Knockdown CD44 Promotes Amyloid-Beta Degradation in Ovarian Cancer Cells By Regulating CD36 Expression

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Abstract

Background: CD44 is highly expressed in many cancers, including ovarian cancer. Its interactions with ligands are involved in tumor progression, prognosis, and metastasis. However, the function of CD44 in the advancement of ovarian cancer remains unclear.

Methods and Results: RNA sequencing was used to investigate the possible molecules and pathways regulated by CD44 in ovarian cancer to compare gene expression in CD44-knockdown SKOV3 cells and control cells. Identify the differentially expressed genes and then proceed to functional enrichment analysis. The results showed that genes differentially expressed were enriched in ECM-receptor interaction, Protein digestion and absorption, Focal adhesion, Notch signaling pathway, microRNA in cancer, and TGF-beta signaling pathway. Furthermore, the analysis of the proteins interaction network revealed the interaction between CD44 and CD36 in SKOV3 cells. Further analysis showed that CD36, a molecule that may be involved in ECM-receptor interaction, was low expressed in CD44-knockdown SKOV3 cells. And the results showed that knockdown CD44 induces amyloid-beta degradation in ovarian cancer cells by regulating CD36 expression. The analyses of the public database demonstrated that the CD36 expression was related to the clinical survival of ovarian cancer.

Conclusions: Our study showed that CD44 might up-regulate the CD36 expression in ovarian cancer, thereby exerting a cancer-promoting effect.

Introduction

Ovarian cancer is one of the malignant tumors that threaten women's health and its mortality rate ranks first among the female reproductive tumors[1]. GLOBOCAN 2020 global cancer statistics show that the mortality rate of ovarian cancer is 5.4 per 100,000, ranking eighth among the global female cancer death rates[2]. The current standard treatment options for ovarian cancer are debulking surgery and postoperative adjuvant chemotherapy based on platinum and paclitaxel[3]. However, 70% of patients with ovarian cancer had already lost the chance of surgery due to extensive metastasis at the first diagnosis, and less than 30% survived in 5 years. For those patients with ovarian cancer who received standard treatment, the tumor progression process can be reversed, and at least 50% of patients still have recurrence and metastasis[4, 5].

The overactivation of oncogenes and the inactivation of tumor suppressor genes are important causes of the occurrence and development of ovarian cancer. Among these genes, CD44, as a kind of cell surface glycoprotein, participates in the process of cell survival, proliferation, differentiation, and motility[6, 7]. Hyaluronic acid combined with CD44 activates various signal pathways involved in cell proliferation, invasion, migration, and adhesion [7-9]. CD44 also participates in multiple essential signal pathways regulating proliferation, invasion, metastasis, and treatment resistance of cancer and regulated by many molecules in cancer cells[7, 10]. Recent studies indicated that CD44-positive ovarian tumor cell subsets express stem cell markers, which can initiate tumorigenesis and promote tumor recurrence[11, 12].

However, the role of CD44 in tumors is complex and only a few reports have systematically described the mechanism of CD44 in the development and progression of ovarian cancer.

Therefore, the purpose of the present study is to investigate the possible molecules and pathways regulated by CD44 in ovarian cancer.

Materials And Methods

Cell culture

The human ovarian cancer cell line SKOV3 was purchased from the ATCC and was authenticated by the analysis of a short tandem repeat (STR). The cell was cultured in RPMI-1640 medium (Hyclone, SH30809.01) with 10% fetal bovine serum (Tianhang, 11012-8611).

Knockdown of CD44

The lentiviruses were constructed according to the target sequence of CD44 for RNAi: 5'- TTG CAG TCA ACA GTC GAA GAA -3' and the negative control sequence: 5'- TTC TCC GAA CGT GTC ACG T-3' (vector: GV493)[13]; by Shanghai Genechem Co., LTD. (Shanghai, China). The lentiviruses (MOI=20) were added to SKOV3 cells for transfection. Puromycin was used to screen infected cells (2.5 μg/ml). The transfection efficiency was observed by GFP expression, and RT-qPCR and western blot determined the knockdown of CD44.

RT-qPCR

RNA was extracted using Trizol (Beyotime, Shanghai, China) and reverse transcribed into cDNA. RT-qPCR analysis used the StepOne™ real-time qPCR system (Applied Biosystems, USA). The primer sequences: CD44 forward, 5’-TCCCAGACGAAGACAGTCCCTGGAT-3' and reverse, 5’-CACTGGGGTGGAATGTGTCTTGGTC-3'; β-actin forward, 5’-TGTGGCATCCACGAAACTAC-3' and reverse 5’-GGAGCAATGATCTTGATCTTCA-3'. The 2−ΔΔCT was used to quantified the expression.

RNA extraction, library construction, and sequencing

RNA was extracted. The mRNA was enriched by Oligo(dT) beads, fragmented into short fragments, and reverse transcripted into cDNA. Then the cDNA fragments were purified, end-repaired, poly(A) added, and ligated to Illumina sequencing adapters. The Illumina Novaseq6000 by Gene Denovo Biotechnology Co. (Guangzhou, China) was used to sequence.

Identification of differentially expressed genes (DEGs)

RNAs differential expression analysis was performed by edgeR[14] between two samples. The gene which a P value of below 0.05 and absolute fold change ≥1.5 was considered differentially expressed genes.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

DEGs were analyzed by differential RNA between 2 groups. Transcripts which a P value of below 0.05 and absolute fold change ≥1.5 were defined as differentially expressed. The KEGG was used to analyze the pathway enrichment.

