved the final manuscript.

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Figures

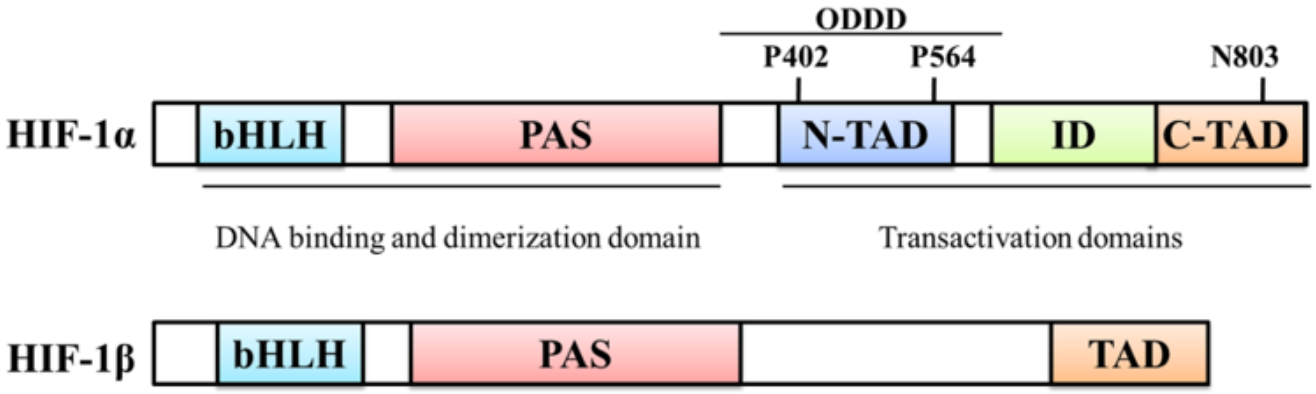

Figure 1

he structure of HIF-1α and HIF-1β. HIF-1α and HIF-1β belong to bHLH-PAS protein family, which contain three residues that are targets for regulatory hydroxylation. P402 and P564 are targeted by the prolyl hydroxylase domain (PHD) enzymes and N803 by FIH. HIF-1α contains two transactivation domains (C-TAD and N-TAD), whereas HIF-1β has only one TAD.

