Potential Candidate Genes for Alveolar Hypoxia Identified by Transcriptome Network Analysis

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Key Words: *alveolar hypoxia; regulatory network; transcriptome network analysis.*

Summary. *Background. Alveolar hypoxia is an important condition related to many disorders such as chronic pulmonary hypertension, pulmonary vasoconstriction, and pulmonary vascular remodeling. The aim of present study was to disclose the biological response and the potential transcriptome networks regulating the hypoxia response in the lungs.*

Materials and Methods. In this study, the microarray dataset GSE11341 was used to construct a regulatory network and identify the potential genes related to alveolar hypoxia. In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) term enrichment analyses were also performed.

Results. Hypoxia inducible factor 1 alpha (HIF-1α), peroxisome proliferator-activated receptor gamma (PPARγ), and nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-κB) were to be the hub nodes in the transcriptome network. HIF-1α may regulate potassium voltage-gated channel, shaker-related subfamily, member (5KCNA5), solute carrier family 2 (facilitated glucose transporter), member (1SLC2A1), and heme oxygenase (decycling) 1 (HMOX1) expression through the regulation of membrane potential, glucose metabolism, and anti-inflammation pathways. HMOX-1 mediates signaling pathways that relate to NF-κB. CCND1 (cyclin D1) expression could be regulated by PPARγ and HIF-1α via the cell cycle pathway. In addition, new transcriptional factors and target genes, such as phosphofructokinase (PFKL, liver), aldolase A (ALDOA, fructose-bisphosphate), and trefoil factor 3 (intestinal) (TFF3), were also identified.

Conclusions. Transcriptome network analysis is a helpful method for the identification of the candidate genes in alveolar hypoxia. The KEGG pathway and GO term analysis are beneficial in the prediction of the underlying molecular mechanism of these identified genes in alveolar hypoxia.

Introduction

Alveolar hypoxia is a pathological condition when alveolar epithelial cells are exposed to much lower oxygen tensions due to high altitude or the consequence of hypoventilation related to a central nervous disorder, obstructive airway diseases, or pulmonary edema from heart failure or acute lung injury (1).

There are 2 general mechanisms that cells invoke during hypoxia to prevent oxygen depletion. First, cells attempt to maintain ATP synthesis by increasing anaerobic glycolysis. Hypoxic cells can upregulate pyruvate kinase and phosphofructokinase, both glycolytic enzymes, and increase lactate production (2). In addition, hypoxia has been shown to stimulate glucose transport into cells, which is in association with an increase in the glucose transporter, GLUT1 (3). In addition to the up-regulation of glycolysis, alveolar epithelial cells induce vascular endothelial growth factor (VEGF) by HIF-1 during hypoxia (4). The increase in VEGF stimulates angiogenesis, which increases oxygen delivery. Second, cells down-regulate ATP consumption pathways, such as Na^+ channels, Na^+-K^+-ATPase activity, NF-κB, and protein synthesis, in order to decrease cellular respiration rates and oxygen demand (5). However, the prolonged exposure (days to weeks) to low oxygen concentrations results in the development of chronic pulmonary hypertension, pulmonary vasoconstriction, pulmonary vascular remodeling, and inflammation, which are often characterized by the accumulation of extracellular matrix proteins and hemoxygenase 1 (HO-1) (6).

However, the molecular mechanism has not been completely understood yet. Advances in molecular genetics and computational biology have led to the development of innovative methods that enable the analysis of differential gene expression profiles. A DNA microarray technology represents a powerful tool for the rapid, comprehensive, and quantitative analysis of gene expression profiles of normal/disease states and developmental processes (7). The expression levels of thousands of genes can thus be quantified simultaneously with this technology (8).

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This technology has successfully been applied to investigate many physiological mechanisms in health and disease (9, 10). A high-throughput microarray experiment has also been designed to analyze genetic expression patterns and identify the potential target genes for alveolar hypoxia (11, 12).

In this study, we aimed to apply the same approach to disclose the biological response and the potential transcriptomics networks regulating the hypoxia response in the lungs. Furthermore, the relevant target genes and pathways in the network were also analyzed to interpret the potential mechanisms in response to the alveolar hypoxia based on previous reports.

