Peptidome analysis of umbilical cord mesenchymal stem cell (hUC-MSC) conditioned medium from preterm and term infants

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Abstract

Background: The therapeutic role of mesenchymal stem cells (MSCs) has been widely confirmed in several animal models of premature infant diseases. Micromolecule peptides have shown promise for the treatment of premature infant diseases. However, the potential role of peptides secreted from MSCs has not been studied. The purpose of this study is to help to broaden the knowledge of the hUC-MSC secretome at the peptide level through peptidomic profile analysis.

Methods: We used tandem mass tag (TMT) labeling technology followed by tandem mass spectrometry to compare the peptidomic profile of preterm and term umbilical cord MSC (hUC-MSC) conditioned medium (CM). Gene Ontology (GO) enrichment analysis and ingenuity pathway analysis (IPA) were conducted to explore the differentially expressed peptides by predicting the functions of their precursor proteins. To evaluate the effect of candidate peptides on human lung epithelial cells stimulated by hydrogen peroxide (H2O2), quantitative real-time PCR (qRT-PCR), western blot analysis, and enzyme-linked immunosorbent assay (ELISA) were, respectively, adopted to detect inflammatory cytokines (TNF-α, IL-1β, and IL-6) expression levels at the mRNA and protein levels.

Results: A total of 131 peptides derived from 106 precursor proteins were differentially expressed in the preterm hUC-MSC CM compared with the term group, comprising 37 upregulated peptides and 94 downregulated peptides. Bioinformatics analysis showed that these differentially expressed peptides may be associated with developmental disorders, inflammatory response, and organismal injury. We also found that peptides 7118TGAKIKLV7127 derived from MUC19 and 508AAAAGPANVH517 derived from SIX5 reduced the expression levels of TNF-α, IL-1β, and IL-6 in H2O2-treated human lung epithelial cells.

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Introduction

The incidence of preterm birth has increased over the past 20 years in most countries [1, 2]. Despite recent advances in perinatal medicine, severe diseases related to premature birth, including periventricular leukomalacia (PVL), bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), and retinopathy of prematurity (ROP), remain major causes of mortality and morbidity, which represent a heavy burden for families and society [3]. Therefore, it is an urgent and significant task to develop new safe and effective treatments to improve the prognosis of these diseases in premature infants.

In the past several decades, the development of mesenchymal stem cell (MSC) therapy and its continuous advancement have gained extensive attention. MSCs are multipotent progenitor cells, which can be raised from different tissues, for instance, adipose tissue, umbilical cord, and bone marrow [4, 5]. Human umbilical cord MSCs (hUC-MSCs) are easily accessible and can be harvested from donors without risks or damage [6]. Additionally, the therapeutic application of MSCs is not limited by the aging-like nature of adult tissues such as bone marrow and adipose tissue [7, 8]. Mechanically, MSCs function in vivo via direct differentiation or paracrine action. The therapeutic potential of MSC engraftment has been proved in premature infant diseases, and early clinical trials in preterm neonates with BPD (NCT01297205 [9], NCT01632475 [10]) and severe intraventricular hemorrhage (NCT02274428 [11]) have been conducted. A myriad of bioactive factors are readily available in the conditioned medium (CM) of MSCs and the medium can mediate multiple known functions of MSCs, such as angiogenesis, anti-fibrosis, and anti-inflammatory effects [12]. Extracellular vesicles such as exosomes have been isolated from CM, and they have been shown to contain microRNAs and proteins, which partially mediated the effects of MSC [13–15]. Many studies have established that the secretome from MSCs can reduce organ damage in animal models of PVL, BPD, NEC, and ROP [16–20]. Other studies have also shown that soluble factors such as heme oxygenase-1 (HO-1) and erythropoietin (EPO) may be principally responsible for the ability of MSC-CM to ameliorate inflammation, angiogenesis, fibrosis, and so on [21, 22]. More types of MSC secreted factors and regulatory mechanisms still need to be established.

Peptides, a type of compound with two or more amino acids connected by peptide bonds, have been shown to play important roles in the treatment of diseases. Glucagon-like peptide-1 (GLP-1), a well-known peptide hormone secreted from the L cells of the duodenum, colon, terminal ileum, and rectal mucosa, has been used in the clinical treatment of type 2 diabetes [23]. Extrinsic calcitonin gene-related peptide (CGRP) could suppress apoptosis, oxidative stress, and ROS production in hyperoxia-induced alveolar epithelium type II (AECII) cells [24]. WKYVM6 hexapeptide could attenuate hyperoxia-induced lung injuries in newborn mice [25]. Additionally, peptides from human milk such as PDC213, β-casein 197, and Casein201 exhibited obvious antimicrobial effects on the common pathogenic bacterial species S. aureus and Y. enterocolitica in neonatal intensive care units [26–28]. These studies indicated that peptides may hold great promise for the treatment of premature infant diseases. However, the secreted peptidomic profile from hUC-MSCs has not been fully characterized.

In the present study, we compared the secreted peptides from preterm and term hUC-MSCs using the tandem mass tag (TMT) labeling method with liquid chromatography (LC)-tandem mass spectrometry (MS/MS) analysis. Moreover, using ingenuity pathway analysis (IPA) software, we predicted that the differentially expressed peptides are associated with developmental disorder, inflammatory response, and organismal injury. And we preliminarily investigated the antiinflammatory effect of differentially expressed peptides on human lung epithelial cells. This study helps to broaden the knowledge of the hUC-MSC secretome at the peptide level and provided potential clues for the treatment of respiratory diseases in premature infants.

Materials and methods

Patients and samples

Umbilical cords were obtained from six infants without genetic or structural anomalies delivered at 27–41 weeks of gestation with parental written consent. Cases involving maternal diabetes, pre-eclampsia, eclampsia, intrauterine growth retardation (IUGR), or infectious diseases were excluded, because these factors may influence cell proliferation, cytokine expression, and other functions [29–33]. This study was approved by the Ethics
Committee of Changzhou Maternal and Child Health Care Hospital (approval number 2019126) and conducted in accordance with the approved guidelines.

