Ghrelin Promote the Ovarian Cancer Cell Autophagy by LncRNA linc00598

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Research Article

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Abstract

Objective

To explore the autophagy effect of ghrelin on the ovarian cancer cell line SK-OV-3. And the lncRNA which regulate the ghrelin effect SK-OV-3 autophagy was showed.

Methods

the expression of ghrelin in the ovarian cancer tissues was analyzed according GEPIA database and HPA database. The CCK-8 was used to detect the the optimal concentration of ghrelin effect on the SK-OV-3. The influence on the SK-OV-3 cell autophagy by ghrelin was showed by detecting the expression of Beclin-1, LC3Ⅱand LC3Ⅰusing western blot. Linc00598 selected as the effecting the SK-OV-3 cells autophagy by ghrelin using RNA-Seq. And the Linc00598 which was silenced or overexressed promote the SK-OV-3 cells autophagy treated by ghrelin though western blot.

Results

Ghrelin was expressed low in the ovarian cancer tissues. Ghrelin concentratio of 600 ng/ml was the optimal concentration o and 24 h was the optimal time. Ghrelin can promote the SK-OV-3 cell autophagy. Ghrelin mainly through linc00598 to promote the SK-OV-3 cells autophagy. When the linc00598 silenced, ghrelin promote SK-OV-3 cells autophagy was inhibited. And When the linc00598 overexpressed, ghrelin promote SK-OV-3 cells autophagy was enhanced.

Conclusions

Ghrelin promote SK-OV-3 cells autophagy. Additionally, we proved that ghrelin regulated the progression of SK-OV-3 cells autophagy by linc00598/ Beclin1 axis.

Introduction

Ovarian cancer is the common gynecologic cancer death in worldwide. It threat to women's health(Wu, Gao et al. 2021; Xie, Wang et al. 2021). And a large number of women was died from ovarian cancer each year. The overall 5-year survival rate of ovarian cancer is about 47%. However, in the early stage, the typical symptoms was lacked in the patients of ovarian cancer(Wang, Chen et al. 2021; Wei, Lv et al. 2021). More than 2/3 cancer cases was diagnosed at the advanced stage, and the 5-year survival rate was less than 25%. Hence, the diagnosis and treatment on ovarian cancer gave a big challenge(Wang and Zheng 2020; Venkatappa and Sarkar 2021; Wang, Yan et al. 2021; Wei, Feng et al. 2021). To find the biomarkers and therapeutic targets were the useful way to improve the diagnosis and prognosis in the early stage ovarian cancer(Wang, Peng et al. 2018; Sun, Hu et al. 2020; Takagi, Yagi et al. 2021; van der Wel, Uijterwaal et al. 2021).
Ghrelin is one of the endogenous ligands of growth hormone secretagogue receptor, which can promote secretion of growth hormone and is proven to be with the orexigenic and adipogenic effects (Leng, Zhao et al. 2021). Moreover, the positive effect of Ghrelin on metabolism of bone has been observed through regulation of by proliferation ovarian cancer cells (Lin and Hsiao 2017; Khatib, Shankar et al. 2018; Asadi, Farahani et al. 2021).

Long non coding RNA (IncRNA) is about 200nt, and cannot translated into proteins (Meng, Zhou et al. 2020; Wang, Ding et al. 2021; Yang and Dong 2021). However, IncRNA can regulate many cell physiological process, such as cell proliferation, autophagy, apoptosis. IncRNA is also the potential prognostic marker for many tumors (Chen, Xie et al. 2021; Guo, Du et al. 2021).

Cell autophagy can clean out the damaged organelles, proteins in normal (Sun, Hu et al. 2020). However in the apoptotic cells, autophagy can prevent the necrosis, and local inflammation (Meng, Zhou et al. 2020; Cai, An et al. 2021). Hence, in this study, the autophagy effect of ghrelin on the ovarian cancer cell line SK-OV-3 was explored. And the IncRNA which regulate the ghrelin effect SK-OV-3 autophagy was showed.

