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Peptidomics analysis of umbilical cord blood reveals potential preclinical biomarkers for neonatal respiratory distress syndrome

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ABSTRACT

Aims: The purpose of this study was to investigate the pathophysiology and discover novel predictors of neonatal respiratory distress syndrome (NRDS) from a peptidomics perspective.

Main methods: Comparative profiling of umbilical cord blood from NRDS and control patients was performed by liquid chromatography tandem mass spectrometry technology. The underlying biological functions of the differentially expressed peptides (DEPs) were predicted by Gene Ontology (GO) and KEGG pathway analyses. The interactions of DEPs and their precursor proteins were explored by ingenuity pathway analysis (IPA). The sources and stability of DEPs were determined by online databases, including UniProt, SMART and ProtParam tool.

Key findings: A total of 251 DEPs were identified, of which 139 peptides were upregulated, and 112 peptides were downregulated (fold change ≥ 2.0, $P < 0.05$). These DEPs were predicted to be associated with respiratory failure, atelectasis, and morphogenesis of endothelial cells. These processes indicated that DEPs may play a role in NRDS. Among them, eleven stable DEPs might be used as preclinical biomarkers.

Significance: Our findings improve our understanding of NRDS and facilitate the discovery of candidate diagnostic biomarkers for NRDS from the perspective of peptidomics.

1. Introduction

NRDS is one of the most common respiratory disorders in preterm infants and occurs in up to 7% of newborn infants [1]. With the increasing number of premature infants, especially extremely low birth weight infants [2], the incidence of NRDS is increasing each year. In addition to causing respiratory insufficiency, NRDS increases the risk of intraventricular hemorrhage, tension pneumothorax, bronchopulmonary dysplasia, sepsis, and even death. There is still an urgent need to find preclinical markers for the prevention and early treatment of NRDS. Previous studies have indicated that the components of cord blood are important clues for the early diagnosis of newborn diseases. For example, Protein S100B and IL-16 in umbilical cord blood could be used as biomarkers for hypoxic-ischemic encephalopathy in asphyxiated newborns [3,4]. This finding encouraged us to find novel markers derived from umbilical cord blood.

Peptidomics is an emerging branch of proteomics that targets endogenously produced protein fragments. This approach has been widely applied to identify specific biomarkers in bodily fluids. Circulating functional protein fragments are cleaved from precursor proteins by endogenous proteases, through a complex formation and degradation process [5,6]. In addition, these protein fragments can modulate signaling pathways through the involvement of multiple biological processes, including metabolism [7], protein synthesis [8], DNA damage

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[9] and cell protection [10]. Umbilical cord plasma has a high protein content and constantly perfuses the fetal tissue, which suggests that it could reflect the biological state and provide diagnostic information. Studies of umbilical cord blood have shown that a low content of placental growth factor indicates fetal growth restriction [11], and the content of NT-proB-type natriuretic peptide could reflect congenital heart disease [12]. Hence, early diagnostic biomarkers for NRDS may be discovered by exploring the peptide contents in umbilical cord blood.

In the present study, we performed a comparative peptidomics analysis of umbilical cord blood between NRDS and control samples to identify novel biomarkers related to NRDS. Tandem mass tag (TMT)-labeled liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to quantify the differentially expressed peptides (DEPs). We also analyzed the bioinformatics features, cleavage sites, gene ontology (GO), canonical pathways, and downstream relations of the down and up regulated peptides. We aimed to reveal the potential biofunctions of these DEPs and to explore the novel preclinical markers for the prevention and treatment of NRDS.

2. Materials and methods

2.1. Sample collection

Umbilical cord blood samples were obtained from newborns at a gestational age of 32–36 weeks between April 1, 2016 and April 29, 2016 at the Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University. Gestational age was determined based on the first day of the last menstrual period and confirmed by ultrasound. All samples were collected after obtaining signed consent forms and ethics board approval from Nanjing Obstetrics and Gynecology Hospital Affiliated with Nanjing Medical University. This study was performed in accordance with the World Medical Association Declaration of Helsinki. The NRDS and control groups were identified from electronic medical records by experienced neonatologists after combining X-ray results and clinical symptoms. Infants who had any other anatomical anomalies (such as Trisomy 18 or 21 chromosomal abnormalities or renal dysplasia) were excluded from this study. The maternal and neonatal clinical features of the two groups are compared in Table 1. The umbilical cord blood samples were collected and placed on ice immediately during transport to the laboratory (within 1 h). Cellular debris was removed by centrifugation at 1000*g* for 10 min at 4 °C. Protease inhibitor cocktail (EDTA-free, Roche, Basel, Switzerland) was added to samples, which were subsequently stored at 80 °C.

