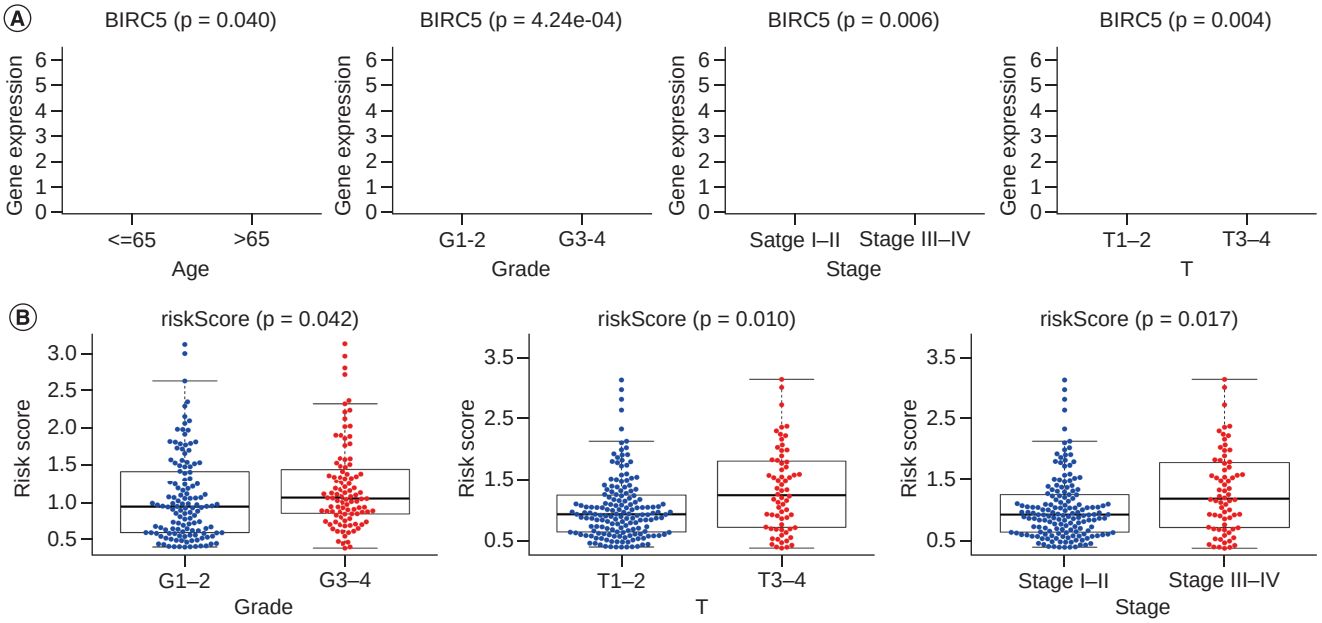

Figure 6. Clinicopathological significance of BIRC5**and risk score in HCC. (A)** Clinicopathological correlation of BIRC5 in HCC. **(B)** Clinicopathological correlation of risk score in HCC.

inflammation, metabolic dysfunction and liver alcoholic disease. However, autophagy may also play an important role in the occurrence and development of HCC in an inflammatory environment [36].

In this study, we profiled the mRNA expression of 232 autophagy-related genes in the TCGA HCC cohort. Among them, seven autophagy-related genes associated with the survival of HCC. We also performed multifactor Cox regression analysis on prognosis-related autophagy genes and found that three genes, *BIRC5, HSPB8* and *TMEM74*, exhibited significant prognostic value for HCC. Gene signatures based on autophagy-related genes have been reported in a variety of tumors [26,37,38], which successfully distinguished patients at a high risk of early relapse from those at low risk [39]. The seven prognosis-related autophagy genes obtained through screening from TCGA were all upregulated in HCC tissues, which indicates that autophagy genes are strictly related to the occurrence and development of HCC. Because of that, we used |Log (fold change) |>2 as the cutoffs and three significant autophagy genes could be used as independent prognostic factors. The risk score and prognostic model based on the three prognosis-related autophagy gene can provide a reference value for evaluating the prognosis of patients with HCC. Chaudhary *et al.* proposed a deep learning model for HCC, which can effectively distinguish patients' survival subgroups in six cohorts [40], This was the first study to use deep learning to identify multigroup characteristics related to different survival rates of HCC patients. Studies have confirmed that gene expression signatures based on autophagy-related genes (*KRT19, EPCAM* and *BIRC5*) and Wnt signaling pathways are closely related to the poor prognosis of patients with HCC. Interestingly, we also screened and analyzed the autophagy gene *BIRC5*, which is significantly related to the prognosis of HCC. We found that *BIRC5* was firmly related to the age, histological grade and pathological stage of HCC patients. The risk score established based on three autophagy genes such as *BIRC5* was significantly related to the pathological stage and histological grade, which has an excellent prognostic significance.