Material and Methods

Data Source

Affymetrix Microarray Data. One transcription profile of GSE11341 (13) was obtained from a public functional genomics data repository Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/, accessed on February 19, 2011), which is based on the Affymetrix GPL96 platform data (Affymetrix Human Genome U133A Array). Human pulmonary microvascular endothelial cells were grown on sterile tissue culture dishes in EGM-2MV endothelial growth medium according to the manufacturer's instructions (cat. no. CC-3202, Lonza, formerly Cambrex). At the beginning of the experiment, the medium was changed, and the dishes were transferred to a hypoxia chamber (Coy Labs) and cultured at 1% O_2, 5% CO_2, and 94% N_2 for 3, 24, or 48 hours. The cells cultured in a normoxia incubator (21% O_2, 5% CO_2, and 74% N_2) were used as control cells. At the end of the experiment, the cell medium was removed, and the cells were washed with a phosphate buffer solution and thoroughly lysed in RLT buffer (Rneasy Mini Kit, Qiagen). Three independent hypoxic time course experiments were carried out in lung cells. Eight hypoxia (1% O_2, 2 for 3 hours, 3 for 24 hours, and 3 for 48 hours) and 3 normoxia (21% O_2) chips were applied to identify differentially expressed genes (DEGs) (13).

Pathway Data. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a collection of online databases dealing with genomes, enzymatic pathways, and biological chemicals (14). The PATHWAY database records the networks of molecular interactions in the cells and variants of them specific to particular organisms (http://www.genome.jp/kegg/). A total of 130 pathways, involving 2287 genes, were collected from the KEGG.

Regulation Data. There are approximately 2600 proteins in the human genome that contain DNA-binding domains, and most of them are presumed to function as transcription factors (TFs) (15). The combinatorial use of a subset of the approximately 2000 human TFs easily accounts for the unique regulation of each gene in the human genome during development (16).

These TFs are grouped into 5 super class families based on the presence of conserved DNA-binding domains. The TRANSFAC database contains data on TFs, their experimentally proven binding sites, and regulated genes (17).

The Transcriptional Regulatory Element Database (TRED) has been built in response to the increasing needs of an integrated repository for both cis- and trans-regulatory elements in mammals (18). The TRED has done the curation for transcriptional regulation information, including TF binding motifs and experimental evidence. The curation is currently focusing on the target genes of 36 cancer-related TF families.

A total of 774 pairs of regulatory relationships among 219 TFs and 265 target genes were collected from the TRANSFAC (http://www.gene-regulation.com/pub/databases.html); 5722 pairs of regulatory relationships among 102 TFs and 2920 target genes were collected from the TRED (http://rulai.cshl.edu/TRED/). After combining these 2 regulation datasets, 6328 regulatory relationships among 276 TFs and 3002 target genes were collected.

Methods

Analysis of Differentially Expressed Genes. The limma method (19) was used to identify DEGs for the GSE11341 dataset. The original expression datasets from all conditions were processed into expression estimates using the Robust Multiarray Average method with the default settings implemented in Bioconductor, and then the linear model was constructed. The DEGs only with the fold change value greater than 2 and the *P* value less than 0.05 were selected. The corrected *P* value (FDR) was calculated by using the Benjamini and Hochberg method (20).

Coexpression Analysis. For demonstrating the potential regulatory relationships, the Pearson correlation coefficients (PCC) were calculated for all pair-wise comparisons of gene expression values between TFs and the DEGs. The regulatory relationships with a PCC of greater than 0.6 were considered significant.

Analysis of Gene Ontology. The Biological Networks Gene Ontology tool (BiNGO) (21) determines the significant enrichments of GO categories among a class of genes of interest using the hypergeometric distribution. The BiNGO was used to identify the overrepresented GO terms of a biological process in a set of genes with the *P* value of <0.05 and the count of >2.

Construction of Regulation Network. Using the regulation data that were collected from the TRANSFAC and TRED databases, the relationships between differentially expressed TFs and their differentially expressed target genes were matched.