**Preparation of hUC-MSCs and CM**

Human umbilical cords were collected after preterm \((n = 3)\) or full term \((n = 3)\) deliveries hUC-MSCs were obtained by the tissue explants adherent method, as previously reported [17, 34]. Each umbilical cord (about 10 cm) was washed in phosphate-buffered saline (PBS; Gibco, Grand Island, CA, USA) and with 1% penicillin/streptomycin (P/S; Gibco) to remove residual blood from the vein and arteries. After the cord was cut longitudinally, and the arteries and vein were removed, Wharton’s jelly was finely dissected into small pieces. The pieces were individually placed on 100-mm\(^2\) tissue culture dishes with Dulbecco’s modified Eagle medium/nutrient mixture F-12 (DMEM/F-12; Gibco) containing 10% fetal bovine serum (FBS; Gibco), and 1% P/S (Gibco) and incubated for 10–12 days at 37 °C with 5% CO\(_2\). The medium was subsequently exchanged every 2–3 days. The cultures were passaged when they reached 80–90% confluency after carefully removing the umbilical cord tissues. hUC-MSCs at passage 3 were cultured to 80–90% confluence in a T75 culture flask (about 10\(^6\) cells). The complete medium was replaced with serum-free DMEM/F-12 medium (5 ml) to avoid peptides contamination from FBS. The collected serum-free medium was centrifuged for 10 min at 3000×g at 4 °C to remove cell debris, and protease inhibitor (Roche, Basel, Switzerland) was added. Lastly, the mixture was snap-frozen in liquid nitrogen and stored at − 80 °C until used.

**Peptide extraction and purification**

Before peptide extraction, the protein integrity of the CM samples was appraised. The collected CM samples were concentrated by centrifugation under vacuum (LaboGene, Allerød, Denmark), boiled in sodium deoxycholate sulfate (SDS)-sample buffer at 95 °C for 10 min and then subjected to 12% SDS-polyacrylamide gel electrophoresis (PAGE). The SDS-PAGE gel was stained using a Pierce Silver Stain Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer’s protocol. Thereafter, the samples were filtered through an ultrafiltration tube (Amicon Ultra-15, Millipore, MA, USA) with a molecular weight cutoff (MWCO) of 10 kDa to acquire the filtered liquid containing the peptides. The protein concentration of the supernatant was also measured using the Bradford protein assay [35].

**TMT labeling and LC-MS/MS analysis**

The peptides from preterm and term hUC-MSC CM were reduced with 10 mM DL-dithiothreitol (DTT; Promega, WI, USA) for 1 h at 56 °C and alkylated with 55 mM iodoacetamide (Promega) for 1 h in the dark at RT. Thereafter, precooled acetone was added, and the peptides were precipitated over 3 h at − 20 °C. After centrifuging for 20 min at 20,000×g and 4 °C, the precipitate was dissolved in 300 μl of the following buffer: 50% triethylamine borane ( Sigma) and 0.1% SDS (Sigma). Next, the peptide solution was desalted using a Strata-X C18 column (Phenomenex, Torrance, CA, USA) and dried and labeled with TMT reagent (TMT 6-plex Label Reagent; Thermo Fisher Scientific) for 1 h [36]. Next, the preterm and term samples were mixed at a 1:1 ratio on the basis of the total peptide amount. Analysis of labeled peptides was performed on a Q Exactive Orbitrap LC-MS/MS system (Thermo Fisher Scientific). Qualitative and relative quantitative analyses of the detected peptides were performed using the SWISSPROT\_human database and Mascot software (version 2.3.01). Peptides with absolute fold change ≥ 1.5 and \(P\) value < 0.05 were considered differentially expressed.

**Bioinformatics analysis**

The molecular weight (MW) and isoelectric point (PI) of the identified peptides were calculated using the online tool PI/MW (http://web.expasy.org/compute.pi/). A Gene Ontology (GO) analysis (http://www.blast2go.com/b2ghome) was carried out to explore the possible cellular components, biological processes, and molecular functions related to the precursor proteins. Diseases and regulator effects networks analysis of the differentially expressed peptides and their precursors were performed using ingenuity pathway analysis (IPA) software v7.1 (Ingenuity Systems, Mountain View, CA, USA) [37]. The UniProt database (http://www.uniprot.org/) was used to detect the predominant subcellular locations of the precursors of the differentially expressed peptides. The Open Targets Platform database (http://www.targetvalidation.org/) was applied to study the diseases associated with the protein precursors [38].

**Synthetic peptides**

All the peptides used in this study were synthesized by GenScript (Nanjing, Jiangsu, China) through the solid-phase method. The purity of each peptide was 95% detected by HPLC-MS method. All the used peptides were preserved in freeze-drying at − 20 °C until immediately dissolved in aseptic water for cell treatment in vitro.

**Cell culture**

Two peptides TGAKIKLGVGT and AAAAGPANVH were selected with high fold change, and we investigated the effects on human lung epithelial cells A549 (Meiyan, Shanghai, China) stimulated by hydrogen peroxide \((H_2O_2; Kelong, Chengdu, Sichuan, China)\). A549 cell is a human lung carcinoma cell line from an adult with
similar characteristics of human alveolar basal epithelial cells, and was often used for researches in BPD. A549 cells were cultured in Dulbecco’s modified Eagle medium (Gibco) with 10% FBS and 1% P/S. The A549 cells were exposed to 1 mM H₂O₂ with or without peptides (1 μM, 10 μM, and 100 μM) for 24 h in serum-free DMEM with 1% P/S. The sample size was 3 biological independent samples per group.

Quantitative real-time polymerase chain reaction (QRT-PCR)
Total RNA was extracted with Trizol reagent (Invitrogen; Thermo Fisher Scientific Inc., Shanghai, China) according to the manufacturer’s instructions. The RNA concentration was detected by ultraviolet spectrophotometer and the appropriate OD value at 260 to 280 nm was 1.8 to 2.0. Then, qRT-PCR was carried out to measure the gene expression levels of inflammatory factors: tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) mRNAs in A549 cells using SYBR Green qPCR method (Thermo Fisher Scientific, Waltham, MA). Primer sequences were the following: human TNF-α: forward, 5′-CCTCTCTCTAATCAGCCTCTG-3′, reverse, 5′-GAGGACCTGGGAGTAGT-GAG 3′; human IL-1β: forward, 5′-AGCTACGAATCTCCGACCAC-3′, reverse, 5′-CGTATCCCATGTCGAAGA-3′; human IL-6: forward, 5′-ACTCACCTCTCCGACCAC-3′, reverse, 5′-CCATCTTTGGAAGGTTT-3′; mouse IL-6: forward, 5′-AGCGAGCATCCCCCAAAGTT-3′, reverse, 5′-GGGCACGAAGGCTCATCATT-3′. To calculate fold change in the expression of these genes, \( \Delta \Delta Ct = Ct \) of individual genes – Ct of β-actin was first obtained. \( \Delta \Delta Ct = \Delta Ct \) of treated groups – \( \Delta Ct \) of control groups was then obtained. Fold change was calculated as \( 2^{-\Delta \Delta Ct} \), with control groups as 1.0-fold.

