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Autophagy-related long non-coding RNA signature for ovarian cancer

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Abstract

Objective: Ovarian cancer (OC) affects nearly 22,000 women annually in the United States and ranks fifth in cancer deaths, largely because of being diagnosed at an advanced stage. Autophagy is the cellular process of self-degrading damaged or degenerate proteins and organelles. Long non-coding RNAs (lncRNAs) are a group of RNA molecules whose transcripts are greater than 200 nt but are not translated into proteins. However, just a small number of autophagy-related lncRNAs have been explored in depth.

Methods: We used RNA sequencing data from The Cancer Genome Atlas (TCGA) and autophagy datasets to identify dysfunctional autophagy-related lncRNAs and provide potential useful biomarkers for OC diagnosis and prognosis.

Results: Seventeen differentially expressed lncRNAs (AC010186.3, AC006001.2, LBX2-AS1, SNHG17, AC011445.1, AC083880.1, MIR193BHG, AC025259.3, HCG14, AC007114.1, AC108673.2, USP30-AS1, AC010336.5, LINC01132, AC006333.2, LINC00665 and AC027348.1) were selected as independent prognostic factors for OC patients. Functional annotation of the data was performed through gene set enrichment analysis (GSEA). The results suggested that the high-risk group was mainly enriched in specific tumor-related and metabolism pathways.

Conclusion: Based on the online databases, we identified novel autophagy-related lncRNAs for the prognosis of ovarian cancer.

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Ovarian cancer, autophagy, long non-coding RNAs, The Cancer Genome Atlas, prognosis, biomarker

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Introduction

Ovarian cancer (OC) affects nearly 22,000 women annually in the United States and ranks fifth in cancer deaths, largely because of being diagnosed at an advanced stage.[1] Epithelial ovarian cancer (EOC) constitutes about 90% of all ovarian cancer cases.[2] Despite recent advances in cyto-reductive surgery and chemotherapy, the 5-year survival rate of EOC patients is only 30% and the prognosis of EOC remains poor. If effective early detection is possible, the survival rate can usually be increased to 70%.[3] The leading cause of death in these patients was extensive peritoneal metastasis, but the specific molecular mechanism controlling this remains unclear.[4] Several aspects affect the progression of the disease, including important epigenetic and genetic factors. Therefore, finding effective biomarkers is necessary for the diagnosis and treatment of OC patients.

Autophagy is the process of self-degradation of damaged or degraded proteins and organelles. Autophagy is thought to be related to malignant tumors,[5] neurodegenerative diseases,[6] immune diseases,[7] infection,[8] aging[9] and other diseases. Especially in tumors, autophagy plays different roles. Under physiological conditions, autophagy can prevent the accumulation of damaged substances and inhibit tumorigenesis. However, once a tumor is formed, autophagy can promote tumor growth, invasion and metastasis in some cases.[10,11] There is increasing evidence that autophagy-mediated cell survival plays a key role in the etiology and progression of OC.

Long non-coding RNAs (lncRNAs) are a group of RNA molecules whose transcripts are greater than 200 nt but are not translated into proteins. LncRNAs can impact the expression levels of genes, and also participate in various biological regulatory processes. Therefore, they are closely related to the occurrence, development and metastasis of tumors.[12,13] In addition, lncRNAs have multiple functions involving chromatin organization and post-transcriptional regulation, and occupy about 4% to 9% of the human genome.[14,15] A recent study found that lncRNA taurine up-regulated 1 (TUG1), via targeting miR-29b-3p, induces autophagy and consequently leads to paclitaxel resistance in OC.[16] Similarly, lncRNA RP11-135L22 was expressed at low levels in OC, related to TMN stage and tumor size, and also inhibited cisplatin-induced autophagy.[17] However, just a small number of autophagy-related lncRNAs have been explored in depth. Additionally, there is no comprehensive way to systematically evaluate autophagy-related lncRNAs and predict overall survival (OS) in OC patients. Thus, we used RNA sequencing (RNA-seq) data from The Cancer Genome Atlas (TCGA) and autophagy datasets to display dysfunctional lncRNA microenvironments, understand their potential molecular function and clinical significance, and provide potential useful biomarkers for OC prognosis. In this

report, we have identified prognostic autophagy-related lncRNAs and built a prognostic prediction model for OC patients.

Materials and methods

Data gathering

Gene expression information was obtained and downloaded from Genotype Tissue Expression (GTEx) projects and TCGA via the University of California Santa Cruz (UCSC) database.[18] Ensembl BioMart was applied to gain the mapping between each gene symbol and Ensembl transcript ID. The collected clinical pathological data included gender, age, stage, TMN classification, survival status and number of days of survival. The list of autophagy-related genes obtained from the Human Autophagy Database[19] (HADb), a web-based resource, provided a comprehensive and up-to-date list of human genes and proteins related to autophagy. HADb included 232 autophagy-related genes. Pearson correlation was used to calculate the correlations between the autophagy-related genes and lncRNAs. A lncRNA was considered autophagy-related if it had a correlation coefficient $|R^2|>0.3$ and $P<0.001$.

KEGG and GO enrichment analyses

The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a resource for exploring high-level gene functions and associating genomic data from large-scale molecular datasets. Gene ontology (GO) functional analysis (biological processes (BP), cellular components (CC) and molecular functions (MF)) is a powerful bioinformatics tool to analyze biological processes and annotate genes. To explore the function of the identified 232 autophagy-related genes, biological analyses were carried out using GO and KEGG pathway analysis via the R language ggplot2 package to generate figures.

Building the prognostic autophagy-related lncRNA signature

After normalizing the mRNA expression profiles through edgerR (R package), false discovery rate (FDR)<0.05 and $|\log_2 FC|\geq 1$ were determined as differentially expressed autophagy-related genes. Univariate and multivariate Cox regression analyses were used to assess the prognostic worth of autophagy-related lncRNAs in the training cohort. LncRNA levels that were significant in both univariate and multivariate Cox regression analysis were chosen as autophagy-related lncRNAs. The prognostic signature as a risk score = (Coefficient $\text{lncRNA}_1 \times$ expression of lncRNA_1) + (Coefficient $\text{lncRNA}_2 \times$ expression of lncRNA_2) + $\cdots$ + (Coefficient $\text{lncRNA}_n \times$ expression lncRNA_n). A receiver operating characteristic (ROC) curve was plotted to predict the accuracy of the prognostic signatures for OC patients.