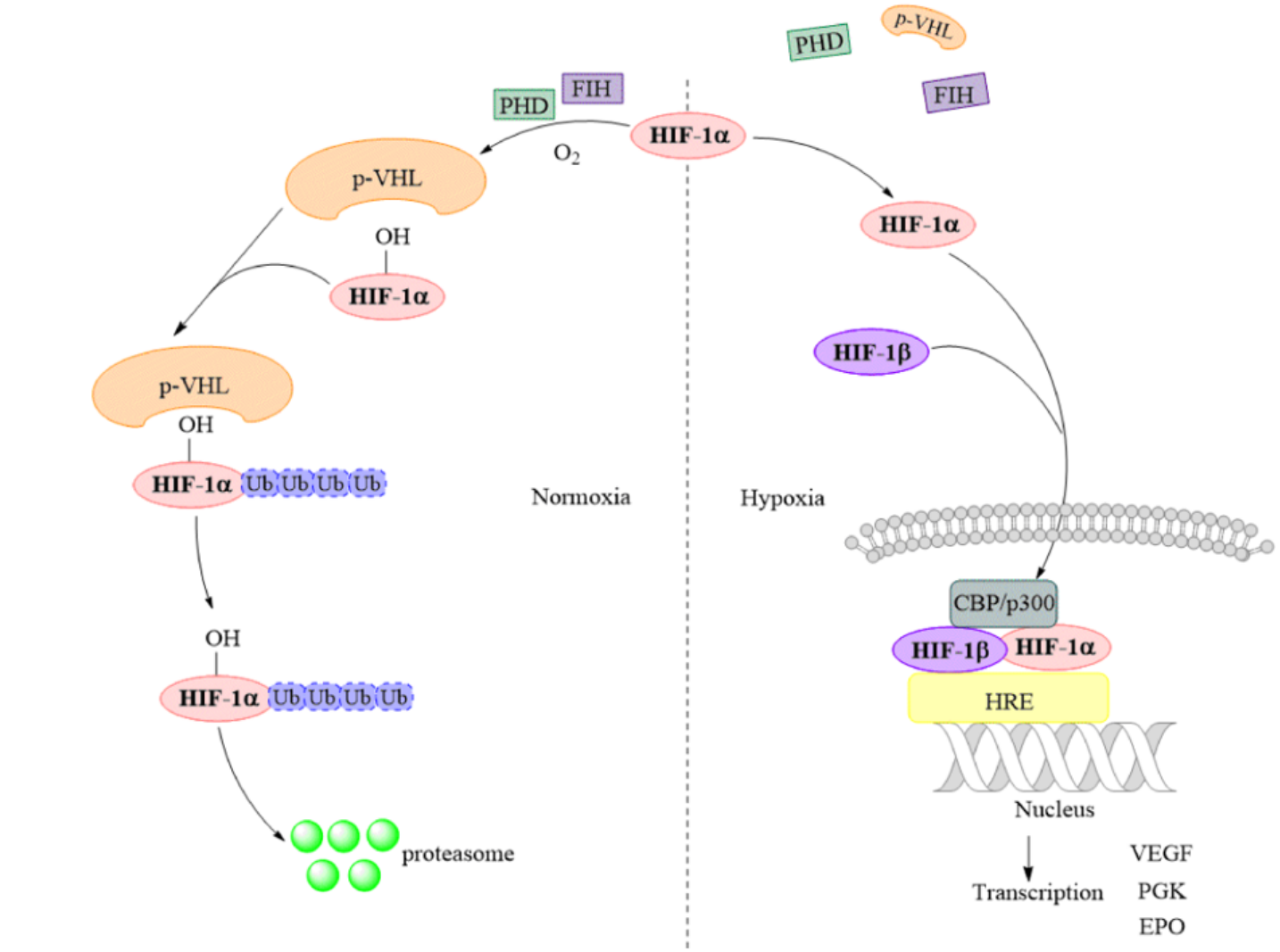

Figure 2

Regulation of HIF-1 by oxygen-dependent prolyl and asparaginyl hydroxylation respectively