Based on the above 2 regulation datasets, we built the regulation networks by Cytoscape (22) to combine with the DEGs. Based on the significant relationships (PCC>0.6 or PCC<−0.6) between TFs and their target genes, 33 putative regulatory relationships were predicted between 7 TFs and 29 target genes.

Significance Analysis of Pathway. We adopted an impact analysis that includes the statistical significance of the set of pathway genes but also considers other crucial factors such as the magnitude of change of each gene expression, the topology of the signaling pathway, and their interactions (23). The impact factor (*IF*) is a parameter proposed for pathway analysis that attempts to capture the impact of gene expression profile changes on specific pathways. Individual genes often cannot play a role on their own, but they might play a regulatory role through the interaction with other genes. Therefore, it is meaningful to analyze the significant dysfunctional pathways. *IF*s correspond to the negative log of the global probability of having both a statistically significant number of DEGs and a large perturbation in the given pathway.

The *IF* of a pathway P_i is calculated as the sum of 2 constituents:

$$IF(P_i) = \log(\frac{1}{P_i}) + \frac{\sum_{g \in P_i} |PF(g)|}{|\Delta E| \cdot N_{de}(P_i)} \qquad [1]$$

The first constituent in the equation [1] is a probabilistic component that captures the significance of the given pathway P_i from the perspective of the set of genes contained in it. The P_i value corresponds to the probability of obtaining a value of the statistic used at least as extreme as the one observed, when the null hypothesis (that the pathway is not significant) is true. It is obtained by using the hypergeometric model in which P_i is the probability of obtaining at least the observed number of differentially expressed gene, N_{de}, just by chance (24, 25).

The second constituent in the equation [1] is a functional component that depends on the identity of the specific genes that are differentially expressed as well as on the interactions described by the pathway (i.e., its topology).

The second constituent sums up the absolute values of the perturbation factors (*PF*s) for all genes g in the given pathway P_i.

The PF of a gene g is calculated as follows:

$$PF(g) = \Delta E(g) + \sum_{u \in USg} \beta_{ug} \cdot \frac{PF(u)}{N_{ds}(u)} \qquad [2]$$

In this equation, the first constituent $\Delta E(g)$ captures the quantitative information measured in the experiment of gene expression. The factor $\Delta E(g)$ represents the normalized measured expression change (log2 fold change) of the gene g. The first constituent in the above equation is a sum of all *PF*s of the genes u directly upstream of the target gene g, normalized by the number of downstream genes of each such gene $N_{ds}(u)$ and weighted by a factor βug, which reflects the type of interaction: $\beta ug=1$ for induction and $\beta ug=-1$ for repression (KEGG supplies this information about the type of interaction of 2 genes in the description of the pathway topology). *USg* is the set of all such genes upstream of g. For a given pathway, the value of *PF(u)* needs to be normalized by dividing it by $N_{de}(P_i)$, which is the number of DEGs in the given pathway. In order to make the *IF*s as independent as possible from the technology and comparable between problems, we also divided the second constituent in the equation 1 by the mean absolute fold change ΔE, calculated across all DEGs. Then, the top 10 significant pathways with the *IF* of >5 and the *P* value of <0.05 were selected.

Regulation Network Between Transcriptional Factors and Pathways. To further investigate the regulatory relationships between TFs and pathways, DEGs were mapped to the KEGG pathways, and a regulation network was obtained between TFs and pathways. The number of regulatory relationships between a TF and its target genes in the same pathway were then calculated. Once more than 66.6% of the regulatory relationships of TF to target genes were activated or depressed simultaneously in one pathway, it was considered that the pathway was activated or depressed by the TFs.