2.2. Peptide extraction and purification

Samples were centrifuged at 12,000*g* at 4 °C for 15 min. The supernatants were collected and mixed with acetonitrile (20% (v/v)) for 20 min at room temperature to disrupt interactions between proteins and peptides. Next, molecular weight cut-off (MWCO, 10 kDa) filters (Millipore, Amicon Ultra-15) were used to remove the abundant proteins. Flow-through was collected, and peptides were purified by C18 columns (Acclaim PepMap C18, 75 μm × 150 mm, LC Packings). The obtained filtrates containing the peptides were recovered and lyophilized (SCAN SPEED 40, Labogene, Denmark).

Table 1
Clinical features of the control and NRDS groups.

Group[a]	GA	MA	T-P-A-L	BPD	FL	AFV	BW
NC-1	32^{+5}	38	1-0-3-1	8.1	5.8	10.4	1630
NC-2	34^{+6}	32	0-0-1-0	8.1	6.2	4.4	1950
NC-3	35^{+4}	28	0-0-0-0	9	6.4	8.3	2320
NRDS-1	32^{+6}	39	1-0-2-1	8.3	6.1	7.7	1950
NRDS-2	33^{+6}	27	0-0-0-0	8.1	6.4	5.9	2040
NRDS-3	34^{+6}	29	0-0-0-0	9.1	6.2	4.7	2730

[a] GA, gestational age (weeks); MA, maternal age (years); T, term infants; P, preterm infants; A, abortuses; L, living children; BPD, biparietal diameter (cm); FL, femur length (cm); AFV, amniotic fluid volume (cm); and BW, fetal birth weight (g).

2.3. TMT labeling and LC-MS/MS analysis

Freeze-dried peptides were dissolved in 0.1% formic acid and filtered through 0.45 μM membranes. Then, 10 mM dithiothreitol (DTT) was used for 1 h at 60 °C to reduce the samples, and 100 μg of peptides were subsequently alkylated with 50 mM iodoacetamide for 45 min at room temperature. Next, peptides were desalted and lyophilized according to the manufacturer's instructions (TMT 6-plex Label Reagent, Thermo Scientific) to label 20 μg of sample with 5 μl of TMT reagent. Nanoflow LC (Easy-nLC, Thermo Fisher Scientific, Inc., San Jose, CA) combined with mass spectrometry (LTQ-Orbitrap Velos, Thermo Fisher Scientific, Inc.) was used to analyze the labeled samples. The labeled samples were then identified by mass spectrometry.

We used the LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, USA) and directly injected the samples. Full-scan analysis was performed over the *m/z* range of 600–5000 at two spectra per second. The capillary voltage and cone voltage were maintained at 3.9 kV and 40 V, respectively. For mass spectrometric analysis, 2000 single shot spectra were accumulated from 10 random positions on each sample, irradiating each position with 200 laser pulses. Compounds with S/N 15 from the mass spectrometric analysis were selected for MS analysis. All data were collected, converted, and analyzed using MassLynx™ Software (version 4.1, Waters Corporation).

2.4. Qualification and selection criteria

The in-house PEAK software database (version 7.0, Bioinformatics Solutions, Waterloo, Canada) containing 20,972 human protein sequences (released December 2015) was used to search the extracted MS/MS spectra data. The error tolerance was set to 15 ppm for precursor ion masses and 0.6 Da for fragment ion masses. The false discovery rate was estimated by the fusion target-decoy approach and was set at ≤1% at the peptide level.

2.5. Bioinformatics analyses

We calculated the molecular weight (MW) and isoelectric point (pI) of each peptide by using the ExPASy online tool (http://web.expasy.org/compute_pi). GO and pathway analyze (https://david.ncifcrf.gov/) were carried out to predict the putative functions of the identified peptides. The identified peptides and their precursor proteins were analyzed by Ingenuity Pathway Analysis (IPA) Software v7.1 (Ingenuity Systems, Mountain View, CA). The identified peptides and their precursor proteins were mapped to the associated network functions that were produced from the literature included in the Ingenuity System Knowledge Base. We used the online tools UniProt (http://www.uniprot.org/) and SMART (http://smart.embl-heidelberg.de/) to determine whether the peptide sequences were located within structural domains of their protein precursors.

2.6. Statistical analysis

We performed Student's *t*-test for comparisons between two groups and one-way ANOVA for comparisons among multiple groups. $^{*}P < 0.05$ was considered significant.

