Original Article

# Peptidome characterization of ovarian cancer serum and the identification of tumor suppressive peptide ZYX$_{36-58}$

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**Background:** Several serum biomarkers, including miRNA, mRNA, protein and peptides in cancer patients are also important mediators of cancer progression.

**Methods:** The differentially expressed peptides between the serum of ovarian cancer patients and healthy controls were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The function of the peptides was analyzed by CCK8, transwell, wound healing, and flow cytometry analysis. And the mechanism of the peptides was analyzed by peptide pull down, and high-throughput RNA-sequencing.

**Results:** A total of 7 and 46 peptides were significantly up-regulated and down-regulated in the serum of ovarian cancer patients, respectively. The precursor proteins of the differentially expressed peptides mainly involved in the complement and coagulation cascades, platelet activation, phagosome and focal adhesion pathways. Interestingly, focal adhesion, platelet activation, platelet-cancer cell interaction, complement activation, coagulation cascades and phagosome formation are all critical factors for cancer initiation or progression, which indicated that the peptides may play a crucial role in cancer development. And we identified one peptide, ZYX$_{36-58}$, which was down-regulated in the serum of ovarian cancer patients, significantly inhibited invasion and migration and promoted the apoptosis of ovarian cancer cells. Mechanistic study indicated that ZYX$_{36-58}$ interacted with and increased the protein level of the antiangiogenic protein thrombospondin-1 (TSP1), which has a tumor suppressive effect on ovarian cancer.

**Conclusions:** ZYX$_{36-58,}$ which was significantly down-regulated in the serum of ovarian cancer patients can significantly inhibit cell invasion, migration and promote apoptosis of ovarian cancer cells by binding and up-regulating TSP1 protein expression.

**Keywords:** Peptidome; ZYX$_{36-58}$; ovarian cancer (OC); migration; TSP1

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Page 2 of 16

Wang et al. Discovery of tumor suppressive peptide ZYX$_{36-58}$

## Introduction

Ovarian cancer (OC) is the leading cause of gynecological cancer deaths. Due to the lack of early symptoms, physical signs, and effective early screening approaches, approximately 70% of OC cases are diagnosed at advanced stages, and the 5-year survival rate is less than 50% (1). Although great progress has been made in targeted therapy and immunotherapy of ovarian cancer during the past decade, overall survival has not significantly changed thus far (1). It therefore remains critical to discover new therapeutic targets and develop drugs with high-efficacy and low side effects for the treatment of ovarian cancer.

Due to the small molecular weight, short half-life, high targeting efficiency, low immunogenicity, peptides have been considered as promising drugs for the treatment of diseases (2). For example, ALM201, a therapeutic peptide derived from FKBPL, could disrupt angiogenesis and inhibit self-renewal of cancer stem cells and thus inhibit ovarian cancer growth, and has completed a Phase Ia clinical trial in ovarian cancer patients (3). The octapeptides LR and its more rigid diastereomer [d-Gln4]LR, could reduce the catalytic activity of human thymidylate synthase, and thus inhibit ovarian cancer growth (4). TAT-p+p-8 peptide (PPPPPPPPVDYEDEE) could efficiently disrupt the ABI1-EPS8 interaction, and block the invasion and metastasis of ovarian cancer cells (5). However, few studies have focused on endogenous peptides against ovarian cancer.

Indeed, endogenous peptides that exist naturally *in vivo*, such as oxytocin, thymosin, and insulin, are involved in development or reproduction, and are often clinically useful. Some endogenous peptides with anti-tumor properties have been reported (6-8) and even applied in clinic (9,10). With the development of liquid chromatography-tandem mass spectrometry (LC-MS/MS), more and more studies have focused on the endogenous peptides and their functions *in vivo*. Indeed, the identification of differentially expressed peptides in serum using LC-MS/MS has provided important clues for the diagnosis of various diseases, such as autism spectrum disorder of children (11), gestational diabetes mellitus (12), and colorectal cancer (13).

Interestingly, we noticed that several serum biomarkers are also regulators of cancer progression, such as miR-206 in cervical cancer (14,15), circPRMT5 in urothelial carcinoma (16). Besides, as important diagnostic biomarkers in ovarian cancer, CA125, which was also expressed in ovarian cancer cells can interact with mesothelin and thus contribute to the metastasis of ovarian cancer cell to the peritoneum (17), HE4 overexpression in ovarian cancer cells could promote ovarian cancer cell progression and metastasis *in vitro* and *in vivo* (18). These findings indicated that the differentially expressed endogenous peptides in serum may provide therapeutic targets for ovarian cancer treatment.

In this study, we first used LC-MS/MS to identify differentially expressed peptides in the serum of ovarian cancer patients and healthy controls, three peptides derived from the precursor proteins F13A1, ZYX and SNP23, respectively, which were down-regulated in the serum of ovarian cancer patients were chosen for the functional analysis. We found that only ZYX$_{36-58}$ could significantly inhibit the invasion and migration of ovarian cancer cells. Therefore, we chose ZYX$_{36-58}$ for further research. Our results show that the endogenous peptide ZYX$_{36-58}$ has great promise in the treatment of ovarian cancer.

We present the following article in accordance with the MDAR checklist (available at http://dx.doi.org/10.21037/atm-20-2018).

## Methods

### Sample collection

Serum from three epithelial ovarian cancer patients (48±25 years old, women) and three age-matched healthy controls (42±16 years old, women) were collected from Women's Hospital of Nanjing Medical University (Nanjing Maternity and Child Health Care Hospital), and all experiments performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013), approved by the ethics committee of Women's Hospital of Nanjing Medical University {Nanjing Maternity and Child Health Care Hospital, Ethics Approval Number: 2016 [16]} and all patients signed informed consent. Blood samples were centrifuged at 3,000 ×g for 10 min at 4 ℃ immediately after collection, then the serum supernatant was collected, supplemented with protease inhibitor (EDTA-free, Roche Applied Science, China), and quickly stored at –80 ℃.

### Peptide extraction

Peptide extraction and LC-MS/MS was performed as previously described (12). In brief, samples were thawed on ice, then centrifuged at 12,000× g for 15 min at 4 ℃. The supernatants were collected and protein concentration was determined using the bicinchoninic acid kit (BCA,

pierce, Rockford, IL, USA). Acetonitrile (ACN, 20% v/v) was then added to the samples and briefly vortexed, then incubated at room temperature for 30 min. Samples were then transferred to 10 kDa molecular weight cut-off centrifugal filter units (Millipore, USA), which was washed with 0.5 mL $H_2O$ before use. The peptides were extracted according to the manufacturer's protocol of 10 kDa molecular weight cut-off centrifugal filter, then samples were dried in a SpeedVac (Scan Speed Maxi Vac, Labogene, Denmark). Tandem mass tag (TMT) labeling of the samples was conducted according to the manufacturer's protocol (TMT 6-plex label reagent, Thermo Fisher Scientific, CA, USA). Finally, analysis was performed using nanoflow liquid chromatography coupled with LTQ-Qrbitrap Velos mass spectrometry.

### Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

A 15 cm×75 µm i.d. (flow rate 300 nL/min) silica capillary column in series packed with 3.6 µm Jupiter C18-bonded particles was used for the analysis of the peptides in the gel. The separation was performed according to the effective gradient profiles, 5% mobile phase B (98% ACN, 0.1% FA) for 0–8 min, 8–35% mobile phase B for 8–43 min, 35–60% mobile phase B for 43–48 min, 60–80% mobile phase B for 48–50 min, 80% mobile phase B for 50–55 min, and 5% mobile phase B, 55–56 min. The separated peptide was then ionized by nanoESI and operated in the data-dependent acquisition mode by LTQ Orbitrap Velos (Thermo Fisher Scientific). The main parameters are as follows: voltage 1.8 V, scanning range of primary mass spectrometry 350≤1,500 m/z, resolution set to 30,000, starting m/z of secondary mass spectrometry fixed to 100, and resolution set to 7,500. The screening conditions of the parent ions for secondary fragmentation are as follows: the charge ≥2 and the first 12 parent ions with peak intensity of more than 1,000. The collision energy of higher energy collision conditions (HCD) is set to 35, and the fragment ions are detected in Orbitrap. Finally, the data are matched with theoretical mass spectrometric data obtained by the database simulation.

### Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis of the protein precursors of the differentially expressed peptides

Gene ontology (GO) and Kyoto Encyclopedia of Genes and

Genomes (KEGG) pathway analysis of the differentially expressed peptide precursor proteins was analyzed by Databases for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/).

### Synthetic peptides

The following 3 differentially expressed peptides between the serum of ovarian cancer patients and healthy controls were synthesized by Shanghai Science Peptide Biological Technology Co., Ltd and dissolved in water or DMSO at the desired concentrations: $ZYX_{36-58}$: VNPFRPGDSEPPPAPGAQRAQMG; $F13A_{18-38}$: NNSNAAEDDLPTVELQGVVPR; $SNP23_{123-131}$: TNGQLQQPT; and the scrambled peptide for $ZYX_{36-58}$: PPNMAVADERPQQPRSFPGGAGP.