Western blot analysis
Lysates from cultured cells were similarly prepared using a cell scraper. Homogenates were clarified by centrifugation (10,000xg, 4 °C, 10 min). Protein concentration was quantified with a BCA protein assay (Beyotime, Shanghai, China). Cell extracts resolved on a 10% or 12% reducing SDS-PAGE gel were transferred to a nitrocellulose membrane according to molecular weight. Blots were probed with the following antibodies: rabbit anti-TNF-α (1:2000; Proteintech, Wuhan, China), anti-IL-1β (1:1000; Proteintech, Wuhan, China), and mouse anti-IL-6 (1:5000; Proteintech, Wuhan, China), whereas mouse GAPDH (1:5000; Proteintech, Wuhan, China) served as a loading control.

Enzyme-linked immunosorbent assay (ELISA)
ELISA was also performed to detect the expression levels of inflammatory factors in A549 cells by ELISA kits for human TNF-α, IL-1β, and IL-6 (4A Biotech, Beijing, China), according to the manufacturer’s instructions. Optical density was measured at 450 nm using an ELISA microplate reader. No significant cross-reactivity or interference was observed.

Statistical analysis
Student’s t test or one-way ANOVA was employed for statistical comparisons. The results of the bioinformatics analysis were visualized using GraphPad Prism 5/7 software. The statistical significances were calculated as \( P \) values, and \( P < 0.05 \) was considered statistically significant.

Results
Isolation and characterization of MSCs derived from human umbilical cords
The range of gestational ages (GA) was 31–32 weeks of gestation for the preterm umbilical cords and 40–41 weeks for the term cords (Table 1). The hUC-MSCs (obtained by the tissue explants adherent method) had a typical fibroblast phenotype (Fig. S1A). Both preterm and term hUC-MSCs were positive for CD29, CD73, and CD90 and negative for CD31, CD34, and HLA-DR staining (Fig. S1B). Furthermore, these isolated cells had the potential to differentiate into adipocytes and chondrocytes (Fig. S1A). These results confirmed that MSCs from human umbilical cords were successfully isolated, without significant differences in morphology, expression of cell surface markers or differentiation capacities between the preterm and term groups.

Identification of differentially expressed peptides in hUC-MSC CM from preterm and term infants
We verified the protein integrity of the hUC-MSC CM by silver staining (Fig. S2). The peptides from preterm and term hUC-MSC CM were directly analyzed by the TMT labeling method combined with LC-MS/MS. We identified a total of 3099 peptides in hUC-MSC CM from both groups. A total of 131 peptides were observed to be significantly differentially expressed (absolute fold change ≥ 1.5, \( P \) value < 0.05) in the hUC-MSC CM from the preterm group compared with the term group, comprising 37 upregulated peptides (Fig. 1a) and 94 downregulated peptides (Fig. 1b). The top 20 upregulated and top 20 downregulated peptides are shown with their precursor proteins in Fig. 1c and d. All the differentially expressed peptides are shown in Table 2.
Basic characteristics of the differentially expressed peptides in hUC-MSC CM from preterm and term infants

The MW and PI of the differentially expressed peptides were analyzed. The MW of most peptides ranged from 500 to 700 Da (Fig. 2a), and the PI ranged from 3 to 11 (Fig. 2b). We also investigated the distribution of the MW relative to the PI (Fig. 2c). Peptides are cleaved from their precursor proteins by specific enzymes [37], so we analyzed the cleavage sites at the N- and C-terminals of the identified peptides. Lysine (K) was the most common N-terminal amino acid (accounting for 13.7% of the peptides), while asparagine (N) was the most common C-terminal amino acid (accounting for 16.0% of the peptides) (Fig. 2d).

| Sample | Gestational age (weeks) | Birth weight (g) | Sex | Apgar score 1 min | Apgar score 5 min | Maternal age (years) | Gravidity | Parity | Perinatal history |
|--------|------------------------|------------------|-----|-------------------|-------------------|----------------------|------------|--------|------------------|
| P 1    | 32 + 5                 | 1630             | Female | 10               | 10                | 28                   | 2          | 1      | Cesarean section due to placental abruption |
| P 2    | 32 + 5                 | 1850             | Male  | 10               | 10                | 29                   | 2          | 1      | Vaginal delivery due to premature rupture of fetal membranes |
| P 3    | 31                     | 1600             | Female | 8                | 8                 | 33                   | 5          | 3      | Cesarean section due to active premature labor |
| T 1    | 40 + 3                 | 4670             | Male  | 10               | 10                | 23                   | 1          | 1      | Cesarean section due to primary uterine atony |
| T 2    | 41 + 1                 | 3360             | Female | 10               | 10                | 29                   | 2          | 2      | Repeated cesarean section |
| T 3    | 40 + 6                 | 4250             | Female | 10               | 10                | 27                   | 2          | 1      | Cesarean section due to non-reassuring fetal status |