Results

Construction of Regulatory Network in Alveolar Hypoxia. To get pathway-related DEGs of alveolar hypoxia, publicly available microarray datasets GSE11341 were obtained from the GEO. After microarray analysis, the DEGs with the fold change value of greater than 2 and the *P* value of less than 0.05 were selected. Fifty-seven genes (Table 1) were selected as DEGs from the GSE11341. To get the regulatory relationships, the coexpressed value (PCC⩾0.6) was chosen as the threshold. Finally, we got 33 regulatory relationships between 7 differentially expressed TFs and their 29 differentially expressed target genes. By integrating the above regulatory relationships, a regulatory network of alveolar hypoxia was built between TFs and their target

Table 1. The List of Differentially Expressed Genes

Gene_Symbol	logFC	*P* Value	FDR
NDRG1	−1.475735312	0.000261	0.006982
HIF-1a	1.052216325	0.001939	0.028803
DUSP1	−1.354317867	1.32E-06	8.07E-05
BHLHE40	−1.940694786	2.71E-07	5.01E-05
SLC2A1	−2.084750448	5.08E-05	0.00121
ENO2	−1.455878832	5.95E-05	0.002781
TGFBI	−1.292338829	0.004839	0.047639
BNIP3	−1.133049677	2.07E-05	0.000421
MXI1	−1.018848919	0.00165	0.027806
SLC2A3	−2.284931989	1.45E-07	5.01E-05
PLOD2	−1.567059472	0.0005	0.01079
TNFSF10	1.312928069	0.010143	0.069833
ADM	−2.131820379	6.81E-05	0.002781
FAM13A1	−1.038141985	0.029089	0.099997
STC2	−1.344661506	0.032433	0.476391
NFIL3	−1.041953825	3.08E-08	3.43E-05
HMOX1	1.107798422	0.032674	0.502874
CHST2	−1.302524486	0.000896	0.021998
ARG2	−1.635797873	0.00924	0.062882
CEBPD	−1.010387116	0.000685	0.017649
FABP4	1.097613378	0.006174	0.050287
DUSP4	1.029762867	0.000256	0.006287
BACH1	−1.432415703	1.14E-05	0.000421
AK3L1	−1.749690775	0.001245	0.02696
STC1	−1.601142552	0.00054	0.011436
HSD17B2	−1.184083276	0.014084	0.07586
ADORA2A	−1.025750437	0.001664	0.027946
SLC6A6	−1.13524485	4.42E-05	0.000807
LDB2	1.290180313	0.0086	0.062872
SLC16A6	−1.577351173	0.017649	0.078799
P4HA1	−1.452817836	0.000126	0.004917
GADD45B	−1.014154572	0.001112	0.022178
METTL7A	1.027985404	0.017954	0.099997
PPARG	−1.023557534	0.000143	0.006157
ID1	1.286480675	0.017693	0.082036
C10orf10	−1.227935765	1.51E-06	0.000121
VLDLR	−1.480715285	0.017869	0.084357
TGFB2	−1.283518883	0.026586	0.099997
INHBA	−1.125616337	0.008509	0.061569
VEGFA	−2.507940626	0.000627	0.017134
AKAP12	−1.040477779	0.000627	0.017134
LIMCH1	−1.079596402	0.044843	0.857777
EPB41L3	−1.392658734	0.023107	0.099997
JMJD1A	−1.304426577	3.28E-05	0.000501
TMEM158	−1.462687237	0.030233	0.179462
LOX	−2.237838767	0.001986	0.037782
CXCR4	−1.604643802	1.25E-05	0.000421
GREM1	−1.155183981	0.017268	0.396378
LYVE1	1.196798605	0.015217	0.077735
EGLN3	−1.0272714	0.005515	0.050106
C4orf18	1.13596533	0.013053	0.069873
SPAG4	−1.14748353	0.005424	0.049168
ANGPTL4	−2.476902739	6.75E-08	3.43E-05
APOLD1	−1.564719119	0.000365	0.007586
TNS1	−1.691417265	0.001324	0.027071
UGCG	−1.629526411	0.000378	0.008204
SLC2A14	−1.808674541	8.10E-05	0.003608

Gene_Symbol indicates the gene name; logFC, the log fold change value of differentially expressed genes. The *P* value was calculated using the *t* test, and FDR indicates the corrected *P* value using the Benjamini method (26).

genes (Fig. 1). In this network, hypoxia inducible factor 1 alpha (HIF-1α), peroxisome proliferator-activated receptor gamma (PPARγ), and nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-κB) with higher numbers of interactions with other proteins formed a local network, suggesting that these TFs might play important roles in alveolar hypoxia. Besides, the heme oxygenase (decycling) 1 (HMOX1) target gene was regulated by 4 TFs in this network.