### Culture of human ovarian cancer cell lines

The SKOV3 cell line (Homo sapiens, human, RRID: CVCL_0532) was purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China), and the HO8910 cell line (Homo sapiens, human, RRID: CVCL_6868) was purchased from Jiangsu Kaiji Biotechnolgy (Jiangsu, China). SKOV3 cells were cultured in McCoy's 5A medium supplemented with 10% FBS, 2200 mg/L sodium bicarbonate and 2 mM L-glutamine, HO8910 cells were cultured in DMEM medium supplemented with 10% FBS. Both cell lines were maintained at 37 ℃ in a humidified incubator under 5% $CO_2$.

### Cell counting kit 8 assay

A suspension of about 1,000 cells and 100 µM $ZYX_{36-58}$ peptide or peptide solvent in 100 µL culture medium were seeded in 96-well plate and incubated as above. Four hours after seeding, most cells were adherent to the surface of the plate, and this time point was considered as 0h. After 0, 12, 24 and 48 h of culture, the medium was supplemented with 10% CCK8 agent (Cell counting kit 8; Jiangsu Kaiji Biotechnolgy, Jiangsu, China) and continue to maintain at 37 ℃ in humidified incubator under 5% $CO_2$ for 1 h. The absorbance at 450 nm was measured using a BioTek Synergy H4 instrument (BioTek Instruments, Vermont, USA).

### Cell invasion and migration assay

About 800 µL medium containing 20% FBS was added to

**Page 4 of 16**

Wang et al. Discovery of tumor suppressive peptide $ZYX_{36-58}$

the lower chamber of a transwell (8-µm pore size, Millipore, Billerica, MA, USA) and $5\times10^4$ cells in 200 µL of medium supplemented with 100 µM $ZYX_{36-58}$ peptide or peptide solvent were seeded in the upper chamber with or without Matrigel (Matrigel: medium =1:6, 60 µL/well) for invasion and migration assays, respectively, and incubated as above. After culturing for 24 h (for migration assay of SKOV3 cells) or 48 h (for invasion assay of SKOV3 cells, and for migration and invasion assay of HO8910 cells), the chamber was fixed in 4% paraformaldehyde for 60 min, followed by staining with 0.1% crystal violet for 30 min, washing two times in PBS, and removing the cells in the surface of the upper chamber by cotton swab. Three randomly selected areas of the lower surface of the chamber were selected for photography under Axio Observer D1 fluroscence microscope (Carl Zeiss, Germany). Finally, the cells on the lower surface were lysed in RIPA buffer, and the absorbance at 562 nm of the cell lysate was analyzed by the BioTek Synergy H4 instrument.

### Fluorescence microscopy

SKOV3 cells were grown in 6-well plates, then FITC-labeled $ZYX_{36-58}$ peptide was added to the culture medium at a concentration of 100 µM. Cell fluorescence was visualized and photographed at 1, 2, 4, 24 and 48 h after seeding under Axio Observer D1 fluroscence microscope (Carl Zeiss, Germany).

### Peptide pull-down assay

Biotin-labeled $ZYX_{36-58}$ or biotin-labeled scrambled peptide at a concentration of 100 µM was added to the medium of SKOV3 cells at 50% confluency, after 24 h culture, 1 ml of RIPA was added and total protein was extracted according to the manufacturer's protocol. Then the total protein of each sample was incubated with 20 µL streptavidin-conjugated immunomagnetic beads overnight on a rotary shaker at 4 ℃. The protein lysate containing the magnetic beads was placed in a Magna GrIP™ magnetic stand (Merck KGaA, Darmstadt, Germany) and the magnetic beads were washed by the protein lysate (RIPA: PMSF =100:1) 4–5 times. After 30 µL protein loading buffer (1×) was added to the magnetic beads, the mixture was heated in a 100 ℃ water bath for 5 min. Then the magnetic beads were discarded and the protein samples were used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After SDS-PAGE was completed, the gel was

stained with silver according to the manufacturer's protocol for Thermo Fisher Scientific Pierce silver stain kits, and the silver stained lane of $ZYX_{36-58}$ and scrambled peptide treated group were separately excised for subsequent mass spectrometry analysis.

### Protein lysis of the gel

Protein from the gel was extracted after washing, dehydration in acetonitrile, disulfide bond reduction in DTT, alkylation cysteine blocking by iodoacetamide, proteolysis, 50% acetonitrile extraction, and 100% acetonitrile extraction. After freeze-drying in the SpeedVac (Scan Speed Maxi Vac, Labogene, Denmark), the freeze-dried peptide powder was dissolved in mobile phase A (2% ACN, 0.1% FA), centrifuged at 20,000 ×g for 10 min, the peptides in the supernatant was separated by the Shimadzu LC-20AD liquid chromatograph system (Shimadzu, Kyoto, Japan).

### Apoptosis assay

Cells were stained with PE and Annexin V using the PE-Annexin V Apoptosis Detection kit (BD Biosciences, San Diego, USA) after culturing with $ZYX_{36-58}$ or peptide solvent and apoptosis inducer from the kit for 24 h. Cell apoptosis was analyzed with a flow cytometer (Beckman, CA, USA).

### Wound healing assay

HO8910 and SKOV3 cells were cultured in 6-well plates in a medium containing 10% FBS and incubated for 24–36 h until the cell density reached 90–100%. The cells were maintained in serum-free medium for 6–8 h and a linear scratch was created on the cell layer using a 200 µL pipette tip, then the serum-free medium supplemented with peptide was added and replaced after 24 h of culture. The wound healing process was observed under EVOS XL Core digital microscope (Thermo Fisher Scientific, Waltham, USA) and analyzed using Image J software (Bethesda, USA).

### Next-generation sequencing

About 1 mL TRIzol was added to the SKOV3 cells treated with $ZYX_{36-58}$ or peptide solvent for 48 h. Three replicates of the TRIzol-treated cells in each group were used for next-generation sequencing. RNA was extracted according

to the manufacturer's protocol of TRIzol and RNA quality was assessed by Agilent 2100. The qualified RNA was then treated with the following procedures: fragmented after rRNA removal or mRNA enrichment, reverse transcribed by random N6 primer, synthesis of the double-stranded DNA and ER/A-tailing, ligating of the bubble adapter, PCR amplification using the specific primer, and then the library was constructed and sequenced by using the BGISEQ-500 system (BGI, Shenzhen, China).

### Quantitative real-time PCR (qRT-PCR)

After culturing of cells with the $ZYX_{36-58}$ or peptide solvent for 24 h, the cells were lysed in 1 mL TRIzol reagent, total RNA was isolated according to the manufacturer's protocol, and cDNAs were synthesized using the First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). An aliquot of 20 ng of sample was subjected to qPCR analysis using the primers listed in *Table S1*. Raw data were analyzed according to the $2^{-\Delta\Delta CT}$ method.

### Western blot

Total protein was extracted after lysing cells with RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitor. The protein was then denatured by mixing with sodium dodecyl sulfonate (SDS) and boiled at 95 ℃ for 5 min. Next, an aliquot of 40 mg of protein sample was separated by 10% PAGE gel and immunoblots were visualized using electrochemiluminescence. The antibodies used in this study were anti-EEF1A2 (Proteintech, 16091-1-AP, RRID: AB_2096979), anti-YBX1 (Proteintech, 20339-1-AP, RRID: AB_10665424), anti-PCLO (Invitrogen, MA5-28620, RRID: AB_2745579), anti-TSP1 (Cell Signaling Technology, #37879, RRID: AB_2799123), anti-ZYX (Proteintech, 60254-1-AP, RRID: AB_2221279), anti-β-ACTIN (Santa Cruz, sc-47778, RRID: AB_626632), goat anti-mouse IgG (H+L) (Proteintech , SA00001-1, RRID: AB_2722565), and goat anti-rabbit IgG (H+L) (Millipore, AP132P, RRID: AB_92486).

### Statistical analysis

Statistical analysis of the differentially expressed peptides used the two-tailed Students' *t*-test, with P<0.05 and fold change ≥1.5 was considered statistically significant. The differentially expressed genes were analyzed by DEGseq software (19), with *q*<0.01 and fold change ≥1.5 was considered statistically significant. The proliferation, invasion, migration, apoptosis, qRT-PCR and WB were performed at least in triplicate and repeated at least three times with similar results. The pull down experiment was performed at least in duplicate. The data are presented as mean ± standard deviation in bar charts from at least three technical replicates, and the difference were analyzed by Student's *t*-test, with P<0.05 considered statistically significant.

