Quantitative Proteomics Analysis of Differentially Expressed Proteins in Serum of Former Uranium Miners by Isobaric Tags for the Relative and Absolute Quantitation

Xuhong Dang[1] ⑩**, Haipeng Lin**[1]**, Yayi Yuan**[1]**, Biao Yang**[1]**, Juancong Dong**[1]**, Zhongxin Zhang**[1]**, Kai Yang**[1]**, Youchen Li**[1]**, Xiaozhen Li**[1]**, Dongliang Chai**[1]**, and Yahui Zuo**[1]

Abstract

The carcinogenicity of radon has been convincingly documented through epidemiological studies of underground miners. However, there is a lack of early warning indicators for radon radiation damage. In this study, mixed serum samples of 3 groups were collected from 27 underground uranium miners and seven aboveground miners according to the radiation exposure dose. The differentially expressed proteins in the serum were identified using the isobaric tags for the relative and absolute quantitation (iTRAQ)-based method. Some differentially expressed proteins were validated by enzyme-linked immunosorbent assay (ELISA) in 84 underground and 32 aboveground miners. A total of 25 co-differentially expressed proteins in 2 underground miner groups were screened, of which 9 were downregulated and 13 were upregulated. Biological process analysis of these proteins using Metascape showed that 5 GO terms were enriched, such as negative regulation of very-low-density lipoprotein particle clearance, endocytosis, and regulated exocytosis. The results of the ELISA for the expression levels of GCN1, CIP2A, and IGHV1-24 in the serum of 116 miners' serum showed that the levels of GCN1 and CIP2A were consistent with the iTRAQ results. In conclusion, APOC1, APOC2, APOC3, ORM1, ORM2, ANTXR1, GCN1, and CIP2A may be potential early markers of radon radiation damage.

Keywords

radon, former uranium miners, serum, isobaric tags for the relative and absolute quantitation, biomarker

Introduction

Estimating the long-term biomedical effects of small doses of ionizing radiation is a complicated issue. It has been reported that more than 60% of the ionizing radiation a person receives every year can be caused by natural sources of radiation.[1] Radon, an imperceptible naturally occurring radioactive noble gas, is the largest single fraction of radiation exposure from natural sources.[2] It is the second most common cause of lung cancer after smoking. A correlation between radon and the frequency of lung cancer has been consistently demonstrated in several studies of indoor exposure in dwellings, as well as for uranium miners.[3] It is important to note that the current models estimating the risk of radiation-related hazards are based on the analysis of data collected from irradiated miners.

In addition to using dosimetry methods to evaluate the risk of harmful radiation effects, the severity of such a risk should be evaluated using a biological marker. Biological test systems can evaluate the risks of radiation and provide a thorough assessment of the quality of the environment and its suitability for humans.[1] Due to its good liposolubility, radon and its progeny can easily reach the lung, enter blood circulation, and

[1]China Institute for Radiation Protection, Taiyuan, China

Corresponding Author:
Yahui Zuo, China Institute for Radiation Protection, No. 102, Xuefu Street, Taiyuan City, Shanxi Province, China.
Email: yahuiz@163.com

act on the human body.[4] Therefore, with increases in radon exposure, we investigate whether there are potential biomarkers in the peripheral blood that can reflect the changes corresponding to radon damage.

Proteomics is an emerging discipline that studies the composition and functions of all proteins in a particular type of cell, tissue, or body fluid in a large-scale, high-throughput, and systematic manner. Novel proteomics named isobaric tags for relative and absolute quantification (iTRAQ) combined with mass spectrometry technology (LC-MS/MS) now represents a powerful tool for the identification of cancer biomarkers. The iTRAQ technology has been successfully applied to biomarker screening of multiple tumors and diseases in both tissue and serum samples.[5] The aim of this study was to screen for differentially expressed proteins in serums from former uranium miners exposed to high doses of radon using iTRAQ based method, and to provide basic experimental support for the study of early warning molecular biomarkers of radon damage.

Materials and Methods

Study Population and Sample Preparation

This cohort study involved 116 male former uranium miners aged from 50 to 86 yr old at a uranium mine in Hunan Province, China, who had no acute infectious diseases, malignant tumors, or serious family history of genetic diseases. Miners were divided into 2 groups according to their workplace: 32 were aboveground miners (controls) and 84 were underground miners (the cumulative exposure dose of radon was 2.10–251.70 working level months [WLM]). All serum samples from miners were frozen following venipuncture and centrifugation and stored in liquid nitrogen until analysis.

Isobaric Tags for the Relative and Absolute Quantitation Analysis

Based on the smoking factors of uranium miners, 34 serum samples of uranium miners who were smokers were selected for iTRAQ detection and analysis. Three mixed serum samples were prepared according to the radiation exposure dose. The mixed serum group 1 consisted of serum samples from 11 underground uranium miners with an exposure dose of 21.00–54.57 WLM. The mixed serum group 2 consisted of serum samples from 16 underground uranium miners with an exposure dose of 82.32–217.05 WLM. Group 3 was a mixed serum sample of 7 aboveground miners and was used as the control group.