We performed an enrichment analysis of prognosis-related autophagy genes. Surprisingly, the prognosis-related autophagy genes of patients with HCC which were mainly enriched were involved in the process of utilizing autophagic mechanism and autophagy, which showed noticeable enrichment differences. So, we have reasons to believe that the risk score based on prognosis-related autophagy genes and the construction of prognostic models have essential reference value for the prognosis evaluation of HCC. In addition, signal pathways such as DNA damage, cell cycle, mitosis and tumor metabolism all showed significant enrichment of prognosis-related autophagy genes by GSEA enrichment analysis. Autophagy involved in cell cycle regulating through multiple

signaling pathways [41]. Studies have shown that autophagy promotes the growth of tumor cells mainly through PI3K/AKT/mTOR pathway to degrade unfolded proteins and damaged organelles [42]. Our enrichment analysis also found that PI3K/AKT/mTOR signaling pathway plays an important role in the functional enrichment of autophagy-related genes in HCC. However, to our surprise, the prognosis-related autophagy genes of HCC were significantly enriched in the autophagy pathway and the enrichment difference was extremely significant.

Studies have shown that the correlation between valuable biomarkers and clinicopathological parameters can provide important references for prognostic analysis of tumors [43,44]. For example, in the evaluation of clinicopathological factors of digestive system tumors (DSTs), the overexpression of autophagy-related genes *NME1* is related to tumor differentiation and lymph node status, but not to tumor-regional lymph node-metastasis (TNM) stage [45]. In addition, increased expression of miR-133α is associated with overall survival in patients with DSTs, suggesting a good prognosis and a mild clinical stage [46].

We have established a prognostic analysis line chart for patients with HCC from two aspects of prognosis-related autophagy genes and clinicopathological characteristics, in order to comprehensively evaluate the prognosis of patients with HCC from different aspects. Nomogram is a stable and reliable tool for quantitative risk assessment of individuals by combining and delineating risk factors, which have been used in prognostic analysis, including DSTs [47]. The RNA-based signature showed higher prognostic accuracy than the TNM stage. In addition, as shown in the calibration curve, the 3- and 5-year survival rate could be accurately predicted by using the nomogram of both RNA signal characteristics and conventional prognostic factors [48]. With this model, we can accurately calculate the patient's risk score and by comparing the data, we can scientifically predict the patient's 1-, 3- and 5-year survival rates. More importantly, through the ROC curve comparison of patient survival analysis, we found that the risk score based on autophagy-related genes showed more accurate predictive ability than clinicopathological indicators with the increase of follow-up years.

There were some limitations to the present study. First, the clinical information downloaded from TCGA was incomplete and limited. We deleted part of the information due to incomplete information, which caused information bias to a certain extent. What is more, due to conditional constraints, we have not performed experimental verification on the selected prognostic autophagy related genes. Besides, multicenter, large-scale clinical trials of prediction model needed further validation.

In summary, by multivariate regression analysis of prognostic-associated autophagy genes, we screened three prognostic autophagy genes with significant differences in HCC patients and constructed a risk score and prognostic nomogram model based on these genes. To a certain extent, the prognosis of HCC patients was scientifically evaluated, which provided a reference for personalized clinical treatment strategies.

Conclusion

In conclusion, we presented a comprehensive identification of prognosis-related autophagy genes, constructed a seven autophagy gene model and their molecular functions analysis in HCC. Our study also highlights the important roles of the risk score was an independent prognostic biomarker for HCC patients. The risk score was an independent prognostic biomarker and associated with the clinical characteristics of HCC. Besides, GO and KEGG pathway functional enrichment analysis based on the prognosis-related autophagy genes demonstrated that these genes mainly enriched in autophagy. These findings provide a comprehensive outlook for further studies into roles of autophagy genes in the pathogenesis of HCC and as potential biomarkers for HCC diagnosis and therapeutics.