Fig. 1 Differentially expressed peptides in hUC-MSC conditioned medium (CM) from preterm infants compared with term infants. a, b Upregulated and downregulated peptides visualized using heatmaps ($n = 3$ per group, P1–3 represent preterm infants and T1–3 represent term infants). c, d Top 20 upregulated and top 20 downregulated peptides visualized using heatmaps.
| Accession | Gene | Protein | Peptide | MW (kD) | Fold change | −10lgP |
|-----------|------|---------|---------|---------|-------------|--------|
| Q6PEZ8-3  | PODNL1| Podocan-like protein 1 | PSLERLHQNNLISKVPR | 5.94 | 6.96 |        |
| A0A0A0MTS7| TTN  | Titin   | ESQSG   | 2.92 | 13.62 | 9.66   |
| Q8BYJ1-2  | ALOXE3| Hydroperoxide isomerase ALOXE3 | LNGRQQY | 0.54 | 7.95 | 10.08  |
| Q15772-1  | SPEG | Striated muscle preferentially expressed protein kinase | SCTVAVARPGLAPEVPQ | 0.76 | 5.96 | 10.05  |
| Q8N196    | SIX5 | Homeobox protein SIX5 | AAAAGPANVH | 1.00 | 5.93 | 11.82  |
| P49815-3  | TSC2 | Tuberin | PAGPAVRL | 0.57 | 4.52 | 10.67  |
| A0A06YA3  | CDHR1| Cadherin-related family member 1 | RVLKRRPSAPRTIRIE | 0.69 | 3.82 | 9.34   |
| Q7Z5P9-2  | MUC19| Mucin-19 | DDFMSSQKN | 2.05 | 3.46 | 5.01   |
| P54259    | ATN1 | Atrophin-1 | GPARPYHP | 0.67 | 3.28 | 11.27  |
| A0A140TA73| SNTB2| Beta-2-syntrophin | NGLPNGGAGDS | 0.88 | 2.98 | 7.03   |
| Q9UQD0-2  | SCN8A| Sodium channel protein type 8 subunit alpha | EAGID | 1.81 | 2.67 | 16.18  |
| Q5TZA2-2  | CROCC| Rootletin | RLLKGEASLEV | 0.68 | 2.67 | 9.78   |
| E7EMZ9    | TACC2| Transforming acidic coiled-coil-containing protein 2 | RMSESPTPC | 1.29 | 2.57 | 8.92   |
| A0A075B756| KLF14| Krueppel-like factor 14 | TKHARRHP | 0.94 | 2.52 | 17.50  |
| A0A0A0MTS7| TTN  | Titin   | LEDGG   | 3.06 | 2.50 | 12.31  |
| Q15Q27-2  | SEC16A| Protein transport protein Sec16A | KSLTQ | 2.31 | 2.49 | 13.78  |
| Q9NRA0-3  | SPHK2| Sphingosine kinase 2 | EWDGIVTSGDGLHEVLN | 0.56 | 2.46 | 10.14  |
| Q8WXG9    | ADGRV1| Adhesion G-protein coupled receptor V1 | EAGLD | 2.02 | 2.23 | 19.57  |
| Q9Y4D8    | HECTD4| Probable E3 ubiquitin-protein ligase HECTD4 | KLAKLQRIARQAVAALCAGG | 1.02 | 2.21 | 6.58   |
| A0A087W7F8| PDE4D1P| Myomegalin | KSMMAV | 1.01 | 2.18 | 21.26  |
| Q01167    | FOXK2| Forkhead box protein K2 | QTHVHHV | 0.67 | 2.18 | 12.99  |
| Q5U48     | CRB2 | Protein crumbs homolog 2 | LLEAVAAPACCLLLLLLGLGLGLAARK | 0.78 | 2.18 | 5.08   |
| Q9U4EL3   | DENND2A| DENN-domain-containing protein 2A | FLHRRK | 1.70 | 2.17 | 21.46  |
| P53420    | COL4A4| Collagen alpha-4(V) chain | PGEQGVLGPPQPGRPG | 0.84 | 2.14 | 7.51   |
| Q7Z5P9-2  | MUC19| Mucin-19 | FLGGS | 2.11 | 2.11 | 13.79  |
| P01833    | PIGR | Polymeric immunoglobulin receptor | QAGDSRASVD | 0.49 | 2.10 | 5.11   |
| Q96666    | AHNAK| Neuroblast differentiation-associated protein AHNAK | KLKGDI | 1.57 | 2.08 | 15.35  |
| P36776    | LONP1| Lon protease homolog, mitochondrial | KHKPR | 0.99 | 2.07 | 8.69   |
| A0A0A0MTS7| TTN  | Titin   | VPEAPKEWPEKKVPVTQQ | 2.94 | 1.98 | 10.42  |
| Q8NEZ4    | KMT2C| Histone-lysine N-methyltransferase 2C | QQNLNSNP | 1.03 | 1.96 | 10.44  |
| A0A0A0MTS7| TTN  | Titin   | SPSSP | 2.94 | 1.93 | 11.70  |
| Q7Z5P9-2  | MUC19| Mucin-19 | AGTSI | 2.24 | 1.89 | 23.15  |
| Q75592-2  | MYCBP2| E3 ubiquitin-protein ligase MYCBP2 | QLLYR | 2.05 | 1.79 | 11.10  |
| Q9H6K5-2  | PRR36| Protein-rich protein 36 | PPSLQTLPSATPQVQPTQ | 0.81 | 1.78 | 9.94   |
| P080913   | ADRA2A| Alpha-2A adrenergic receptor | ISAVSFPPLISIEKKGCGG | 0.93 | 1.71 | 9.59   |
| Q9UJ5S5   | MAGEL2| MAGE-like protein 2 | PPRIPGPG | 1.10 | 1.65 | 15.90  |
| Q4V328-4  | GRIPAP1| GRIP1-associated protein 1 | LCSQMEQLE | 0.63 | 1.60 | 6.91   |

**Downregulated peptides**

| Accession | Gene | Protein | Peptide | MW (kD) | Fold change | −10lgP |
|-----------|------|---------|---------|---------|-------------|--------|
| O14526-3  | FCHO1| F-BAR domain only protein 1 | AGAVRFV | 0.53 | −1.56 | 9.90   |