## Results

### The identification of the differentially expressed peptides in the serum of ovarian cancer patients and healthy controls

First, liquid chromatography-tandem mass spectrometry (LC-MS/MS) were performed for the identification of the differentially expressed peptides in the serum of ovarian cancer patients (n=3) and healthy controls (n=3). A total of 1,623 peptides derived from 495 precursor proteins were identified, with 1,331±80 peptides from 474 precursor proteins identified in control serum and 1,344±42 peptides from 461 precursor proteins identified in serum of ovarian cancer patients (*Figure 1A* and Table online: http://cdn.amegroups.cn/static/application/a02 ebfdcb099287f46f5a43296c43bc5/atm-20-2018-1.pdf). Although 71% of the precursor proteins contained only 1 peptide, 22% of the precursor proteins contained 2–4 peptides, 1% of the precursor proteins contained 5–7 peptides, and 6% of the precursor proteins contained ≥8 peptides (*Figure 1B*). About 99.4% of the peptides were 5 to 26 amino acids in length and 98.6% of the peptides were 700 to 3,199 daltons in molecular weight (*Figure 1C*). In the serum of ovarian cancer patients, 7 peptides that were derived from 6 precursor proteins were up-regulated and 46 peptides that were derived from 23 precursor proteins were down-regulated (P<0.05 and fold change ≥1.5, *Figure 1D* and *Table 1*). We also noticed that two peptides from FIBA protein and 1 peptide from ITIH4 protein were up-regulated whereas 5 peptides from FIBA protein and 2 peptides from ITIH4 protein were down-regulated in the serum of the ovarian cancer patients (*Table 1*), indicating that these peptides

**Page 6 of 16**

Wang et al. Discovery of tumor suppressive peptide ZYX$_{36-58}$
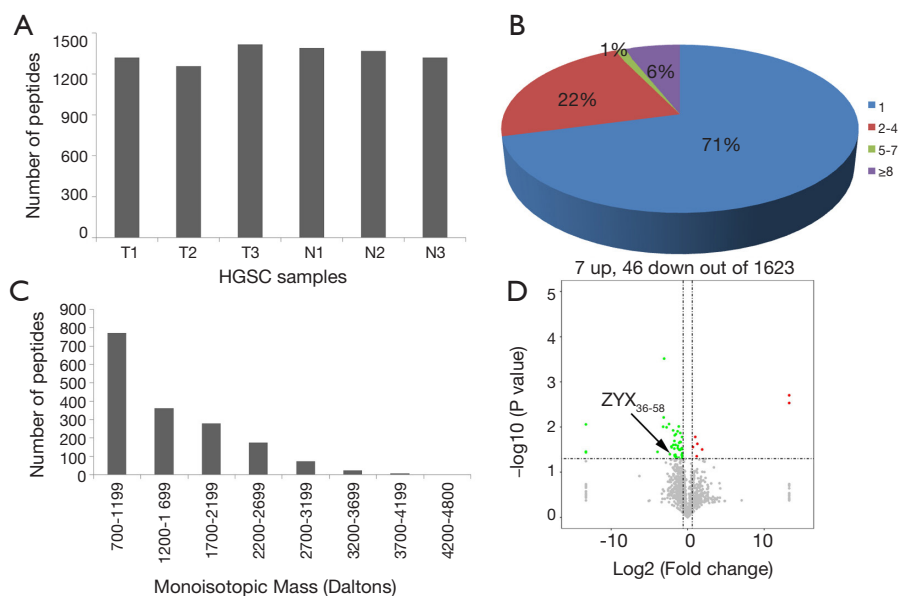
**Figure 1** Peptide ZYX$_{36-58}$ is significantly decreased in the serum of ovarian cancer patients. (A) The number of peptides detected in each tissue was between 1,200 and 1,400; (B) There are 1 to 4 peptides present in the same precursor protein, which accounts for 93% of all precursor proteins, and more than 8 peptides present in the same precursor protein, which account for 6% of all precursor proteins. (C) Most of the peptides were 700 to 3,199 daltons in molecular weight. (D) Seven peptides were significantly up-regulated and 46 peptides were significantly down-regulated in the serum of ovarian cancer patients compared with the serum of healthy controls (Fold change ≥1.5, P<0.05).

have different half-life in different systems, so we propose that they may also perform biological functions and are strictly regulated.

### *Gene ontology and pathway analysis of the precursor proteins of the differentially expressed peptides*

It was once thought that only intact proteins are functional units in the body. Then we found that the activation of a variety of proteins depends on proteolytic processes. As a product of the proteolytic products, endogenous peptides can also directly interact with target cells or target proteins and thus regulate the targets' function (2). Indeed, most of the peptides' function was closely related with their precursor proteins (2). So gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the precursor proteins of the differentially expressed peptides were performed. GO analysis indicated that the precursor proteins were mainly involved in the biological processes, such as platelet degranulation, platelet activation, the acute-phase response, blood coagulation, fibrinolysis and the negative regulation of endopeptidase activity (*Figure 2A*), involved in the molecular functions,

such as endopeptidase inhibition, cytoskeletal structure, receptor binding, structural molecule activity and the serine-type endopeptidase activity (*Figure 2B*). KEGG pathway analysis indicated that the precursor proteins mainly involved in the complement and coagulation cascades, platelet activation, phagosome, and focal adhesion pathways (*Figure 2C*).

### *ZYX$_{36-58}$ inhibits the invasion, migration and promotes apoptosis of ovarian cancer cells*

Previous study has shown that the platelet activation and platelet-cancer cell interaction are critical factors for cancer metastasis (20), complement system activation in tumor microenvironment promotes tumor growth and metastasis (21), and several molecules in focal adhesion pathway are involved in ovarian cancer cell proliferation and migration (22). Besides, several coagulation factors contribute to cancer initiation, growth and metastasis (23) and the molecules essential for the phagosome are important regulators of autophagy and cancer progression (24). Besides, although none of the dys-regulated peptides have been reported to be the diagnostic biomarker, peptides

**Table 1** The dys-regulated peptides in the serum of ovarian cancer patients compared with control (P<0.05 and fold change ≥1.5)

| Protein | Accession | Peptide | Fold change (Exp/Con) | Up/down | P value |
|---|---|---|---|---|---|
| Q9HCU4 | CELR2_HUMAN | NDGDWHHAQLALGASGGPGHAIL | #DIV/0! | Down | 0.036575 |
| P02675 | FIBB_HUMAN | DKKREEAPSLRPAPPPISGGGY | #DIV/0! | Down | 0.035085 |
| P02671 | FIBA_HUMAN | DEAGSEADHEGTHST | #DIV/0! | Down | 0.008773 |
| P00734 | THRB_HUMAN | FEKKSLEDKTERELLESY | −15.1636 | Down | 0.035594 |
| P00734 | THRB_HUMAN | SLEDKTERELLESY | −9.12038 | Down | 0.009882 |
| P00488 | F13A_HUMAN | NNSNAAEDDLPTVELQGVVPR | −8.70484 | Down | 0.006157 |
| P00734 | THRB_HUMAN | LEDKTERELLESY | −8.33199 | Down | 0.000307 |
| P00734 | THRB_HUMAN | EDKTERELLESY | −6.6876 | Down | 0.010167 |
| P01833 | PIGR_HUMAN | ADAAPDEKVLDSGFREIEN | −5.34658 | Down | 0.008607 |
| P60608 | EFC2_HUMAN | PIAFLGLK | −4.95581 | Down | 0.040054 |
| P00734 | THRB_HUMAN | DKTERELLESY | −4.31751 | Down | 0.027049 |
| Q14624 | ITIH4_HUMAN | NFRPG | −4.22144 | Down | 0.028265 |
| P02765 | FETUA_HUMAN | SLGSPSGEVSHPRKT | −3.69737 | Down | 0.011908 |
| P02671 | FIBA_HUMAN | SYKMADEAGSEADHEGTHST | −3.65124 | Down | 0.024903 |
| Q8WZ42 | TITIN_HUMAN | EEVVV | −3.32132 | Down | 0.030125 |
| Q14624 | ITIH4_HUMAN | QGAKIPKPEAS | −3.30605 | Down | 0.020436 |
| Q15942 | ZYX_HUMAN | VNPFRPGDSEPPPAPGAQRAQMG | −3.27098 | Down | 0.04204 |
| P00734 | THRB_HUMAN | SLEDKTERELL | −3.19064 | Down | 0.015295 |
| P02671 | FIBA_HUMAN | DEAGSEADHEGTH | −3.13457 | Down | 0.044595 |
| Q9BXJ8 | T120A_HUMAN | FKLYLTII | −3.01185 | Down | 0.041241 |
| P01024 | CO3_HUMAN | SEETKENEGFTVTAEGK | −2.97348 | Down | 0.024854 |
| P01024 | CO3_HUMAN | TKENEGFTVTAEGK | −2.87275 | Down | 0.014382 |
| P02671 | FIBA_HUMAN | DGHKEVTKEVV | −2.83653 | Down | 0.048455 |
| P0C0L4 | CO4A_HUMAN | QLNNRQI | −2.77262 | Down | 0.045834 |
| P00734 | THRB_HUMAN | GDGLDEDSDRAIEG | −2.45483 | Down | 0.012483 |
| P00734 | THRB_HUMAN | EDKTERELL | −2.42458 | Down | 0.029486 |
| P01024 | CO3_HUMAN | NEGFTVTAE | −2.40987 | Down | 0.026103 |
| P00734 | THRB_HUMAN | SLEDKTERELLESYIDG | −2.21135 | Down | 0.009743 |
| P00734 | THRB_HUMAN | DEDSDRAIEG | −2.19274 | Down | 0.048079 |
| P02452 | CO1A1_HUMAN | DDGEAGKPG | −2.14112 | Down | 0.031919 |
| P00734 | THRB_HUMAN | DKTERELL | −2.13598 | Down | 0.02241 |
| P02461 | CO3A1_HUMAN | DGVPGKDGPRGPT | −1.96876 | Down | 0.032337 |
| P58215 | LOXL3_HUMAN | AKYGPG | −1.94901 | Down | 0.021372 |
| O00161 | SNP23_HUMAN | TNGQLQQPT | −1.92509 | Down | 0.013627 |
| P02671 | FIBA_HUMAN | MADEAGSEADHEGTHST | −1.89567 | Down | 0.031302 |