Future perspective

The autophagy gene as prognostic marker has been verified in HCC and the prognosis model is more accurate. Although the pathogenesis is not clear, the study of cancers in autophagy will become a hot topic and could bring have significance in the next 5–10 years.

Author contributions

QL Guan and DH Mi designed the study, JT Wang and YD Miao drafted the article. JT Ran and Y Yang downloaded the data, JT Wang and YD Miao analyzed the data. All authors approved the paper.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Data sharing statement

The authors certify that all the original data in this research could be obtained from public database.

Summary points

- Hepatocellular carcinoma (HCC) is one of the most prevalent cancers and the second most lethal tumor after pancreatic cancer in the world with little being known about the autophagy gene as the prognostic biomarker in HCC.
- For the purpose of improving diagnostic accuracy and treatment efficacy for the HCC patients, it is crucial to identify novel biomarkers and therapeutic targets.
- Seven prognosis-related autophagy genes were significantly related to prognosis of hepatocellular carcinoma by univariate Cox regression analysis.
- Gene Ontology terms and Kyoto Encyclopedia of Genes and Genomes pathway functional enrichment analysis demonstrated that these genes mainly enriched in autophagy.
- *BIRC5, HSPB8* and *TMEM74*, filtrated by multifactor Cox regression analysis, exhibited significant prognostic value for hepatocellular carcinoma.
- The risk score was highly consistent with the clinical characteristics of the tumor.
- The prognostic analysis nomogram of hepatocellular carcinoma patients can evaluate the prognosis of HCC patients from different levels.
- A single-gene analysis showed that the expression of *BIRC5* has significant statistical significance with age, tissue grade, pathological stage and tumor volume.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Wang H, Lu Z, Zhao X. Tumorigenesis, diagnosis and therapeutic potential of exosomes in liver cancer. *J. Hematol. Oncol.* 12(1), 133 (2019).
2. Craig AJ, von Felden J, Garcia-Lezana T, Sarcognato S, Villanueva A. Tumour evolution in hepatocellular carcinoma. *Nat. Rev. Gastroenterol. Hepatol.* 17(3), 139–152 (2020).
3. Chen S, Cao Q, Wen W, Wang H. Targeted therapy for hepatocellular carcinoma: challenges and opportunities. *Cancer Lett.* 460, 1–9 (2019).
4. Ringelhan M, Pfister D, O'Connor T, Pikarsky E, Heikenwalder M. The immunology of hepatocellular carcinoma. *Nat. Immunol.* 19(3), 222–232 (2018).
5. Hollebecque A, Malka D, Ferté C, Ducreux M, Boige V. Systemic treatment of advanced hepatocellular carcinoma: from disillusions to new horizons. *Eur. J. Cancer* 51(3), 327–339 (2015).
6. Gao Q, Zhu H, Dong L *et al.* Integrated proteogenomic characterization of HBV-related hepatocellular carcinoma. *Cell* 179(2), 561–577.e22 (2019).
7. Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. *Nat. Rev. Cancer* 17(9), 528–542 (2017).
8. Reuken PA, Lutz P, Casper M *et al.* The ATG16L1 gene variant rs2241880 (p.T300A) is associated with susceptibility to HCC in patients with cirrhosis. *Liver Int.* 39(12), 2360–2367 (2019).
9. Amaravadi R, Kimmelman AC, White E. Recent insights into the function of autophagy in cancer. *Genes Dev.* 30(17), 1913–1930 (2016).
10. Martens S, Behrends C. Molecular mechanisms of selective autophagy. *J. Mol. Biol.* 432(1), 1–2 (2019).
11. Mao DL, Zhang Z, Zhao X *et al.* Autophagy-related genes prognosis signature as potential predictive markers for immunotherapy in hepatocellular carcinoma. *PeerJ* 8, 16 (2020).

•• **Prognosis signature of ATGs is closely related to immune cell infiltration and PD-L1 expression.**

12. Mazza T, Fusilli C, Saracino C. Functional impact of autophagy-related genes on the homeostasis and dynamics of pancreatic cancer cell lines. *IEEE/ACM Trans. Comput. Biol. Bioinform.* 12(3), 667–678 (2015).
13. Tan P, Ren Y, Zhang Y. Dissecting dynamic expression of autophagy-related genes during human fetal digestive tract development via single-cell RNA sequencing. *Autophagy* 15(11), 2019–2021 (2019).