GO Analysis of the Regulatory Network in Alveolar Hypoxia. Several GO categories were enriched among these genes in the regulatory network (Table 2 only list the top 10 enriched GO terms with the cutoff of corrected *P* value <0.05 and count >2), including response to chemical stimulus, chemical homeostasis, homeostatic process and response to hypoxia.

Significant Pathway in Alveolar Hypoxia. To identify the relevant pathways changed in alveolar hypoxia, a statistical approach at the pathway level was used. Significance analysis at single gene level may suffer from the limited number of samples and experimental noise that can severely limit the power of the chosen statistical test. Because one pathway always includes many genes, significance analysis at the pathway level may raise the reliability by relaxing the significance threshold applied to single genes. Therefore, we adopted a pathway based impact analysis method that contained many factors, such as the statistical significance of the set of DEGs in the pathway, the magnitude of change of each gene expression, the topology of the signaling pathway, and their interactions. The impact analysis method yielded several significant pathways, such as phosphatidylinositol signaling system, cell cycle, and p53 signaling pathway (Table 3).

Regulation Network Between Transcriptional Factors and Pathways in Alveolar Hypoxia. To further investigate the regulatory relationships between TFs and pathways, we mapped DEGs to pathways and got a regulation network showing the associations between TFs and different pathways (Fig. 2). In the network, HIF-1α, PPARγ, and CCAAT/enhancer binding protein delta (CEBPδ) were shown as hub nods linked to many pathways of alveolar hypoxia. For example, the JAk-STAT signaling pathway, cell cycle, ABC transporters, endocytosis, hematopoietic cell lineage, cytokine-cytokine receptor interaction, and phagosome were all regulated by HIF-1α.

Discussion

From the result of regulatory network construction in alveolar hypoxia, we could find that many TFs

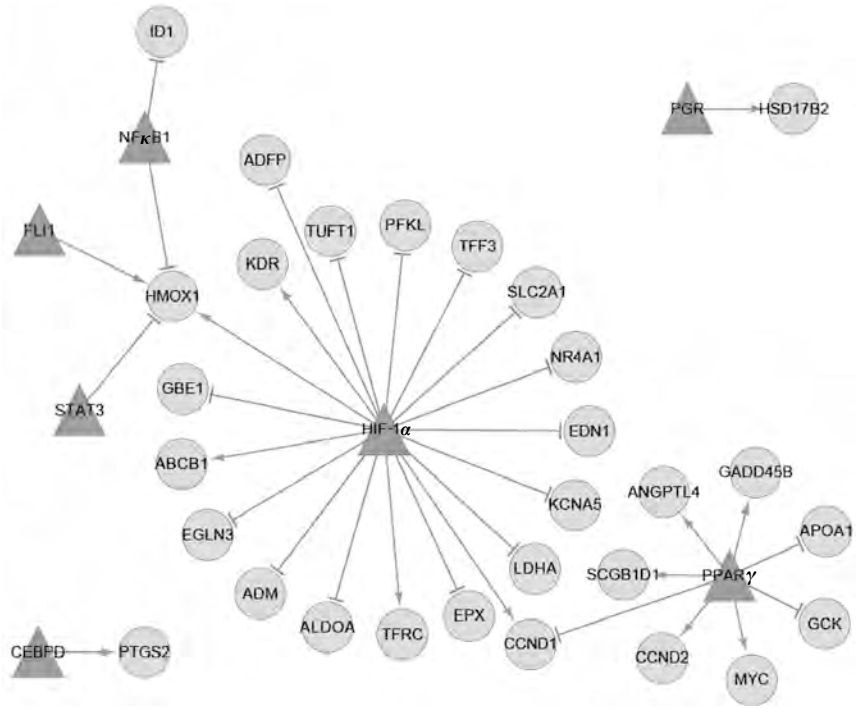

Fig. 1. Construction of regulatory network in alveolar hypoxia

The triangles denote transcription factors, and the circles denote target genes. The arrow suggests that the transcription factor could activate their target genes. In contrast, the another indicator suggests that the transcription factor could inhibit the expression of their target genes.