**Materials And Methods**

**Cell culture**

The ovarian cancer cell line SK-OV-3 () containing 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 100 U/mL penicillin and 0.1 mg/mL streptomycin, with 5% CO2.

**Cell counting kit-8 (CCK-8) assay**

Cell proliferation after ghrelin treatment was assessed using the CCK-8 (K1018, Apexbio, Houston, TX, USA) kit. Cells (1 × 104 cells per well) were plated in the 96-well plates (100 µL/well) and 10 µL of CCK-8 solution was added at each time point (1d, 2d, 3d, 4d, 5d, 6d, and 7d) for 2 h at 37°C. The absorbance values at 450 nm were then measured using an enzyme marker.

**RNA extraction and sequencing**

Sequencing libraries were generated using the TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA, USA). Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in an Illumina proprietary fragmentation buffer. First strand cDNA was synthesize dusing random oligonucleotides and SuperScript II. Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities and the enzymes were removed. After adenylation of the 3’ ends of the DNA fragments, Illumina PE adapter oligonucleotides were ligated to prepare for hybridization. The library fragments were purified using the AMPure XP system (Beckman Coulter, Beverly, CA, USA). DNA fragments with ligated adaptor molecules on both ends were selectively enriched using Illumina PCR Primer Cocktail in a 15 cycle PCR
reaction. Products were purified (AMPure XP system) and quantified using the Agilent high sensitivity DNA assay on a Bioanalyzer 2100 system (Agilent). The sequencing library was then sequenced on a HiSeq platform (Illumina) by Seqhealth Technology Co., Ltd., Wuhan, China. Differentially expressed genes were then identified by applying a FDR cutoff of 0.05.

**Gene function annotation and pathway analysis**

Identification of enriched KEGG pathways in the upregulated and downregulated gene lists was performed with DAVID (Database for Annotation, Visualization, and Integrated Discovery) v6.8. Pathway analysis was performed using Ingenuity Pathway Analysis (IPA), version to infer the functional roles and relationships of the differentially expressed genes based on the log2 fold-change value of each gene.

**Cell transfection**

The mimic of miR-378b as well as negative control (NC mimic) were purchased from Invitrogen (Carlsbad, CA, USA). The full length of SNHG10 was ligated in pcDNA3.1 vector (pcDNA3.1SNHG10) was purchased from GenePharma (Shanghai, China) with empty plasmid as negative control (pcDNA3.1). Cell transfection was conducted using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). After 48 h, cells were harvested for following experiments.

**Real-Time qPCR analysis**

The total RNA was extracted from the SK-OV-3 cells by using the commercial TRIzol reagent (Invitrogen, USA) following the manufacturer’s instruction. The quality of the total RNA was determined by agarose electrophoresis, and the RNA was reversely transcribed into complementary DNA (cDNA) by using the TIANScript RT kit (Tiangen Biotech, China). Then, the SYBR Premix Ex Taq TM II Kit (Takara, Japan) was used to determine relative gene expression levels and enrichment. The up-primer sequences for linc00598 is CCTCCCCTACTATCAACATCCC and down primer is TGCCAAGAACGAGCCCTA. The expression levels were normalized by GAPDH (up primer CAATGCCTCCTGCACCACCAACTGC and down primer GCAGTTGGTGGTGCAGGAGGCATTG )

**Western blot**

Total tissues or cellular proteins were extracted using high performance radio immunoprecipitation analysis lysis buffer (C0481, Sigma-Aldrich) containing 1% protease inhibitor and 1% phosphatase inhibitor (Beyotime). The protein concentration of each sample was determined by a bicinchoninic acid kit (23227, Thermo Fisher Scientific). The proteins were separated by polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA) which were blocked with 5% bovine serum albumin (BSA) at room temperature for 1 h. Primary antibodies including rabbit anti-Beclin-1 (1:1000, 3495, CST) rabbit anti-LC3 and (1:1000, 4108, CST), and rabbit anti-GAPDH (1:3000, a5174, CST) were incubated with membranes overnight at 4°C. On the second day, the membranes were washed with TBST, and incubated with horseradish peroxide (HRP)-labeled goat anti-
rabbit immunoglobulin G (IgG; 1:20000, ab205718, Abcam) dilutions at room temperature for 1.5 h. After incubation, the membranes were developed by developing liquid (NCI4106, Pierce, Rockford, IL, USA).