**Table 1** (*continued*)

Page 8 of 16

Wang et al. Discovery of tumor suppressive peptide ZYX$_{36-58}$

**Table 1** (*continued*)

| Protein | Accession | Peptide | Fold change (Exp/Con) | Up/down | P value |
|---------|-----------|---------|----------------------|---------|---------|
| O60673 | DPOLZ_HUMAN | PYLRQ | −1.84589 | Down | 0.021651 |
| P02775 | CXCL7_HUMAN | NLAKGKEESLDSD | −1.69837 | Down | 0.029394 |
| P01024 | CO3_HUMAN | TLDPER | −1.69714 | Down | 0.041666 |
| P01024 | CO3_HUMAN | SEETKENEGF | −1.64204 | Down | 0.016716 |
| P01024 | CO3_HUMAN | EGFTVTAEGK | −1.63584 | Down | 0.048265 |
| P01024 | CO3_HUMAN | ENEGFTVTAEGK | −1.6276 | Down | 0.038204 |
| P02452 | CO1A1_HUMAN | DGKTGPPGP | −1.62456 | Down | 0.045696 |
| Q96A19 | C102A_HUMAN | NIEKL | −1.62081 | Down | 0.030902 |
| Q13402 | MYO7A_HUMAN | NLEKL | −1.62081 | Down | 0.030902 |
| P00734 | THRB_HUMAN | NIEKI | −1.62081 | Down | 0.030902 |
| P68363 | TBA1B_HUMAN | DVVPK | −1.62022 | Down | 0.041681 |
| Q9H4Q3 | PRD13_HUMAN | GDPPPP | −1.62629 | Up | 0.027741 |
| P04632 | CPNS1_HUMAN | GGGGGGLGGGLG | 2.00767 | Up | 0.016592 |
| Q6UX71 | PXDC2_HUMAN | DTNRASVGQDSPEPR | 2.294694 | Up | 0.044462 |
| P02671 | FIBA_HUMAN | GDSTFESKS | 2.43554 | Up | 0.023656 |
| P02671 | FIBA_HUMAN | DFLAEGGGV | 3.758263 | Up | 0.031511 |
| A7KAX9 | RHG32_HUMAN | NHLLVH | #DIV/0! | Up | 0.002947 |
| Q14624 | ITIH4_HUMAN | QLGLPGPPDVPDH | #DIV/0! | Up | 0.00199 |

#DIV/0! indicated the peptides that were only existed in the serum of ovarian cancer patients or control.

(DGEA and DGEAGK) with the recognition motif DEGA from type I collagen were identified with the ability to bind α2β1-integrin, so cy5.5 labeled DEGA and DGEAGK was used for optical imaging in mice with α2β1 positive prostate tumors (25). Fibrinogen derived peptides alphastatin (ADSGEGDFLAEGGGVRGPRVVERH) and AHN419 (DFLAEGGGVRG) could inhibit endothelial cell tubule formation and migration *in vitro* and subcutaneous breast tumor growth *in vivo* (26). In our results, one of the down-regulated peptides from COL1A1 (DDGEAGKPG) contains the recognition motif, and one of the up-regulated peptide derived from FIBA protein (DFLAEGGGV) share the sequence DFLAEGGGV with alphastatin and AHN419 peptides. So we propose that some of the differentially expressed peptides may also function in the progression of ovarian cancer.

Three peptides which were down-regulated in ovarian cancer patients (ZYX$_{36-58}$, F13A$_{18-38}$, and SNP23$_{123-131}$,

detailed information of these peptides are presented in *Table 1*) were selected and synthesized. They were derived from the focal adhesion protein Zyxin (ZYX), Coagulation factor XIII A chain (F13A), Synaptosomal-Associated Protein 23 [SNP23, an important regulator of phagosome formation (27)], respectively. The cell proliferation, invasion, migration and apoptosis of cultured HO8910 and SKOV3 cells were analyzed after treatment with these peptides or peptide solvent. Our results showed that cell invasion and migration was significantly inhibited in HO8910 and SKOV3 cells treated with ZYX$_{36-58}$ peptide (*Figure 3*) but not with other peptides (data not shown). Meanwhile, proliferation of HO8910 and SKOV3 cells were not altered after treatment with ZYX$_{36-58}$ peptide (*Figure 4A,B*). But apoptosis of HO8910 and SKOV3 was significantly increased after treatment with ZYX$_{36-58}$ peptide (*Figure 4C,D,E,F*). In conclusion, ZYX$_{36-58}$ treatment *in vitro* can significantly inhibit cell invasion and migration, and
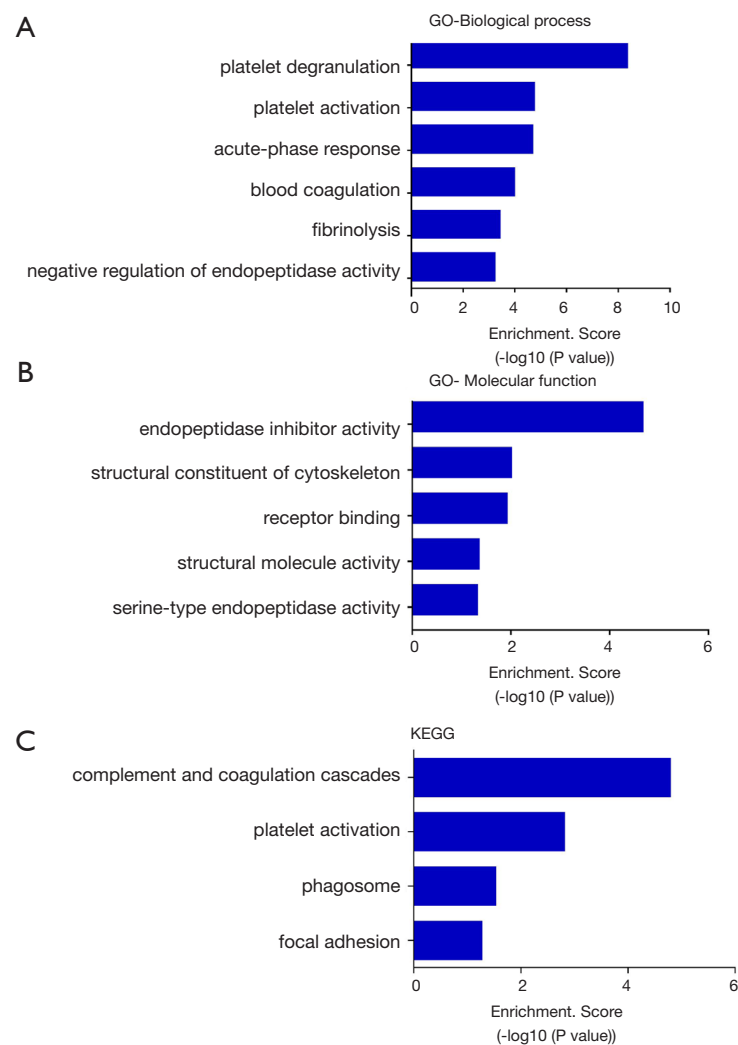
**Figure 2** Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the precursor proteins of the differentially expressed peptide. (A) The top 6 enriched biological processes of the precursor proteins of the differentially expressed peptide. (B) The enriched molecular function of the precursor proteins of the differentially expressed peptide. (C) The enriched KEGG pathway analysis of the precursor proteins of the differentially expressed peptide.

promote cell apoptosis of ovarian cancer cells, but has no effect on ovarian cancer cell proliferation.

