Alternative splicing events implicated in carcinogenesis and prognosis of thyroid gland cancer

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Abstract

Background: Alternative splicing events (ASEs), a critical post-transcriptional regulatory mechanism, expands gene expression patterns, resulting in increased protein diversity and more than 95% of human genes experience AS and encode splice variants in the regular physiological processes. While the role of AS in the thyroid cancer as yet missing, therefore, it was necessary to carry out this study to provide more information about the combination of splicing and clinical parameters, as well as potential mechanism of the survival-related splicing events in thyroid cancer.

Materials and methods: Here, we draw all-around AS profiles of thyroid cancer by analyzing RNA-seq data. We also constructed prognostic models via combining splicing signatures and clinicopathological parameters. Splicing network was constructed as a way to offer functional insight into the full practical knowledge of AS in the initiation and development of thyroid cancer.

Results: There were 10446 genes, and 45150 AS events in 506 TC patients, which indicates that ASEs are universal in TC. Moreover, 1819 AS signatures were identified to be significantly related to OS of TC patients and among the seven types of ASES, ES was the most common, followed by AP and AT. Kaplan-Meier survival curves results suggested that seven types of ASEs were related to bad prognosis in TC patients (P<0.05). In TC, AA (AUC: 0.937), AD (AUC: 0.965), AT (AUC: 0.964), ES (AUC: 0.999), ME (AUC: 0.999), RI (AUC: 0.837) all demonstrated an AUC over 0.6, of which ES and ME best predict the incidence of TC. We found that age and risk score (All) were risk factors for TC patients. As for ASEs is regulated by SFs, we study if the TC-ASEs were regulated by various SFs and the results demonstrated that the expression of 90 SFs was

related to 469 ASEs OS in the TC cohort. **Conclusions:** In sum, the findings in the current study may provide a basis for spliceosomes in TC, and the methods used in this study could provide novel perspectives in other fields of tumor study to help shed light on future oncology research.

Keywords: Alternative splicing events, thyroid gland cancer, Bioinformatical analysis, RNA sequencing.

Background

Thyroid cancer (TC) is the most well-known endocrine neoplasia as well as a common malignant tumor in the head and neck, with an incidence rate accounting for 1% of all the malignant tumors[1-2]. In recent decades, the remarkable increase in TC morbidity has aroused substantial public concern. Meanwhile, high-incidence patients often develop distant metastasis and lymph node metastasis, which in turn result in high mortality[3]. Pathologically, thyroid cancer can be divided into four pathological type which includes papillary thyroid carcinoma (PTC), anaplastic thyroid carcinoma (ATC), follicular thyroid carcinoma (FTC) and medullary thyroid carcinoma (MTC)[4]. PTC is the widely recognized pathological types accounts for approximately 90% of thyroid cancer[5]. Most of PTC patients with over 80% of 35-year survival or 40-year survival after effective treatment[6]. However, the patients who are not sensitive to radioiodine therapy or accompanied with cervical lymph node metastasis at the time of diagnosis suffer from poor prognosis, with lower than 10% of 10-year survival[7]. Therefore, it is important to assess for biomarkers to characterize TC recurrence and metastasis for useful prognostic monitoring. It has become widely accepted that gene regulation dysfunction is a critical factor in the initiation and progression of tumors.

Alternative splicing events (ASEs), a critical post-transcriptional regulatory mechanism, expands gene expression patterns, resulting in increased protein diversity and more than 95% of human genes experience AS and encode splice variants in the regular physiological processes[8]. AS is widely involved in biological processes such as cell differentiation, proliferation, and apoptosis, studies exhibited that unnormal alternative splicing events play an important role in cancer metastasis, progression, immunotherapy**,** therapeutic resistance, and may provide opportunities for novel cancer therapeutics[9-13]. Alternative processing of mRNA may offer the potential of a broadened target space for cancer immunotherapy[12]. AS included seven fundamental splicing patterns[14-15], includes alternate acceptor sites (AA), alternate promoter (AP), alternate donor sites (AD), alternate terminator (AT), mutually exclusive exons (ME), exon skipping (ES), and retained intron (RI). More and more studies reported that aberrant AS is everywhere event in development and progression of cancer such as gastrointestinal adenocarcinomas, urogenital malignancies[16-19]. Xie et al[20] constructed a novel combined prognostic model of ASEs and clinicopathological parameters in esophageal carcinoma. Wang et al[21] analysis