**Statistical analysis**

Statistical analyses of data were performed using Graphpad Prism 8.0 (GraphPad Software, San Diego, CA, USA). The measurement data were expressed as mean ± standard deviation. Independent sample t test were used in comparisons between groups, and one-way analysis of variance (ANOVA) was used for comparison among multiple groups, followed by Tukey's post hoc tests. Comparison of data between groups at different time points was performed using two-way ANOVA. A p < 0.05 indicated statistically significant.

**Results**

**Ghrelin down expression in the ovarian cancer tissues**

To obtain the ghrelin expression in the ovarian cancer tissues, the GEPIA database was used to analyse. The ghrelin expression was lower in the ovarian cancer tissues than the normal tissues (Fig. 1A). And in HPA database ghrelin was lowly expressed in ovarian cancer tissues compared with normal tissues (P<0.05, Fig. 1B). Hence, the ghrelin was expressed low in the ovarian cancer tissues.

**Ghrelin promoted the cell autophagy and inhibited the cell viability in SK-OV-3 cells**

To obtain the optimal concentration of ghrelin effect on the SK-OV-3, the SK-OV-3 cells were treated with ghrelin with the concentrations of 400, 500, 600, 700 and 800 ng/ml. Compared with the blank control group (control), the cell survival rate of 400, 500, 600, 700 and 800 ng/ml group decreased at 24 h, 48 h and 72 h (P < 0.01). When SK-OV-3 cells were treated with 600 ng/ml ghrelin for 24 h, 48 h and 72 h, the cell survival rates were around 50%(Fig. 2A). Hence, the ghrelin concentratio of 600 ng/ml was the optimal concentration o and 24 h was the optimal time.

The influence on the SK-OV-3 cell autophagy by ghrelin was showed by detecting the expression of Beclin-1, LC3δ and LC3δ(Fig. 2B). The expression of Beclin-1and LC3δin SK-OV-3 cell treated with the 600 ng/ml ghrelin 24h was higher than without ghrelin treated(Fig. 2C&D). To explore the function of ghrelin on the SK-OV-3 cell autophagy further, the D-Lys3-GHRP6, a ghrelin receptor antagonist, was used. The expression of Beclin-1and LC3δin SK-OV-3 cell treated with ghrelin was higher than ghrelin+ D-Lys3-GHRP6 treatment (Fig. 2E-G).

**Linc00598 selected as the effecting the SK-OV-3 cells autophagy by ghrelin using RNA-Seq**

To found the LncRNAs which effected the SK-OV-3 cells autophagy by ghrelin, the SK-OV-3 cells treated with ghrelin was analyzed by RNA-Seq. The differential expression of LncRNA was analyzed by DESeq. The conditions for screening differentially expressed genes were as follows: the multiple of expression difference | log2foldchange | > 1, P < 0.05. Compared with the control group, 236 LncRNAs were
differentially expressed (up-regulated 130 and down regulated 106), in the ghrelin treatment group. The volcanic map shows the distribution of LncRNAs, the difference of LncRNAs expression fold and significance results (Fig. 3A). In Fig. 3A, the red dot represents the differentially up-regulated LncRNAs, the blue dot represents the differentially down-regulated LncRNAs, and the gray dot represents the non significant differentially expressed LncRNAs.