Enrichment analysis of prognostic autophagy-related lncRNAs

Cytoscape was used to visualize the interaction network between lncRNAs and genes. Gene set enrichment analysis (GSEA) was applied to elucidate gene expression data. GSEA can determine whether a predefined set of genes can show significant differences in consistency between two biological states.[20] We confirmed if the genes that were differentially expressed among the two groups were enriched in autophagy. A two-sided P-value <0.05 was deemed statistically significant.

Results

KEGG and GO enrichment analyses

We first performed functional enrichment analysis of these 232 autophagy-related genes. GO analysis results suggested that changes in biological processes were significantly enriched in regulation of autophagy, cellular response to protein, macroautophagy and cellular response to external stimulus. Changes in cellular components were mainly enriched in the membrane region, membrane raft and membrane microdomain. Changes in molecular functions were mainly enriched in ubiquitin protein ligase binding, protein binding and kinase activity. KEGG pathway analysis suggested enrichment mainly in carcinoma, virus infection, NOD-like receptor/FoxO/PI3K-Akt signaling pathway, and platinum drug resistance. These results are shown in Figure 1, and may provide important information for the further related functional analysis of autophagy-related genes.

Prognostic autophagy-related lncRNAs in OC

According to the screening criteria, we identified 992 autophagy-related lncRNAs. LncRNA protein kinase C theta antisense RNA 1 (PRKCQ-AS1) had the largest correlation coefficient and the target gene was *PRKCQ* (cor=0.817; P=2.42e-92). To screen the prognostic lncRNAs, differentially expressed lncRNAs were examined with univariate COX analysis. Next, 51 lncRNAs of great significance ($P<0.05$) in univariate COX analysis were examined with multivariate COX analysis. As a result, 17 differentially expressed autophagy-related lncRNAs (AC010186.3, AC006001.2, LBX2-AS1, SNHG17, AC011445.1, AC083880.1, MIR193BHG, AC025259.3, HCG14, AC007114.1, AC108673.2, USP30-AS1, AC010336.5, LINC01132, AC006333.2, LINC00665 and AC027348.1) were selected as independent prognostic factors of OC patients. In addition, the risk score of individual patients in our study cohort was calculated. The cohort was divided into two groups (high- and low-risk) in relation to the median risk score value. Thus, the formula for our model was: Risk Score = $(0.596 \times \text{expression}_{\text{AC010186.3}}) + (0.580 \times \text{expression}_{\text{AC006001.2}}) + (0.410 \times \text{expression}_{\text{LBX2-AS1}}) + (0.395 \times \text{expression}_{\text{SNHG17}}) + (0.348 \times \text{expression}_{\text{AC011445.1}}) + (0.323 \times \text{expression}_{\text{AC083880.1}}) + (0.323 \times \text{expression}_{\text{MIR193BHG}}) + (0.298 \times \text{expression}_{\text{AC025259.3}}) + (0.268 \times \text{expression}_{\text{HCG14}}) - (0.246 \times \text{expression}_{\text{AC007114.1}}) - (0.286 \times \text{expression}_{\text{USP30-AS1}}) - (0.317 \times \text{expression}_{\text{AC010336.5}}) - (0.317 \times \text{expression}_{\text{LINC01132}}) - (0.364 \times \text{expression}_{\text{AC006333.2}}) - (0.441 \times \text{expression}_{\text{LINC00665}}) - (0.590 \times \text{expression}_{\text{AC027348.1}})$.

Survival results and multivariate examination

We next analyzed the effect of these 17 lncRNAs on patient survival through Kaplan–Meier curves. The results demonstrated that all 17 lncRNAs significantly affected patient OS (Figures 2-3). As shown in Figure 4a, the Kaplan–Meier curves of the OC cohorts suggest the predictive OS value of the signature based on these 17 lncRNAs. Patients with high risk have poorer survival compared with the low-risk group (P=1.11e−16). We also established a patient risk survival status plot, which suggested that as the patient's risk score increases, the number of deceased patients also increases (Figure 4b). We also used the ROC curves to investigate if the expression patterns of survival-related lncRNAs could serve as biomarkers for early prediction OC occurrence. Here, we