ASEs through whole genome methods and develop a prognostic model of endometrial cancer. Chen et al[22] indicated that a prognostic index based on ASEs is prognostic for overall survival in hepatocellular carcinoma. While the role of AS in the thyroid cancer as yet missing, therefore, it was necessary to carry out this study to provide more information about the combination of splicing and clinical parameters, as well as potential mechanism of the survival-related splicing events in TC. Here, we draw all-around AS profiles of thyroid cancer by analyzing RNA-seq data. We also constructed prognostic models via combining splicing signatures and clinicopathological parameters. Splicing network was constructed as a way to offer functional insight into the full practical knowledge of AS in the initiation and development of thyroid cancer. This study will help us knowing the regulatory mechanisms of AS events in TC and may facilitate the therapeutic of clinical practice.

Materials and Methods

Download raw data

The targeted records of RNA sequencing (RNA-seq) data in TC patients was get from The Cancer Genome Atlas (TCGA), a web-based resource, provides a user-friendly interface for detailed views of alternative mRNA splicing based on the TCGA database and Percent Spliced In (PSI) degrees from 0 to 1, which will be used to quantify ASEs. In our study, data of 58 normal and 495 thyroid cancer tissues as well as clinicopathological data was acquired to explore the changes of ASEs connection to the carcinogenesis and prognosis of TC. PSI values of ASEs in TC samples were gotten from TCGA SpliceSeq[23], a resource for explore of tumor-normal and cross-tumor alterations in mRNA splicing patterns of TCGA RNASeq information.

Identification of survival-associated splicing events and clinical parameters

Related clinical data of TC patients were also downloaded and only patients with an overall survival (OS) of 90 days or longer were enrolled in our study. Basis its median number, each parameter has been isolated low-risk (<median number) groups and high-risk (≥ median number). Cox regression was used to analyze the relationship between AS events and OS and found prognostic value of demographic and clinicopathological parameters of TC. A prognosis risk score was confirmed based on the linear mix of AS PSI multiplied by a corresponding regression coefficient (β) speaking the degree of the correlation and the value was calculated based on a univariate Cox proportional hazards regression model[24]. Kaplan–Meier survival analysis was used to analyze the survival significance between the study and control groups. A time-dependent receiver operating characteristic (ROC) curve was carried out to evaluate the predictive correctness of prognostic signatures in patients with TC. The top 20 in each type of splicing and seven combined events were selected.

Construction of gene network and correlation analysis

As for AS is regulated via splicing factors (SFs), we investigated if the ASEs were regulated by way of a subset of SFs. We investigated the links between TC-associated SFs and the PSI values for ASEs utilizing the Spearman correlation method. The connection network between SFs and ASEs was constructed in Cytoscape (version 3.7.1).

Statistical Analysis

All statistical analyses have been finished using R 3.5.3 and the aggregates and intersections between seven types of AS were showed using the UpSetR package[25]. We used hazard ratios (HRs) and 95% confidence intervals (CIs) were to evaluate relative risk of TC patients with seven PSI values of ASEs and of different risk groups. Univariate and multivariate Cox regressions were then conducted to identify survival-associated SFs. Two-tailed $P<0.05$ was viewed statistically significant.

Results

Overview of ASEs in TCGA-TC.

Each ASEs were allocated a completely unique annotation that was a mixture of the gene name, ID number and the AS type in the SpliceSeq database (AS ID). Such as, in the annotation term “FNTA-83754-AD”, the gene name is FNTA, AS ID is 83754 and the splicing pattern is AD. We must note that one gene can experience different types ASEs; thus, we used UpSet image to match the genes with ASEs which can tell us quantitative results of different interactive sets. In general, there were 45150 ASEs from 10446 genes in 506 TC patients and the median value of ASEs for every gene was 4.322. Among the seven kinds of ASEs, ES was the majority common, followed by AP and AT. Among the ASEs, 4481 genes in the 8594 AT events, 2449 genes in the 3189 AD events, 4793 genes in the 9126 AP events, 2799 genes in the 3684 AA events, 7485 genes in the 17536 ES events, 2035 genes in the 2786 RI events, 2449 genes in the 3189 AD events and 217 genes in the 232 ME events **(Figure 1(a)).**