The go enrichment analysis results of differentially expressed LncRNAs are classified, according to molecular function MF, biological process BP and cell component CC. The top 10 go term items with the smallest p-value, i.e. the most significant enrichment, are selected for display in each go classification (Fig. 2B). In the figure 2B, the orange histogram represents the top 10 cell components with the most significant enrichment, the green histogram represents the top 10 molecular functions with the most significant enrichment, and the blue histogram represents the top 10 biological processes with the most significant enrichment. According to the go enrichment results, the enrichment degree is measured by rich factor, FDR value and the number of LncRNA enriched on the GO term. The larger the rich factor, the greater the degree of enrichment. The general value range of FDR is 0-1. The closer is closed to zero, the more significant the enrichment. The first 20 GO term entries with the lowest FDR value, are selected for display (Fig. 2C). Go enrichment analysis showed that these differentially expressed LncRNAs were mainly related to arginine and lysine transmembrane transport, oxidative stress response and developmental process.

The top 20 pathways with the lowest p-value value are displayed, according to the KEGG enrichment analysis the differentially expressed LncRNAs (Fig. 3D). The path in the figure mainly involves four aspects: environmental information processing (orange part), human diseases (green part), metabolism (blue part) and organic systems (purple part). According to the KEGG enrichment results, the enrichment degree, the top 20 KEGG pathways with the lowest FDR value are selected. (Fig. 3E). KEGG enrichment analysis showed that these differentially expressed LncRNAs were mainly enriched in cytokine receptor signaling pathway, glucagon and insulin signaling pathway and cancer-related signaling pathway.

According to the prediction of target genes and the enrichment analysis results of go and KEGG pathway, the linc00598 was selected as the potential relationship with autophagy, and the linc00598 was differential up-regulation of fold change > 2 times. The expression of linc00598 in SK-OV-3 cells was detected by qPCR. The expression of linc00598 in the ghrelin treated group was higher that blank group.

**Linc00598 promote the SK-OV-3 cells autophagy treated by ghrelin**

To show the function of linc00598 on the SK-OV-3 cells autophagy, the linc00598 was silenced and overexpressed (Fig. 4A&B). And the expression of Beclin1 and LC3 was in the linc00598 silence group (si-linc00598) was lower than the control group and si-NC group (the blank plasmid, Fig. 4C,D,F ). However, in the linc00598 overexpressed group (h-linc00598), the expression of Beclin1 and LC3 was high (Fig. 4C,E,G). The effect of linc00598 silenced or overexpressed on the SK-OV-3 cells autophagy treated by ghrelin was also explored. The expression of Beclin1 and LC3 in the Ghrelin + Si-linc00598 group was
lower than the ghrelin group (Fig. 4A,B,D). And the expression of Beclin1 and LC3 in the Ghrelin + h-linc00598 was higher than ghrelin group (Fig. 4A,C,E).

**Discussion**

Ovarian cancer has a high mortality rate (Rogalska, Gajek et al. 2019; Shao, Liu et al. 2019; Quan, Xiong et al. 2021). Patients have chemotherapy resistance highly and therapeutic effect poorly. (Badi, Khaleel et al. 2020; Chen, Sun et al. 2020) Hence, to show the molecular mechanism is very urgently. Ghrelin as a growth hormone secretagogue receptor has many function such as promote the oocyte (Bai, Zhao et al. 2012; Sirini, Anchordoquy et al. 2019), anti-cancer and the osteocyte differentiation (Ye and Jiang 2015; Ye, Wang et al. 2018; Liu, Pan et al. 2020; Ye, Yang et al. 2020). In study, we found ghrelin can promote the SK-OV-3 cells autophagy. In the GEPIA database and HPA database, the ghrelin expression was lower in the ovarian cancer tissues than the normal tissues. Hence, the ghrelin was used to treat the ovarian cancer cell line SK-OV-3. And the ghrelin can promote the SK-OV-3 cell autophagy.

Many study has show the LncRNA can influence the development of ovarian cancer (Meng, Zhou et al. 2020). Particular, inhibiting growth, promoting autophagy were the focus of anti cancer research (Gu, Li et al. 2020; Guo, Du et al. 2021; Wang, Ding et al. 2021). In this study, the RNA was sequenced in the SK-OV-3 cells treated by ghrelin. And the linc00598 was selected as the closely related to cell autophagy effected by ghrelin. Linc00598 can regulate the transcription of specific target genes, including those for cell cycle regulators and autophagy (Jeong, Chae et al. 2016). To identify linc00598 can regulate the SK-OV-3 cells autophagy, the linc00598 was silenced and overexpressed. And the linc00598 can promote the SK-OV-3 cells autophagy.