•• **Autophagy may play an essential role in the development of the digestive tract, especially for the small intestine, in early human embryos.**

14. Xu Z, Han X, Ou D. Targeting PI3K/AKT/mTOR-mediated autophagy for tumor therapy. *Appl. Microbiol. Biotechnol.* 104(2), 575–587 (2020).

15. Yang LY, Greig NH, Tweedie D. The p53 inactivators pifithrin-mu and pifithrin-alpha mitigate TBI-induced neuronal damage through regulation of oxidative stress, neuroinflammation, autophagy and mitophagy. *Exp. Neurol.* 324, 113135 (2019).

16. Cheng Z, Yi Y, Xie S *et al.* The effect of the JAK2 inhibitor TG101209 against T cell acute lymphoblastic leukemia (T-ALL) is mediated by inhibition of JAK-STAT signaling and activation of the crosstalk between apoptosis and autophagy signaling. *Oncotarget* 8(63), 106753–106763 (2017).

17. Kim SH, Yu HS, Park S *et al.* Electroconvulsive seizures induce autophagy by activating the AMPK signaling pathway in the rat frontal cortex. *Int. J. Neuropsychopharmacol.* 23(1), 42–52 (2020).

• **Repeated electroconvulsive seizure treatments activated *in vivo* autophagy in the rat frontal cortex through the AMPK signaling pathway.**

18. Yazdani HO, Huang H, Tsung A. Autophagy: dual response in the development of hepatocellular carcinoma. *Cells* 8(2), 91 (2019).

19. Kwanten WJ, Martinet W, Michielsen PP, Francque SM. Role of autophagy in the pathophysiology of nonalcoholic fatty liver disease: a controversial issue. *World J. Gastroenterol.* 20(23), 7325–7338 (2014).

20. Alvur O, Tokgun O, Baygu Y. The triazole linked galactose substituted dicyano compound can induce autophagy in NSCLC cell lines. *Gene* 712, 143935 (2019).

21. Li C, Ma L, Liu Y *et al.* TLR2 promotes development and progression of human glioma via enhancing autophagy. *Gene* 700, 52–59 (2019).

•• **TLR2 promotes development and progression of human glioma via enhancing autophagy.**

22. Wang X, Zhang Y, Feng T *et al.* Fluid shear stress promotes autophagy in hepatocellular carcinoma cells. *Int. J. Biol. Sci.* 14(10), 1277–1290 (2018).

23. Nagashima K, Sato Y. Information criteria for Firth's penalized partial likelihood approach in Cox regression models. *Stat. Med.* 36(21), 3422–3436 (2017).

24. Abraham G, Malik R, Yonova-Doing E *et al.* Genomic risk score offers predictive performance comparable to clinical risk factors for ischaemic stroke. *Nat. Commun.* 10(1), 5819 (2019).

25. Liu Y, Wu L, Ao H *et al.* Prognostic implications of autophagy-associated gene signatures in non-small cell lung cancer. *Aging (Albany NY)* 11(23), 11440–11462 (2019).

26. Wang Z, Gao L, Guo X *et al.* Development and validation of a nomogram with an autophagy-related gene signature for predicting survival in patients with glioblastoma. *Aging (Albany NY)* 11(24), 12246–12269 (2019).

• **The nomograms are well calibrated and internally and externally valid.**

27. Xu WP, Liu J-P, Feng J-F *et al.* miR-541 potentiates the response of human hepatocellular carcinoma to sorafenib treatment by inhibiting autophagy. *Gut* 69(7), 1309–1321 (2020).

28. Limpert AS, Lambert LJ, Bakas NA *et al.* Autophagy in cancer: regulation by small molecules. *Trends Pharmacol. Sci.* 39(12), 1021–1032 (2018).

29. White E, Mehnert JM, Chan CS. Autophagy, metabolism and cancer. *Clin. Cancer Res.* 21(22), 5037–5046 (2015).

30. Peixoto P, Grandvallet C, Feugeas JP, Guittaut M, Hervouet E. Epigenetic control of autophagy in cancer cells: a key process for cancer-related phenotypes. *Cells* 8(12), 1656 (2019).

31. Di Malta C, Cinque l, Settembre C. Transcriptional regulation of autophagy: mechanisms and diseases. *Front. Cell Dev. Biol.* 7, 114 (2019).