***The mechanism of ZYX$_{36-58}$ function on ovarian cancer cell invasion, migration and apoptosis***

To explore the mechanism whereby ZYX$_{36-58}$ inhibits invasion and migration of ovarian cancer cells, we began by analyzing the peptide distribution using the FITC-labeled ZYX$_{36-58}$. The results showed that FITC-labeled ZYX$_{36-58}$ was observed within 1 h in both the cell matrix and nucleus; and the fluorescence intensity peaked at 24 h, but was

diminished by 48 h (*Figure S1*). Then we first analyzed the interaction between ZYX$_{36-58}$ and its precursor protein ZYX by biotin-labeled ZYX peptide pull down and western blot, however, ZYX was not detected in the eluent of ZYX$_{36-58}$ (*Figure S2A*). Then how does ZYX$_{36-58}$ function in inhibiting cell invasion, migration and promoting cell apoptosis of ovarian cancer cells?

Mass spectrometry followed by biotin-labeled peptide pull down assay was an important strategy for identifying the peptide interacting proteins. The protein pull-down by biotin-labeled ZYX$_{36-58}$ and biotin-labeled scrambled peptide were first analyzed by sodium dodecyl sulfate

Page 10 of 16

Wang et al. Discovery of tumor suppressive peptide ZYX$_{36-58}$
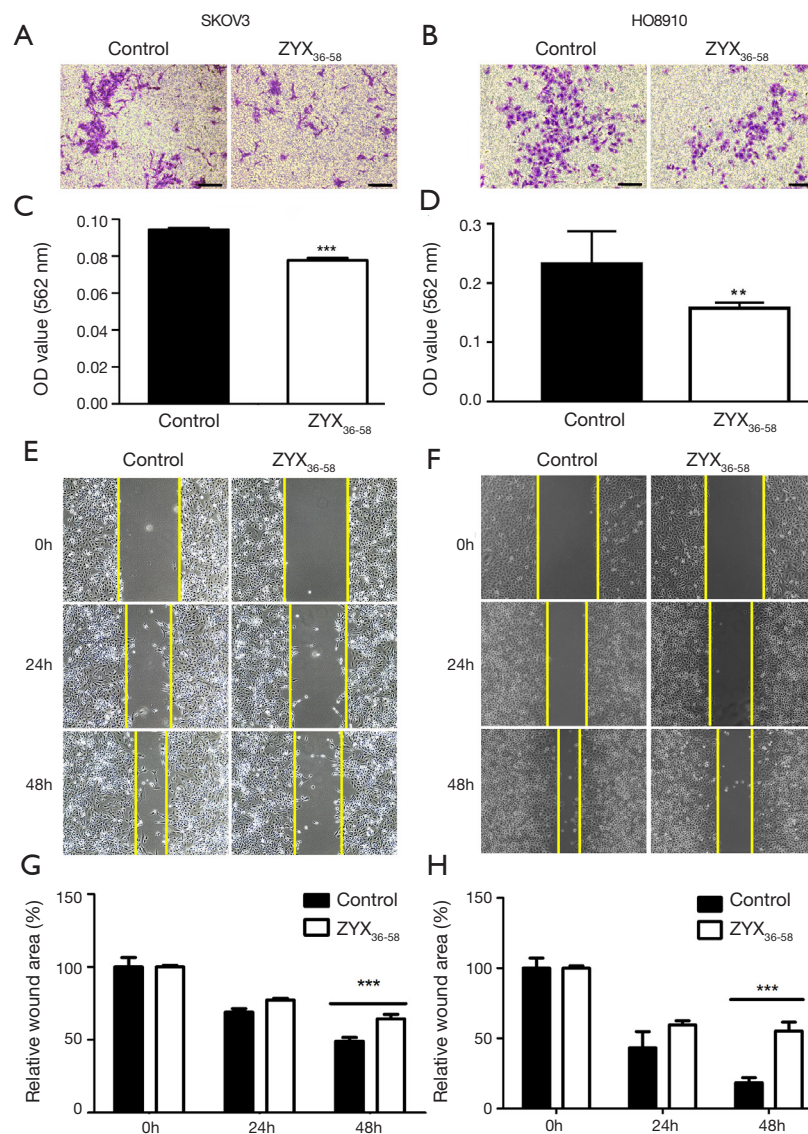


**Figure 3** Peptide ZYX$_{36-58}$ significantly inhibits the invasion and migration of ovarian cancer cells. (A) Representative images of the invasive assay of ZYX$_{36-58}$ peptide and control solvent treated SKOV3 cells. Scale Bar: 200 μm. (B) Representative images of the invasive assay of ZYX$_{36-58}$ peptide and control solvent treated HO8910 cells. Scale Bar: 200μm. (C) Quantitative analysis of the invasive cells of ZYX$_{36-58}$ peptide and control solvent treated SKOV3 cells. (D) Quantitative analysis of the invasive cells of ZYX$_{36-58}$ peptide and control solvent treated HO8910 cells. (E) The representative images of the wound healing assay of ZYX$_{36-58}$ peptide and control solvent treated SKOV3 cells. (F) The representative images of the wound healing assay of ZYX$_{36-58}$ peptide and control solvent treated HO8910 cells. (G) Quantitative analysis of the relative wound area of ZYX$_{36-58}$ peptide and control solvent treated SKOV3 cells. (H) Quantitative analysis of the relative wound area of ZYX$_{36-58}$ peptide and control solvent treated HO8910 cells. **, $P<0.01$, ***, $P<0.001$.

polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining (*Figure 5A*). As a result, several protein bands were specifically found in biotin-labeled ZYX$_{36-58}$ group. So the in-gel digested proteins from the band of biotin-labeled ZYX$_{36-58}$ and biotin-labeled scrambled peptide as illustrated in *Figure 5A* were analyzed by LC-MS/MS. Finally, 22 proteins were specifically identified in the biotin-labeled ZYX$_{36-58}$ pull down lysate (*Table S2*), and 68
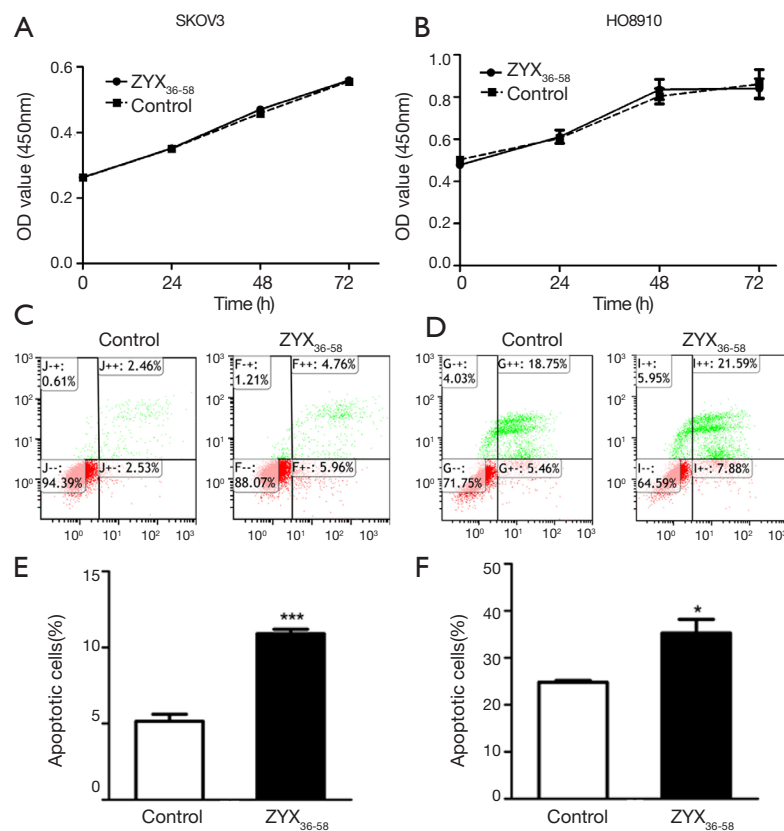
**Figure 4** Peptide $ZYX_{36-58}$ significantly promotes the cell apoptosis of ovarian cancer cells. (A) Cell proliferation of $ZYX_{36-58}$ peptide and control solvent treated SKOV3 cells were examined by cell counting kit 8 (CCK8) assays at the indicated time points. (B) Cell proliferation of $ZYX_{36-58}$ peptide and control solvent treated HO8910 cells were examined by CCK8 assays at the indicated time points. (C) Cell apoptosis of $ZYX_{36-58}$ peptide and control solvent treated SKOV3 cells were analyzed by flow cytometry. (D) Cell apoptosis of $ZYX_{36-58}$ peptide and control solvent treated HO8910 cells were analyzed by flow cytometry. (E) Quantitative analysis of the apoptotic cells of $ZYX_{36-58}$ peptide and control solvent treated SKOV3 cells. (F) Quantitative analysis of the apoptotic cells of $ZYX_{36-58}$ peptide and control solvent treated HO8910 cells. *, $P<0.05$, ***, $P<0.001$.

proteins were specifically identified in the biotin-labeled scrambled peptide pull down lysate. Among the proteins specifically identified in the biotinylated $ZYX_{36-58}$ group, EEF1A2, YBX1, PCLO, PROF1, RAP1L, TSP1, RACK1, A2MG and TLN1 were also involved in the invasion and migration of cancer cells. So further western blot analysis of the top four enriched proteins (EEF1A2, TSP1, YBX1 and PCLO) followed by biotin-labeled $ZYX_{36-58}$ pull down assay was performed, our results showed that only physical interaction between TSP1 (Thrombospondin-1) and $ZYX_{36-58}$ was detected, but no interaction was observed between $ZYX_{36-58}$ and the other three proteins (*Figure 5B* and *Figure S2B,C,D*). Intriguingly, the protein level of TSP1 was also significantly increased in $ZYX_{36-58}$ treated ovarian cancer cells (*Figure 5C,D*), which indicated that $ZYX_{36-58}$ may function through binding and upregulating TSP1 protein level.