Protein-Protein interaction

String v10 was used to identify the network of proteins interaction[15], which defined genes as nodes and interaction as lines in a network. Cytoscape (v3.7.1) was used to display gene biological interaction.

Immunofluorescence

Cells were plated on a 15 mm circle microsope cover glass (NEST, China) placed in the 24-well culture plate. Cells were fixed for 15 min and blocked for 30 min. Cells were incubated with antibodies against CD36 (1:200, Proteintech, 18836-1-AP) overnight at 4°C. CY3-Goat Anti-rabbit IgG (BOSTER, Wuhan, China) was used as the secondary antibody. DAPI (BOSTER, Wuhan, China) was used to stain the nucleus. Cells were observed by a Leica TCS SP8 confocal microscope (Leica Microsystems GmbH, Mannheim, Germany).

Western blot analysis

Cells were lysed by RIPA Lysis Buffer (BOSTER, Wuhan, China). The antibodies against CD44 (1:1,000, Proteintech, 60224-1-Ig), CD36 (1: 1,000, Proteintech, 18836-1-AP), and beta-amyloid (1:50, Proteintech, 25524-1-AP) were used as primary antibodies and Actin (1:5,000, Bioss, bs-0061R) used as internal control. Image Lab™ Software 4.1 (Bio-Rad, USA) was used to analyze the expression of proteins.

Statistical analysis

Use GraphPad Prism 8 to analyze the data. Use unpaired Student's t-test to compare the two groups, and the results showed by mean ± SEM. The in vitro experiments were repeated at least three times. A value of $P<0.05$ was considered statistically significant. The progress Free Survival (PFS) and overall survival (OS) rates in different cohorts of serous ovarian cancer patients were assessed by Kaplan-Meier plot (http://kmplot.com/), and the databases calculated the hazard ratio (HR) and log-rank P-values. The correlation of CD44 and CD36 expression in ovarian cancer was analyzed by The Gene Expression Profiling Interactive Analysis database (http://gepia2.cancer-pku.cn/), while CD36 mRNA levels in different stages were analyzed. The correlation between different genes was calculated by Pearson's correlation coefficient.

Results

Generation of CD44-knockdown SKOV3 cells

The Gene Expression Profiling Interactive Analysis database (http://gepia2.cancer-pku.cn/) shows that CD44 is highly expressed in ovarian cancer (Fig. S1).To investigate the function of CD44 in ovarian cancer cells, we constructed SKOV3 ovarian cancer cells interfered with by lentiviruses. The results showed that the transfected SKOV3 cells expressed GFP (Fig. 1A). The western blot (Fig. 1B) and RT-qPCR (Fig. 1C) demonstrated that CD44 expression levels in CD44 knockdown sh-CD44-SKOV3 cells were significantly downregulated compared with control sh-NC cells. The results revealed that the sh-CD44 SKOV3 cell line was successfully constructed.

Identification of DEGs between sh-CD44 SKOV3 and sh-NC SKOV3 cells

To identify which genes are regulated by CD44, the total RNA of sh-CD44 SKOV3 cells and sh-NC SKOV3 cells were extracted for RNA-seq analysis. We compared sh-CD44 SKOV3 cells and sh-NC SKOV3 cells and performed a global gene expression analysis, and constructed a heat map of DEGs to display genes that expressions were changed (Fig. 2A). The volcano plot shows that 670 genes were dysregulated, including 230 upregulated genes and 440 downregulated genes (Fig. 2B).

KEGG analysis of DEGs

To further explore the related pathways involving CD44 and the functions of CD44 in ovarian cancer, we performed the KEGG database analysis to find enriched pathways. The results of KEGG analysis of DEGs showed that genes differentially expressed were enriched in 'ECM-receptor interaction', 'Protein digestion and absorption, 'Focal adhesion', 'Notch signaling pathway', 'MicroRNAs in cancer', 'TGF-beta signaling pathway', and other signaling pathways in ovarian cancer(Fig. 2C). The genes involved in each pathway are shown in Table 1. These pathways had been reported to play an essential role in the adhesion, movement, metastasis, and proliferation of tumors. As one of the top 20 gene enrichment signaling pathways, the ECM-receptor interaction pathway plays a crucial role in the adhesion, movement, and proliferation of tumors[16-19]. Moreover, cancer cells could participate in the tumor metastasis passing through the ECM, while the suppress factors could prevent the migration of cancer cells by interacting with some proteins[20].

CD36 expression was decreased in CD44 knockdown SKOV3 cell

As above, CD44 might regulate the ECM-receptor interaction pathway. To identify the mechanism of CD44 in ovarian cancer progression, we further constructed a regulatory network of protein-protein interaction in response to the knockdown of CD44 in SKOV3 cells (Fig. 3A). It was revealed that CD36, a molecule that may interact with CD44, was downregulated in sh-CD44 SKOV3 cells(Fig. 3B). The results revealed that CD44 might regulate CD36 expression in SKOV3 cells. Western blot was used to analyze CD36 expression to determine further the influence of CD44 to CD36 expression in SKOV3. The results showed that CD36 expressed low when CD44 was knockdown (Fig. 3C). Then, the expression and location of CD36 in sh-CD44 SKOV3 cells or sh-NC SKOV3 cells were observed by Immunofluorescence analysis. It found that the CD36 protein of the sh-NC group was mainly distributed in the cell membrane and cytoplasm rather

than the nucleus. In contrast, the CD36 protein fluorescence signal of the sh-CD44 group was weakened, and the protein appeared in the nucleus (Fig. 3D).