Table 2: Differentially expressed peptides in hUC-MSC conditioned medium from preterm and term infants
| Accession | Gene | Protein                                      | Peptide                      | MW (kD) | Fold change | −10lgP |
|-----------|------|----------------------------------------------|------------------------------|---------|-------------|--------|
| Q7ZSP9-2  | MUC19| Mucin-19                                     | KTLAAGS                      | 2.15    | −1.57       | 14.06  |
| Q9H8I4    | EPAS1| Endothelial PAS domain-containing protein 1 | TPLSSMGGRS                   | 1.00    | −1.58       | 18.34  |
| Q6ZNL6    | FGDS | FLYE, RhoGEF and PH domain-containing protein 5 | EDHAQ                        | 0.77    | −1.58       | 13.24  |
| Q9BW04-2  | SARG | Specifically androgen-regulated gene protein | LTPPKPRKLPPN                  | 0.61    | −1.60       | 5.40   |
| O15027-2  | SEC16A| Protein transport protein Sec16A              | QACAAASGS                    | 2.41    | −1.61       | 17.59  |
| S4R393    | ZSWM8| Zinc finger SWIM domain-containing protein 8 | QTHKPQQT                     | 0.99    | −1.64       | 13.03  |
| Q2VWA4    | SKOR2| SKI family transcriptional corepressor 2     | GGSGGDCSAG                   | 0.50    | −1.65       | 8.13   |
| Q9Y6V0-6  | PCLO | Protein piccolo                              | QOPGPAPKPP                    | 1.00    | −1.69       | 6.21   |
| P28329    | CHAT | Choline O-acetyltransferase                  | GLPKLVPPLQQ                   | 0.66    | −1.70       | 5.12   |
| Q8WXH0    | SYNE2| Nesprin-2                                    | KYKFLKKAQQLTSLLKEL           | 2.04    | −1.71       | 5.55   |
| P13611-5  | VCAN | Versican core protein                        | QPEFSS                       | 1.97    | −1.74       | 29.72  |
| Q8TE85    | GRHL3| Grainyhead-like protein 3 homolog            | LFIPNVHFSSLQRSQ              | 0.54    | −1.78       | 9.85   |
| A0A1B0GUF7| IQCM | IQ domain-containing protein M                | KTFKT                        | 0.88    | −1.79       | 14.67  |
| A0A087WXW9| COLS1A| Collagen alpha-1(V) chain                    | PPGEV                        | 2.70    | −1.83       | 8.75   |
| A0A0AMTS7 | TTN  | Titin                                        | KACDPVF                      | 2.92    | −1.87       | 8.46   |
| A0A0AMTS7 | TTN  | Titin                                        | IVASDVTIRKLKANLLANN          | 2.78    | −1.87       | 5.62   |
| H0YS7     | SFI1 | Protein SFI1 homolog                         | QQLAARRQEQRATVRALW           | 0.82    | −1.88       | 6.99   |
| A6NM27    | COL6A6| Collagen alpha-6(VI) chain                   | RRAIN                        | 0.91    | −1.89       | 13.43  |
| Q8NEZ4    | KMT2C| Histone-lysine N-methyltransferase 2C        | EGCVK                        | 1.08    | −1.89       | 12.10  |
| A0A087WXW9| COLS1A| Collagen alpha-1(V) chain                    | GPRGTTGKPGBK                 | 2.70    | −1.90       | 10.79  |
| Q9Y6W6    | DUSP10| Dual specificity protein phosphatase 10       | DNQAQT                       | 1.21    | −1.91       | 9.85   |
| A0A0J9XV3 | N/A  | Uncharacterized protein                      | KIGGLY                       | 0.94    | −1.91       | 14.05  |
| A0A0AMTS7 | TTN  | Titin                                        | EGKNDD                       | 3.08    | −1.91       | 12.34  |
| Q92616    | GCN1 | eIF-2-alpha kinase activator GCN1            | ILDVASLVLN                   | 0.66    | −1.92       | 5.74   |
| Q9NR09    | BIRC6| Baculoviral IAP repeat-containing protein 6  | DNESCNT                      | 1.47    | −1.95       | 6.90   |
| Q8TEP8    | CEP192| Centrosomal protein of 192 kDa               | LLSTTK                       | 1.70    | −1.96       | 17.83  |
| A0A0AMTS7 | TTN  | Titin                                        | DPPGKPVPLN                   | 3.22    | −2.08       | 14.35  |
| Q8TE73    | DNAH5| Dynnein heavy chain 5, axonomal              | QRVKSKIPAAIEQVPHLAKV         | 1.84    | −2.10       | 7.15   |
| P10827-4  | TRHA | Thyroid hormone receptor alpha               | LHRAV                        | 0.51    | −2.11       | 9.87   |
| Q92D3     | HEATR5B | HEAT repeat-containing protein 5B            | HAKGK                        | 0.83    | −2.12       | 12.50  |
| O60423-3  | ATP8B3| Phospholipid-transporting ATPase 1K         | YGLVI                         | 0.98    | −2.13       | 11.77  |
| P06401-2  | PGR  | Progerin receptor                            | GPLLKGGKPRALGGAAAGGG         | 0.77    | −2.14       | 5.47   |
| A0A14OT8Y3| TNXB | Tenasin-X                                    | HGRGRCEEGRLCDPGYTGPTCA       | 1.71    | −2.14       | 5.92   |
| P276S8    | COL8A1| Collagen alpha-1(VIII) chain                 | GIDGVKPKPHYAGKKGKN           | 0.65    | −2.14       | 6.82   |
| Q9C093    | SPEF2| Sperm flagellar protein 2                   | ESLECKVJXELITIEAKKKN        | 0.69    | −2.14       | 7.26   |
| Q8NAC3-3  | IL17RC| Interleukin-17 receptor C                    | AAALSLLLKDKHAKGWRLKQ         | 0.48    | −2.15       | 5.00   |
| Q82771    | DDX12| Putative ATP-dependent RNA helicase DDX12    | KGGLGLRLAARKKIFQEPK         | 0.67    | −2.16       | 6.09   |
| Q6PG9     | LRFN4| Leucine-rich repeat and fibronectin          | VAVGGLVIVALVFTVALLVRG        | 0.69    | −2.17       | 5.32   |
Table 2  Differentially expressed peptides in hUC-MSC conditioned medium from preterm and term infants (Continued)

| Accession | Gene   | Protein                                                                 | Peptide            | MW (kD) | Fold change | 10lgP |
|-----------|--------|-------------------------------------------------------------------------|--------------------|---------|-------------|-------|
| Q9HD67    | MYO10  | type-III domain-containing protein 4                                   | RGAGNGRL           | 0.82    | -2.23       | 14.68 |
| Q96DN2    | VWCE   | von Willebrand factor C and EGF domain-containing protein              | KTSCVE             | 0.50    | -2.26       | 9.34  |
| Q96QD8    | SLC38A2| Sodium-coupled neutral amino acid transporter 2                         | KVLSNADVGLSL       | 0.60    | -2.31       | 12.68 |
| A0A0C4DG66| NPC1L1 | NPC1-like intracellular cholesterol transporter 1                       | WAFTILLVGFRAVQAR    | 0.68    | -2.32       | 12.53 |
| Q9UQDO2-3 | SCNA   | Sodium channel protein type 8 subunit alpha                            | VSLVSLIAB          | 1.71    | -2.33       | 12.11 |
| Q9UPA5    | BSN    | Protein bassoon                                                         | KGGPRR             | 2.05    | -2.34       | 10.76 |
| Q9UKV8    | AGD2   | Protein argonaute-2                                                    | KLGQAN             | 1.17    | -2.36       | 13.86 |
| Q9UQDO2-3 | SCNA   | Sodium channel protein type 8 subunit alpha                            | YLALL              | 1.72    | -2.36       | 14.90 |
| I6L894    | ANK2   | Ankyrin-2                                                               | KHKLNVP            | 0.89    | -2.38       | 11.67 |
| Q95996    | APC2   | Adenomatous polyposis coli protein 2                                   | PAAEAATKPLPRLH     | 0.78    | -2.39       | 5.35  |
| P36776    | LONP1  | Lon protease homolog, mitochondrial                                     | TIAAKRAGVT         | 0.99    | -2.46       | 12.42 |
| H7BX25    | KALRN  | Kalrin                                                                  | VVCKCITKATKDVAVFKVS  | 0.91    | -2.46       | 9.58  |
| Q8NF91-4  | SYNE1  | Nesprin-1                                                               | QKAVDHRKAILSIN      | 1.02    | -2.56       | 8.45  |
| J3KQC6    | TMRRS13| Transmembrane protease serine 13                                       | LPLIGCVLLALWSSL    | 0.45    | -2.60       | 5.00  |
| Q8TE73    | DNAH5  | Dynnein heavy chain 5, axonemal                                        | AQTRRLVGEVLLATAFLS | 1.82    | -2.62       | 5.48  |
| J3L2R4    | SLC2A4 | Solute carrier family 2 (Facilitated glucose transporter), member 4, isoform CRA_b | IGAVGVNTFVTLVSV    | 0.62    | -2.63       | 7.43  |
| P42167    | TMPO   | Lamina-associated polypeptide 2, isoforms beta/gamma                    | KSEKTKKGRISPVKI     | 0.49    | -2.66       | 6.27  |
| Q9BW11-4  | MXD3   | Max dimerization protein 3                                              | GPIHRK             | 0.50    | -2.66       | 7.43  |
| E7EPG1    | MRRN1  | Multimerin-1                                                            | LPDIQLLKQGLTEFV    | 0.66    | -2.70       | 6.46  |
| GSEA42    | TMOD2  | Tropomodulin 2 (Neuronal), isoform CRA_a                                 | HKWKF              | 0.67    | -2.76       | 12.93 |
| A0A0A0MTS7| TTN    | Titin                                                                   | SSRLECKI           | 2.84    | -2.77       | 7.13  |
| Q7Z5P9-2  | MUC19  | Mucin-1                                                                 | QNGIVI             | 2.18    | -2.78       | 18.23 |
| Q588A4    | PIGG   | GPI ethanolamine phosphate transferase 2                                | WLAAGGVMVNASALCVVS | 1.18    | -2.80       | 9.72  |
| Q9NRR8    | BIRC6  | Baculoviral IAP repeat-containing protein 6                             | TRIGKLIDILLRNCAS   | 1.36    | -2.82       | 5.14  |
| A0A0A0MTS7| TTN    | Titin                                                                   | WHAVGVRIRAYY       | 2.83    | -2.89       | 5.92  |
| Q8NG04    | SLC26A10| Solute carrier family 26 member 10                                      | EPVKALTSGAALHV     | 0.59    | -2.91       | 11.40 |
| J3KNF3    | TET3   | Methylcytosine dioxygenase TET3                                         | GPQGCSA            | 1.65    | -2.94       | 15.44 |
| Q92835-2  | INPP5D | Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1                | PLPVKSPA           | 1.01    | -2.97       | 22.11 |
| Q9Y6R1    | SLC4A4 | Electrogenic sodium bicarbonate cotransporter 1                        | HHTHYGVHPKSYR      | 0.78    | -2.99       | 8.24  |
| Q99707    | MTR    | Methionine synthase                                                     | KSARVMMKAVG        | 1.66    | -3.12       | 21.33 |
| Q8WXG9    | ADGRV1 | Adhesion G-protein coupled receptor V1                                  | RFLQSIYLVPEDEHLIIP | 1.97    | -3.13       | 8.73  |
| P43243-2  | MATR3  | Matrin-3                                                                | HUIJLN             | 0.67    | -3.21       | 8.71  |
| O75970-3  | MPDZ   | Multiple PDZ domain protein                                             | FISLLKKT          | 0.58    | -3.22       | 11.08 |
| Q96N23-2  | CFAP54 | Cilia- and flagella-associated                                          | HLKFPKIKSGPLTLKPPRLR | 0.62    | -3.25       | 6.96  |
GO analysis and subcellular location analysis of the differentially expressed peptide precursors