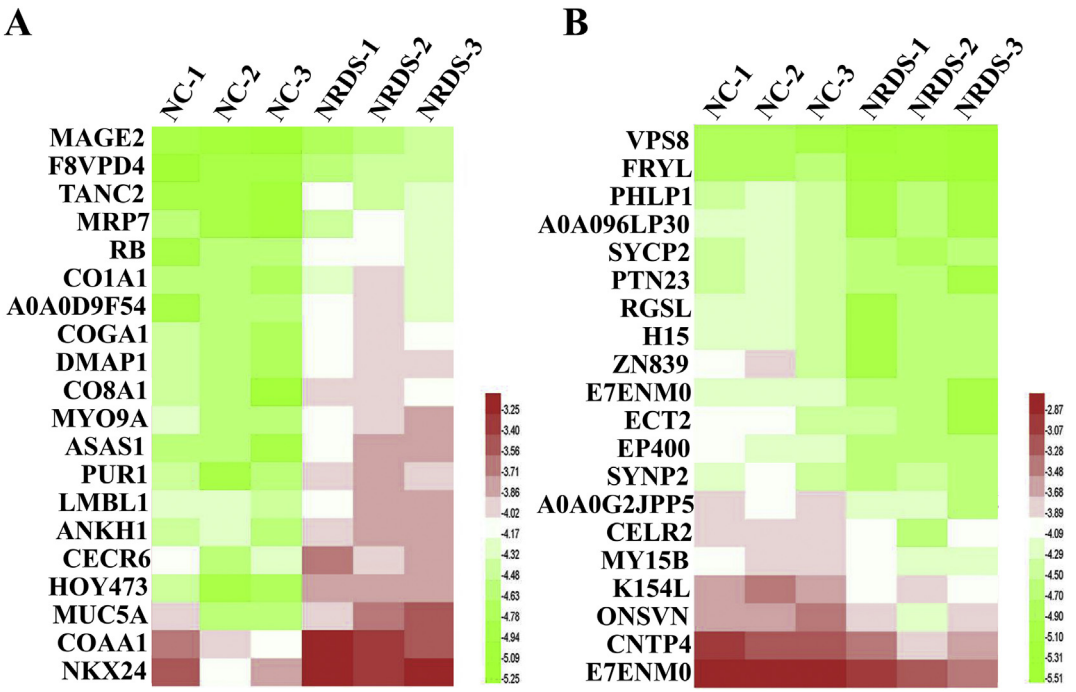

Fig. 1. Heat map for the distribution of the 40 peptides with the greatest fold changes between the NRDS and control groups detected in all the samples. (A) Upregulated peptides; (B) downregulated peptides. The peptide abundance is colored according to the heat map scale. Values are based on log10(values). (NC: normal control, NRDS: neonatal respiratory distress syndrome, high expression: red, low expression: green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Differentially expressed peptides derived from the NRDS and control groups

The umbilical cord blood peptides in the NRDS and control groups were analyzed by LC-MS/MS after TMT labeling. A total of 2878 non-redundant peptides were identified in umbilical cord blood from these two groups and quantified, with 215 differentially expressed peptides (fold change ≥ 2.0 and *P-value* < 0.05). All raw data are presented in Supplementary Table 1, and the differentially expressed peptides are listed in Supplementary Table 2. The twenty upregulated and downregulated peptides with the highest fold changes detected in all samples were graphically represented (Fig. 1A and B). The six columns represent three NRDS samples and three controls. The magnitude of peptide expression is indicated by color, according to the scale at the bottom right.

3.2. Characteristics of differentially expressed peptides in umbilical cord blood

We analyzed the composition distribution characteristics of the differentially expressed peptides from the NRDS and control groups, including MW, isoelectric point (pI) and the number of amino acids. Most peptides had a MW range of 500–3000 Da (Fig. 2A), which is consistent with the general characteristics of peptides. The pI ranges of the most upregulated and downregulated peptides were 8–9 and 10–12, respectively (Fig. 2B). The relative distribution of MW versus pI was also investigated (Fig. 2C). The number of amino acids in each peptide was also determined, and most peptides were < 25 amino acids in length.

3.3. Cleavage patterns of differentially expressed peptides

The regulation of peptide levels occurs according to the properties of the cleavage enzymes that release the peptides from their precursor proteins in particular tissues [13]. To further analyze the differentially expressed peptides, we combined peptidomics analysis of the infants' umbilical cord blood with bioinformatics analysis of the cleavage sites in the N-termini and C-termini. Four cleavage sites (C-terminal amino acid of the preceding peptide; N-terminal amino acid of the identified peptide; C-terminal amino acid of the identified peptide; N-terminal amino acid of the subsequent peptide) were investigated for each up- and downregulated peptide (Fig. 2E and F). The four dominant cleavage sites in the upregulated peptides were alanine (A), lysine (K), threonine (T), and glycine (G), while in the downregulated peptides, the four dominant cleavage sites were alanine (A), lysine (K), lysine (K), and proline (P).