Survival related ASEs in TCGA-TC

Consequently, 1819 ASEs signatures were identified to be significantly related to OS of TC patients ($P<0.05$)**.** The vol plot survival related to ASEs was shown in **Figure 2H.** The survival associated ASEs among the seven types of AS were shown by UpSet plot, the results demonstrate that ES was the most common, followed by AP and AT (**Figure 1B**). The top 20 significant survival associated ASEs of each types were provide in **Figure 2A-2G**. In order to determine the prognostic value of AS, and to identified AS that significantly associated with survival ($P<0.05$), which we chosen for further functional analysis and development of a capability risk signature with the LASSO Cox regression

algorithm[25-26] **(Figures 3)**.

Prognostic Predictors of ASEs in TCGA-TC

The cohorts were then divided into high- and low-risk groups using the median risk score value as a cut-off. All the seven prognostic models built with different types of ASEs and the results showed significant to predict the prognostic of TC patients **(Figures 4)**. We next study whether AS patterns could serve as an early predictor of incidence of TC by ROC curve. In TC, AA (AUC: 0.937), AD (AUC: 0.965), AT (AUC: 0.964), ES (AUC: 0.999), ME (AUC: 0.999), RI (AUC: 0.837) all showed an AUC over 0.6, of which ES and ME best predict the incidence of TC and the integrated predictor model of TC demonstrated an AUC of 0.882. Overall, aberrant active AFs was a specific event in TC as most models exhibited a relatively high specificity value. Detailed prognostic signature information of TC groups is visualized in **Figure 5**, which indicated that the mortality rate was higher for TC patients in the high-risk group and related to the lower OS. We next used Cox regression analysis to assess prognostic value of the all AS and other clinicopathological parameters including gender, age and tumor stage. The hazard ratios (HRs) for AS-ALL in the univariate and multivariate Cox regression analyses were 2.798 (95% CI: 2.286–3.424) and 2.603 (95% CI: 2.108–3.215), respectively **(Figure 6A– 6B).**

Correlation between TC-ASEs and SFs expression

Univariate Cox regression analysis result shown that the expression of 90 SFs was related to OS in the TC cohort. Correlation plots were then generated using Cytoscape**.** These results indicated that the expression of 90 survival-associated SFs (triangular nodes) was related to 469 TC-ASEs. Of the 469 OS-ASEs, 260 were associated with poor OS (green ovals) and 209 that were related to favorable OS (red ovals). The majority of the ASEs related to favorable OS were negatively correlated with SFs expression (blue lines), whereas the majority of the ASEs related to poor OS were positively correlated with SFs expression (red lines). The 10 most significant connections between genes and SFs by *P* value were HSPB1, ZC3H11A, NOSIP, SNRPB, SNRPF, WDR83, ZNF346, THOC6, FAM50A and CLK1 **(Figure 7)**. In addition, we found that SF NOSIP was positively related to PSI value of CABIN1-61386-AP and negatively of HAS3-37253-AT. In addition, SF ZNF346 demonstrated different connection between different ASEs types of the same gene PCNA ($P<0.001$). The results indicated that different SFs played different roles in different ASEs. The plug-in Molecular Complex Detection (MCODE) of Cytoscape was applied to detect densely connected regions in the networks. The result show that UBL5 and PTCD2-72456-AT were identified as hub gene or AS event with degrees ≥10.

Discussion

AS is a critical biological process for producing protein variety. Aberrant ASEs in cancers are nearly linked to cancer initiation and progression. A gene can

experience various types of ASEs and can be regulated by sorts of SFs, therefore complicating explore of the regulatory networks between ASEs and AFs. We recognized ASEs and regulatory SFs in TC through the analysis of TCGA program to get comprehensive knowledge into varies RNA splicing patterns. Consequently, 1819 AS signatures were identified to be significantly related to OS of TC patients. Of the 469 OS-ASEs, 209 that were linked to favorable OS and 260 with poor OS.