Next, molecular functions, cellular components and biological processes of the corresponding precursor proteins were determined by GO analysis to predict the latent functions of the differentially expressed peptides. Binding and catalytic activity were the most highly enriched molecular functions (Fig. 3a). Cell part, organelle part, and intrinsic component of membrane were the most highly enriched cellular components (Fig. 3b). Cellular process, biological regulation, and cellular component organization were the most highly enriched biological processes (Fig. 3c). Furthermore, we categorized the subcellular locations of the precursor proteins of all 131 peptides in accordance with their annotations in the UniProt database. The analysis revealed that the nucleus (25%), plasma membrane (16%), and cytoskeleton (15%) were the predominant subcellular locations of the differentially expressed peptide precursors. About 10% of the precursors were types of proteins that are located in the extracellular region of hUC-MSCs (Fig. 3d).

Diseases and regulator effects networks associated with the differentially expressed peptide precursors

We further evaluated the diseases and regulator effects networks associated with the differentially expressed peptide precursors using IPA software. Disease and functional protein network analysis indicated that several precursor proteins were involved in developmental disorders and inflammatory responses (Fig. 4a, b). More precisely, precursor proteins including Alpha-2A adrenergic receptor (ADRA2A), Protein argonaute-2 (AGO2), Baculoviral IAP repeat-containing protein 6 (BIRC6), Kalirin (KALRN), and Histone-lysine N-methyltransferase 2C (KMT2C) were involved in developmental disorders, and KMT2C,
**Fig. 2** Basic features of the differentially expressed peptides in hUC-MSC CM from preterm and term infants. **a, b** Molecular weights (MW) and isoelectric points (PI) of the differentially expressed peptides. **c** Scatter plot of MW versus PI of the differentially expressed peptides. **d** Distributions of the N- and C-terminals of the differentially expressed peptides.

**Fig. 3** Gene Ontology (GO) and subcellular location analysis of the differentially expressed peptide precursors. **a** Molecular functions. **b** Cellular components. **c** Biological processes. **d** Subcellular locations.
Solute carrier family 2 (SLC2A4), Electrogenic sodium bicarbonate cotransporter 1 (SLC4A4), STIM1L (STIM1), and Rootletin (CROCC) were involved in inflammatory responses. All the putative precursor proteins associated with diseases are shown in Table S1. Furthermore, the regulator effects network analysis showed that some of the protein precursors participated in the networks of cellular development, embryonic development, organismal development, and organismal injury and abnormalities (Fig. 4c, d). For example, AGO2, CROCC, DENN domain-containing protein 2A (DENND2A), Krueppel-like factor 14 (KLF14), and Lon protease homolog (LONP1) were involved in the networks of cellular development, embryonic development, and organismal development. Additionally, Atrophin-1 (ATN1), Collagen alpha-1 (VIII) chain (COL8A1), Protein jagged-2 (JAG2), KMT2C, and Mucin-19 (MUC19) were related to the network of organismal injury and abnormalities. All the precursor proteins involved in regulator effects networks are shown in Table S2.

Putative bioactive peptides associated with respiratory diseases

It is well known that peptides with biological functions have functions that are related to the functions of their precursor proteins, with domains playing key roles in the biological functions [39, 40]. The UniProt database was used to analyze the peptides and their precursors and the results showed that 25 peptides were located in the functional domains of their corresponding precursors (Table S3). The preceding results suggested that these precursors are mainly associated with inflammatory responses and abnormal organ development, which contribute to premature infant respiratory diseases. Therefore, we focused on peptides and their precursors related to respiratory diseases. Using the Open Targets Platform database, we investigated whether these peptides might play potential roles in respiratory diseases. All told, 17 precursor proteins were found to be closely related to respiratory diseases (association score ≥ 0.5) (Table S4).
TNF-α, IL-1β, and IL-6 in H2O2-treated lung epithelial cells with differentially expressed peptides