3.4. GO and canonical pathway analysis

Because peptides may have biological functions similar to those of their precursor proteins, we attempted to evaluate the biological functions of the identified peptides according to their protein precursors through GO and pathway analysis. The GO results consisted of three categories: biological process, cellular component and molecular function. Enriched categories within these larger categories were obtained (Fig. 3A–C). Regarding biological processes, extracellular matrix organization, collagen catabolic process, cellular response to amino acid stimulus, cell adhesion, cell proliferation, endothelial cell morphogenesis, activation of GTPase activity, and microtubule-based movement were the most highly enriched subcategories. For cellular components, the most highly enriched sub-categories were endoplasmic reticulum lumen, extracellular matrix, basement membrane, proteinaceous extracellular matrix, cell junction, presynaptic membrane, extracellular region, etc. The most enriched molecular functions were extracellular matrix structural constituent, platelet-derived growth factor binding, metalion binding, calmodulin binding, ATP binding, poly (A) RNA binding, microtubule motor activity, and ATPase activity. The results were mapped to canonical pathways by IPA software (Fig. 3D). The results revealed that the differentially expressed peptides

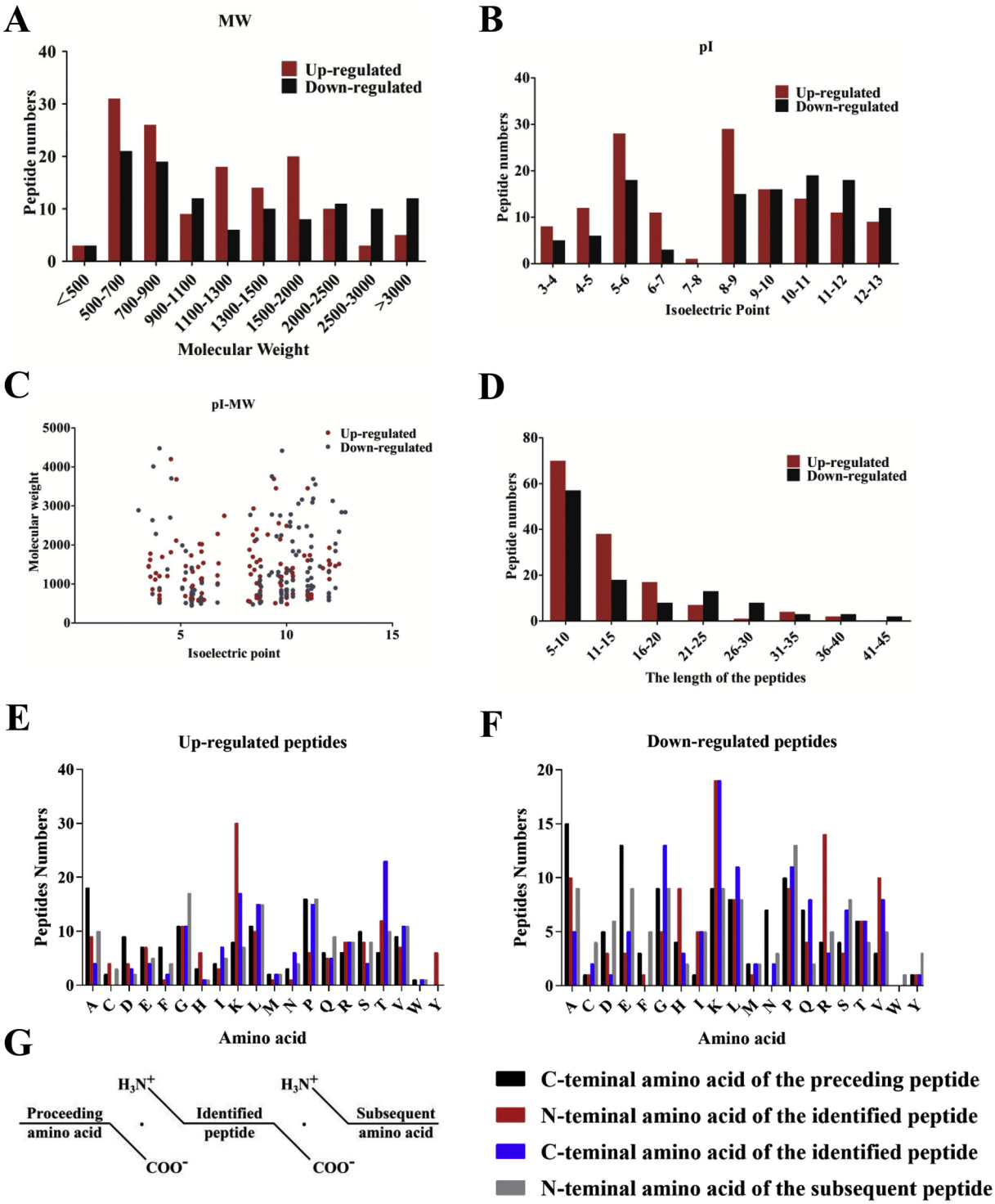

Fig. 2. Features of differentially expressed peptides identified by LC-MS/MS. (A) Molecular weight (MW); (B) isoelectric point (pI); (C) scatter plot of MW versus pI; (D) the amino acid number of peptides; (E) four cleavage sites in upregulated peptides (F) four cleavage sites in downregulated peptides; (G) diagram of the cleavage site distribution.

and their corresponding precursor proteins were involved in protein digestion and absorption, ECM-receptor interaction, amoebiasis, focal adhesion, the PI3K-Akt signaling pathway, small cell lung cancer, and pyrimidine metabolism.