• **The transcriptional regulation of autophagy could be targeted for the treatment of human genetic diseases, such as lysosomal storage disorders and neurodegeneration.**

32. Sanaei M, Kavoosi F. Histone deacetylases and histone deacetylase inhibitors: molecular mechanisms of action in various cancers. *Adv. Biomed. Res.* 8, 63 (2019).

33. Takamura A, Komatsu M, Hara T *et al.* Autophagy-deficient mice develop multiple liver tumors. *Genes Dev.* 25(8), 795–800 (2011).

34. Mulcahy Levy JM, Thorburn A. Autophagy in cancer: moving from understanding mechanism to improving therapy responses in patients. *Cell Death Differ.* 27(3), 843–857 (2020).

35. Huang F, Wang BR, Wang YG. Role of autophagy in tumorigenesis, metastasis, targeted therapy and drug resistance of hepatocellular carcinoma. *World J. Gastroenterol.* 24(41), 4643–4651 (2018).

36. Hazari Y, Bravo-San Pedro JM, Hetz C, Galluzzi L, Kroemer G. Autophagy in hepatic adaptation to stress. *J. Hepatol.* 72(1), 183–196 (2020).

37. Wang Y, Zhang Q, Gao Z *et al.* A novel 4-gene signature for overall survival prediction in lung adenocarcinoma patients with lymph node metastasis. *Cancer Cell Int.* 19, 100 (2019).

38. Mo S, Dai W, Xiang W *et al*. Prognostic and predictive value of an autophagy-related signature for early relapse in stages I-III colon cancer. *Carcinogenesis* 40(7), 861–870 (2019).

- **Autophagy-related signature, a credible approach to early relapse prediction in stages I–III colon cancer patients.**

39. Huang Z, Liu J, Luo L *et al.* Genome-wide identification of a novel autophagy-related signature for colorectal cancer. *Dose Response* 17(4), 1559325819894179 (2019).

40. Chaudhary K, Poirion OB, Lu L, Garmire LX. Deep learning-based multi-omics integration robustly predicts survival in liver cancer. *Clin. Cancer Res.* 24(6), 1248–1259 (2018).

41. Anand SK, Sharma A, Singh N, Kakkar P. Entrenching role of cell cycle checkpoints and autophagy for maintenance of genomic integrity. *DNA Repair (Amst.)* 86, 102748 (2020).

42. Reddy D, Kumavath R, Ghosh P, Barh D. Lanatoside C induces G2/M cell cycle arrest and suppresses cancer cell growth by attenuating MAPK, Wnt, JAK-STAT and PI3K/AKT/mTOR signaling pathways. *Biomolecules* 9(12), 792 (2019).

43. Zhang Y, Liu W, Xu J. Prognostic utility and clinical significance of lysyl oxidase-like 2 protein expression in digestive system cancers. *J. Cell. Physiol.* 234(11), 20713–20720 (2019).

44. Li J, Cui Z, Li H *et al.* Clinicopathological and prognostic significance of long noncoding RNA MALAT1 in human cancers: a review and meta-analysis. *Cancer Cell Int.* 18, 109 (2018).

45. Han W, Shi C-T, Cao F-Y *et al.* Prognostic value of NME1 (NM23-H1) in patients with digestive system neoplasms: a systematic review and meta-analysis. *PLoS ONE* 11(8), e0160547 (2016).

46. Zhu W, Ji X. The impact of MicroRNA-133a on prognosis and clinicopathological parameters for digestive system cancers: a comprehensive study based on meta-analysis and TCGA database. *Pathol. Oncol. Res.* 26(2), 771–781 (2020).

47. Wang YY, Xiang BD, Ma L *et al.* Development and validation of a nomogram to preoperatively estimate post-hepatectomy liver dysfunction risk and long-term survival in patients with hepatocellular carcinoma. *Ann. Surg.* doi: 10.1097/SLA.0000000000003803 (2020).

48. Xiong Y, Wang R, Peng L *et al.* An integrated lncRNA, microRNA and mRNA signature to improve prognosis prediction of colorectal cancer. *Oncotarget* 8(49), 85463–85478 (2017).

- **Multi-RNA-based classifier may have important clinical implications in the selection of patients with colorectal cancer who are at high risk of mortality and add prognostic value to the current stage system.**