Isobaric Tags for the Relative and Absolute Quantitation analysis was conducted at Shanghai Applied Protein Technology Co.(Shanghai, China). Total proteins were extracted from samples using SDT lysis (4% SDS, 0.1 M DTT, 100 mM Tris/HCl pH7.6). Serum pools were depleted of the most abundant proteins using an Agilent Human 14 Multiple

Affinity Removal System Column (Applied Biosystems, USA) following the manufacturer's instruction. Ultrafiltration tubes were used for desalination and concentration of low-abundance components. Protein concentrations were quantified using the bicinchoninic acid (BCA) Protein Assay Kit (Bio-Rad, USA). Subsequently, samples were labeled using an 8-plex iTRAQ labeling kit (Applied Biosystems) according to the manufacturer's protocol. iTRAQ-labeled peptides were fractionated by strong cation exchange (SCX) chromatography using the AKTA purifier system (GE Health care, USA). Each fraction was injected into the nanoscale liquid chromatography coupled to tandem mass spectrometry (nano-LC-MS/MS) system for analysis. High-resolution LC-MS/MS analysis was performed on a Q Exactive mass spectrometer (Thermo Fisher Scientific) operated in positive ion mode coupled to an EASY-nLC liquid chromatograph (Thermo Fisher Scientific). The MS data were acquired in a data-dependent acquisition mode. The raw files were processed using Proteome Discoverer 1.4 (Thermo Fisher Scientific) and searched using the Mascot search engine (version 2.2, Matrix Science, UK) against the UniProt protein human database. The false discovery rate (FDR) for the peptides was set to 1%. Among the identified proteins, proteins with more than 1.2-fold and $P < .05$ compared to control were defined as up-regulated expression, while proteins with less than 0.83-fold and $P < 0.05$ were defined as down-regulated expression.

Bioinformatics Analyses

Metascape (http://metascape.org) is a web-based portal designed gene-list tool that provides gene annotation, enrichment analysis, and a protein-protein interaction (PPI) network based on over 40 independent knowledge bases.[6] In our study, Metascape was employed for GO, KEGG, and PPI analysis to further explore the complex mechanism of co-differentially expressed proteins in 2 underground miner groups. We selected term enrichment significance according to P-value < 0.01, minimum count of 3, and enrichment factor > 1.5.

Enzyme-Linked Immunosorbent Assay

The human CIP2A enzyme-linked immunosorbent assay (ELISA) Kit, human GCN1 ELISA kit, and human IgHV1-24 ELISA kit were all purchased from AndyGene Biotechnology Co. (China) and used to detect the levels of CIP2A, GCN1, and IgHV1-24, respectively, according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed using the Statistical Program for Social Sciences (SPSS, Inc., Chicago, IL, USA). The data are reported as the mean ± standard deviation (SD), and the quantitative variables were analyzed using Student's t-tests between 2 groups. Statistical significance was set at < 0.05.

Results

Differential Expression Analysis of Serum Proteins in Underground Miners Using iTRAQ

Serum protein expression was tested using iTRAQ. Relative to group 3, groups 1 and 2 had 43 and 34 upregulated and 42 and 23 downregulated proteins, respectively. A total of 25 co-differentially expressed proteins in 2 underground miner groups (groups 1 and 2) were screened (Tables 1-3).

Functional Enrichment Analysis of Differentially Expressed Proteins

The differentially expressed serum proteins of groups 1 and 2 were considered for gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway

enrichment analysis using Metascape.[6] The results showed that 9 biological process terms were significantly enriched in the 2 groups: regulated exocytosis, hemostasis, protein-lipid complex remodeling, immunoglobulin production, endocytosis, zymogen activation, post-translational protein phosphorylation, cell-substrate adhesion, and contractile actin filament bundle assembly (Figure 1). To further investigate the relationship between the differentially expressed proteins in groups 1 and 2, a PPI network was constructed based on the information from the Metascape online database. The PPI network and molecular complex detection (MCODE) components identified in the gene lists are shown in Figure 2, and the top 4 modules with the highest scores were obtained from the PPI network. Figure 2 shows that the biological functions mainly related to MCODE1 were post-translational protein phosphorylation, regulation of Insulin-like Growth Factor (IGF), transport and uptake by Insulin-like Growth Factor

Table 1. The Differentially Expressed Proteins in Group 1 Compared to Group 3.