CD36 promotes amyloid-beta degradation and is negatively correlated with ovarian cancer progression

Previous studies have shown that CD36 is a key mediator of amyloid aggregation [21, 22]. Hence, the expression of amyloid-beta in sh-CD44 SKOV3 cells or sh-NC SKOV3 cells was analyzed by western blot. The results indicated that sh-CD44 SKOV3 cells expressed lower beta-amyloid than sh-NC (Fig. 4A). The Kaplan-Meier plots were plotted using the public database to analyze the association between CD36 level and the five years survival in ovarian cancer. High CD36 expression was a destructive factor for ovarian cancer PFS (HR=1.37(1.18-1.59); log-rank $P=3.1\times10^{-5}$) (Fig. 4B) and OS (HR=1.39(1.16-1.65); log-rank P=0.00026) (Fig. 4C). In addition, CD36 expression was no significant difference in each ovarian cancer stage (Fig. 4D). Moreover, in the ovarian cancer samples of the Gene Expression Profiling Interactive Analysis database, the expression of CD44 was positively correlated with CD36 (R=0.21; $P=1.4\times10^{-6}$)(Fig. 4E). To sum up, all of these results demonstrated that CD36 might have a promoting function in ovarian cancer progression.

Discussion

Ovarian cancer is a common and deadly malignant tumor of the female reproductive system, which seriously threatens women's life and health[23]. It is urgent to study the pathogenesis of ovarian cancer to find new therapeutic targets to improve the clinical outcome of patients with ovarian cancer[24]. Previous studies indicated that CD44 might participate in cancer progression, metastasis, and resistance to therapy[7, 13]. And CD44 has been reported to play a vital role in cancer stem cells and promote cancer progressions involving ovarian cancer[9, 25, 26]. Hence, the exploration to understand the target genes regulated by CD44 in ovarian cancer is necessary to support our discovery of novel treatment strategies.

To explore the function of CD44 in ovarian cancer progression, we constructed the CD44-knockdown SKOV3 cells. RNA-seq revealed that genes relating to many pathways were significantly enriched in CD44-knockdown SKOV3 cells, such as the ECM-receptor interaction pathway. Several studies reported that the ECM-receptor interaction pathway is involved in cancer cell migration and tumor adhesion, movement, and proliferation [16-20]. Furthermore, our data demonstrated that amyloid beta-related genes were significantly enriched in CD44-knockdown SKOV3 cells, which suggests that CD44 may play a role in the amyloid-beta of ovarian cancer cells. Most studies found that many cancer types are intrinsically associated with specific types of amyloidosis, in which amyloid is accumulated locally inside tumors or systemically. Usually, this condition relates to the hyperproduction of specific amylogenic proteins[27-30]. And studies reveal that amyloid precursor protein affects migration and invasion in ovarian cancer[31, 32]. Studies have revealed that CD36 is a key molecule involved in the endocytosis of oxidized phospholipids, apoptosis, and many biological processes of amyloid[33-35]. CD36 increases amyloid clearance in the Alzheimer's disease animal models [21]. In the tumor microenvironment, increased lipid deposition and inflammatory factors may change the expression and distribution of CD36, which may

promote metastasis[36]. The amyloid precursor protein is a transmembrane glycoprotein that could be post-translationally processed to create the amyloid-beta peptides eventually. Amyloid precursor protein was crucial to neurotransmission and neuronal homeostasis and development. The protein highly expresses in the brain and other organs and is over-expressed in various cancers[37]. Our data demonstrated that CD36 is lowly expressed in CD44-knockdown SKOV3 cells and knockdown CD44 induces amyloid-beta degradation in ovarian cancer cells by regulating CD36 expression. The result suggests that there might be CD44-CD36 interaction in ovarian cancer to accelerate tumor development.

In conclusion, compared with wild-type SKOV3 cells, DEGs were identified in CD44 knockdown SKOV3 cells by RNA-Seq analysis. The KEGG pathway analysis was used to initially explore the function of CD44 in ovarian cancer. The use of bioinformatics analysis proved that CD44 is related to the ECM-receptor interaction pathway. In addition, it has been confirmed that CD36 expression is related to amyloid aggregation[21], while this study found that CD36 and beta-amyloid expression can both be upregulated by CD44. Hence, this study displays new insights into the cancer-promoting function of CD44 in ovarian cancer, showing that CD44 might participate in the procession of ovarian cancer by regulating the expression of CD36 and beta-amyloid.

Declarations

Funding

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Conflicts of interest

The authors declare that they have no competing interests.

Availability of data and material

Data are available on request to the authors.

Code availability

Not applicable.