3.5. Functional clustering by IPA

The differentially expressed peptides between the two groups were entered into IPA software to perform a comprehensive analysis of the precursor proteins and their related biological processes. The data analysis indicated that some of the precursor proteins that differed with respect to content in umbilical cord blood were related to respiratory system development and function. These precursor proteins were predicted to have roles in respiratory failure ($P = 3.12E-03$), atelectasis ($P = 8.53E-04$), morphogenesis of endothelial cells ($P = 1.35E-03$), structural integrity of basement membranes ($P = 2.63E-04$), and cell

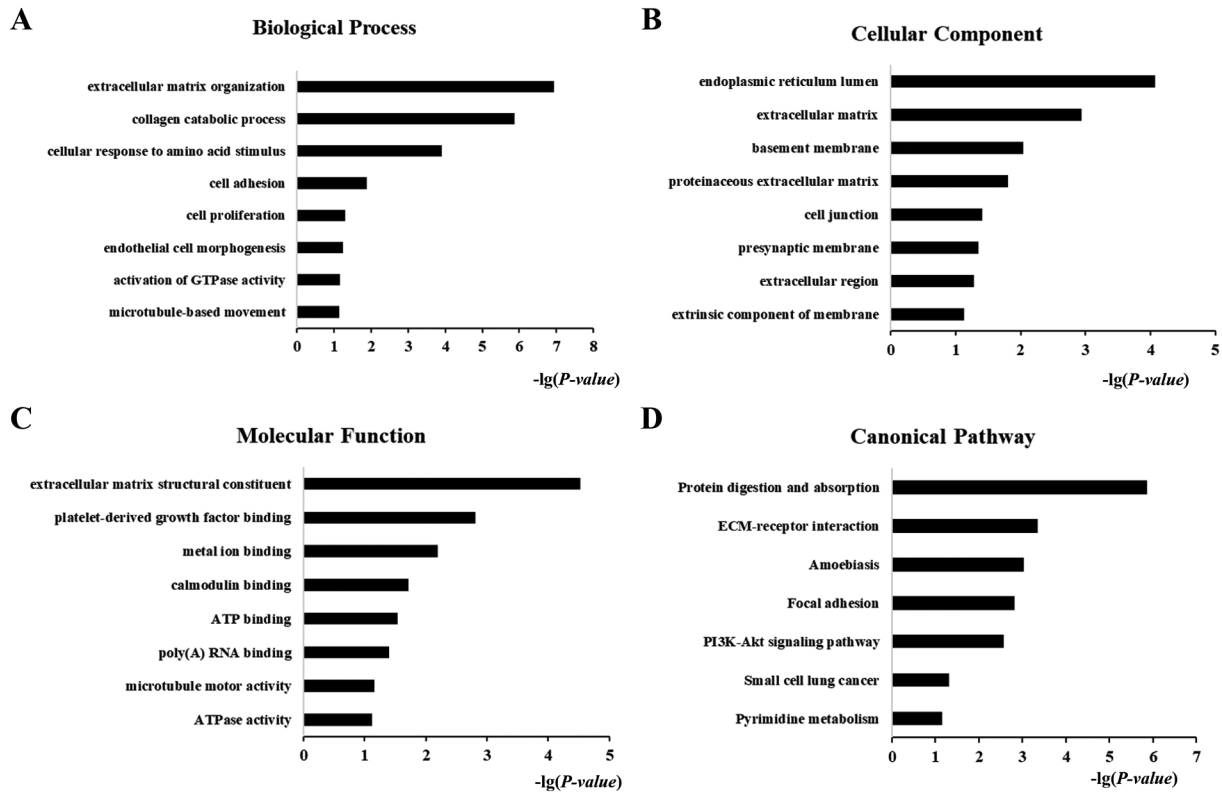

Fig. 3. Gene ontology (GO) and canonical signaling pathway analysis of the precursor proteins from which the differentially expressed peptides were derived. (A) GO analysis of the biological process; (B) GO analysis of the cellular component; (C) GO analysis of the molecular function; (D) mapping of differentially expressed proteins to canonical signaling pathways by Ingenuity Pathway Analysis (IPA).

movement of endothelial cell lines ($P = 7.34E-03$). Precursor proteins related to pulmonary development were also identified (Fig. 4A). The intensity of the node color indicates the degree of upregulation (red) or downregulation (green). MADD is the precursor protein most closely related to atelectasis from the network of upregulated peptides. MADD has already been shown to be associated with glucose metabolism, cell proliferation and metastasis in non-small-cell lung cancer [14,15].

IPA software was also used to generate the interaction network of the precursor proteins of 251 differentially expressed peptides, and highly scoring networks that may be associated with the respiratory system. Both "posttranslational modification, cellular function and maintenance, cell morphology" and "cell-to-cell signaling and interaction, cellular assembly and organization, cellular function and maintenance" were identified (Fig. 4B and C).