NO.	Expression Status	The Differentially Expressed Proteins
1	Up regulated expression	(von Willebrand factor, VWF), (Keratin, type I cytoskeletal 10, KRT10), (Apolipoprotein(a), LPA), (Actin, cytoplasmic 1, ACTB), (Immunoglobulin heavy variable 1-3, IGHV1-3), (Immunoglobulin lambda-like polypeptide 1, IGLL1), (Neogenin,NEO1), (Matrix metalloproteinase-9, MMP9), (Trypsin-1, TRY1), (Ferritin light chain, FRIL), (Roundabout homolog 4, ROBO4), (Receptor-type tyrosine-protein phosphatase S, PTPRS), (Syndecan-4, SDC4), (Protein C8orf37, CH037), (Platelet glycoprotein VI, GPVI), (Protein CIP2A, CI2A), (Myocilin, MYOC), (Leukocyte immunoglobulin-like receptor subfamily A member 3, LIRA3), (Immunoglobulin lambda variable 2-23, IGLV2-23), (BDNF/NT-3 growth factors receptor, NTRK2), (Myeloperoxidase, MPO), (Phosphoinositide-3-kinase-interacting protein 1, PIK3IP1), (Proteasome subunit alpha type-4, PSMA4), (Ankyrin repeat and protein kinase domain-containing protein 1, ANKK1), (Anthrax toxin receptor 1, ANTXR1), (Ribonuclease pancreatic, RNASE1), (Kinesin-like protein KIF7, KIF7), (eIF-2-alpha kinase activator GCN1, GCN1), (Transitional endoplasmic reticulum ATPase, VCP), (Histone H3.1, HIST1H3A), (N-acetylglutamate synthase, mitochondrial, NAGS), (Heterogeneous nuclear ribonucleoprotein Q, SYNCRIP), (Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform, PIK3CB), (Coiled-coil domain-containing protein 170, CCDC170), (Myosin light polypeptide 6, MYL6), (Leucine-rich repeat flightless-interacting protein 2, LRRFIP2), (Osteopontin, OSTP), (Complement component C1q receptor, C1QR1), (Death domain-containing protein 1, DTHD1), (Chondroitin sulfate proteoglycan 4, CSPG4), (Protein YIF1B, YIF1B), (Immunoglobulin lambda variable 2-11, IGLV2-11), (Cell adhesion molecule 1, CADM1)
2	Down regulated expression	(Serotransferrin, TF), (Haptoglobin, HPT), (Haptoglobin-related protein, HPTR), (Hemoglobin subunit beta, HBB), (Hemoglobin subunit alpha, HBA), (Immunoglobulin lambda constant 7, IGLC7), (Serum albumin, ALBU), (Alpha-1-acid glycoprotein 1, ORM1), (Alpha-1-acid glycoprotein 2, ORM2), (Apolipoprotein C-II, APOC2), (Apolipoprotein C-III, APOC3), (Serum paraoxonase/arylesterase 1, PON1), (Apolipoprotein L1, APOL1), (Actin, gamma-enteric smooth muscle, ACTH), (Apolipoprotein C-I, APOC1), (Immunoglobulin heavy variable 4-4, IGHV4-4), (Complement factor H-related protein 3, CFHR3), (Immunoglobulin heavy variable 1-24, IGHV1-24), (Immunoglobulin heavy variable 5-10-1, IGHV5-10-1), (C-reactive protein, CRP), (Immunoglobulin lambda variable 4-69, IGLV4-69), (Immunoglobulin kappa variable 3-15, IGKV3-15), (Immunoglobulin kappa variable 1-8, IGKV1-8), (Eukaryotic translation initiation factor 5A-2, IF5A2), (Phosphoglucomutase-1, PGM1), (Immunoglobulin heavy variable 3-73, IGHV3-73), (Immunoglobulin lambda variable 5-45, IGLV5-45), (AP-3 complex subunit beta-2, AP3B2), (Galectin-related protein, LEGL), (THAP domain-containing protein 4, THAP4), (Arf-GAP with Rho-GAP domain, ANK repeat and PH domain-containing protein 1, ARAP1), (Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1, SVEP1), (ADP-ribosylation factor GTPase-activating protein 1,ARFG1), (IQ domain-containing protein E, IQCE), (Tenascin-N, TENN), (Melanocyte protein PMEL, PMEL), (C-type lectin domain family 2 member A, CLC2A), (Crk-like protein, CRKL), (Pro-cathepsin H, CATH), (Glucocorticoid modulatory element-binding protein 2, GMEB2), (Immunoglobulin lambda variable 2-14, IGLV2-14), (Prothymosin alpha, PTMA), (Immunoglobulin lambda variable 3-22, IGLV3-22), (Tyrosine-protein kinase receptor UFO, UFO)

Table 2. The Differentially Expressed Proteins in Group 2 Compared to Group 3.

No.	Expression Status	The Differentially Expressed Proteins
1	Up regulated expression	(Immunoglobulin heavy constant gamma 3, IGHG3), (Immunoglobulin heavy constant gamma 2, IGHG2), (Immunoglobulin delta heavy chain, IGD), (Alpha-1-acid glycoprotein 1, ORM1), (Alpha-1-acid glycoprotein 2, ORM2), (Immunoglobulin kappa variable 2D-29, IGKV2D-29), (Immunoglobulin heavy variable 3-7, IGHV3-7), (Immunoglobulin lambda variable 3-10, IGLV3-10), (Immunoglobulin heavy variable 5-10-1, IGHV5-10-1), (Immunoglobulin kappa variable 2-24, IGKV2-24), (Immunoglobulin heavy variable 1-8, IGHV1-8), (Immunoglobulin lambda variable 1-51, IGLV1-51), (Serum amyloid A-1 protein, SAA1), (Immunoglobulin lambda-like polypeptide 1, IGLL1), (Immunoglobulin lambda variable 3-16, IGLV3-16), (Chitinase-3-like protein 1, CHI3L1), (Protein C8orf37, C8orf37), (Protein CIP2A, CIP2A), (Neuroblastoma suppressor of tumorigenicity 1, NBL1), (Myocilin, MYOC), (Leukocyte immunoglobulin-like receptor subfamily A member 3, LILRA3), (Immunoglobulin lambda variable 2-23, IGLV2-23), (Ankyrin repeat and protein kinase domain-containing protein 1, ANKK1), (Anthrax toxin receptor 1, ANTXR1), (Secretogranin-1, CHGB), (Ribonuclease pancreatic, RNASE1), (eIF-2-alpha kinase activator GCN1, GCN1), (Coiled-coil domain-containing protein 170, CCDC170), (Osteopontin, SPP1), (Bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 4, NDST4), (Complement component C1q receptor, CD93), (Adhesion G protein-coupled receptor L4, ADGRL4), (Immunoglobulin lambda variable 4-3, IGLV4-3), (Immunoglobulin heavy variable 1-69-2, IGHV1-69-2)
2	Down regulated expression	(Serotransferrin, TF), (Thrombospondin-1, THBS1), (Beta-Ala-His dipeptidase, CNDP1), (Apolipoprotein C-II, APOC2), (Apolipoprotein C-III, APOC3), (Apolipoprotein C-I, APOC1), (Profilin-1, PROF1), (Coagulation factor XI, FA11), (Immunoglobulin heavy variable 1-24, IGHV1-24), (Transgelin-2, TAGL2), (Complement C1q subcomponent subunit A, C1QA), (Immunoglobulin heavy variable 1-18, IGHV1-18), (Tropomyosin alpha-4 chain, TPM4), (Cofilin-1, CFL1), (Pantetheinase, VNN1), (SH3 domain-binding glutamic acid-rich-like protein 3, SH3BGRL3), (Extracellular serine/threonine protein kinase FAM20 C, FAM20 C), (Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1, SVEP1), (Zyxin, ZYX), (60S ribosomal protein L26, RPL26), (Pro-cathepsin H, CTSH), (Glucocorticoid modulatory element-binding protein 2, GMEB2), (Prothymosin alpha, PTMA)

Binding Proteins (IGFBPs), and regulated exocytosis. In MCODE2, these were plasma lipoprotein particle remodeling, protein–lipid complex remodeling, and protein-containing complex remodeling. In MCODE3, these were platelet degranulation, platelet degranulation, and response to elevated platelet cytosolic Ca^{2+}. The biological function related to MCODE4 was leukocyte migration.