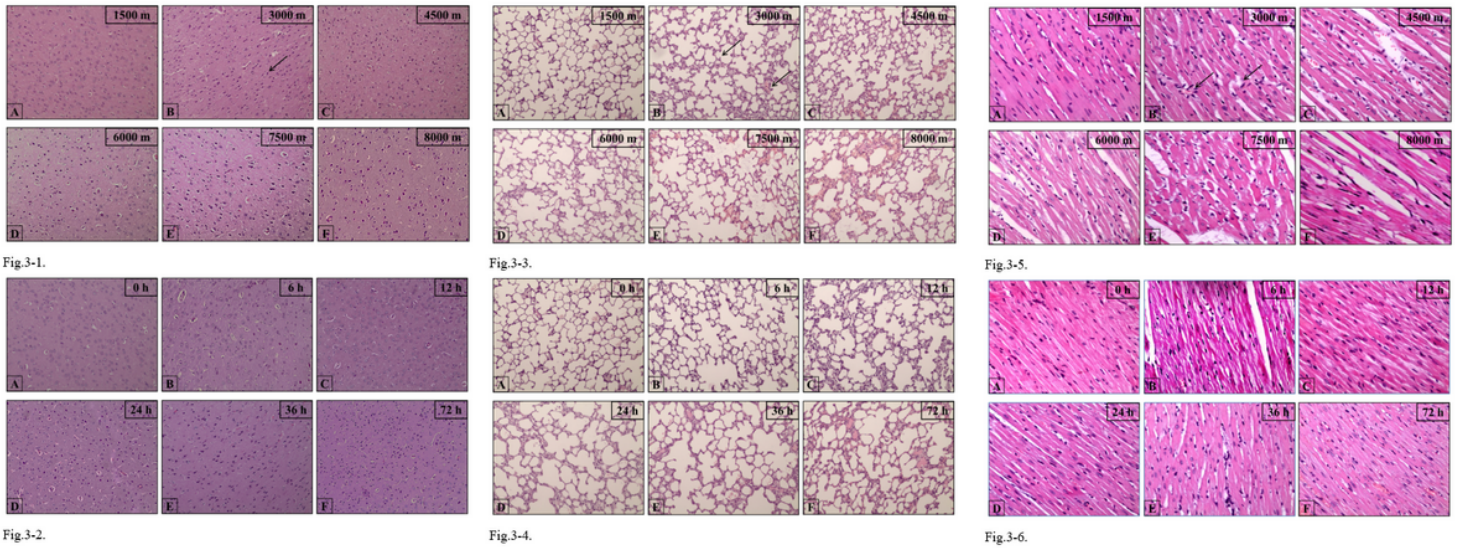

Figure 3

3-1. Histochemical HE staining of brain tissue in rats after simulated hypoxia exposure at different altitudes (HE, 100×). (A): Normoxic group; (B): Hypoxia group at 3000m; (C): Hypoxia group at 4500m; (D): Hypoxia group at 6000m; (E): Hypoxia group at 7500m; (F): Hypoxia group at 8000m. 3-2. Histochemical HE staining of brain tissue in rats after simulated hypoxia exposure at different time (HE, 100×). (A): Normoxic group; (B): Hypoxia for 6 hours group; (C): Hypoxia for 12 hours group; (D): Hypoxia for 24 hours group; (E): Hypoxia for 36 hours group; (F):Hypoxia for 72 hours group. 3-3. Histochemical HE staining of lung tissue in rats after simulated hypoxia exposure at different altitudes (HE, 100×). (A): Normoxic group; (B): Hypoxia group at 3000m; (C): Hypoxia group at 4500m; (D): Hypoxia group at 6000m; (E): Hypoxia group at 7500m; (F): Hypoxia group at 8000m. 3-4. Histochemical HE staining of lung tissue in rats after simulated hypoxia exposure at different time (HE, 100×). (A): Normoxic group; (B): Hypoxia for 6 hours group; (C): Hypoxia for 12 hours group; (D): Hypoxia for 24 hours group; (E): Hypoxia for 36 hours group; (F): Hypoxia for 72 hours group. 3-5. Histochemical HE staining of heart tissue in rats after simulated hypoxia exposure at different altitudes (HE, 200×). (A): Normoxic group; (B): Hypoxia group at 3000m; (C): Hypoxia group at 4500m; (D): Hypoxia group at 6000m; (E): Hypoxia group at 7500m; (F): Hypoxia group at 8000m. 3-6. Histochemical HE staining of heart tissue in rats after simulated hypoxia exposure at different time (HE, 200×). (A): Normoxic group; (B): Hypoxia for 6 hours group; (C): Hypoxia for 12 hours group; (D): Hypoxia for 24 hours group; (E): Hypoxia for 36 hours group; (F): Hypoxia for 72 hours group.