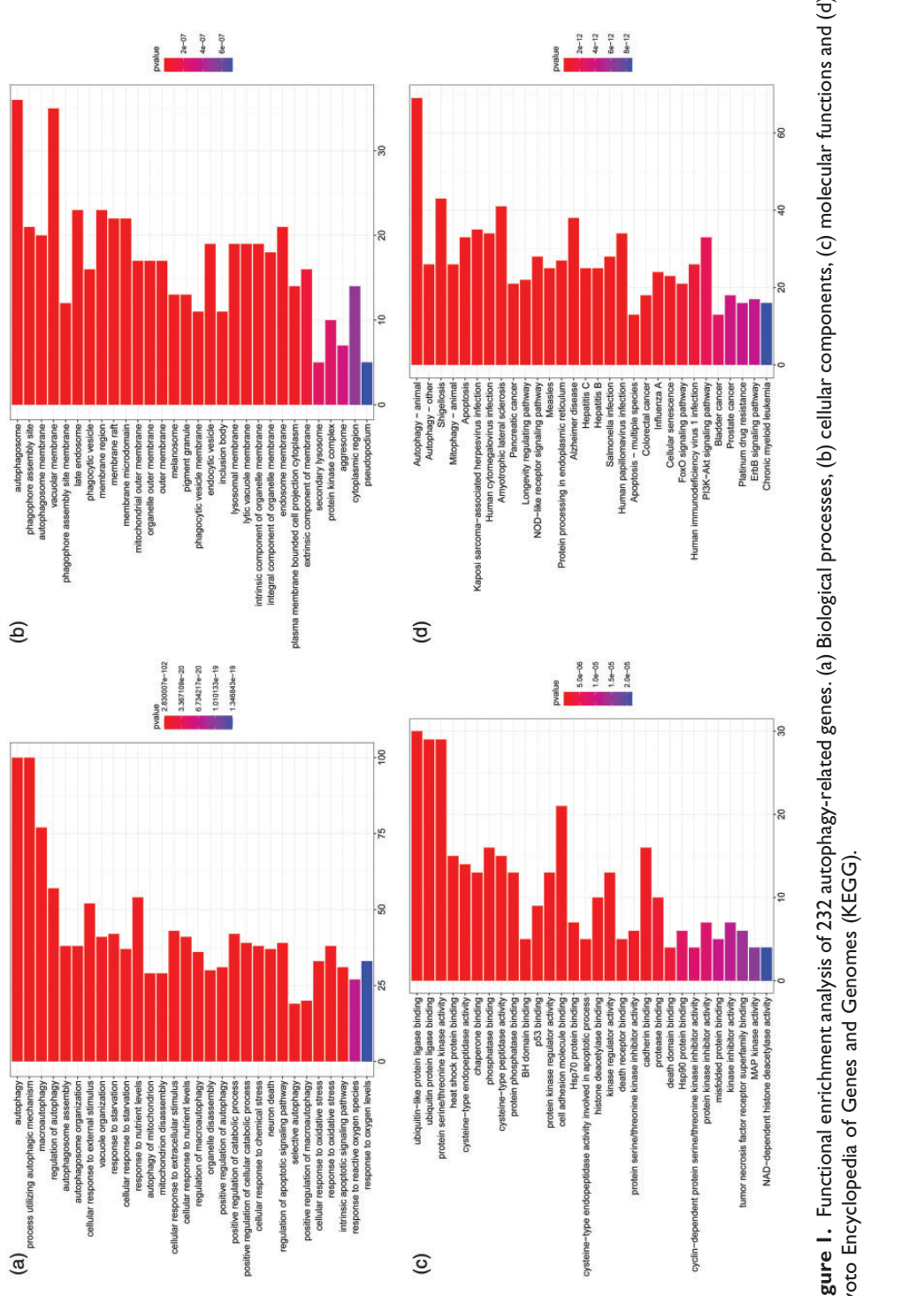

Figure 1. Functional enrichment analysis of 232 autophagy-related genes. (a) Biological processes, (b) cellular components, (c) molecular functions and (d) Kyoto Encyclopedia of Genes and Genomes (KEGG).

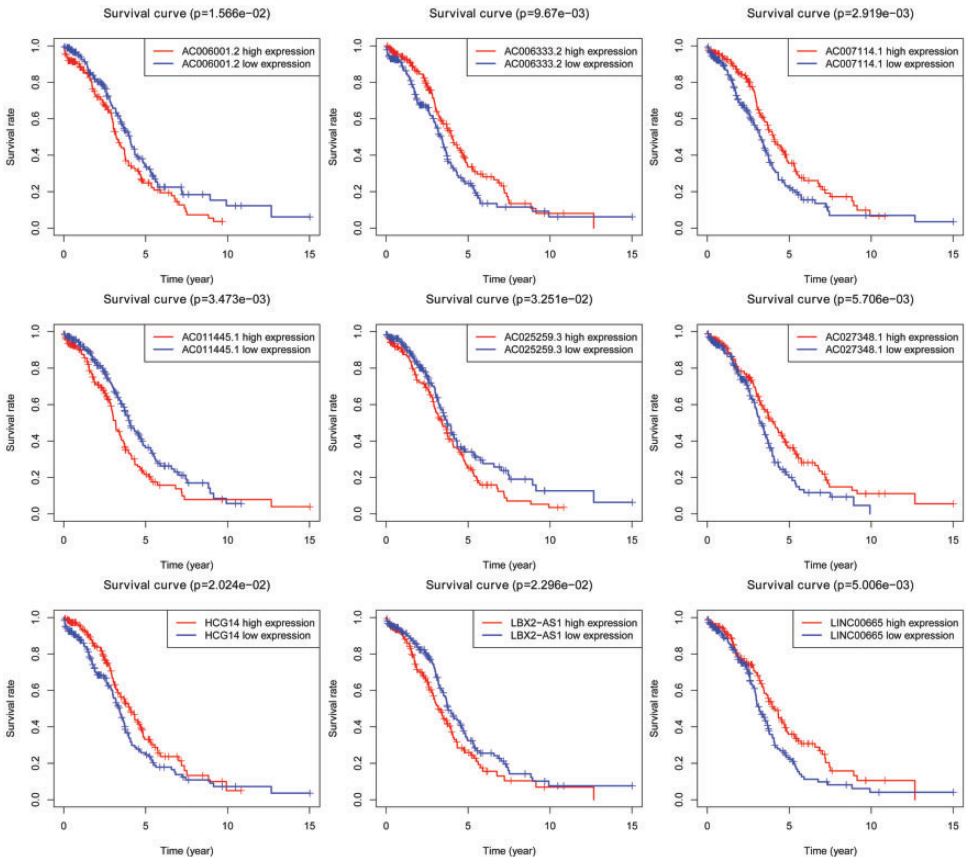

Figure 2. Kaplan–Meier curve results demonstrated that long non-coding RNA (lncRNA) expression significantly affects ovarian cancer patient overall survival.

found an area under the curve (AUC) of 0.731 in our dataset, meaning that the sensitivity and specificity of this prognostic model are moderate (Figure 4c). Next, to establish a prognostic model based on 17 lncRNAs, we used univariate and multivariate COX analyses to determine risk factors. Finally, a lncRNA-based signature including nine lncRNAs (USP30-AS1, LINC00665, AL163051.1, SNHG17, LINC02014, AC010186.3, AC069120.1, AC011445.1 and AC006001.2) was established as independent prognostic factors for OS.