To further understand the mechanism and function of co-differentially expressed proteins, bioinformatic analyses were performed using Metascape. As summarized in Figure 3, 5 GO terms were mainly enriched: negative regulation of very-low-density lipoprotein particle clearance, endocytosis, regulated exocytosis, tissue homeostasis, and cell morphogenesis involved in differentiation (Figure 3A and 3B, and Table 4). Moreover, to investigate the relationship between co-differentially expressed proteins, a PPI network was constructed based on the information from the Metascape online database. The identified PPI network and MCODE1 component are shown in Figure 3C. The biological function related to the PPI network was very-low-density lipoprotein particle clearance, involving proteins such as apolipoprotein C1 (APOC1), C2 (APOC2), and C3 (APOC3). DisGeNET GO analysis in Metascape showed that co-differentially expressed proteins were associated with 30 GO terms, such as hyperlipoproteinemia type III, hyperinsulinism, drug toxicity, etc. The top 20 GO enrichment pathways are shown in Figure 4 and Table 5.

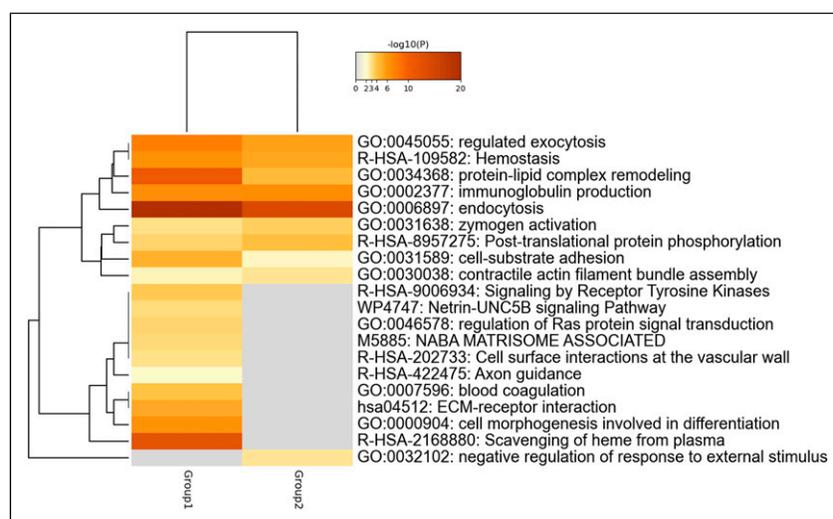## Validation of Differentially Expressed Proteins in Serum Using ELISA

To validate the iTRAQ results, ELISA was used to measure 3 candidate proteins: GCN1, IGHV1-24, and CIP2A in the serum of 116 former uranium miners. Figure 5 illustrates that GCN1 and CIP2A levels were significantly increased in underground miners (U-M) compared to aboveground miners (A-M). There were no significant differences in serum IGHV1-24 levels between underground and aboveground miners. Thus, GCN1 and CIP2A levels were consistent with the iTRAQ results.

Discussion

Uranium minerals are associated with radioactive elements such as radium and radon in the ore which arises from the radioactive decay. There are mainly 3 parts of total effective dose of uranium miners which contains radon exposure, gamma radiation, and long-lived alpha emitters. Uranium underground miners are primarily exposed to internal ionizing radiation from radon decay products especially α-emitters. Radon is a naturally occurring radioactive noble gas that contributes the largest single fraction of radiation exposure from natural sources. Underground uranium miners are primarily exposed to ionizing radiation internally through inhalation of radon decay products, especially α-emitters such

Table 3. Co-differentially Expressed Proteins in the 2 Underground Miner Groups (groups 1 and 2).

NO.	Accession	Protein	Fold Change group1/group3	group2/group3
1	P15814	Immunoglobulin lambda-like polypeptide 1 (IGLL1)	2.33	1.51
2	Q96NL8	Protein C8orf37 (C8orf37)	2.76	1.56
3	Q8TCG1	Protein CIP2A (CIP2A)	19.85	5.13
4	Q99972	Myocilin (MYOC)	1.30	1.44
5	Q8N6C8	Leukocyte immunoglobulin-like receptor subfamily A member 3 (LILRA3)	1.30	1.44
6	P01705	Immunoglobulin lambda variable 2-23 (IGLV2-23)	1.90	1.55
7	Q8NFD2	Ankyrin repeat and protein kinase domain-containing protein 1 (ANKK1)	3.12	1.69
8	Q9H6X2	Anthrax toxin receptor 1 (ANTXR1)	2.68	1.43
9	P07998	Ribonuclease pancreatic (RNASE1)	1.68	1.45
10	Q92616	eIF-2-alpha kinase activator GCN1 (GCN1)	6.24	3.01
11	Q8IYT3	Coiled-coil domain-containing protein 170 (CCDC170)	1.45	1.99
12	P10451	Osteopontin (SPP1)	2.12	1.41
13	Q9NPY3	Complement component C1q receptor (CD93)	2.05	1.41
14	P02787	Serotransferrin (TF)	0.51	0.66
15	P02763	Alpha-1-acid glycoprotein 1 (ORM1)	0.53	1.80
16	P19652	Alpha-1-acid glycoprotein 2 (ORM2)	0.56	1.59
17	P02655	Apolipoprotein C-II (APOC2)	0.69	0.71
18	P02656	Apolipoprotein C-III (APOC3)	0.48	0.68
19	P02654	Apolipoprotein C-I (APOC1)	0.67	0.74
20	A0A0C4DH33	Immunoglobulin heavy variable 1-24 (IGHV1-24)	0.17	0.21
21	A0A0J9YXX1	Immunoglobulin heavy variable 5-10-1 (IGHV5-10-1)	0.57	1.77
22	Q4LDE5	Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1(SVEP1)	0.56	0.75
23	P09668	Pro-cathepsin H (CTSH)	0.49	0.71
24	Q9UKD1	Glucocorticoid modulatory element-binding protein 2 (GMEB2)	0.75	0.64
25	P06454	Prothymosin alpha (PTMA)	0.56	0.46