Authors' contributions

Study Design: Ying Xu, Hong Yang and Jianfang Zhang

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Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Ledermann JA, Raja FA, Fotopoulou C, Gonzalez-Martin A, Colombo N, Sessa C et al (2018) Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 29(Suppl 4):iv259. .https://doi.org/10.1093/annonc/mdy157
2. Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020. CA Cancer J Clin 70(1):7–30. https://doi.org/10.3322/caac.21590
3. Lee JY, Kim S, Kim YT, Lim MC, Lee B, Jung KW et al (2018) Changes in ovarian cancer survival during the 20 years before the era of targeted therapy. BMC Cancer 18(1):601. https://doi.org/10.1186/s12885-018-4498-z
4. Li J, Pan C, Boese AC, Kang J, Umano AD, Magliocca KR et al (2020) DGKA Provides Platinum Resistance in Ovarian Cancer Through Activation of c-JUN-WEE1 Signaling. Clin Cancer Res 26(14):3843–3855. https://doi.org/10.1158/1078-0432.CCR-19-3790
5. Reverdy T, Sajous C, Peron J, Glehen O, Bakrin N, Gertych W et al.(2020) Front-Line Maintenance Therapy in Advanced Ovarian Cancer-Current Advances and Perspectives. Cancers (Basel), 12(9).https://doi.org/10.3390/cancers12092414
6. Senbanjo LT, Chellaiah MA (2017) CD44: A Multifunctional Cell Surface Adhesion Receptor Is a Regulator of Progression and Metastasis of Cancer Cells. Front Cell Dev Biol 5:18. https://doi.org/10.3389/fcell.2017.00018
7. Ponta H, Sherman L, Herrlich PA (2003) CD44: from adhesion molecules to signalling regulators. Nat Rev Mol Cell Biol 4(1):33–45. https://doi.org/10.1038/nrm1004
8. Banerjee S, Modi S, McGinn O, Zhao X, Dudeja V, Ramakrishnan S et al (2016) Impaired Synthesis of Stromal Components in Response to Minnelide Improves Vascular Function, Drug Delivery, and

Survival in Pancreatic Cancer. Clin Cancer Res 22(2):415–425. https://doi.org/10.1158/1078-0432.CCR-15-1155

9. Zoller M (2011) CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? Nat Rev Cancer 11(4):254–267. https://doi.org/10.1038/nrc3023
10. Xu H, Niu M, Yuan X, Wu K, Liu A (2020) CD44 as a tumor biomarker and therapeutic target. Exp Hematol Oncol 9(1):36. https://doi.org/10.1186/s40164-020-00192-0
11. Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T et al (2009) Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle 8(1):158–166. https://doi.org/10.4161/cc.8.1.7533
12. Bapat SA, Mali AM, Koppikar CB, Kurrey NK (2005) Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res 65(8):3025–3029. https://doi.org/10.1158/0008-5472.CAN-04-3931
13. Gao Y, Xu Y, Zhao S, Qian L, Song T, Zheng J et al (2021) Growth differentiation factor-15 promotes immune escape of ovarian cancer via targeting CD44 in dendritic cells. Exp Cell Res 402(1):112522. https://doi.org/10.1016/j.yexcr.2021.112522
14. Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26(1):139–140. https://doi.org/10.1093/bioinformatics/btp616
15. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A et al.(2013) From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinformatics, 43:11 10 11–11 10 33.https://doi.org/10.1002/0471250953.bi1110s43
16. Andersen MK, Rise K, Giskeodegard GF, Richardsen E, Bertilsson H, Storkersen O et al (2018) Integrative metabolic and transcriptomic profiling of prostate cancer tissue containing reactive stroma. Sci Rep 8(1):14269. https://doi.org/10.1038/s41598-018-32549-1
17. Cui X, Morales RT, Qian W, Wang H, Gagner JP, Dolgalev I et al (2018) Hacking macrophage-associated immunosuppression for regulating glioblastoma angiogenesis. Biomaterials 161:164–178. https://doi.org/10.1016/j.biomaterials.2018.01.053
18. Rahbari NN, Kedrin D, Incio J, Liu H, Ho WW, Nia HT et al (2016) Anti-VEGF therapy induces ECM remodeling and mechanical barriers to therapy in colorectal cancer liver metastases. Sci Transl Med 8(360):360ra135. .https://doi.org/10.1126/scitranslmed.aaf5219
19. Yan P, He Y, Xie K, Kong S, Zhao W (2018) In silico analyses for potential key genes associated with gastric cancer. PeerJ 6:e6092. .https://doi.org/10.7717/peerj.6092
20. Alahari SK (2003) Nischarin inhibits Rac induced migration and invasion of epithelial cells by affecting signaling cascades involving PAK. Exp Cell Res 288(2):415–424. https://doi.org/10.1016/s0014-4827(03)00233-7
21. Dobri AM, Dudau M, Enciu AM, Hinescu ME (2021) CD36 in Alzheimer's Disease: An Overview of Molecular Mechanisms and Therapeutic Targeting. Neuroscience 453:301–311. https://doi.org/10.1016/j.neuroscience.2020.11.003