Gene set enrichment analysis

Cytoscape was used to explore the potential link between the lncRNAs and target genes. As shown in Figure 5, AC083880.1 was the largest node in the network, while LINC01132, ladybird homeobox 2 antisense RNA 1 (LBX2-AS1), small nucleolar RNA host gene 17 (SNHG17), AC006001.2 and AC108673.2 had the smallest matched target genes. Functional annotation was performed through GSEA. The results suggested that the high-risk group was mainly enriched in cancer-related and metabolism-related pathways. Cancer-related pathways

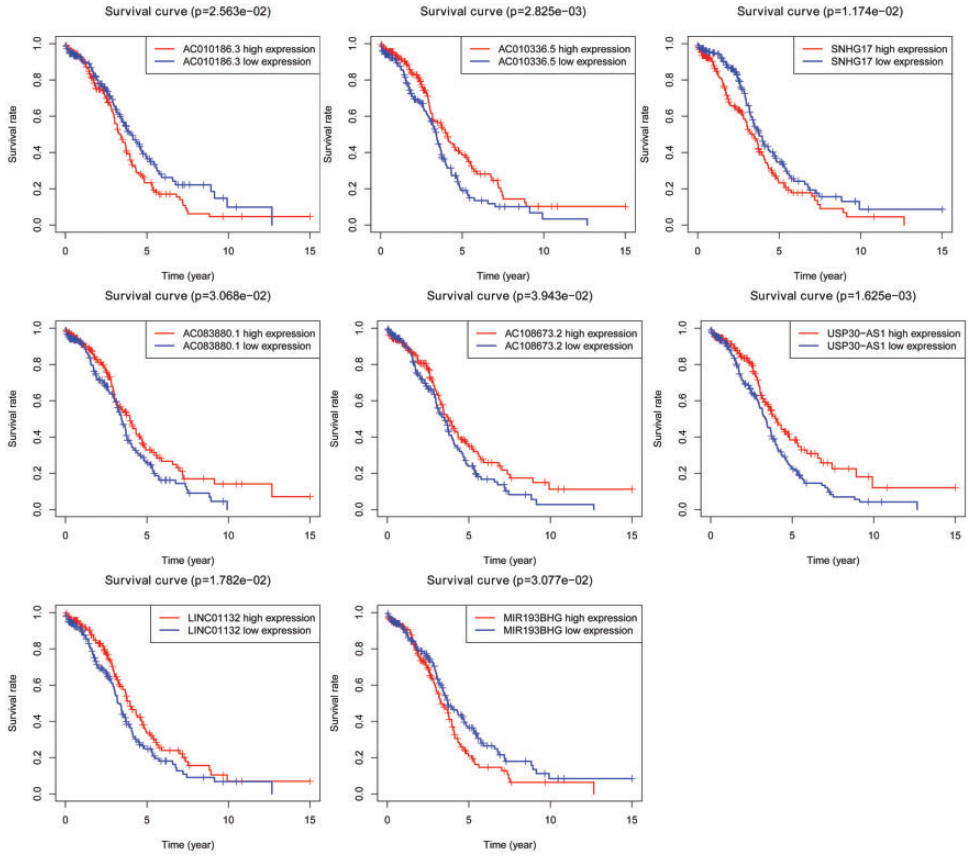

Figure 3. Kaplan–Meier curve results demonstrated that long non-coding RNA (lncRNA) expression significantly affects ovarian cancer patient overall survival.

mainly involved regulation of the mammalian target of rapamycin (MTOR), peroxisome proliferator activated receptor (PPAR), and receptor tyrosine kinase (ERBB) signaling pathways (Figure 6). Metabolism-related pathways mainly involved amino sugar and nucleotide sugar metabolism, purine metabolism, fructose and mannose metabolism, inositol phosphate metabolism and arachidonic acid metabolism Figure 7. Overall, these results provide useful information for signaling pathway analysis regarding autophagy-related lncRNAs.

Discussion

Exploring the molecular mechanisms related to OC pathogenesis has important clinical significance for the early diagnosis, treatment and improvement of prognosis of OC. LncRNAs play a role in the promotion or inhibition of tumor growth, invasion and metastasis through a variety of molecular mechanisms. Many lncRNAs are involved in the regulation of autophagy and affect cell signaling pathways related to autophagy.[21] Therefore, understanding the basic mechanism of autophagy-related lncRNA regulation may provide useful insights for the development of novel