Figure 1. Heatmap of enriched pathway terms of differentially expressed proteins in groups 1 and 2, colored according to P-values.

as radon-222 (^{222}Rn), polonium-218 (^{218}Po), plumbum-214 (^{214}Pb), bismuth-214(^{214}Bi), and polonium-214 (^{214}Po), along with dust with high contents of crystalline silica.[7,8] Upon inhalation, radon progeny in a conjugated state are mainly deposited in the lung, whereas most of its unconjugated forms are deposited in the nasal pharynx and trachea bronchus. Due to
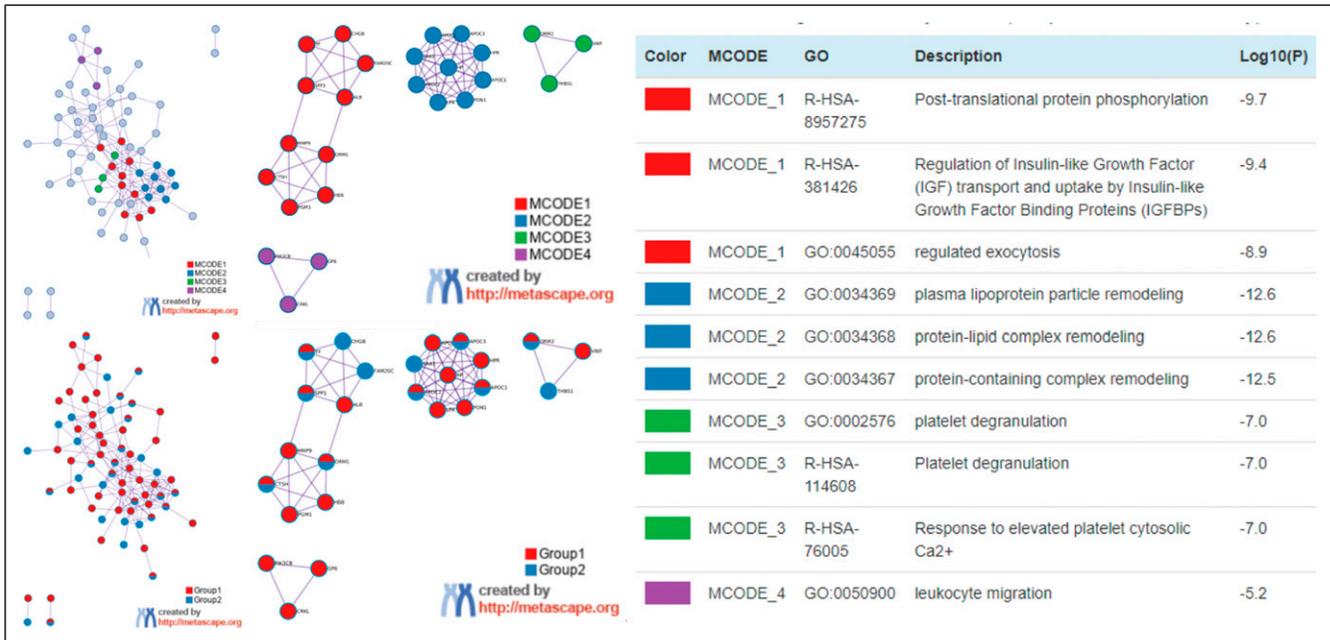
Figure 2. Protein-protein interaction network and MCODE components identified in the protein list of groups 1 and 2.
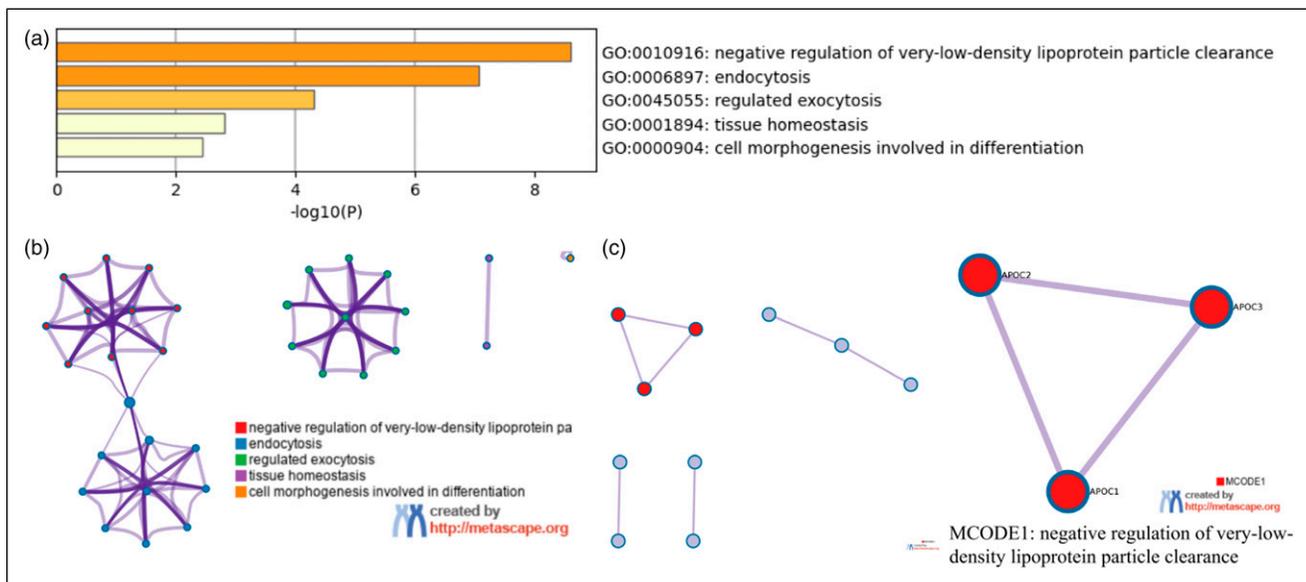