Next, the differentially expressed mRNA in $ZYX_{36-58}$ treated and untreated ovarian cancer cells were analyzed by high throughput sequencing. Altogether, 169 genes were significantly upregulated and 49 genes were significantly down-regulated in $ZYX_{36-58}$ treated ovarian cancer cells [fold change >1.5, FDR <0.01, *Figure 5E* and Table (online: http://cdn.amegroups.cn/static/appl ication/42a7b771d63b6a426459e247cf00fdd4/atm-20-2018-2.pdf]. Gene ontology analysis of the biological pathway indicated that the differentially expressed genes are involved in the regulation of macrophage chemotaxis and macrophage migration, and the positive regulation of the leukocyte apoptotic process (*Figure 5F*). Further, qRT-PCR showed that gene expression of interleukin-1 alpha (IL1A), peptidase inhibitor 16 (PI16), and amelotin

Page 12 of 16

Wang et al. Discovery of tumor suppressive peptide ZYX$_{36-58}$
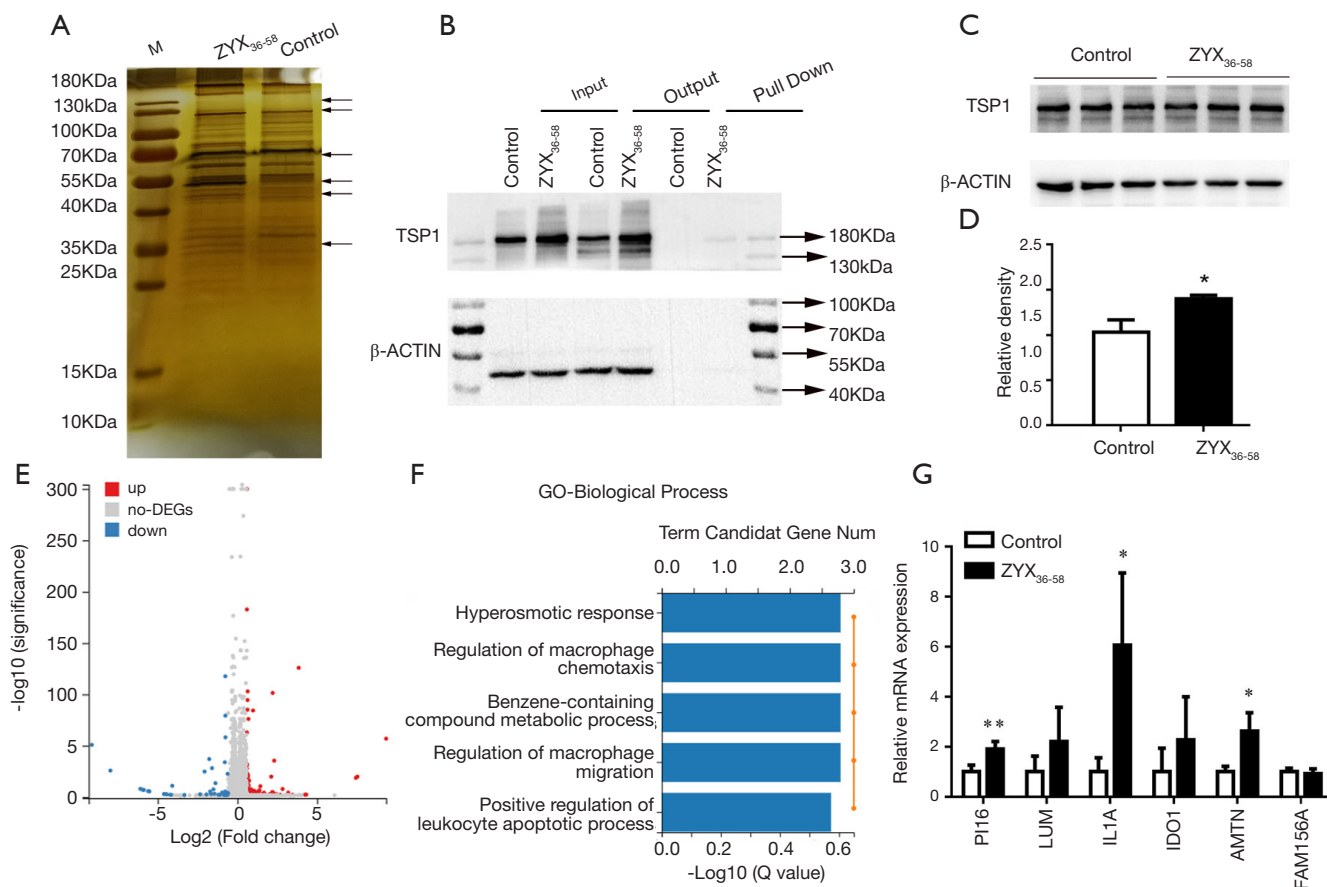


**Figure 5** ZYX$_{36-58}$ binds thrombospondin-1 (TSP1) and increases the TSP1 protein level in the ZYX$_{36-58}$ treated ovarian cancer cells. (A) Silver staining of the biotin labeled ZYX$_{36-58}$ and scrambled peptides pull down protein in the ZYX$_{36-58}$ and scrambled peptide treated SKOV3 cells, the arrowhead indicated the different band between ZYX$_{36-58}$ and scrambled peptide treated group. (B) Western blot verification of TSP1 expression in the biotin labeled ZYX$_{36-58}$ and scrambled peptide pull down eluent. Input: the total protein of biotin labeled ZYX$_{36-58}$ and scrambled peptide treated SKOV3 cells, Output: The protein which could not interact with the biotin labeled ZYX$_{36-58}$ and scrambled peptide; Pull down: the protein which interacts with the biotin labeled ZYX$_{36-58}$ and scrambled peptide. (C) Western blot analysis of TSP1 in the ZYX$_{36-58}$ and scrambled peptide treated SKOV3 cells. (D) Quantitative analysis of TSP1 expression in ZYX$_{36-58}$ and scrambled peptide treated SKOV3 cells. (E) Volcanic plot of the differentially expressed genes in ZYX$_{36-58}$ and peptide solvent treated ovarian cancer cells [fold change ≥1.5 and False Discovery Rate (FDR) <0.01]. (F) Gene ontology (GO, Biological Process) analysis of differentially expressed genes in ZYX$_{36-58}$ peptide and control treated SKOV3 cells. (G) Quantitative Real-time PCR (qRT-PCR) analysis of the six differentially expressed genes which were associated with tumor progression in ZYX$_{36-58}$ and scrambled peptides treated SKOV3 cells. *, $P<0.05$, **, $P<0.01$.

(AMTN), all of which are important inhibitors of cancer progression, was significantly up-regulated in ZYX$_{36-58}$ treated ovarian cancer cells, which is consistent with the sequencing result (*Figure 5G*).

Interestingly, TSP1 exerts its antitumor effects through its widely established antiangiogenic ability (28), by inducing ovarian cancer apoptosis (6), via the regulation of

immune system (29), and by activating TGF-β signaling pathway (30), and so on. Previous study indicated that thrombin can directly activate IL-1α (31) as well as *de novo* synthesis of TSP1 (32). Besides, PI16 is highly expressed in homing CD8+ T cells (33) and is considered as a CD4 subset biomarker (34). The secreted protein AMTN is induced at the initiation of apoptosis by TGF-β1 (35).

These results indicated that $ZYX_{36-58}$ may inhibit ovarian cancer invasion and migration through targeting TSP1 and its downstream signaling pathway.