22. Doens D, Valiente PA, Mfuh AM, A X T V, Tristan A, Carreno L et al (2017) Identification of Inhibitors of CD36-Amyloid Beta Binding as Potential Agents for Alzheimer's Disease. ACS Chem Neurosci 8(6):1232–1241. https://doi.org/10.1021/acschemneuro.6b00386
23. Jiang Y, Wang C, Zhou S (2020) Targeting tumor microenvironment in ovarian cancer: Premise and promise. Biochim Biophys Acta Rev Cancer 1873(2):188361. https://doi.org/10.1016/j.bbcan.2020.188361
24. Martincuks A, Li PC, Zhao Q, Zhang C, Li YJ, Yu H et al (2020) CD44 in Ovarian Cancer Progression and Therapy Resistance-A Critical Role for STAT3. Front Oncol 10:589601. https://doi.org/10.3389/fonc.2020.589601
25. Yan Y, Zuo X, Wei D (2015) Concise Review: Emerging Role of CD44 in Cancer Stem Cells: A Promising Biomarker and Therapeutic Target. Stem Cells Transl Med 4(9):1033–1043. https://doi.org/10.5966/sctm.2015-0048
26. Chen C, Zhao S, Karnad A, Freeman JW (2018) The biology and role of CD44 in cancer progression: therapeutic implications. J Hematol Oncol 11(1):64. https://doi.org/10.1186/s13045-018-0605-5
27. Zayas-Santiago A, Diaz-Garcia A, Nunez-Rodriguez R, Inyushin M (2020) Accumulation of amyloid beta in human glioblastomas. Clin Exp Immunol 202(3):325–334. https://doi.org/10.1111/cei.13493
28. Pavliukeviciene B, Zentelyte A, Jankunec M, Valiuliene G, Talaikis M, Navakauskiene R et al (2019) Amyloid beta oligomers inhibit growth of human cancer cells. PLoS One 14(9):e0221563. https://doi.org/10.1371/journal.pone.0221563
29. Reddy PH, Manczak M, Yin X, Grady MC, Mitchell A, Tonk S et al (2018) Protective Effects of Indian Spice Curcumin Against Amyloid-beta in Alzheimer's Disease. J Alzheimers Dis 61(3):843–866. https://doi.org/10.3233/JAD-170512
30. Bahlis NJ, Lazarus HM (2006) Multiple myeloma-associated AL amyloidosis: is a distinctive therapeutic approach warranted? Bone Marrow Transplant 38(1):7–15. https://doi.org/10.1038/sj.bmt.1705395
31. Soragni A, Janzen DM, Johnson LM, Lindgren AG, Thai-Quynh Nguyen A, Tiourin E et al (2016) A Designed Inhibitor of p53 Aggregation Rescues p53 Tumor Suppression in Ovarian Carcinomas. Cancer Cell 29(1):90–103. https://doi.org/10.1016/j.ccell.2015.12.002
32. Rangel LP, Ferretti GDS, Costa CL, Andrade S, Carvalho RS, Costa DCF et al (2019) p53 reactivation with induction of massive apoptosis-1 (PRIMA-1) inhibits amyloid aggregation of mutant p53 in cancer cells. J Biol Chem 294(10):3670–3682. https://doi.org/10.1074/jbc.RA118.004671
33. Moore KJ, Freeman MW (2006) Scavenger receptors in atherosclerosis: beyond lipid uptake. Arterioscler Thromb Vasc Biol 26(8):1702–1711. https://doi.org/10.1161/01.ATV.0000229218.97976.43
34. Jones RS, Minogue AM, Connor TJ, Lynch MA (2013) Amyloid-beta-induced astrocytic phagocytosis is mediated by CD36, CD47 and RAGE. J Neuroimmune Pharmacol 8(1):301–311. https://doi.org/10.1007/s11481-012-9427-3

35. Wu X, Chen S, Lu C (2020) Amyloid precursor protein promotes the migration and invasion of breast cancer cells by regulating the MAPK signaling pathway. Int J Mol Med 45(1):162–174. https://doi.org/10.3892/ijmm.2019.4404
36. Wang J, Li Y (2019) CD36 tango in cancer: signaling pathways and functions. Theranostics 9(17):4893–4908. https://doi.org/10.7150/thno.36037
37. Pandey P, Sliker B, Peters HL, Tuli A, Herskovitz J, Smits K et al (2016) Amyloid precursor protein and amyloid precursor-like protein 2 in cancer. Oncotarget 7(15):19430–19444. https://doi.org/10.18632/oncotarget.7103

Tables

Table 1. Significantly enriched signaling pathways and dysregulated genes.

Pathway	Pvalue	Up_genes	Down_genes
ECM-receptor interaction	0.001004	LAMA3, AMC2	THBS1, CD44, CD36, ITGB6, ITGB7
Protein digestion and absorption	0.005628	–	CPA3, CPB2, KCNE3, SLC7A9
Focal adhesion	0.010179	–	MYLK2, RASGRF1, EGF, VAV3, ITGB6, ITGB7, CCND2, THBS1, PGF
Notch signaling pathway	0.014670	–	LFNG, MFNG, DTX3, MAML2, NOTCH2NLC, ATXN1
MicroRNAs in cancer	0.016917	CDC25A	CDKN2A, CYP24A1, CCND2, THBS1, BMF, NOTCH2NLC
TGF-beta signaling pathway	0.016307	–	SMAD6, ID1, THBS1, ID2, ID3, ID4, GREM1