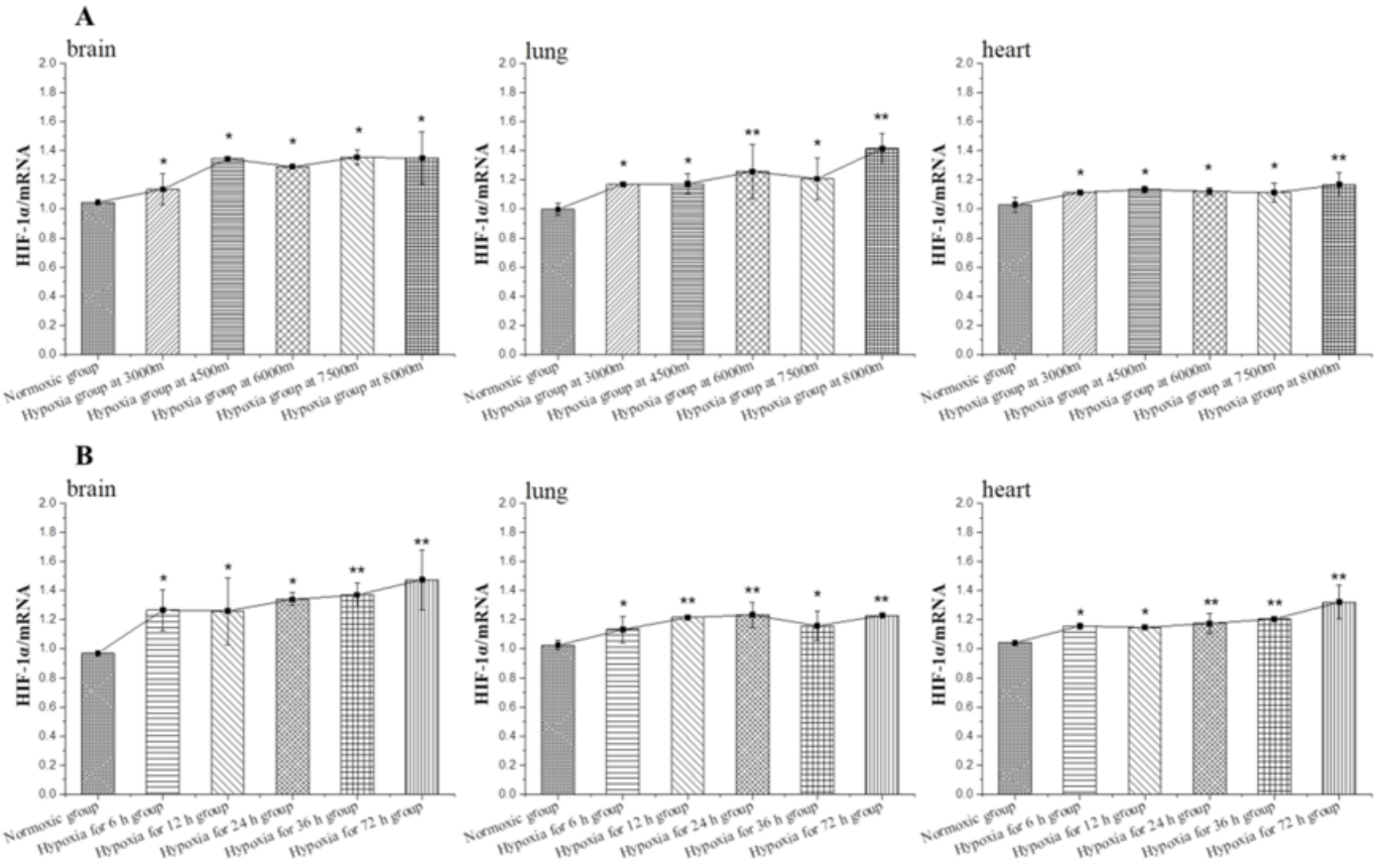

Figure 4

(A). The mRNA levels of HIF-1α in brain, lung and heart tissues after simulated hypoxia exposure at different altitudes (duration 12 h). (B). The mRNA levels of HIF-1α in brain, lung and heart tissues after simulated hypoxia exposure at different duration (7500 m altitude). All experiments were performed at least three times. Results are presented as the mean ± standard deviation (n=10). *P<0.05, **P<0.01, hypoxia groups vs. normoxic group.