## Discussion

With the development of mass spectrometry, several studies have focused on the identification of the peptide biomarkers in diseases (11-13). Up to now, the peptidome of the urine from healthy control and ovarian cancer patients and the peptidome of the ascites from benign gynecological conditions and ovarian cancer patients have been analyzed (36,37), the serum peptidomes of control and ovarian cancer patients have not been studied before. However, none of the differentially expressed peptides in this study were found in the preceding studies. This could be caused by the different systems in different studies and may further reflect the peptide distribution specificity in different systems.

In this study, KEGG pathway analysis of the differentially expressed peptides indicated that although most of the peptides' precursors are important regulators of the coagulation system, there are also some regulators of focal adhesion, platelet activation and phagosome formation, which are important for cancer initiation and progression (21,38). Besides, two of the dys-regulated peptides in our study also share the same sequence with the functional peptides reported before (24,25), so we accordingly think that some of the differentially expressed peptides are involved in ovarian cancer progression.

Endogenous peptides are increasingly seen to be important regulators of biological processes. For example, the production of endogenous peptide apelin, induced by muscle contraction, can significantly enhance muscle function by inducing the formation of mitochondria in muscle fibers and influencing autophagy and anti-inflammatory pathways (39). The peptide PAF, released by diverse therapeutic sensitive cancer cells following therapy, can promote apoptosis of therapy-resistant cancer cells (8). Serum A-type natriuretic peptide (ANP) in the circulatory system can significantly inhibit the proliferation of cancer cells *in vitro* and inhibit the cancer growth *in vivo* (40). Additionally, the C-type natriuretic peptide (CNP) and its precursor are considered as biomarkers of human prostate cancer (41), and CNP can also inhibit the cell proliferation of prostate cancer cells by inhibiting DNA synthesis (42). For this reason, we synthesized three peptides that were differentially expressed in serum of control *vs.* ovarian cancer patients and analyzed the function of these peptides on proliferation, invasion and migration of ovarian cancer cells. Our results clearly indicated that one of these peptides, $ZYX_{36-58}$, can inhibit the invasion and migration of ovarian cancer cells and thus may become an important target of ovarian cancer therapy.

TSP1 is an extracellular matrix protein that can bind fibronectin, collagen and integrin, mediate cell adhesion and migration (43), bind VEGF and regulate the internalization and expression of VEGF and promotes the apoptosis of endothelial cells (28). A higher plasma TSP1 level predicts longer overall survival of ovarian cancer patients (44). TSP1 derived peptides such as ABT-510, ABT-526, DI-TSP, which mimic the thrombospondin-1/CD47 epitope, and the prosaposin-derived peptide targeting TSP1, all can inhibit the growth and metastasis of ovarian cancer and other types of cancer (6,45-47). This has caused secreted TSP1 to be considered an important therapeutic target of ovarian cancer. Interestingly, our results show that $ZYX_{36-58}$ can bind and increase the protein level of TSP1.

TSP1, also known as the thrombin-sensitive protein, was first separated from thrombin-induced platelets (48). TSP1 regulates macrophage recruitment and the production of inflammatory factors such as IL-1β (49), and IL-10 (50). Gene ontology showed that the differentially expressed genes between $ZYX_{36-58}$ and peptide solvent treated ovarian cancer cells are involved in the regulation of macrophage chemotaxis, macrophage migration, and leukocyte apoptosis, which is consistent with the peptide pull-down result. IL-1A, which was also upregulated in the $ZYX_{36-58}$ treated ovarian cancer cells, whose activation directly links the coagulation system and immune system; this is also consistent with KEGG analysis of the precursor proteins of the differentially expressed peptides in the $ZYX_{36-58}$ and peptide solvent treated ovarian cancer cells. Thus, although the mechanism by which $ZYX_{36-58}$ increases TSP1 protein level is still unknown and the binding efficiency is low, our study provides a new strategy for identifying the therapeutic molecules.

In future, strategies for increasing the stability and target efficiency of $ZYX_{36-58}$, such as N-terminal acetylation, C-terminal amination, cyclization and amino acid substitution, may also be used to increase its stability or ability and thereby improve the feasibility of the clinical application of $ZYX_{36-58}$.

## Conclusions

With small molecular weight, short half-life and low

**Page 14 of 16**

Wang et al. Discovery of tumor suppressive peptide ZYX$_{36\text{-}58}$

immunogenicity, peptides drugs are considered as one of the development directions with wide market prospect in the pharmaceutical industry. Indeed, several peptide drugs have been used in clinic, such as thymic pentapeptide (the peptide derived from thymopoietin II), Leuprorelin (GnRH analogue) and octreotide (Somatostatin octapeptide). With the development of mass spectrometry and peptide synthesis technology, more and more endogenous peptides have been identified, although only few of the peptide's function have been characterized, we believe that most of the bioactive peptides' function will be comprehensively analyzed, and the peptide drugs will be used in clinic in the future.

## Acknowledgments

## Footnote

*Reporting Checklist*: The authors have completed the MDAR checklist. Available at http://dx.doi.org/10.21037/atm-20-2018

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*Peer Review File*: Available at http://dx.doi.org/10.21037/atm-20-2018

*Conflicts of Interest*: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-2018). XW, GL, XP, XJ and JX report a patent of a polypeptide ZYX$_{36\text{-}58}$ for the treatment or adjuvant treatment of ovarian cancer pending. JX serves as an unpaid Section Editor of *Annual of Translational Medicine* from Oct 2019 to Mar 2020. The other authors have no conflicts of interest to declare.

*Ethical Statement*: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All experiments performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013), approved by the ethics committee of Women's Hospital of Nanjing Medical University (Nanjing Maternity and Child Health Care Hospital, Ethics Approval Number: 2016[16]) and all patients signed informed consent.

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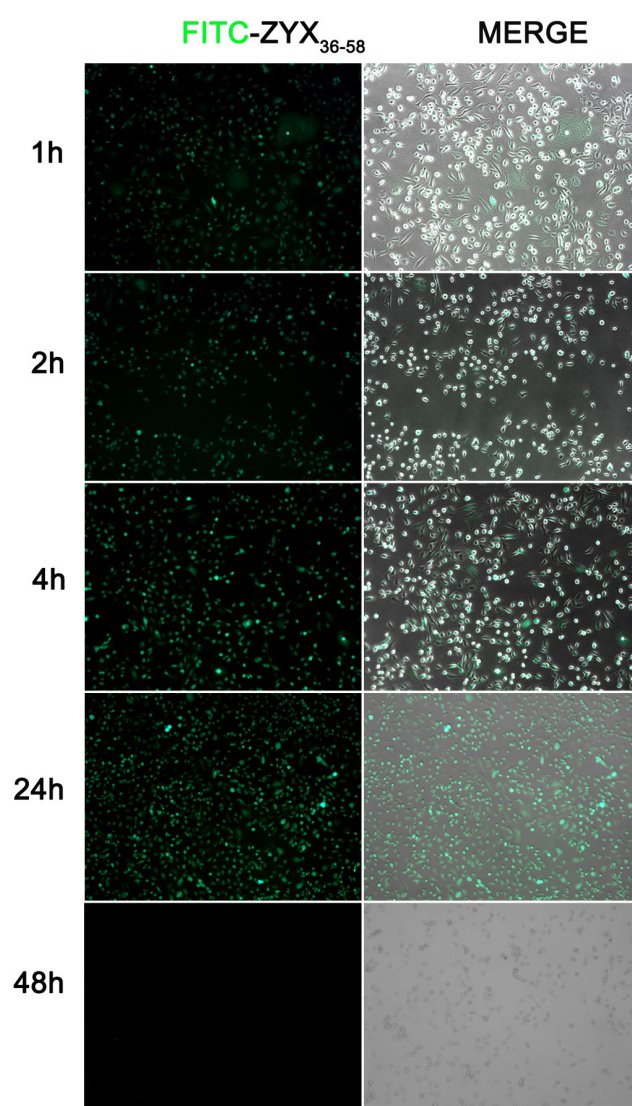
**Figure S1** The distribution of FITC-labeled $ZYX_{36-58}$ peptides in ovarian cancer cells at different time point.
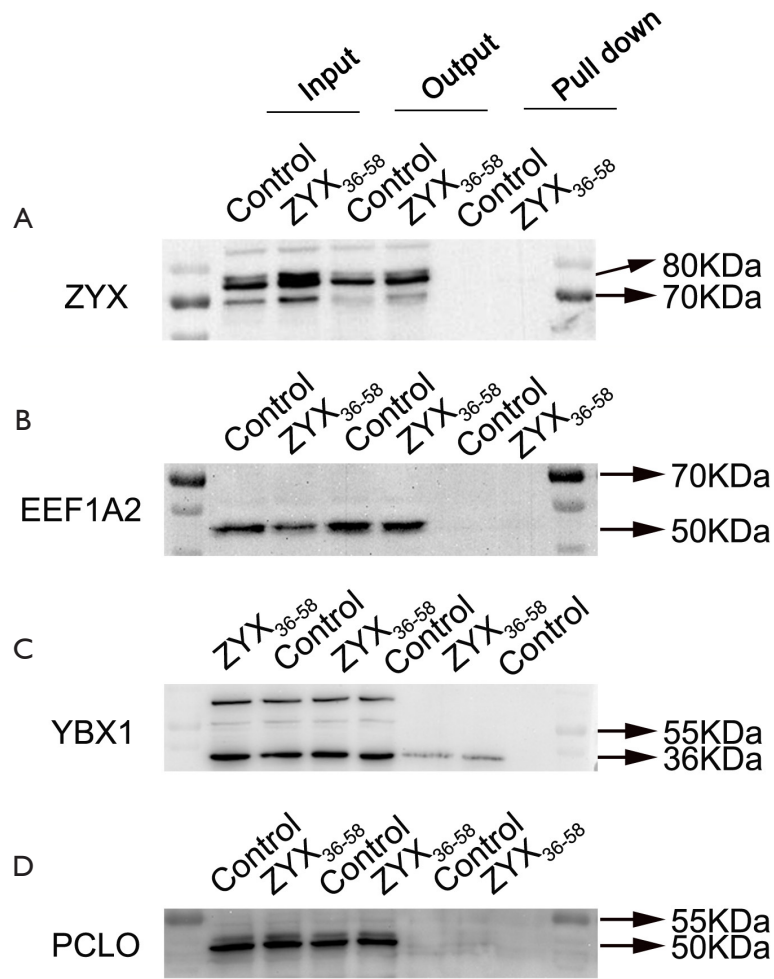
**Figure S2** The expression of YBX1, EEF1A2, PCLO and ZYX in the eluent of the biotin-labeled ZYX$_{36-58}$ and scrambled peptide pull down lysate. Input: the total protein of biotin labeled ZYX$_{36-58}$ and scrambled peptide treated SKOV3 cells, Output: The protein which could not interact with the biotin labeled ZYX$_{36-58}$ and scrambled peptide, pull down: the protein which interacts with the biotin labeled ZYX$_{36-58}$ and scrambled peptide.