Ghrelin and linc00598 can promote the SK-OV-3 cells autophagy. The linc00598 was silenced and overexpressed in the SK-OV-3 cells. Then the ghrelin was used to effect the SK-OV-3 cells. We found ghrelin mainly through linc00598 to promote the SK-OV-3 cells autophagy. When the linc00598 silenced, ghrelin promote SK-OV-3 cells autophagy was inhibited. And When the linc00598 overexpressed, ghrelin promote SK-OV-3 cells autophagy was enhanced.

**Conclusions**

In conclusion, ghrelin promote SK-OV-3 cells autophagy. Additionally, we proved that ghrelin regulated the progression of SK-OV-3 cells autophagy by linc00598/ Beclin1 axis.

**Declarations**

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**Authors’ contributions**
Ruixia Bai1a, Wanying Song and Pengwei Zhao wrote this article. Xiumei Wang, Pengwei Zhao designed, organized and reviewed this article. All authors have read and agreed to the published version of the manuscript.

**Availability of data and materials**

All data generated or analyzes during this study are included in this published article.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Declaration of Interest Statement**

The authors declare that they have no competing interests.

**References**

1. Asadi, A., H. Farahani, et al. (2021). "Circulating Ghrelin Levels and Susceptibility to Colorectal Cancer." *Arq Gastroenterol* **58**(3): 316-321.

2. Badi, R. M., E. F. Khaleel, et al. (2020). "Exendin-4 Induces Cytotoxic Autophagy in Two Ovarian Cancer Cell Lines through Inhibition of Mtorc1 Mediated by Activation of AMPK and Suppression of Akt." *Folia Biol (Praha)* **66**(5-6): 186-203.

3. Bai, R., P. Zhao, et al. (2012). "Ghrelin promotion of oocyte maturation via ERK1/2 pathway in ovis aries." *Cell Mol Biol (Noisy-le-grand)* **58** Suppl: OL1797-1802.

4. Cai, Y., B. An, et al. (2021). "MicroRNA miR-30a inhibits cisplatin resistance in ovarian cancer cells through autophagy." *Bioengineered*.

5. Chen, Q., J. Xie, et al. (2021). "Long non-coding RNA NRSN2-AS1 facilitates tumorigenesis and progression of ovarian cancer via miR-744-5p/PRKX axis." *Biol Reprod*.

6. Chen, X., Y. Sun, et al. (2020). "Prognostic significance of autophagy-related genes Beclin1 and LC3 in ovarian cancer: a meta-analysis." *J Int Med Res* **48**(11): 300060520968299.

7. Gu, L., Q. Li, et al. (2020). "Long Noncoding RNA TUG1 Promotes Autophagy-Associated Paclitaxel Resistance by Sponging miR-29b-3p in Ovarian Cancer Cells." *Onco Targets Ther* **13**: 2007-2019.

8. Guo, F., J. Du, et al. (2021). "IncRNA OR3A4 Promotes the Proliferation and Metastasis of Ovarian Cancer Through KLF6 Pathway." *Front Pharmacol* **12**: 727876.

9. Jeong, O. S., Y. C. Chae, et al. (2016). "Long noncoding RNA linc00598 regulates CCND2 transcription and modulates the G1 checkpoint." *Sci Rep* **6**: 32172.
10. Khatib, M. N., A. H. Shankar, et al. (2018). "Ghrelin for the management of cachexia associated with cancer." Cochrane Database Syst Rev 2: CD012229.

11. Leng, Y., C. Zhao, et al. (2021). "Ghrelin enhances cisplatin sensitivity in HO-8910 PM human ovarian cancer cells." J Ovarian Res 14(1): 162.