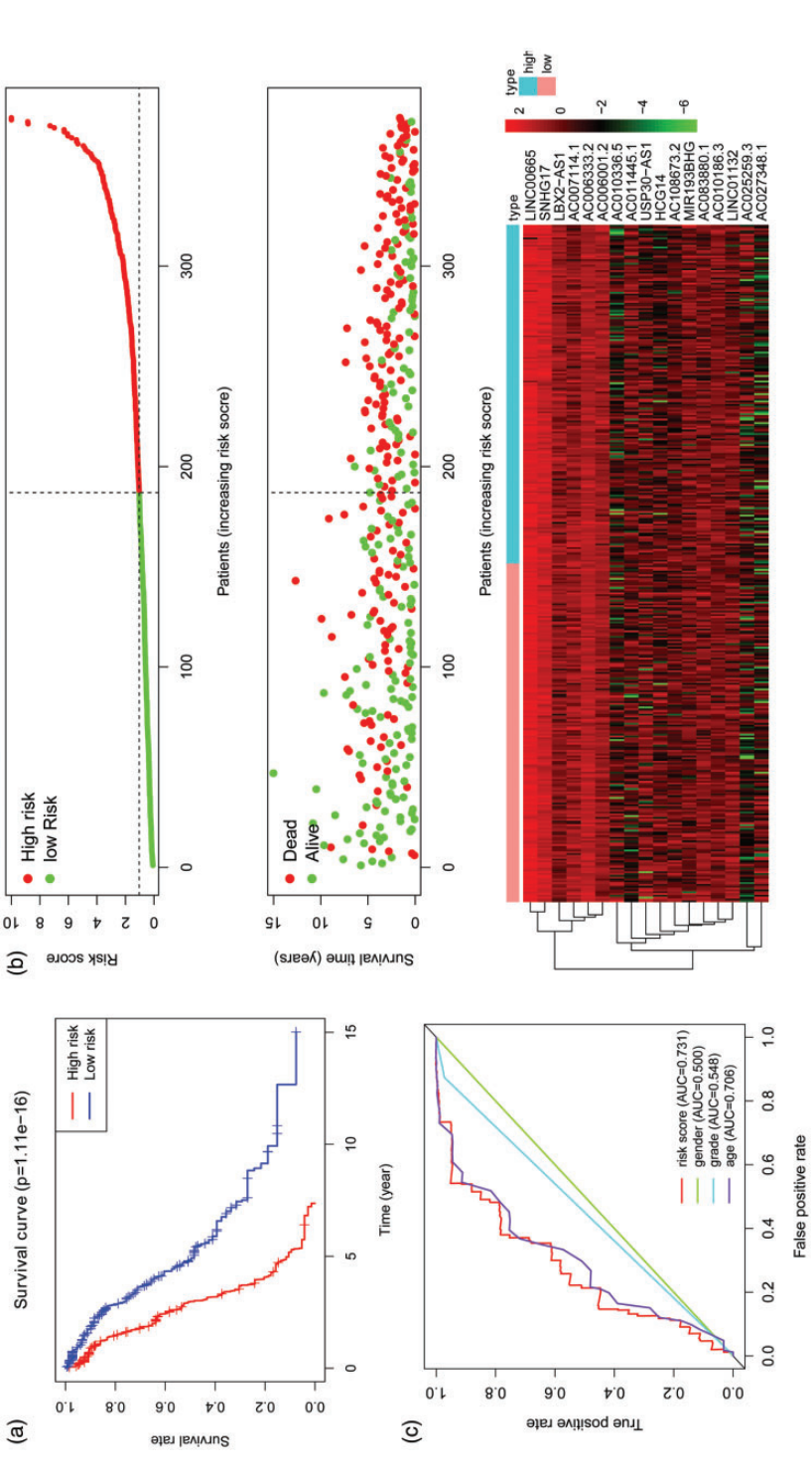

Figure 4. Survival results and multivariate analysis. (a) Survival results, (b) risk survival status plot and (c) receiver operating characteristic (ROC) results.

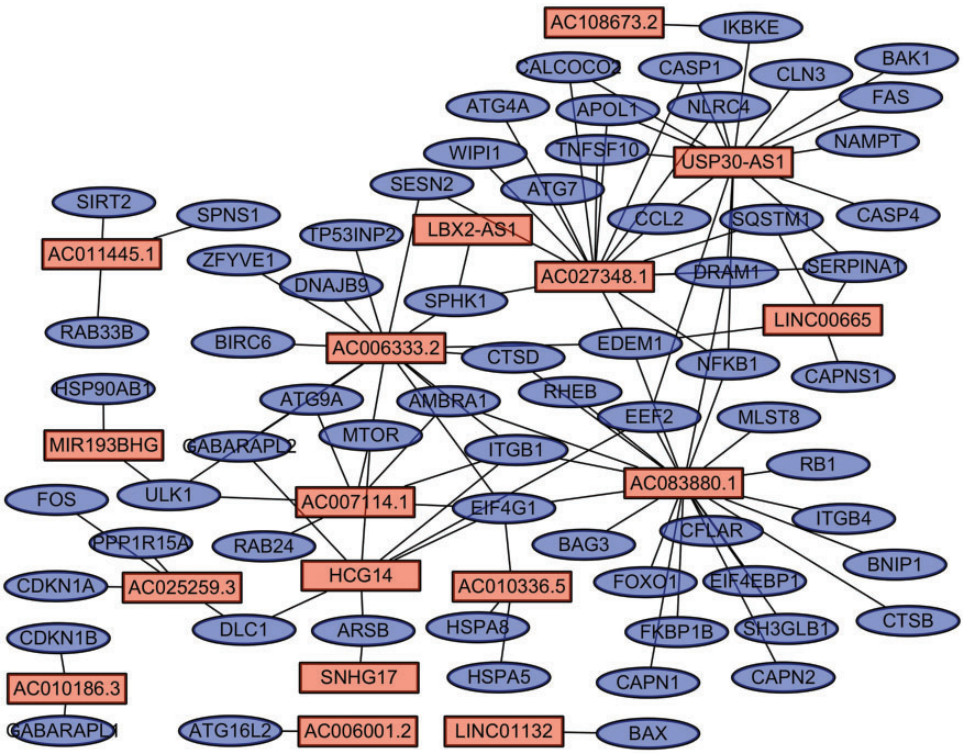

Figure 5. Cytoscape was used to explore the potential link between long non-coding RNAs (lncRNAs) and target genes.

cancer treatments. In this study, we used an online dataset to determine a new and effective autophagy-related lncRNA signature for OC prognosis. Our signature may affect the autophagy-related lncRNA status of OC patients and provide potential biomarkers for clinical therapeutic intervention.

In our study, we performed a comprehensive analysis of autophagy-related lncRNAs, and also obtained OC clinical data from TCGA. First, we identified 17 autophagy-related lncRNAs, and the GSEA results suggested that the differentially expressed lncRNAs were mainly enriched in cancer-related and metabolism-related pathways. Because tumor cells mainly obtain energy for growth through glycolysis, inhibiting glycolysis can reduce colonization and kill tumor cells.[22] Glycolytic rate-limiting enzymes and hypoxia-inducible factors are expected to become new targets for cancer treatment.[23] Therefore, we hypothesize that these target lncRNAs may play an indispensable role in cancer metabolic pathways, and our analysis may provide potential useful biomarkers for cancer treatment.