**Table S1** Primer sequences

| Name | Primer F (sequence 5'-3') | Primer R (sequence 5'-3') | Length (bp) |
|------|---------------------------|---------------------------|-------------|
| PI16 | GCGTGAGCACTACAACCTCAG | GCACACCAGTAATTCGATGTTGG | 163 |
| LUM | TAACTGCCCTGAAAGCTACCC | GGAGGCACCATTGGTACACTT | 75 |
| IL1A | TGGTAGTAGCAACCAACGGGA | ACTTTGATTGAGGGCGTCATTC | 215 |
| IDO1 | GCCAGCTTCGAGAAAGAGTTG | ATCCCAGAACTAGACGTGCAA | 96 |
| AMTN | TGTCTTCTAGGATCAACTCGGT | TGGTTTGGTAGTGTTCCCTGA | 95 |
| FAM156A | GAATCCAGCATCGCCTTCCAA | CCCATCATCGGCTGTTCTGAG | 105 |
| β-ACTIN | TCCCTGGAGAAGAGCTACGA | AGCACTGTGTTGGCGTACAG | 194 |

**Table S2** The list of the protein specifically identified in the ZYX$_{36-58}$ pull down lysate by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

| Protein_ID | Group_ID | Protein_Qscore | Protein _FDR | Protein_Mass | PeptideSeqs | PepIs Unique | Unique_Peptide_Num | Unique_Spectra_Num | Coverage | Abundance | iBAQ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| sp\|Q9Y6V0\|PCLO_HUMAN | 59_1 | 2.474748207 | NA | 560354.7522 | VDAKVEIIK | 1 | 1 | 1 | 0.0018 | 428189.1517 | 1791.586409 |
| sp\|Q05639\|EF1A2_HUMAN | 4_1 | 10.25249928 | NA | 50438.30624 | IGGIGTVPVGR;LPLQDVYK;QTVAVGVIK | 1;1;1 | 3 | 3 | 0.0605 | 198694.2396 | 8638.879982 |
| sp\|P67809\|YBOX1_HUMAN | 38_1 | 10.25249928 | NA | 35902.67505 | GAEAANVTGPGGVPVQGSK;NGYGFINR; SVGDGETVEFDVVEGEK | 1;1;1 | 3 | 4 | 0.1358 | 93534.62237 | 7194.970952 |
| sp\|P07996\|TSP1_HUMAN | 73_1 | 7.52926131 | NA | 129299.5536 | FVFGTTPEDILR;QHVVSVEEALLATGQWK | 1;1 | 2 | 2 | 0.0248 | 80850.38631 | 1206.722184 |
| sp\|Q4VXU2\|PAP1L_HUMAN | 21_1 | 2.474748207 | NA | 68348.50212 | YQGVNLYVK | 1 | 1 | 1 | 0.0147 | 65299.87899 | 1718.417868 |
| sp\|P01042\|KNG1_HUMAN | 45_1 | 2.72323797 | NA | 71912.1499 | GHGLGHGHEQQHGLGHGHK | 1 | 1 | 1 | 0.0295 | 42162.06619 | 1109.528058 |
| sp\|Q9Y490\|TLN1_HUMAN | 10_1 | 6.239378862 | NA | 269599.0446 | AAAFEEQENETVVVK;GVGAAATAVTQAL NELLQHVK | 1;1 | 2 | 2 | 0.0142 | 37171.24947 | 247.8083298 |
| sp\|P16401\|H15_HUMAN | 25_1 | 3.764630655 | NA | 22566.46766 | ALAAGGYDVEK | 1 | 1 | 1 | 0.0487 | 34208.38958 | 2631.414583 |
| sp\|Q13268\|DHRS2_HUMAN | 69_1 | 3.764630655 | NA | 29907.70664 | TLALELAPK | 1 | 1 | 1 | 0.0321 | 20981.29161 | 1234.193624 |
| sp\|O76021\|RL1D1_HUMAN | 74_1 | 3.764630655 | NA | 54938.92899 | TEQFYR | 1 | 1 | 1 | 0.0122 | 20233.51651 | 778.2121733 |
| sp\|P18621\|RL17_HUMAN | 56_1 | 2.131035571 | NA | 21383.33238 | YSLDPENPTK | 1 | 1 | 1 | 0.0543 | 17073.66359 | 1552.151235 |
| sp\|P01023\|A2MG_HUMAN | 43_1 | 3.764630655 | NA | 163187.889 | SASNMAIVDVK | 1 | 1 | 1 | 0.0075 | 17032.0033 | 227.0933773 |
| sp\|P62424\|RL7A_HUMAN | 65_1 | 3.764630655 | NA | 29977.02345 | VVNPLFEK | 1 | 1 | 1 | 0.0301 | 16159.28796 | 1077.285864 |
| sp\|P07737\|PROF1_HUMAN | 62_1 | 3.764630655 | NA | 15044.55581 | DSLLQDGEFSMDLR | 1 | 1 | 1 | 0.1 | 15813.04493 | 1757.004992 |
| sp\|P62906\|RL10A_HUMAN | 3_1 | 2.474748207 | NA | 24815.53467 | DTLYEAVR | 1 | 1 | 1 | 0.0369 | 13506.52467 | 900.4349778 |
| sp\|P27635\|RL10_HUMAN | 16_1 | 3.764630655 | NA | 24587.86717 | EHVIEALR | 1 | 1 | 1 | 0.0374 | 11996.84742 | 922.834417 |
| sp\|P63244\|RACK1_HUMAN | 66_1 | 3.764630655 | NA | 35054.55748 | DETNYGIPQR | 1 | 1 | 1 | 0.0315 | 11736.2341 | 533.4651865 |
| sp\|Q9NQI0\|DDX4_HUMAN | 5_1 | 2.72323797 | NA | 79257.75184 | YLVLDEADR | 1 | 1 | 1 | 0.0124 | 10731.23008 | 228.3240442 |
| sp\|Q92900\|RENT1_HUMAN | 58_1 | 2.474748207 | NA | 124266.7116 | YGVIIVGNPK | 1 | 1 | 1 | 0.0089 | 8353.668533 | 136.9453858 |
| sp\|P0DMV8\|HS71A_HUMAN | 11_1 | 2.325762101 | NA | 70009.03579 | VEIIANDQGNR | 1 | 1 | 1 | 0.0172 | 5349.037205 | 148.5843668 |
| sp\|P35232\|PHB_HUMAN | 6_1 | 2.72323797 | NA | 29785.90188 | FGLALAVAGGVVNSALYNVDAGHR | 1 | 1 | 1 | 0.0882 | 1995.144597 | 110.8413665 |
| sp\|P04844\|RPN2_HUMAN | 20_1 | 2.131035571 | NA | 69240.96992 | SIVEEIEDLVAR | 1 | 1 | 1 | 0.019 | 0 | 0 |