12. Lin, T. C. and M. Hsiao (2017). "Ghrelin and cancer progression." Biochim Biophys Acta Rev Cancer 1868(1): 51-57.

13. Liu, G. B., Y. M. Pan, et al. (2020). "Ghrelin promotes neural differentiation of adipose tissue-derived mesenchymal stem cell via AKT/mTOR and beta-catenin signaling pathways." Kaohsiung J Med Sci 36(6): 405-416.

14. Meng, C., J. Q. Zhou, et al. (2020). "Autophagy-related long non-coding RNA signature for ovarian cancer." J Int Med Res 48(11): 300060520970761.

15. Quan, Q., X. Xiong, et al. (2021). "Identification of Immune-Related Key Genes in Ovarian Cancer Based on WGCNA." Front Genet 12: 760225.

16. Rogalska, A., A. Gajek, et al. (2019). "Suppression of autophagy enhances preferential toxicity of epothilone A and epothilone B in ovarian cancer cells." Phytomedicine 61: 152847.

17. Shao, Y., X. Liu, et al. (2019). "MicroRNA-1251-5p Promotes Carcinogenesis and Autophagy via Targeting the Tumor Suppressor TBCC in Ovarian Cancer Cells." Mol Ther 27(9): 1653-1664.

18. Sirini, M. A., J. P. Anchordoquy, et al. (2019). "Expression of Ghrelin and Its Receptor mRNA in Bovine Oocyte and Cumulus Cells." Int J Fertil Steril 12(4): 335-338.

19. Sun, T., Y. Hu, et al. (2020). "SRT2183 impairs ovarian cancer by facilitating autophagy." Aging (Albany NY) 12(23): 24208-24218.

20. Takagi, K., T. Yagi, et al. (2021). "Splenic and Peritoneal Metastases with Para-aortic and Virchow Lymph Node Metastases: Late Recurrence of Ovarian Cancer 30 Years after Initial Treatment." JMA J 4(4): 428-431.

21. van der Wel, A. W. T., M. H. Uijterwaal, et al. (2021). "[Chlamydia mimics suspected ovarian cancer in a 21-year-old patient]." Ned Tijdschr Geneeskd 165.

22. Venkatappa, S. and A. Sarkar (2021). "Comment on: hyperthermic intraperitoneal chemotherapy as consolidation treatment of advanced stage ovarian cancer." Obstet Gynecol Sci.

23. Wang, H., Y. Peng, et al. (2018). "Effect of autophagy on the resveratrol-induced apoptosis of ovarian cancer SKOV3 cells." J Cell Biochem.

24. Wang, K. and J. Zheng (2020). "Knockdown of BAG3 synergizes with olaparib to kill ovarian cancer cells via repressing autophagy." J Investig Med.

25. Wang, P., J. Chen, et al. (2021). "Author Correction: Propofol inhibits invasion and enhances paclitaxel-induced apoptosis in ovarian cancer cells through the suppression of the transcription factor slug." Eur Rev Med Pharmacol Sci 25(21): 6446.

26. Wang, Y., M. Ding, et al. (2021). "lncRNA SNHG15 Promotes Ovarian Cancer Progression through Regulated CDK6 via Sponging miR-370-3p." Biomed Res Int 2021: 9394563.
27. Wang, Y., C. Yan, et al. (2021). "miR-874-3p mitigates cisplatin resistance through modulating NF-kappaB/inhibitor of apoptosis protein signaling pathway in epithelial ovarian cancer cells." Mol Cell Biochem.

28. Wei, W., Z. Feng, et al. (2021). "Design, synthesis and biological evaluation of 7-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)oxy)-2,3-dihydro-1H-inden-1-one derivatives as potent FAK inhibitors for the treatment of ovarian cancer." Eur J Med Chem: 113978.

29. Wei, X., H. Lv, et al. (2021). "CircRNA PLOD2 enhances ovarian cancer propagation by controlling miR-378." Saudi J Biol Sci 28(11): 6260-6265.

30. Wu, W., C. Gao, et al. (2021). "Comprehensive analysis of competitive endogenous RNAs networks reveals potential prognostic biomarkers associated with epithelial ovarian cancer." Oncol Lett 22(6): 843.