Among the 17 autophagy-related lncRNAs, high expression of AC006001.2, AC010186.3, SNHG17, AC011445.1, AC025259.3, LBX2-AS1 and MIR193BHG were associated with the high-risk group, and AC083880.1, HCG14, AC007114.1, AC108673.2, USP30-AS1, AC010336.5, LINC01132, AC006333.2, LINC00665 and AC027348.1 were associated with the low-risk group. A

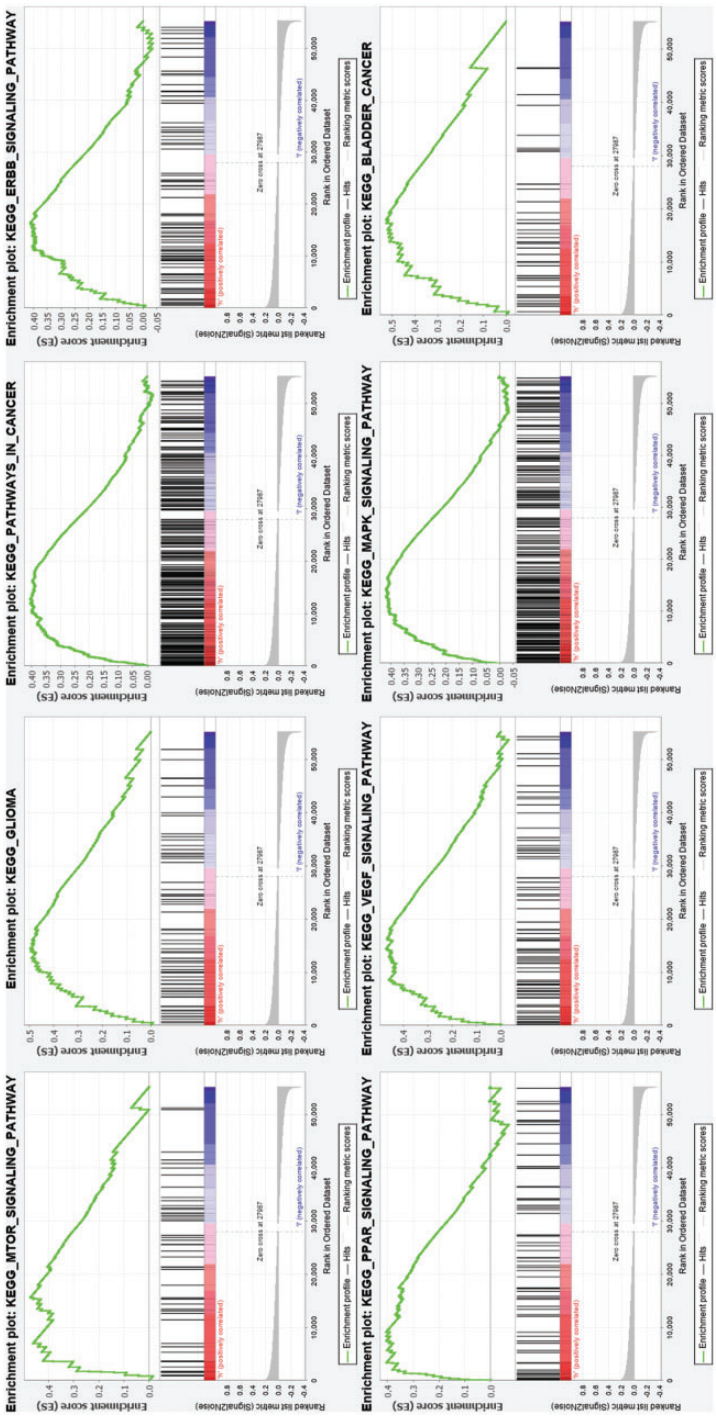

Figure 6. Functional annotation was performed using gene set enrichment analysis (GSEA) for tumor-related pathways.