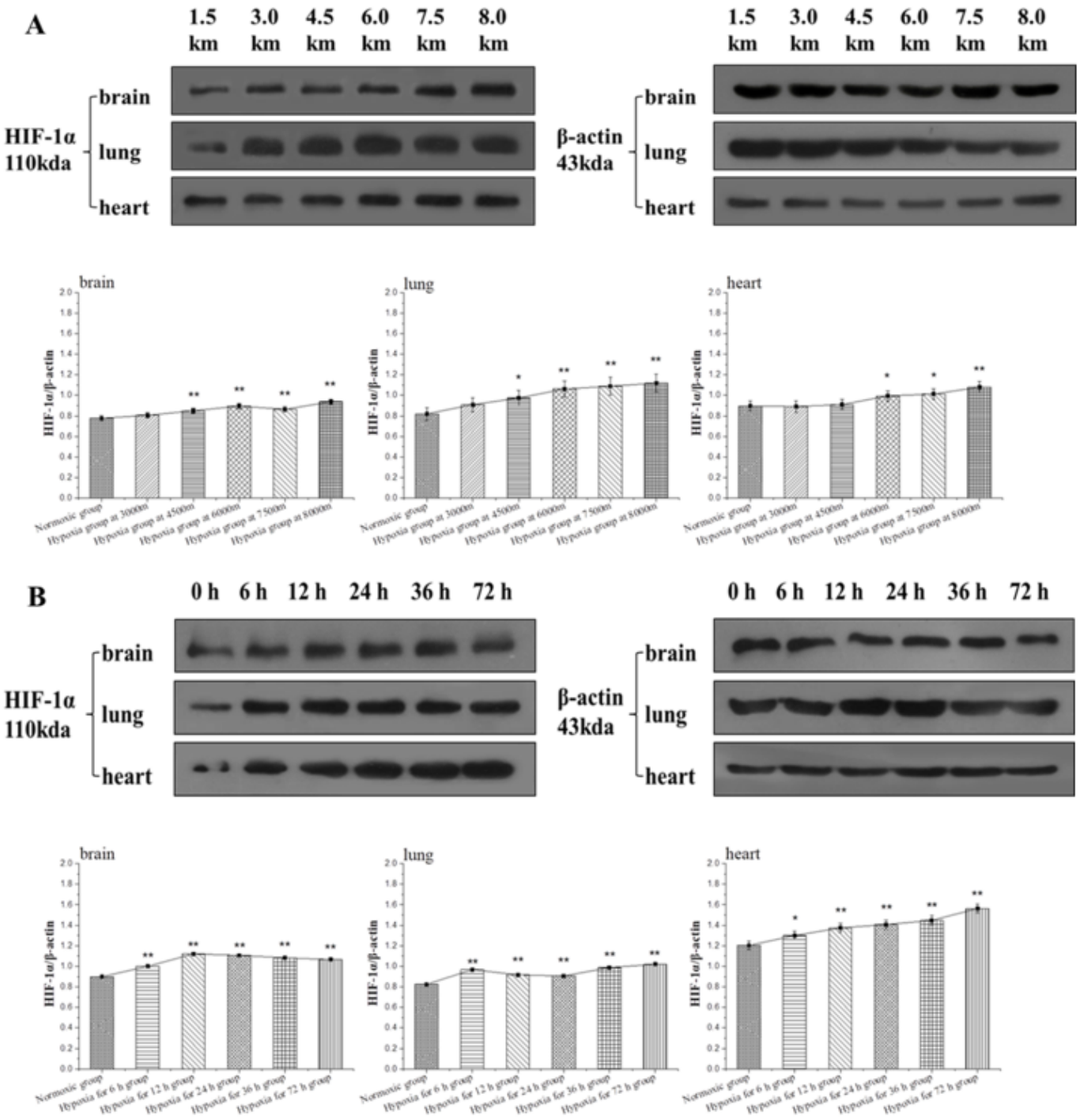

Figure 5

(A). The protein expressions of HIF-1α and β-actin in brain, lung and heart tissues were detected by western blotting after simulated hypoxia exposure at different altitudes (duration 12 h). (B). The protein expressions of HIF-1α and β-actin in brain, lung and heart tissues were detected by western blotting after simulated hypoxia exposure at different duration (7500 m altitude). The relative protein expression was shown as a ratio compared with the internal (n=10, each group).*P<0.05; **P<0.01, hypoxia groups vs. normoxic group.