Figure 3. Enrichment analysis of co-differentially expressed proteins in 2 underground miner groups in Metascape. (a) Heatmap of enriched terms across input gene lists, colored by P-values; (b) Net graph of enriched terms, Different colors represent different cluster ID. (c) PPI network and MCODE components identified in the protein lists. *Note*: PPI, protein-protein interaction; MCODE, molecular complex detection.

good liposolubility, radon and its progeny easily reach the lung and are absorbed into the blood. Thus, lung and blood cells become the main targets of irradiation from radon exposure.[9] Animal and cell studies, clinical trials, and epidemiological investigations have confirmed that long-term exposure to high levels of radon and its progeny can initiate biological effects at the molecular and cellular levels and pose potential health risks to the exposed populations.[10] Radon has already been defined

as a lung carcinogen by the International Agency for Research on Cancer (IARC) based on acohort of radon-exposed miners from 1988.[11] Primary prevention is fundamental in lung cancer, which is characterized by a long latency period. Therefore, it is necessary to explore early warning molecular biomarkers for radon-induced lung cancer.

Serum is an attractive sample for identifying potential protein biomarkers because of its easy accessibility. Moreover,

Table 4. GO Category Enrichment Results.

GO	Description	LogP	Target Proteins
GO: 0010916	negative regulation of very-low-density lipoprotein particle clearance	−8.61	APOC1, APOC2, and APOC3
GO: 0006897	Endocytosis	−7.06	APOC1, APOC2, APOC3, GLL1, TF, IGHV5-10-1, IGHV1-24, and IGLV2-23
GO: 0045055	regulated exocytosis	−4.30	CTSH, ORM1, ORM2, TF, ILRA3, and CD93
GO: 0001894	tissue homeostasis	−2.81	CTSH, SPP1, and TF
GO: 0000904	cell morphogenesis involved in differentiation	−2.45	MYOC, SPP1, ANTXR1, and C8orf37

Note: GO, gene ontology.
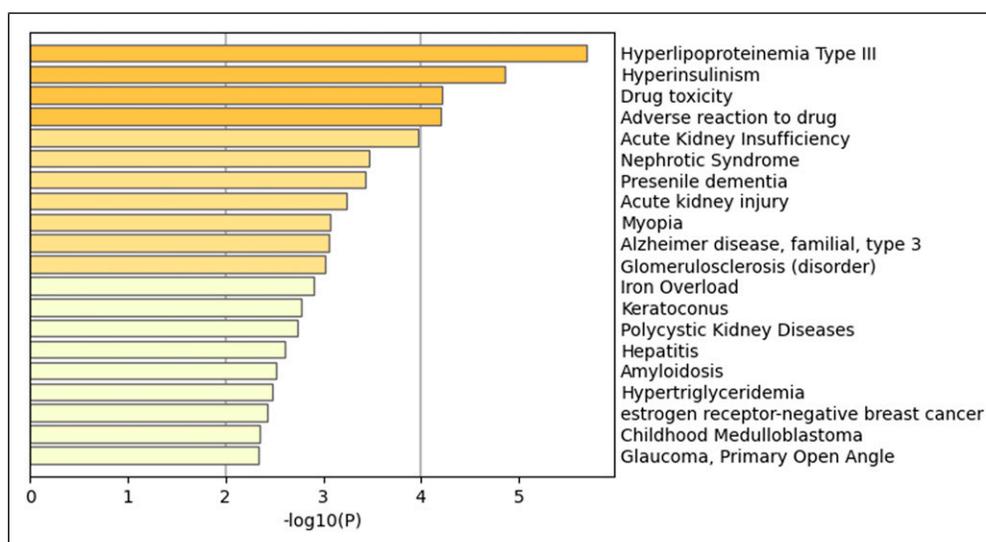
Figure 4. Enrichment analysis of co-differentially expressed proteins related disease in Metascape.

it is an archive of information on physiological and pathological conditions.[12] Compared with conventional techniques for detection of specific proteins, proteomics technology separates and identifies proteins with high throughput and high sensitivity. It has low detection limits and aids discovery of new disease-related proteins.[13] Thus, in the present study, we evaluated the changes in a diverse panel of potential biomarkers in the serums of former uranium miners after long-term radon exposure using iTRAQ technology.

The uranium miners were divided into 2 groups according to the cumulative radon exposure dose, and the mixed serum samples from the 2 groups were detected using iTRAQ. A total of 25 co-differentially expressed proteins were screened, including CIP2A, GCN1, and ANKK1 among others. Biological process analysis of these proteins using Metascape showed that 5 GO terms were enriched, namely, negative regulation of very-low-density lipoprotein particle clearance, endocytosis, regulated exocytosis, tissue homeostasis, and cell morphogenesis involved in differentiation. Many proteins may be biomarkers of some diseases, and most of them are closely related to lipoprotein metabolism. For example, CIP2A functions as an oncoprotein by directly binding to the tumor suppressor PP2AB56a, which is overexpressed in a variety of human cancers.[14-16] Anthrax toxin receptor 1 (ANTXR1) is highly upregulated in the tumor endothelium and expressed in diverse types of cancer. Tissue over-expression and elevated serum ANTXR1 levels play pivotal roles in the development and progression of colorectal cancer (CRC). Thus, detection of serum ANTXR1 may serve as a diagnostic biomarker and clinical predictor of CRC.[17] Meanwhile, high ANTXR1 expression was strongly correlated with stromal and immune infiltration in gastric cancer.[18] APOC1, APOC2, APOC3, Orm1, and Orm2 are associated with lipoprotein metabolism. APOC1, APOC2, and APOC3 have distinct effects on the major metabolic pathways involved in lipoprotein metabolism and play an important role in the etiology of human hyperlipidemia.[19] Orm1 and Orm2 are yeast members of a conserved family of endoplasmic

Table 5. Top 20 Enriched Protein Patway-Related Diseases.