Figures

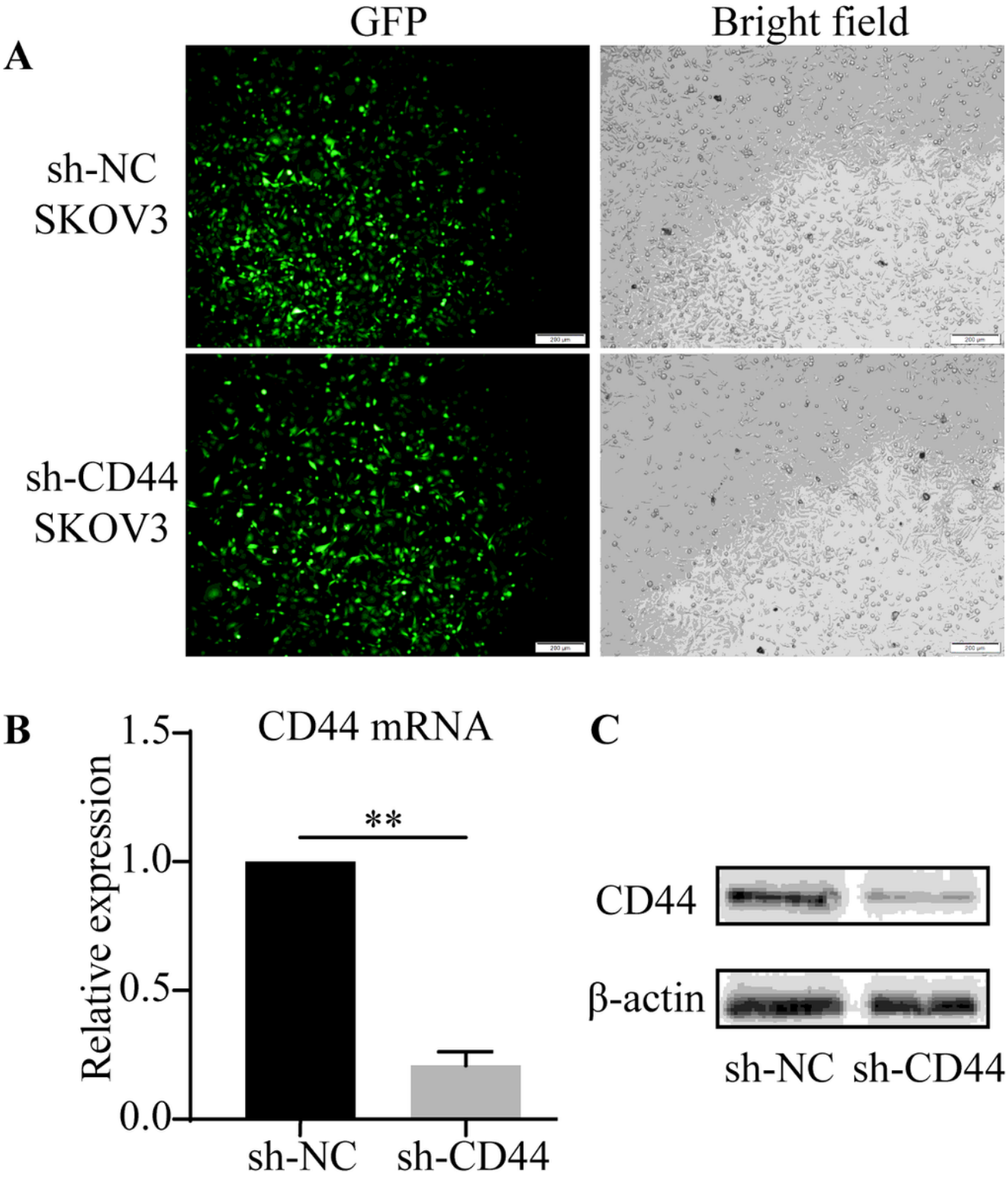

Figure 1

The generation of CD44-knockdownSKOV3 cells (A) SKOV3 cells were transfected by lentiviruses, and the bright field and GFP expression were observed in fluorescence microscopy. Scale bar, 200 μm. (B, C) WB (B) and RT-qPCR (C) analysis of the mRNA and protein expression of CD44 in control(sh-NC) and CD44 knockdown(sh-CD44) SKOV3 cells. Student's t-test, mean ± SD (n = 3), **P < 0.01.