31. Xie, H., W. Wang, et al. (2021). "Targeting DNA Repair Response Promotes Immunotherapy in Ovarian Cancer: Rationale and Clinical Application." Front Immunol 12: 661115.

32. Yang, Q. and Y. J. Dong (2021). "LncRNA SNHG20 promotes migration and invasion of ovarian cancer via modulating the microRNA-148a/ROCK1 axis." J Ovarian Res 14(1): 168.

33. Ye, N. and D. Jiang (2015). "Ghrelin accelerates the growth and osteogenic differentiation of rabbit mesenchymal stem cells through the ERK1/2 pathway." BMC Biotechnol 15: 51.

34. Ye, N., L. Wang, et al. (2018). "Ghrelin accelerates the cartilagic differentiation of rabbit mesenchymal stem cells through the ERK1/2 pathway." Cytotechnology 70(1): 415-421.

35. Ye, N., Y. Yang, et al. (2020). "Ghrelin promotes the osteogenic differentiation of rMSCs via miR-206 and the ERK1/2 pathway." Cytotechnology 72(5): 707-713.

Figures
Figure 1

Expression of ghrelin in different ovarian cancer tissues. A. The expression levels of ghrelin in different cancer tissues were provided by GEPIA database. B. The expression levels of ghrelin in ovarian cancer tissues were provided by HPA database. * $P < 0.05$ was regarded as statistical significance.
Figure 2

Influence of ghrelin on SK-OV-3 Cell viability and the autophagy pathway. A. Effect of ghrelin with 400, 500, 600, 700 and 800 ng / mL on SK-OV-3 Cell viability at 24h, 48h and 72h. B-D Western Blot analysis was conducted to examine the protein levels of Beclin-1, LC3 and LC3 in SK-OV-3 cells after ghrelin added. E-G Western Blot analysis was conducted to examine the protein levels of Beclin-1, LC3 and LC3 in SK-OV-3 cells after ghrelin and D-Lys3-GHRP6 added. Single experiment had 3 repetitions, and * P < 0.05 was regarded as statistical significance.
Figure 3

LINC00598 selected on the SK-OV-3 cells treated by ghrelin using RNA-Seq. A. Volcano Plot of differentially expressed lncRNA. the red dot represents the differentially up-regulated LncRNAs, the blue dot represents the differentially down-regulated LncRNAs, and the gray dot represents the non significant differentially expressed LncRNAs. B&C. Histogram of GO enrichment analysis. the orange histogram represents the top 10 cell components with the most significant enrichment, the green histogram represents the top 10 molecular functions with the most significant enrichment, and the blue histogram represents the top 10 biological processes with the most significant enrichment in fig 3B. D&E. Bubble chart of KEGG enrichment analysis. environmental information processing (orange part), human diseases (green part), metabolism (blue part) and organic systems (purple part) in fig 3D. F. The effect of Ghrelin on the expression of LINC00598 was detect by qRT-PCR. Single experiment had 3 repetitions, and * $P < 0.05$ was regarded as statistical significance.
Figure 4

Effect of LINC00598 on the SK-OV-3 cells autophagy pathway. A&B. the expression of LINC00598 in the SK-OV-3 cells after the LINC00598 silenced or overexpressed. C-F. Western Blot analysis was conducted to examine the protein levels of Beclin-1, LC3Ⅰand LC3Ⅱin SK-OV-3 cells after the LINC00598 silenced or overexpressed. Single experiment had 3 repetitions, and * $P < 0.05$ was regarded as statistical significance.
Figure 5

Influence of ghrelin on autophagy pathway of SK-OV-3 cells after the LINC00598 silenced or overexpressed. A. Western Blot analysis was conducted to examine the protein levels of Beclin-1, LC3Ⅰ, and LC3Ⅱ. B-E. Relative expression of Beclin-1, LC3Ⅰ, and LC3Ⅱ. Single experiment had 3 repetitions, and * $P < 0.05$ was regarded as statistical significance.