3.6. Putative bioactive peptides enriched in NRDS samples

To obtain biomarkers for NRDS in umbilical cord blood, the upregulated peptides were analyzed by the online bioinformatics analysis tool SMART. A domain is an important region of a protein, that performs biological functions and is highly conserved. Several peptides showed biological function and were reported to be derived from protein domains [16]. The ProtParam tool was used to calculate the stability of the upregulated peptides. Among the nineteen upregulated peptides, eight peptides were predicted to be unstable, in contrast, eleven peptides were considered stable (Table 2). Eleven stable NRDS-linked peptides might be bioactive and potential biomarkers.

4. Discussion

NRDS could induce acute respiratory failure or chronically develop into bronchopulmonary dysplasia, which is a leading cause of mortality and disability in preterm infants [17,18]. The discovery of novel biomarkers for NRDS is benefit for its early diagnosis and the clinical outcomes. Recent advances in peptidomics are actively being used to study umbilical cord blood peptides to better understand this complex biological fluid and to discover neonatal-related specific biomarkers. Potential markers of neonatal-specific diseases in cord blood, including growth-restricted fetuses [19], congenital heart disease [12,20], and Down's syndrome [21] have been recently discovered or analyzed. We conducted this study to determine whether there are respiratory, regular, and detectable peptide biomarkers in human cord blood to facilitate early diagnosis of NRDS. The samples were acquired from infants of the same gestational age to exclude preterm biomarkers. We used TMT-labeled analysis to compare the composition of umbilical cord blood peptides from the NRDS and control group and identified 2878 peptides from LC-MS/MS. The 251 peptides showed not only statistically significant changes ($P\text{-value} < 0.05$) but also more than two-fold differences, 139 of these peptides were upregulated relative to the control group, and 112 peptides were downregulated. According to our results, the number of peptides with a pI range of 7–8 was significantly smaller than the number of peptides with other pI ranges, which was consistent with studies of other plasma peptidomes and demonstrated that the results are reliable [22].

The peptidome hypothesis states that many proteins and peptides are shed into the local circulation from the microenvironment [23]. Under varying pathophysiological conditions, cells shed different degradation products which will be pushed to the circulation. Although full-length cellular or tissue proteins are too large to passively penetrate the vessel wall, they can enter the circulation freely by diffusion or convection in the form of cleavage fragments. Circulating protein fragments generated in the body fluid or tissues can reflect biological events and provide emerging information for clinical diagnosis [23]. As products of proteolysis, peptides represent a series of reaction results under different conditions [24], and serve as mediators for mediating environmental changes and biological events [25]. Therefore,