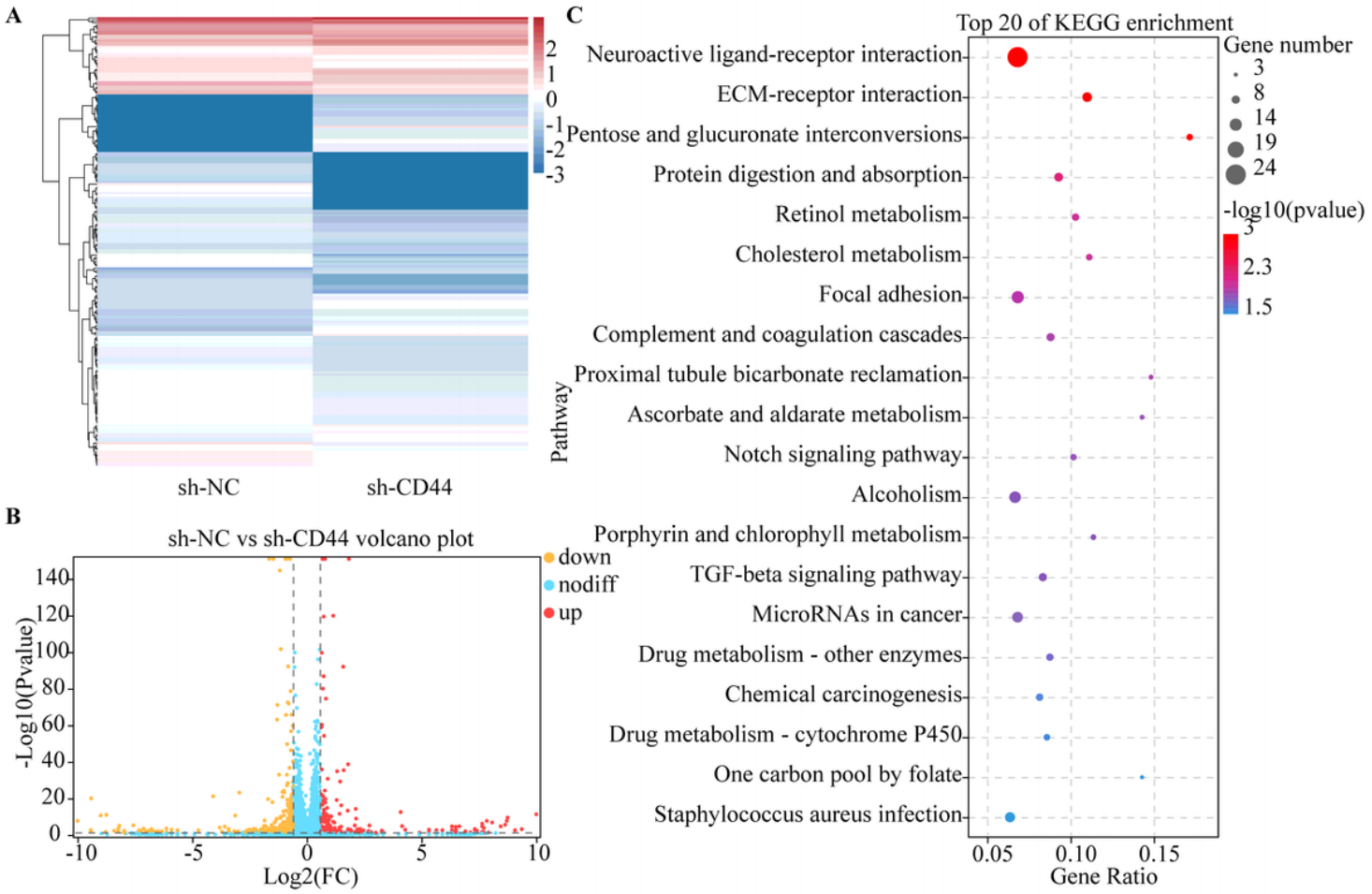

Figure 2

Identification of DEGs between sh-CD44 SKOV3 and sh-NC SKOV3 cells (A) Hot map of the differentially expressed genes between sh-CD44 SKOV3 cells and sh-NC SKOV3 cells. (B) The volcano plot of the differentially expressed genes. Each point represents a gene. FC >1.5. (C)Top 20 significantly enriched KEGG pathways of DEGs associated with CD44 regulation. Each point represents a KEGG signaling pathway. Gene ratio reflects the enrichment level of DEGs enriched in the pathway. Round size represents the gene count of each pathway, and the color represents the significance level.