Table 2. Gene Ontology (GO) Biological Process Analysis

GO-ID	Description	Count	*P* Value	Corr *P* Value
42221	Response to chemical stimulus	21	8.15E-13	1.06E-09
48878	Chemical homeostasis	13	1.46E-10	9.49E-08
42592	Homeostatic process	14	1.32E-09	5.69E-07
1666	Response to hypoxia	8	2.13E-09	6.90E-07
70482	Response to oxygen levels	8	3.39E-09	8.79E-07
50896	Response to stimulus	25	1.41E-08	3.04E-06
50793	Regulation of developmental process	13	1.95E-08	3.55E-06
31325	Positive regulation of cellular metabolic process	14	2.27E-08	3.55E-06
6950	Response to stress	18	2.46E-08	3.55E-06
22603	Regulation of anatomical structure morphogenesis	9	3.14E-08	4.07E-06

GO-ID indicates the GO term ID; count, enriched numbers. The *P* value was calculated using the hypergeometric distribution. The corrected *P* value indicates the *P* value corrected with the Benjamin method (26).

Table 3. Significant Pathway Analysis

Database Name	Pathway Name	Impact Factor	*P* Value	*P* Value
KEGG	Phosphatidylinositol signaling system	21.558	0.703935	9.79E-09
KEGG	Cell cycle	20.026	2.35E-08	4.22E-08
KEGG	p53 signaling pathway	16.005	9.10E-07	1.90E-06
KEGG	Epithelial cell signaling in *Helicobacter pylori* infection	13.569	0.666085	1.86E-05
KEGG	Pathways in cancer	11.697	2.42E-05	1.06E-04
KEGG	Cytokine-cytokine receptor interaction	11.481	4.53E-05	1.29E-04
KEGG	Adherens junction	10.111	0.351794	4.51E-04
KEGG	Renal cell carcinoma	9.002	1.61E-04	0.001231879
KEGG	DNA replication	7.582	0.002234	0.004372882
KEGG	MAPK signaling pathway	6.895	0.009764	0.007996347