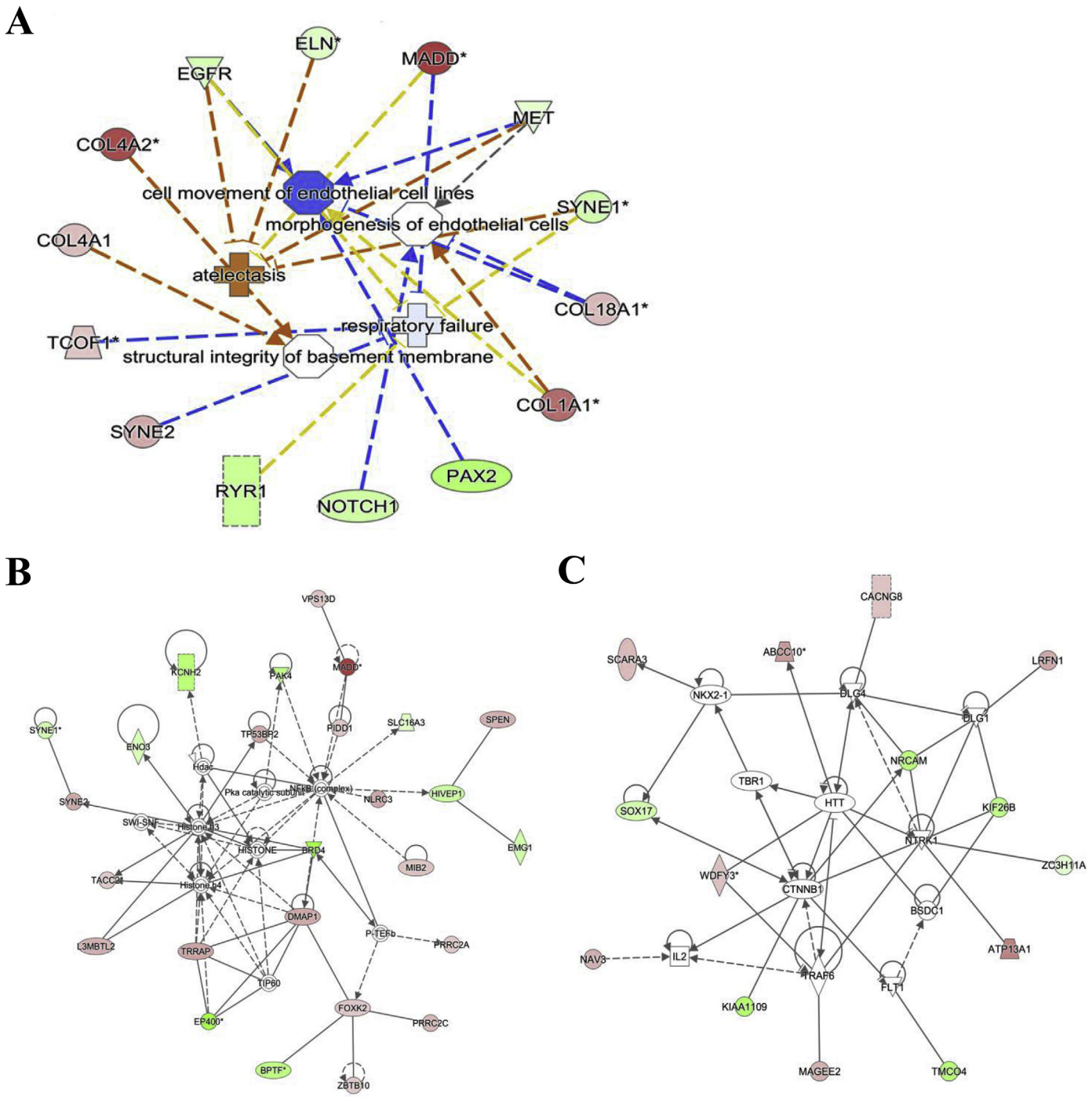

Fig. 4. Network analysis based on the precursor proteins of 251 differentially expressed peptides by IPA. (A) Downstream effect analysis of differentially expressed peptides on respiratory diseases. (Precursor proteins, diseases and functions are represented as nodes, and the biological relationships between nodes are represented as lines with arrows.) (B) Network analysis of "precursor proteins associated with post translational modifications", "cell morphology" and "cellular functions and maintenance". (C) Network analysis of the peptide precursors associated with "cell-to-cell signaling and interactions", "cellular assembly and organization", and "cellular function and maintenance". The intensity of the node color indicates the degree of up or downregulation (upregulation, red; downregulation, green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

peptidomics analysis may help us find early markers of NRDS and further explore its pathophysiological mechanisms.

The biological activity of proteolytic enzymes varies in physiological and pathological conditions. The cleavage site pattern can indicate the cleavage specificity and activity of proteolytic enzymes [26]. The cleavage site distribution at the amino-terminus (N-terminal) and carboxyl-terminus (C-terminal) of the peptides, is shown in Fig. 2E and F. For the overexpressed peptides, lysine is the most common cleavage site. Compared with the gestational diabetes-induced *macrosomia* peptidome in umbilical cord blood, lysine (K) was also the most common cleavage site, but the four peptide cleavage sites were all different [22]. This finding indicates that in different physiological and pathological states, the production of peptides involved cleavage according to certain rules and based on the tight regulation of proteases under specific conditions [27]. Thus, endogenous peptides produced in the same bodily fluid can change during different disease states and present

Table 2
Potential peptide markers for preclinical diagnosis of NRDS.

Peptide sequence	Protein name	Number of amino acids	Molecular weight	Theoretical pI	Stability
DLEVLEGGAATL	OBSCN	12	1187.31	3.57	Stable
ATQDNAHRAEATRRVLERLVLALGPLGPQAVQ	CO7A1	32	3451.94	9.5	Stable
GSPLV	HMCN2	5	471.55	5.52	Unstable
SGKSKGGK	MYH6	8	747.85	10.3	Stable
YLALGLLKLVGTMLG	MRP7	15	1561.99	8.59	Stable
CLKSVTLSLDGAQT	MUC5A	14	1435.66	5.83	Stable
RRLLHRVLMAPV	MRP7	12	1460.85	12.3	Unstable
KTGLLF	LRFN1	6	677.84	8.75	Stable
IITLEDVNDNSPQ	FAT2	13	1457.56	3.49	Unstable
DECSKDNGGCQHECVNTMGSYMCQCRNGFVLHD	E9PD25	33	3682.05	4.8	Stable
ESLDMPKASLL	MAGE2	11	1203.42	4.37	Unstable
YVAAKLALGI	F8VPD4	10	1018.26	8.59	Stable
TKTSRLP	CO1A1	7	801.94	11	Unstable
ETAPL	Q5H8Y1	5	529.59	4	Unstable
GPVGGRGPKGDPGSLGPL	SCAR3	18	1617.83	8.75	Stable
EDHLYMVFELVNQG	KKCC2	14	1693.89	4.13	Unstable
QGPFTTQ	PERQ1	7	777.83	5.52	Stable
TQSNLLSVAGRLGLDW	PIDD1	16	1729.95	5.5	Stable
SGLWSFYFFFAS	ABCA6	12	1458.64	5.24	Unstable

different proteolytic cleavage patterns. Therefore, protein degradation fragments could be biomarker candidates for diseases.