Previous researches have reported that lung epithelial cells A549 can be stimulated by H2O2 to induce inflammatory response [41, 42]. Therefore, we explored the effect of differentially expressed peptides on inflammatory response of H2O2-treated A549 cells. As observed, TNF-α, IL-1β, and IL-6 mRNA and protein expression levels were higher in A549 cells from the H2O2 (1 mM) group compared with the control group (Fig. 5), respectively, measured by qRT-PCR, ELISA, and western blot. According to the high fold change, we selected two peptides 508AAAAGPANVH517 derived from Homeobox protein SIX5 (SIX5, absolute fold change: 5.9) and 7118TGAKIKLVT727 derived from MUC19 (absolute fold change, 6.3) for future study. Peptide 7118TGAKIKLVT727 (MUC19) at 10 and 100 μM significantly attenuated the H2O2-induced increase of TNF-α, IL-1β, and IL-6. Moreover, we observed that the levels of inflammatory cytokines decreased more obviously with the 7118TGAKIKLVT727 at the concentration of 100 μM than 10 μM (Fig. 5). Meanwhile TNF-α, IL-1β, and IL-6 were significantly reduced in H2O2-treated A549 cells with peptide 508AAAAGPANVH517 derived from SIX5 at concentration of 100 μM, but not at 1 μM or 10 μM (Fig. 5). Both the two peptides help to suppress inflammatory response in H2O2-treated A549 cells.

Discussion

The MSC secretome and its therapeutic effects have been extensively demonstrated in preterm diseases, such as BPD [43] and NEC [19]. Previously, most researchers considered soluble factors and extracellular vesicles as the primary components of the secretome derived from MSCs [44–46]. However, the paracrine substances from MSCs are not limited to these biomolecules. Therefore, further studies are required to explore more types of components derived from MSCs and investigate their potential functions.

With the advance of detection technologies, mounting evidence has confirmed the differences in hUC-MSCs between preterm and term groups, which may help to identify the possible regulators or mechanisms underlying MSC function. To compare the global gene expression patterns in hUC-MSCs between these two groups, Iwataini et al. used microarray analysis and revealed that upregulated WNT2B in preterm hUC-MSCs was involved in the control of hUC-MSC proliferation [47]. A very recent study comparing hUC-MSC transcriptomics and proteomics profiles from term and preterm groups showed that Frizzled-2 (FZD2) protein and mRNA expression levels were both higher in preterm hUC-MSCs [48]. Importantly, FZD2 is the receptor of Wnt5a/b and FZD2 mutations influence Wnt signaling, which mediates the epithelial to mesenchymal transition (EMT) during lung development [49]. In addition, by comparing the proteome of microvesicles collected from hUC-MSC CM between preterm and term groups, Bruschi et al. found that 173 proteins were significantly changed, 163 of which were increased in the preterm group [50]. However, there have been no comprehensive comparisons of hUC-MSC CM peptidomic profiles between preterm and term infants. In the present study, we found that 131 peptides derived from 106 precursor proteins were differentially expressed in the preterm hUC-MSC CM compared with the term group by TMT labeling quantification (Fig. 1). Our study provides hUC-MSC CM polypeptide profiles for preterm and term infants. Secreted peptides have been shown to have important biological functions. Neuropeptide Y, a 36-amino acid peptide secreted by the hypothalamus, was found to play key roles in neurodegenerative diseases including modulation of neurogenesis, food intake, and thermogenesis [51]. Mao et al. showed that peptides derived from human beta-defensins are secreted by viable human cryopreserved amniotic membrane and exhibited direct antimicrobial effects against P. aeruginosa [52]. Further studies are needed to understand and explore the functions of secreted peptides from hUC-MSCs. In our study, we used p value to screen differentially expressed peptides between term and preterm groups. Although the false discovery rate (FDR) can detect differentially expressed peptides more accurately than p value, we would get very few peptides by FDR. Additionally, in order to identify more differentially expressed peptides, some studies also applied the p value to the analysis of peptides [53, 54]. Thus, in some condition, it is also appropriate to use the p value.

Subcellular location analysis of precursor proteins can help to better understand the source and potential functions of peptides. As shown in Fig. 3d, most precursor proteins were annotated as being part of organelles or membranes (56%). Notably, a small fraction of peptides were annotated as being derived from proteins located in the extracellular region, termed secreted proteins from hUC-MSCs. Classically, a large proportion of peptides (such as peptide neurotransmitters) are generated by the proteolysis of macromolecular proteins followed by release into the space outside of cells [55]. However, some other peptides containing one or more cleavage sites do not derive from endosomal processing [56]. These peptides are the N- or C-terminal peptides of their precursor proteins, rather than internal fragments [57]. In addition, the identification in our study of several peptides arising from secreted proteins raised the possibility that some bioactive peptides may be produced by enzymatic hydrolysis of extracellular proteins. These observations provide us with more methods to evaluate...
Fig. 5 (See legend on next page.)
the potential functions of differentially expressed peptides in our study; additional studies are needed to ascertain whether these peptides are actually secreted from hUC-MSCs and have biological activities. From the results of IPA analysis, we found that a series of precursor proteins were involved in networks of developmental disorders, inflammatory responses, and organo- 

Based on the above results, we focused on exploring the potential role of differentially expressed peptides in developmental disorders and inflammatory responses. BPD is clearly one of the most common respiratory morbidity in preterm infants, and it may not simply be a consequence of lung immaturity [66]. And inflammation, one key contributor, often initiated by a pulmonary fetal inflammatory response, is aggravated by invasive or non-invasive mechanical ventilation and exposure to hyper- 

As the functions of secretory peptides derived from hUC-MSCs were unclear, we assessed whether the peptides were located in the functional domains of their precursor proteins to analyze their functionality. Utilizing the UniProt database, we discovered that 25 peptides were situated in the functional domains of their corresponding precursors. Peptides derived from Titin [69] were situated in the functional domains of type D and easy to mutate [69]. Thus, we chose human embryonic alveolar epithelial cells are more in line with the purpose of the project and can be better used to evaluate the effects of peptides on the characteristics of lung development. However, this cell is difficult to obtain and easy to mutate [69]. Thus, we chose human lung epithelial cells A549 to explore the effects of differentially expressed peptides in vitro. Accordingly, to make a full understanding of these peptides, animal experiments will be carried out to support our researches in the following experiments.