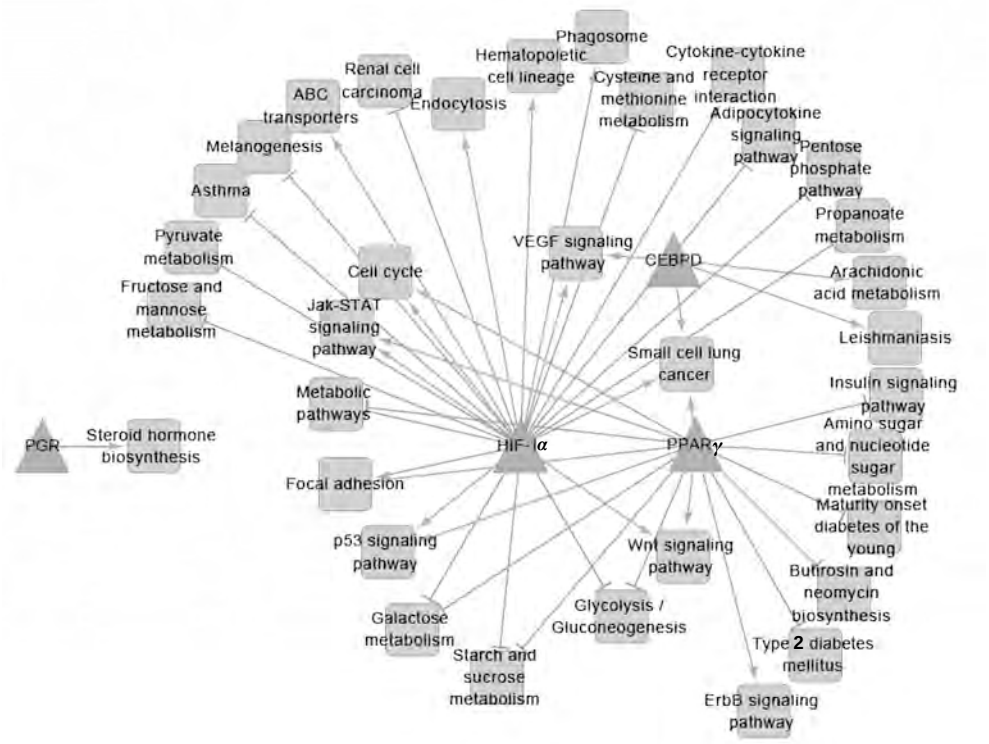

Fig. 2. Regulatory network of TF-PATHWAY

The triangles denote transcription factor and squares denote pathways. The arrow suggests that the transcription factor could activate the pathways in alveolar hypoxia. In contrast, the another indicator suggests that the transcription factor could inhibit the expression of the pathways in alveolar hypoxia.

and pathways closely associated with alveolar hypoxia have been linked by our method. The HIF-1α, NF-κB, and PPARγ genes are also hub nodes in our transcriptome network, which is in agreement with previous studies. Related target genes and pathways were also identified to be involved in alveolar hypoxia in a direct or an indirect manner.

HIF-1 is a basic helix-loop-helix TF that transactivates genes participating in homeostatic responses to hypoxia. HIF-1 is a heterodimer composed of an alpha (α) subunit and a beta (β) subunit. When alveolar epithelial cells are exposed to 1% oxygen for 2 hours, a major increase in HIF-1α protein and mRNA are observed. However, HIF-1α protein and mRNA levels decrease from 8 to 16 h, which may be related to mRNA stability (27). HIF-1α expression could further regulate a series of downstream gene products in our regulatory network (Fig. 1), which may contribute to the pathogenesis of pulmonary hypertension through different pathways (4).

For example, HIF-1α could inhibit KCNA5 expression in alveolar hypoxia. The *KCNA5* gene encodes a member of the potassium channel, voltage-gated, shaker-related subfamily. Acute alveolar hypoxia elicits pulmonary vasoconstriction. Ca^{2+} concentration is an important signal in regulating the contraction and proliferation of pulmonary artery smooth muscle cells. Hypoxia-induced increases in [Ca^{2+}] are, in part, mediated by the selective inhibition of voltage-gated KCNA5 channels in these cells. Therefore, KCNA5 is an important hypoxia-sensitive K^+ channel in pulmonary artery smooth muscle cells, contributing to regulation of membrane potential and intracellular Ca^{2+} homeostasis during hypoxia (28).

Under hypoxia, ATP supply is dependent on the ability of cells to increase anaerobic glycolysis by up-regulating the expression of glycolytic enzymes and increasing glucose transport at the membrane level. SLC2A1, also known as GLUT1, encodes a major glucose transporter in the mammalian blood-brain barrier. Ouiddir et al. found that the level of glucose transporter GLUT1 in alveolar epithelial cells was increased by 3-fold at both protein and mRNA levels when exposed to hypoxia (0% O_2) for 18 hours (29). A previous study has shown that HIF-1α can also regulate the SLC2A1 expression by binding to the SLC2A4 gene promoter (30). Therefore, HIF-1α may be involved in glucose metabolism through regulating the SLC2A1 expression.

HMOX1, an inducible heme oxygenase, belongs to the family of heme oxygenases. The activity of HMOX1 can be induced by various stimuli, such as hyperthermia, hypoxia, endotoxin, ischemic/reper-

fusion injury, and radiation, that have the capacity of provoking oxidative stresses (31). Some reports have shown that transgenic mice overexpressing HO-1 are protected from the development of pulmonary inflammation, as well as pulmonary hypertension and vessel wall hypertrophy induced by hypoxia (6, 32). Therefore, the up-regulated expression of HMOX1 induced by HIF-1α may be conducive to alleviate alveolar hypoxia damage.