Peptide function was predicted by analysis on its precursor protein. In biological processes, extracellular matrix organization, collagen catabolic process, cellular response to amino acid stimulus and cell adhesion were enriched. Damage to alveolar epithelial cells causes a series of structural and membrane protein changes when NRDS occurs [28], including extracellular matrix damage and abnormal expression of epithelial adhesion molecules [29]. Studies have shown that cell adhesion components in the bronchoalveolar lavage fluid are elevated significantly in NRDS patients compared with controls [30]. This result is consistent with the results of our NRDS umbilical cord blood peptides. In the cellular component, the endoplasmic reticulum lumen, extracellular matrix, basement membrane, and proteinaceous extracellular matrix were enriched. These components are associated with tissue fibrosis. The endoplasmic reticulum is involved in protein synthesis, and the extracellular matrix is rich in collagen. When the alveolus is damaged, the basement membrane is exposed and injured. The well-maintained basement membrane provides support for the repair of lung tissue. Additionally, fibrillar collagen proliferation will gradually cause pulmonary fibrosis [31]. In molecular function, extracellular matrix structural constituents, platelet-derived growth factor (PGDF) binding, metal ion binding and calmodulin binding were enriched. When NRDS occurs, the content of magnesium and potassium ions in plasma increases and sodium ions decrease due to acidosis, which is consistent with metal ion binding enrichment. The pathway enrichment analysis revealed protein digestion and absorption, ECM-receptor interaction, focal adhesion and the PI3K-Akt signaling pathway. Protein digestion and absorption impacts peptide concentrations in the blood. During the pathophysiology of NRDS, the alveolar and capillary endothelium are damaged by acidosis, alveolar fluid and protein leakage to form a transparent membrane [32]. Acidosis affects calcium homeostasis. Phosphorylation is essential for the synthesis of dipalmitoyl lecithin, which is a key substance that affects the development of NRDS [32]. The GO analysis results were consistent with the pathological changes associated with NRDS. We also found that some of the precursor proteins of the differentially expressed peptides, such as PAX2 [33], RYR1 [34], and NOTCH1 [35], are closely related to respiratory diseases according to Fig. 4A. Ryanodine Receptor1 (RyR) is a calcium release channel found on the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR). It can rapidly release Ca^{2+} from the ER/SR, which leads to an increase in extracellular calcium concentration, thereby exerting a series of physiological functions. RYR1 also plays a significant role in maintaining intracellular and extracellular calcium balance [36]. This suggests that changes in peptidomics may be related to respiratory function. Peptides derived from cord blood might be used to indicate the occurrence of NRDS.

It was recently recognized that portions of peptides can be considered biomarkers and are amplified in the circulatory system because they accumulate on high-concentration resident proteins, acquire a longer half-life and protect themselves from clearance [23]. For example, endothelin-1 could be used to predict the mortality of acute heart failure [34], besides, plasminogen activator inhibitor-1 could be used as predictors on for insulin resistance in polycystic ovary syndrome patients [35]. As shown in Table 2, nineteen peptides in the precursor protein domain were found to be up-regulated in NRDS umbilical card blood. Notably, eleven peptides derived from the domains were thought to be stable and enriched in the umbilical card blood. Therefore, these peptides might be potentially preclinical biomarkers for NRDS. In addition, analyses on their precursor proteins indicates that they might play important regulatory roles in physiological and pathophysiological processes, including contributions to epithelial basement membrane organization and adhesion, cellular detoxification, mucosa protection, scavenging of oxidative molecules, and regulation of tyrosine kinase receptor signaling. In order to explore their potential roles and the correlation with the occurrence of NRDS, it could be further detected using targeted mass spectrometry in future [37].

Some of the precursor proteins of differentially expressed peptides are known to be involved in pulmonary development, disease and abnormal lung morphology, providing a molecular signature. Combining these findings with bioinformatics analyses indicates that the peptides in umbilical cord blood are closely related to NRDS. In addition, eleven stable and upregulated peptides were identified that may serve as biomarkers. The presented data contain valuable and novel information, which can serve as a background for further study. These findings provide a platform for researchers interested in identifying biomarkers for early diagnosis and improve our understanding of the roles that these peptides may play in NRDS.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116737.

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Declaration of competing interest

No competing financial interests exist.

Author contribution

Shuping Han, Aiqing Zhang and Jun Wang designed this study. Yin Hu, Juan Wang and Yahui Zhou wrote this manuscript, Xiangyun Yan, Xingyun Wan, Wenjuan Chen, Yiwen Liu, Xue Chu carried out the experiments and performed the statistical analysis. Yin Hu, Yahui Zhou, Xiangyun Yan wrote the manuscript.

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