GO	Description	Log P	Target Proteins
C0020479	Hyperlipoproteinemia type III	−5.7	APOC1, APOC2, and APOC3
C0020459	Hyperinsulinism	−4.9	APOC1, APOC3, CTSH, SPP1, CIP2A, and C8orf37
C0013221	Drug toxicity	−4.2	ORM1, SPP1, and TF
C0041755	Adverse reaction to drug	−4.2	ORM1, SPP1, and TF
C1565662	Acute Kidney insufficiency	−4.0	ORM1, SPP1, and TF
C0027726	Nephrotic syndrome	−3.5	MYOC, SPP1, TF, and C8orf37
C0011265	Presenile dementia	−3.4	APOC1, APOC3, SPP1, TF, and ANKK1
C2609414	Acute kidney injury	−3.2	ORM1, SPP1, and TF
C0027092	Myopia	−3.1	CTSH, MYOC, ANTXR1, and C8orf37
C1843013	Alzheimer disease, familial, type 3	−3.1	SPP1, CIP2A, and ANKK1
C0178664	Glomerulosclerosis (disorder)	−3.0	APOC1, MYOC, and SPP1
C0282193	Iron Overload	−2.9	APOC3, SPP1, and TF
C0022578	Keratoconus	−2.8	TF, ANTXR1, and C8orf37
C0022680	Polycystic Kidney diseases	−2.7	CTSH, PTMA, and SPP1
C0019158	Hepatitis	−2.6	APOC3, IGLL1, SPP1, and TF
C0002726	Amyloidosis	−2.5	APOC2, APOC3, MYOC, and SPP1
C0020557	Hypertriglyceridemia	−2.5	APOC1, APOC2, and APOC3
C4733092	Estrogen receptor-negative breast cancer	−2.4	SPP1, CIP2A, and CCDC170
C0278510	Childhood Medulloblastoma	−2.4	SPP1, TF, GCN1, and CIP2A
C0339573	Glaucoma, Primary Open Angle	−2.3	APOC3, MYOC, and SPP1
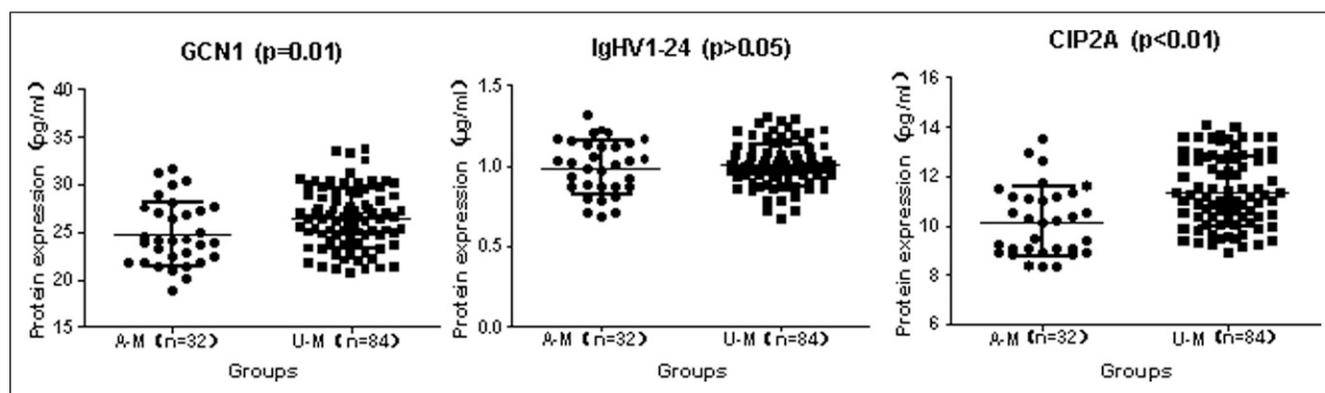
Figure 5. Expression of GCN1, IgHV1-24, and CIP2A in serum of miners.

reticulum membrane proteins whose physiologic significance is emphasized by the linkage of a human family member to asthma susceptibility, which plays a central role in lipid homeostasis and protein quality control.[20,21]

Lipids are widely distributed in cellular organelles and are critical components of all membranes. In addition to their role as structural components, lipids in membranes also serve important functions in different organelles. Dysregulation of lipid metabolism contributes to the progression of various metabolic diseases, including cardiovascular diseases, obesity, hepatic steatosis, and diabetes.[22] Apolipoproteins are structural components of lipoproteins and play a critical role in lipid transport. Genetic mutations in apolipoproteins are widely associated with metabolic and cardiovascular diseases. Increasing evidence suggests that apolipoproteins may also be related to the development and outcomes of certain cancers, such as ovarian, renal, and bladder cancer.[23] For example, the expression of apolipoprotein ApoA-1 and ApoC-III in small cell lung cancer (SCLC) was significantly different from that in normal lung tissue, suggesting that ApoA-1 and ApoC-III may be used as differentiating and predictive markers for SCLC. Based on the results of the proteomic tests, differentially expressed serum proteins related to lipid metabolism, such as APOC1, APOC2, APOC3, Orm1, and Orm2, may reflect the harmful effects of long-term radon exposure.