We also observed that 7118TGAKIKLVGT derived from MUC19 reduced the levels of TNF-α, IL-1β, and IL-6 in H2O2-treated A549 cells and the degree of decrease is related to the concentration of peptide as shown in Fig. 5. As a secreted mucin, MUC19 is released to the extracellular medium and has been identified in respiratory, digestive, and reproductive tracts [71]. It has been reported that MUC19 was differentially regulated after exposure to inflammatory cytokines [72]. And one recent study found that MUC19 peptides may enhance vaginal mucous immunity against infections [73]. These results indicated that 7118TGAKIKLVGT derived from MUC19 may also play a role in protecting from inflammatory response stimulated by H2O2 in human lung epithelial cells. In addition, the other peptide 508AAAAGPANVH derived

Fig. 5 Function analysis of differentially expressed peptides in vitro. (a) TNF-α, IL-1β, and IL-6 mRNA expression assessed by qRT-PCR in A549 cells stimulated by H2O2 (1 mM) with or without peptides 7118TGAKIKLVGT (MUC19) and 508AAAAGPANVH (SIX5) at concentrations of 1, 10, and 100 μM. b The protein levels of TNF-α, IL-1β, and IL-6 measured by ELISA analyze in H2O2-treated A549 cells with or without peptides. c, d The protein levels of TNF-α, IL-1β, and IL-6 measured by western blot in H2O2-treated A549 cells with or without peptides (n = 3 biological independent samples per group in qRT-PCR and WB, technical replication = 3 in ELISA. *P < 0.05, **P < 0.01, ***P < 0.001)
from SIX5 also reduced the expression levels of TNF-α, IL-1β, and IL-6 in H2O2-treated A549 cells only at the maximum concentration of 100 μM. It has been acknowledged that SIX5 was correlated with eye development [74] and myotonic dystrophy [75], which are related to embryonic and organismal development. Thus, we put forward one hypothesis that the hUC-MSCs from preterm infants may secrete protective substances such as peptides under stress. These findings indicated that two peptides may play an anti-inflammatory role in the process of BPD. And previous studies have shown that the regulation of inflammation-related signal pathways such as p38 mitogen activated protein kinases (p38MAPK) signal pathways and nuclear factor-κB (NF-κB) signal pathway can reduce the level of pro-inflammatory cytokines, thus relieving the pulmonary inflammation of BPD [76, 77]. Combined with previous studies, we consider that the two peptides secreted from hUC-MSCs may decrease the production of pro-inflammatory cytokines through inflammation-related signal pathways and further researches need to be conducted to validate the possible pathway.

Conclusion
As far as we know, no large-scale quantitative peptidomic analysis has been carried out on the secretory components of hUC-MSCs. Our study identified the differentially expressed peptides secreted by preterm and term hUC-MSCs using TMT-based LC-MS/MS technology. Furthermore, bioinformatics analysis of precursors predicted the possible functions of peptides that may be useful in the treatment of premature respiratory diseases in connection with inflammatory responses and developmental disorders. And we first investigated the anti-inflammatory effect of the peptides 7118TGAKIKLVGT7127 derived from MUC19 and 508AAAAGPANVH517 derived from SIX5 on human lung epithelial cells. This study expands our knowledge of the hUC-MSC secretome and may provide insights into new therapy for premature respiratory diseases.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s13287-020-01931-0.

Additional file 1: Figure S1. Characterization of hUC-MSCs from preterm and term umbilical cords. (A) hUC-MSCs from preterm and term umbilical cords exhibited a fibroblast phenotype in culture. Adipogenesis capacity was confirmed by oil red O staining, and chondrocytes were evaluated by Alcian blue staining after differentiation. (B) Flow cytometry was used to detect the expression of positive markers (CD29, CD73, CD105) and negative markers (CD31, CD34 and HLA-DR) on hUC-MSCs.

Additional file 2: Figure S2. Identification of protein integrity of hUC-MSC CM from preterm and term infants by silver staining. The protein integrity of hUC-MSC CM was visualized by SDS-PAGE and silver staining (n=3 per group, P1-3 represent preterm infants and T1-3 term represent term infants).

Additional file 3: Table S1. Putative precursor proteins associated with diseases.

Additional file 4: Table S2. Precursor proteins involved in networks.

Additional file 5: Table S3. Differentially peptides located in functional domain based on Uniprot database.

Additional file 6: Table S4. Protein precursors and identified peptides related to respiratory system diseases.

Abbreviations
UC-MSC: Umbilical cord mesenchymal stem cell; TMT: Tandem mass tag; CM: Conditioned medium; GO: Gene Ontology; IPA: Ingenuity pathway analysis; PVL: Periventricular leukomalacia; BPD: Bronchopulmonary dysplasia; NEC: Necrotizing enterocolitis; ROP: Retinopathy of prematurity; Ang-1: Angiopoietin-1; HO-1: Heme oxygenase-1; EPO: Erythropoietin; GLP-1: Glucagon-like peptide-1; CGRP: Calcitonin gene-related peptide; AECII: Alveolar epithelium type II cells; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; DMEM/F-12: Dulbecco’s modified Eagle medium/nutrient mixture F-12; RT: Room temperature; SDS: Sodium dodecyl sulfate; MWCO: Molecular weight cutoff; MW: Molecular weight; PII: Isoelectric point; H2O2: Hydrogen peroxide; TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin 1β; IL-6: Interleukin 6; GA: Gestational ages; ADRA2A: Alpha-2A adrenergic receptor; AGO2: Protein argonaute-2; BIRC6: Baculoviral IAP repeat-containing protein 6; KALRN: Kalirin; KMT2C: Histone-lysine N-methyltransferase 2C; SLCA4A: Solute carrier family 1, multicationic sodium bicarbonate cotransporter 1; CROCC: Rootetin; DENND2A: DENN domain-containing protein 2A; KL14: Kruellpefel-like factor 14; LONP1: Lon protease homolog; ATN1: Atrophin-1; COL8A1: Collagen alpha-1 (VIII) chain; JAG2: Protein jagged-2; MUC19: Mucin-19; MPDZ: Multiple PDZ domain protein; SIX5: Homeobox protein SIX5; FZD2: Frizzled-2; EMT: Epithelial to mesenchymal transition; HCAECs: Human coronary artery endothelial cells; HUVECs: Human umbilical vein endothelial cells; WFD: Type D von Willebrand factor.

Acknowledgements
This study was supported by contract grant sponsors and numbers: Jiangsu Provincial Women and Children Health Research Project (grant no. F201816 and F201744), National Natural Science Foundation of China (grant no. 81600867 and 81701491), Nanjing Medical Science and Technique Development Foundation (grant no. YKK18155), Jiangsu Provincial Medical Youth Talent (grant no. QNRC2016111), and Six Talent Peaks Project of Jiangsu Province (grant no. YY-112). The TMT method followed by mass spectrometry analysis was supported by the Analysis and Testing Center of Nanjing Medical University.

Authors’ contributions
YuW and LZ performed the experiments, interpreted the results of the experiments, and drafted the manuscript. YunW and RPZ prepared the figures. YanW and YC analyzed the data. WL and CBJ participated in the experiments, and drafted the manuscript. YunW and RPZ prepared the figures. YanW and YC analyzed the data. WL and CBJ participated in the experiments, and drafted the manuscript.
Consent for publication
Written informed consent for publication of their clinical details was obtained from the parents of the patients.

Competing interests
The authors declare that they have no competing interests.

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Received: 12 May 2020 Revised: 29 August 2020 Accepted: 9 September 2020

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