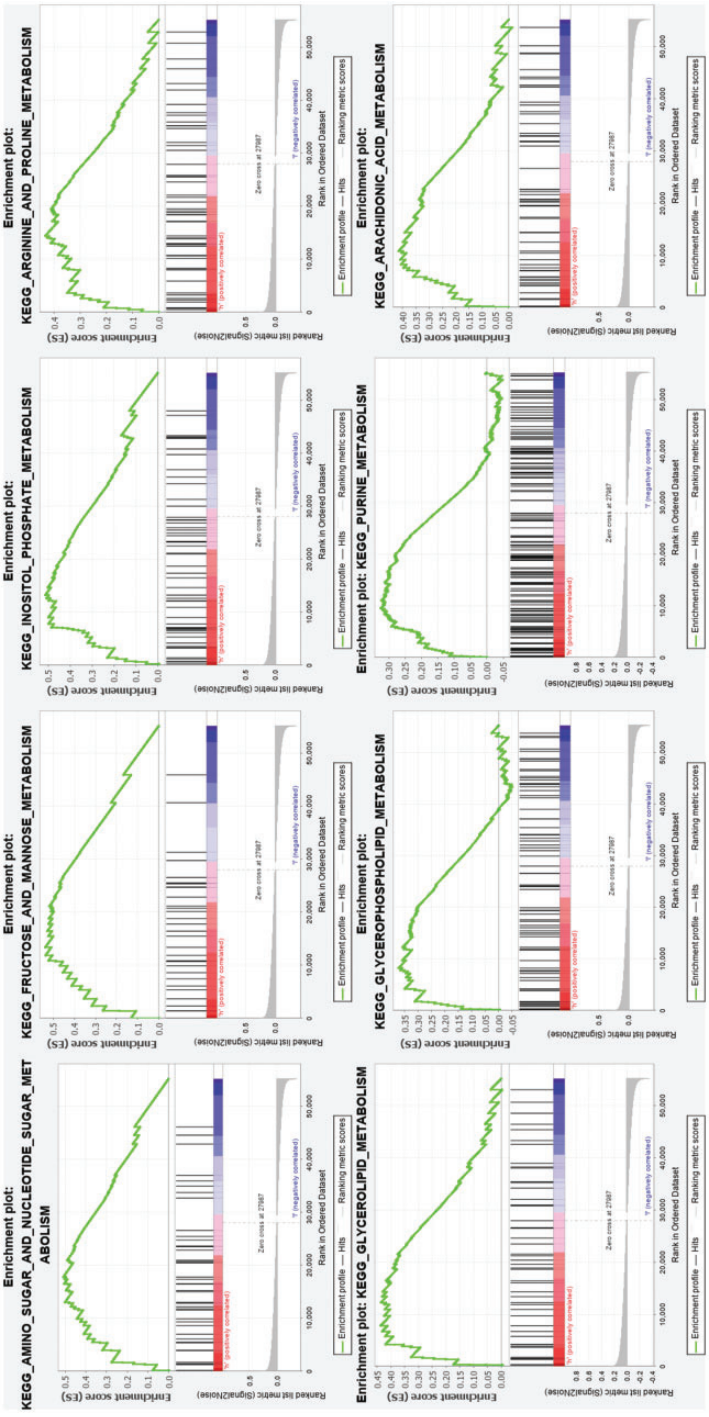

Figure 7. Functional annotation was performed using gene set enrichment analysis (GSEA) for metabolism-related pathways.

recent study reported that lncRNA SNHG17 acts as a competing endogenous RNA with respect to regulating *ZHX1* expression by binding miR-23b-3p and thus promotes glioma progression.[24] Upregulation of SNHG17 promoted the proliferation, migration and invasion of prostate cancer cells *in vitro* and *in vivo*.[25] Another study found that LBX2-AS1, upregulated by *NFIC*, promoted gastric cancer progression via targeting miR-491-5p/zinc finger protein 703 (ZNF703).[26] Higher expression levels of LBX2-AS1 had unfavorable effects on patient OS and promoted activation of the Notch pathway.[27] Zhang *et al*. found that high expression of lncRNA MIR193BHG showed good clinical value for diagnosing early-onset preeclampsia.[28] Additional work suggested that lncRNA AC083880.1 had the most significant prognostic value in pancreatic cancer.[29] Major histocompatibility complex (MHC) genotyping revealed single nucleotide polymorphisms (SNPs) located in the non-coding gene human lymphocyte antigen complex group 14 (HCG14), and showed significant association independent of HLA-DR-DQ loci and related to decreased nucleotide binding oligomerization domain containing 1 (*NOD1*) expression in duodenal intestinal cells.[30] In addition, K674 methylation suppressed hypoxia inducible factor 1 (HIF-1) transcriptional activity and expression of its downstream target gene LINC01132 in human glioblastoma U251MG cells.[31] A recent study found that LINC00665 promoted the proliferation, migration and invasion of colorectal cancer cells, and also inhibited cell apoptosis by sponging miR-9-5p.[32] Similarly, another group indicated that LINC00665 is involved in NF-κB signaling activation in hepatocellular carcinoma cells.[33] LINC00665 also encodes a micropeptide, cellular inhibitor of protein phosphatase 2A binding peptide (CIP2A-BP), whose translation was shown to be downregulated by transforming growth factor-β (TGF-β) in breast cancer cell lines.[34] Until now, there were few published studies regarding the role of these 17 lncRNAs in various cancers. More related research should be conducted to further improve our understanding of these molecules.

Though it has been previously established that autophagy plays a critical role in OC, autophagy-related lncRNAs that affect gene expression is still unclear. In this study, our goal was to integrate lncRNA biomarkers into the current procedure for assessing the prognosis of treatment effects. Our study can help identify novel biomarkers and precise medical targets for OC. Additionally, our study can help with prognosis prediction, diagnosis and treatment strategies for OC patients. However, our approach needs to be performed in further independent cohorts and the predictive autophagy-related lncRNAs functionally confirmed by experiments. The limitations of our research include that our outcomes have not been validated in clinical samples and that our work utilized a relatively small number of patients. Although our research aims to establish a prognostic prediction model for OC, it is still in its infancy and requires further optimization.

Conclusion

Using the TCGA database and other bioinformatics methods, we have identified prognostic autophagy-related lncRNAs and were able to build a prognostic prediction model for OC patients. This model may assist us with identifying novel biomarkers and predicting prognosis, clinical diagnosis and management for OC patients.

Availability of data and materials

RNA-seq data and corresponding clinical data were acquired from the TCGA data portal (https: //portal.gdc.cancer.gov/).