Among the 25 co-differentially expressed proteins, we selected GCN1, CIP2A, and IGHV1-24 for further verification. We selected these 3 proteins because they had a high degree of differential expression in the serum of uranium miners. The results showed that GCN1 and CIP2A levels were

consistent with the iTRAQ results, indicating that the expression levels of GCN1 and CIP2A in serums were related to radon exposure, and the expression of IGHV1-24 in serum was unstable, which may be affected by many factors.

Conclusions

In conclusion, ANTXR1, GCN1, CIP2A, and differentially expressed proteins related to lipid metabolism (APOC1, APOC2, APOC3, ORM1, and ORM2) may be used as early markers of the harmful effects after long-term radon exposure.

ORCID iD

Xuhong Dang ![ORCID] https://orcid.org/0000-0002-2006-2503

References

1. Sinitsky MY, Druzhinin VG. The application of the cytokinesis-block micronucleus assay on peripheral blood lymphocytes for the assessment of genome damage in long-term residents of areas with high radon concentration. *J Radiat Res*. 2014;55(1): 61-66.

2. Maier A, Wiedemann J, Rapp F, et al. Radon exposure-therapeutic effect and cancer risk. *Int J Mol Sci*. 2020;22(1).

3. Rosenberger A, Hung RJ, Christiani DC, et al. Genetic modifiers of radon-induced lung cancer risk: a genome-wide interaction study in former uranium miners. *Int Arch Occup Environ Health*. 2018;91(8):937-950.

4. Sun L, Pan Y, Wang X, et al. Screening for potential biomarkers in peripheral blood from miners exposed to radon radiation. *Dose-response : A Publication of International Hormesis Society*. 2020;18(1):1559325820904600.

5. Zhang Y, Cai Y, Yu H, Li H. iTRAQ-based quantitative proteomic analysis identified HSC71 as a novel serum biomarker for renal cell carcinoma. *BioMed Research International*. 2015; 2015:802153.

6. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun*. 2019;10(1):1523.

7. Schneider J, Presek P, Braun A, Woitowitz H-J. Serum levels of pantropic p53 protein and EGF-receptor, and detection of anti-p53 antibodies in former uranium miners (SDAG Wismut). *Am J Ind Med*. 1999;36(6):602-609.

8. Helmig S, Lochnit G, Schneider J. Comparative proteomic analysis in serum of former uranium miners with and without radon induced squamous lung cancer. *J Occup Med Toxicol*. 2019;14:9.

9. Su S, Jin Y, Zhang W, et al. Aberrant promoter methylation of p16 INK4a and O 6 -Methylguanine-DNA methyltransferase genes in workers at a Chinese uranium mine. *J Occup Health*. 2006;48(4):261-266.

10. Piao C, Tian M, Gao H, et al. Effects of radon from hot springs on lymphocyte subsets in peripheral blood. *Dose-response : A Publication of International Hormesis Society*. 2020;18(1): 1559325820902338.

11. Xu Q, Fang L, Chen B, et al. Radon induced mitochondrial dysfunction in human bronchial epithelial cells and epithelial-mesenchymal transition with long-term exposure. *Toxicology Research*. 2019;8(1):90-100.

12. Ray S, Reddy PJ, Jain R, Gollapalli K, Moiyadi A, Srivastava S. Proteomic technologies for the identification of disease biomarkers in serum: advances and challenges ahead. *Proteomics*. 2011;11(11):2139-2161.

13. Zhang JH, Li Y, Song XB, et al. Differential expression of serum proteins in rats subchronically exposed to arsenic identified by iTRAQ-based proteomic technology-14-3-3 ζ protein to serve as a potential biomarker. *Toxicology Research*. 2016;5(2):651-659.

14. Wang J, Okkeri J, Pavic K, et al. Oncoprotein CIP 2A is stabilized via interaction with tumor suppressor PP 2A/B56. *EMBO Reports*. 2017;18(3):437-450.

15. Soofiyani SR, Hejazi MS, Baradaran B. The role of CIP2A in cancer: a review and update. *Biomed Pharmacother*. 2017;96: 626-633.

16. Mäkelä E, Pavic K, Varila T, et al. Discovery of a novel CIP2A variant (NOCIVA) with clinical relevance in predicting TKI resistance in myeloid leukemias. *Clin Cancer Res*. 2021;27(10): 2848-2860.

17. Pietrzyk Ł, Korolczuk A, Matysek M, Arciszewski MB, Torres K. Clinical value of detecting tumor endothelial marker 8 (ANTXR1) as a biomarker in the diagnosis and prognosis of colorectal cancer. *Cancer Manag Res*. 2021;13:3113-3122.

18. Huang X, Zhang J, Zheng Y. ANTXR1 is a prognostic biomarker and correlates with stromal and immune cell infiltration in gastric cancer. *Frontiers in Molecular Biosciences*. 2020;7: 598221.

19. Jong MC, Hofker MH, Havekes LM. Role of apoCs in lipoprotein metabolism. *Arterioscler Thromb Vasc Biol*. 1999;19(3): 472-484.

20. Moffatt MF, Kabesch M, Liang L, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature*. 2007;448(7152):470-473.

21. Han S, Lone MA, Schneiter R, Chang A. Orm1 and orm2 are conserved endoplasmic reticulum membrane proteins regulating lipid homeostasis and protein quality control. *Proc Natl Acad Sci Unit States Am*. 2010;107(13):5851-5856.

22. Cheng C, Geng F, Cheng X, Guo D. Lipid metabolism reprogramming and its potential targets in cancer. *Cancer Commun*. 2018;38(1):27.

23. Shi J, Yang H, Duan X, et al. Apolipoproteins as differentiating and predictive markers for assessing clinical outcomes in patients with small cell lung cancer. *Yonsei Med J*. 2016;57(3): 549-556.