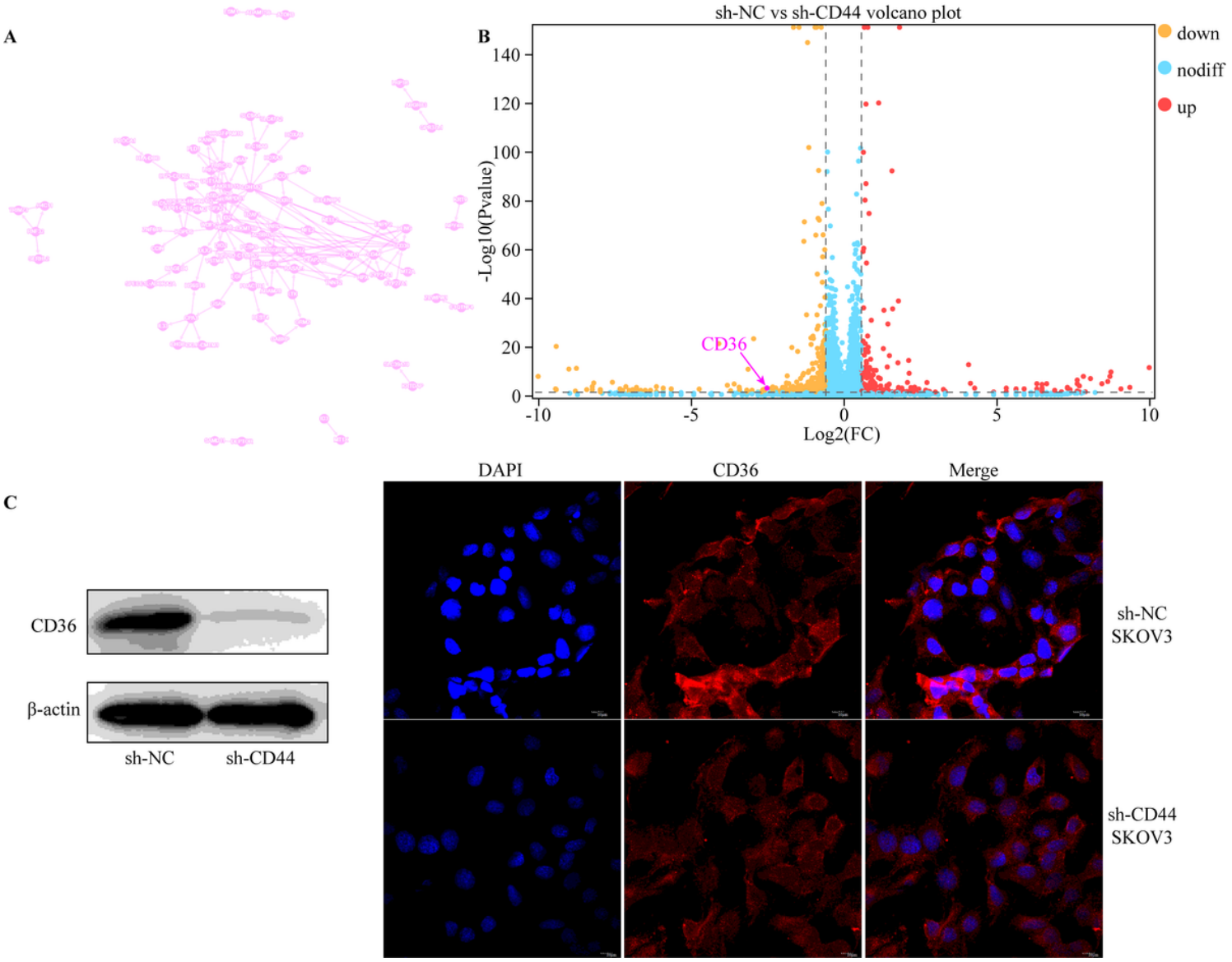

Figure 3

CD44 regulates the CD36 expression (A)The DEG proteins reconstructed the network of proteins interaction in response to CD44 knockdown in SKOV3 cells, such as CD36. (B) Hot map of the differentially expressed genes between sh-CD44 SKOV3 cells and sh-NC SKOV3 cells. CD36 was included in the downregulated genes. (C) WB analysis detects the protein levels of CD36. (D) Immunofluorescence staining showed the expression and location of CD36 in sh-CD44 SKOV3 cells or sh-NC SKOV3 cells. Scale bar, 10 μm.

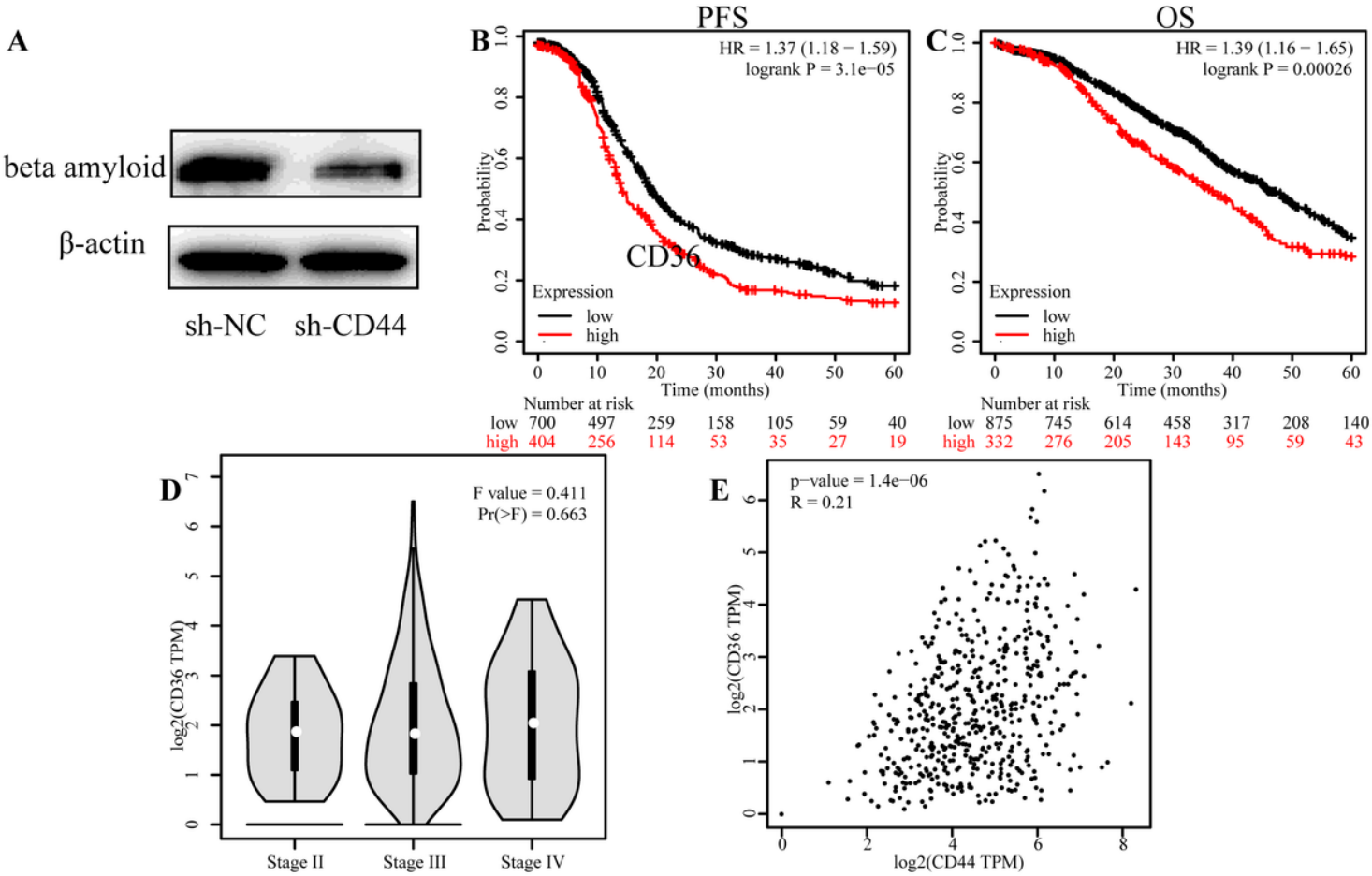

Figure 4

CD36 is related to ovarian cancer progression (A) WB analysis detects the protein levels of beta-amyloid. (B) Kaplan-Meier survival curves for PFS in ovarian cancer patients that CD36 expression was high or low. n=1,104. HR=1.37(1.18-1.59); log-rank P=3.1×10-5. (C) Kaplan-Meier survival curves for OS in ovarian cancer patients that CD36 expression was high or low.n=1,207. HR=1.39(1.16-1.65); log-rank P=0.00026. (D) CD36 mRNA levels in each stage of ovarian cancer. (E) The expression of CD44 was positively correlated with CD36 in the public database. Pearson's correlation coefficient, P=1.4×10-6, R=0.21.

Supplementary Files

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- OnlinefigureS1.png