Author contributions

C.M. designed the research study and analyzed the data. J.Q.Z. wrote and revised the manuscript. Y.S.L. collected the data. All authors have read and approved the manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. U.S. Cancer Statistics Working Group. U.S. Cancer Statistics Data Visualizations Tool, based on November 2017 submission data (1999–2015). U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute. www.cdc.gov/cancer/dataviz (accessed 10 January 2019).
2. Vargas-Hernandez VM, Moreno-Eutimio MA, Acosta-Altamirano G, et al. Management of recurrent epithelial ovarian cancer. *Gland Surg* 2014; 3: 198–202.
3. Lyu T, Guo L, Chen X, et al. Ovarian preservation for premenopausal women with early-stage endometrial cancer: a Chinese retrospective study. *J Int Med Res* 2019; 47(6): 2492–2498.
4. Naora H and Montell DJ. Ovarian cancer metastasis: integrating insights from disparate model organisms. *Nat Rev Cancer* 2005; 5: 355–366.
5. Tan P, Ye Y, Mao J, et al. Autophagy and Immune-Related Diseases. *Adv Exp Med Biol* 2019; 1209: 167–179.
6. Gomes LC and Dikic I. Autophagy in antimicrobial immunity. *Mol Cell* 2014; 54: 224–233.
7. Mizushima N and Komatsu M. Autophagy: renovation of cells and tissues. *Cell* 2011; 147: 728–741.
8. Kroemer G, Mariño G, and Levine B. Autophagy and the integrated stress response. *Mol Cell* 2010; 40: 280–293.
9. Guo JY, Karsli-Uzunbas G, Mathew R, et al. Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. *Genes Dev* 2013; 27: 1447–1461.
10. Zachari M and Ganley IG. The mammalian ULK1 complex and autophagy initiation. *Essays Biochem* 2017; 61: 585–596.
11. Papinski D and Kraft C. Regulation of Autophagy By Signaling Through the Atg1/ULK1 Complex. *J Mol Biol* 2016; 428: 1725–1741.
12. Sneha S, Nagare RP, Sidhanth C, et al. The hedgehog pathway regulates cancer stem cells in serous adenocarcinoma of the ovary. *Cell Oncol (Dordr)* 2020; 43: 601–616.
13. Bindhya S, Sidhanth C, Shabna A, et al. Induced pluripotent stem cells: A new strategy to model human cancer. *Int J Biochem Cell Biol* 2019; 107: 62–68.
14. Pal S, Garg M, and Pandey AK. Deciphering the Mounting Complexity of the p53 Regulatory Network in Correlation to Long Non-Coding RNAs (lncRNAs) in Ovarian Cancer. *Cells* 2020; 9: 527.
15. Gu L, Li Q, Liu H, et al. Long Noncoding RNA TUG1 Promotes Autophagy-Associated Paclitaxel Resistance by Sponging miR-29b-3p in Ovarian Cancer Cells. *Onco Targets Ther* 2020; 13: 2007–2019.
16. Pandya G, Kirtonia A, Sethi G, et al. The implication of long non-coding RNAs in the diagnosis, pathogenesis and drug resistance of pancreatic ductal adenocarcinoma and their possible therapeutic potential. *Biochim Biophys Acta Rev Cancer* 2020; 1874: 188423.
17. Zou SH, Du X, Sun FD, et al. Cisplatin suppresses tumor proliferation by inhibiting autophagy in ovarian cancer via long non-coding RNA RP11-135L22.1. *Eur Rev Med Pharmacol Sci* 2018; 22: 928–935.
18. Casper J, Zweig AS, Villarreal C, et al. The UCSC Genome Browser database: 2018 update. *Nucleic Acids Res* 2017; 46: D762–D769.
19. Galluzzi L, Bravo-San Pedro JM, Levine B, et al. Pharmacological modulation of autophagy: therapeutic potential and

persisting obstacles. *Nat Rev Drug Discov* 2017; 16: 487–511.
20. Li W, Han F, Fu M, et al. High expression of VCAN is an independent predictor of poor prognosis in gastric cancer. *J Int Med Res* 2020; 48(1): 300060519891271.
21. Gao T, Liu X, He B, et al. Long non-coding RNA 91H regulates IGF2 expression by interacting with IGF2BP2 and promotes tumorigenesis in colorectal cancer. *Artif Cells Nanomed Biotechnol* 2020; 48(1): 664–671.
22. Okrah EA, Wang Q, Fu H, et al. PdpaMn inhibits fatty acid synthase-mediated glycolysis by down-regulating PI3K/Akt signaling pathway in breast cancer. *Anticancer Drugs* 2020; 31(10): 1046–1056.
23. Li Y, Xu Q, Yang W, et al. Oleanolic acid reduces aerobic glycolysis-associated proliferation by inhibiting yes-associated protein in gastric cancer cells. *Gene* 2019; 712: 143956.
24. Ge BH and Li GC. Long non-coding RNA SNHG17 promotes proliferation, migration and invasion of glioma cells by regulating the miR-23b-3p/ZHX1 axis [published online ahead of print, 2020 Jun 30]. *J Gene Med* 2020; e3247.
25. Bai M, Lei Y, Wang M, et al. Long Non-coding RNA SNHG17 Promotes Cell Proliferation and Invasion in Castration-Resistant Prostate Cancer by Targeting the miR-144/CD51 Axis. *Front Genet* 2020; 11: 274.
26. Xu G, Zhang Y, Li N, et al. LBX2-AS1 up-regulated by NFIC boosts cell proliferation, migration and invasion in gastric cancer through targeting miR-491-5p/ZNF703. *Cancer Cell Int* 2020; 20: 136.
27. Tang LX, Su SF, Wan Q, et al. Novel long non-coding RNA LBX2-AS1 indicates poor prognosis and promotes cell proliferation and metastasis through Notch signaling in non-small cell lung cancer. *Eur Rev Med Pharmacol Sci* 2019; 23: 7419–7429.
28. Zhang Z, Wang P, Zhang L, et al. Identification of Key Genes and Long Noncoding RNA-Associated Competing Endogenous RNA (ceRNA) Networks in Early-Onset Preeclampsia. *Biomed Res Int* 2020; 2020: 1673486.
29. Wei C, Liang Q, Li X, et al. Bioinformatics profiling utilized a nine immune-related long noncoding RNA signature as a prognostic target for pancreatic cancer. *J Cell Biochem* 2019; 120: 14916–14927.
30. Santin I, Jauregi-Miguel A, Velayos T, et al. Celiac Diasease-associated lncRNA Named HCG14 Regulates NOD1 Expression in Intestinal Cells. *J Pediatr Gastroenterol Nutr* 2018; 67: 225–231.
31. Bao L, Chen Y, Lai HT, et al. Methylation of hypoxia-inducible factor (HIF)-1α by G9a/GLP inhibits HIF-1 transcriptional activity and cell migration. *Nucleic Acids Res* 2018; 46: 6576–6591.
32. Zhao X, Weng W, Long Y, et al. LINC00665/miR-9-5p/ATF1 is a novel axis involved in the progression of colorectal cancer. *Hum Cell* 2020; 33: 1142–1154.
33. Ding J, Zhao J, Huan L, et al. Inflammation-induced LINC00665 increases the malignancy through activating PKR/NF-κB pathway in hepatocellular carcinoma. *Hepatology* 2020; doi: 10.1002/hep.31195.
34. Guo B, Wu S, Zhu X, et al. Micropeptide CIP2A-BP encoded by LINC00665 inhibits triple-negative breast cancer progression. *EMBO J* 2020; 39: e102190.