In addition, our study also showed that HMOX1 expression could be regulated by NF-κB. NF-κB is a transcription regulator that is activated by various intra- and extracellular stimuli, such as cytokines, oxidant-free radicals, ultraviolet irradiation, and bacterial or viral products. The inappropriate activation of NF-κB has been associated with a number of inflammatory diseases. The effects of alveolar hypoxia were studied in rat lungs, exposing rats to 10% oxygen over the periods of 1, 2, 4, 6, and 8 hours. The number of macrophages in bronchoalveolar lavage fluid of hypoxic animals was found to be increased between 1 and 8 hours. NF-κB binding activity was increased within the first 2 hours of exposure to hypoxia. The elimination of alveolar macrophages led to a decreased expression of NF-κB binding activity. In summary, alveolar hypoxia may induce macrophage recruitment and enhance expression of inflammatory mediators, such as NF-κB (33, 34). Because of the inverse effect, the HMOX1 expression may be inhibited by the NF-κB expression. Previous studies from several laboratories suggest that HMOX-1 can mediate signaling pathways that are associated with NF-κB (35).

The PPARγ protein is a member of the peroxisome proliferator-activated receptor family. PPARγ has been implicated in the pathology of numerous diseases including obesity (36–38), diabetes (38, 39), atherosclerosis (40, 41), and cancer (42, 43). Chronic alveolar hypoxia contributes to the development of pulmonary hypertension. PPARγ expression is decreased in the lung tissues of the group that was exposed to hypoxia (44).

The CCND1 protein belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance throughout the cell cycle. Hypoxia can cause G (0)/G (1) cell cycle arrest in human lung adenocarcinoma cell line A549 (45). Increased cyclin D1 expression could promote G_1-S cell cycle transition and cell proliferation showing that the level of cyclin D1 expression is negatively correlated with G (0)/G (1) arrest (46). CCND1 expression could be regulated by PPARG (47) and HIF-1α (48) through the cell cycle pathway in alveolar hypoxia.

Our results also indicated several other pathways to be involved in alveolar hypoxia, such as adhesion and PI3K signaling pathway. Alveolar hypoxia causes pulmonary hypertension, vasoconstriction, and inflammation, which have been related to alveolar vascular remodeling, showing the accumulation of adhesion molecules. Therefore, our results suggest HIF-1α to be involved in the focal adhesion pathway. However, hypoxia-induced mitogenic factor (HIMF) could modulate vascular adhesion molecule 1 (VCAM-1) expression via the PI3K/Akt-NF-κB signaling pathway. The recombinant HIMF protein, intratracheally instilled into adult mouse lungs, led to a significant increase of VCAM-1 production in vascular endothelial, alveolar type II, and airway epithelial cells. A dominant-negative mutant of PI-3K, Δp85, as well as PI-3K inhibitor, LY294002, blocked HIMF-induced NF-κB activation and attenuated VCAM-1 production (49). The PI3K/AKT-NF-κB pathway has also been described to be activated by hypoxia and to regulate HIF-1 expression (50).

It is important to understand the mechanism underlying the function of genes involved in alveolar hypoxia. However, the deeper insight of TFs and their regulated genes remains an area of intense research in the future. These interactions still need to be confirmed by future experimental studies since our results were based on microarray data derived from a small sample size.

Conclusions

In our study, many related transcriptional factors, target genes, and pathways were identified to be linked with alveolar hypoxia. HIF-1α may regulate the expression of KCNA5, SLC2A1, and HMOX1 through the regulation of membrane potential, glucose metabolism, and anti-inflammation. HMOX-1 can mediate signaling pathways that associated with NF-κB. The CCND1 expression could be regulated by PPARγ and HIF-1α through the cell cycle pathway. In addition, some new transcriptional factors and target genes, such as phosphofructokinase (PFKL, liver), aldolase A (ALDOA, fructose-bisphosphate), and trefoil factor 3 (TFF3, intestinal), were shown to be associated with alveolar hypoxia, which have not been identified in previous works. However, further experiments are still indispensable to confirm these conclusions.

Statement of Conflict of Interest

The authors state no conflict of interest.

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