The aberrant regulation of AS in various tumors has been the source of a great deal of research recently. Kozlovski et al[27] present evidence that supporting the idea that AS serves as a molecular switch in many types of cancer that alters metabolism to drive tumorigenesis such as the regulation of the metabolic mTOR pathway and glycolytic pathway/TCA cycle. Additionally, AS may significantly alter the coding region of drug targets, leading to increased drug resistance in some cancer therapies[28], such as BCR-ABL splice variant, imatinib resistance, BCL2-Like 11 (BIM or BCL2L11) splice variant and TKI resistance, BRCA splice variants leading to PARP inhibitor or cytotoxic drug resistance and TP53 splice variants and cisplatin resistance[29]. Over the last few years, an increasing number of AS events have been implicated in the progression of many types of cancers. SRSF1 (also known as SF2/ASF) was the first SF to be identified as a proto-oncogene in human tumors. Previous studies reported that SRSF1 is up-regulated in varies types of human tumors, including colon, thyroid, breast, kidney, small intestine and lung cancers[30-31]. Piqué et al[32] reported that the splicing RNA-binding protein CELF2 is targeted by promoter hypermethylation-linked transcriptional silencing in the breast cancer. Duan et al[32] suggested the aberrant splicing variants in renal cell cancer. AS was also found to regulate some apoptotic genes. The BCL2L1 pre-mRNA related to greater tumor cell survival in various cancer types, including human lymphoma, breast cancer, prostate cancer and human hepatocellular carcinoma[34-35]. AS even found in AIDS, study found AS of HIV-1 mRNAs increases viral coding potential and controls the levels and timing of gene expression[36]. While the role of AS in the thyroid cancer as yet missing, therefore, it was necessary to carry out this study to provide more information about the combination of splicing and clinical parameters, as well as potential mechanism of the survival-related ASEs in TC.

In our study, we downloaded seven types of AS from the TCGA SpliceSeq database. There were 10446 genes, and 45150 AS events in 506 TC patients, which indicates that ASEs are universal in TC. Moreover, 1819 AS signatures were identified to be significantly related to OS of TC patients and among the seven types of ASES, ES was the most common, followed by AP and AT. We also demonstrated top 20 significant survival related ASEs of the seven types. In order to evaluate the diagnostic significance of aberrant ASEs in the prognosis of TC, we constructed prognostic models based on risk score and ASEs types (AA, AP, AD, AT, ES, ME, RI and ALL). We then plotted Kaplan-Meier survival curves of risk score and the risk scores of each types of ASEs. The results

suggested that seven types of ASEs were related to bad prognosis in TC patients (P<0.05). We subsequently explored if AS patterns could use as an early predictor of incidence of TC by ROC curve. In TC, AA (AUC: 0.937), AD (AUC: 0.965), AT (AUC: 0.964), ES (AUC: 0.999), ME (AUC: 0.999), RI (AUC: 0.837) all demonstrated an AUC over 0.6, of which ES and ME best predict the incidence of TC. The integrated predictor model of TC showed an AUC of 0.882. Cox regression was used to explore the impacts of clinicopathological parameters and risk score on the prognosis of TC patients. We found that age and risk score (All) were risk factors for TC patients. As for ASEs is regulated by SFs, we study if the TC-ASEs were regulated by various SFs and the results demonstrated that the expression of 90 SFs was related to 469 ASEs OS in the TC cohort. The 10 most significant related between genes and SFs by P value were HSPB1, ZC3H11A, NOSIP, SNRPB, SNRPF, WDR83, ZNF346, THOC6, FAM50A and CLK1. In addition, UBL5 and PTCD2-72456-AT were identified as hub gene or AS event with degrees ≥10. Studies reported that UBL5 plays an evolutionary conserved role in pre-mRNA splicing, the integrity of which is important for the fidelity of chromosome segregation[37]. Xu et al[38] found that the PTCD2 protein is involved in processing RNA transcripts involving cytochrome b derived from mitochondrial DNA. These findings provide detailed information about the mechanisms by which ASEs function in TC development and progression.

Conclusions

Although our study has limitations (e.g. lack of therapeutic strategies, sample size, TC subtype research and lack of validation studies), our study indicate that ASEs are frequent in TC and are related to patient prognosis. These ASEs may be part of a prognostic signature in TC. In sum, the findings in the current study may provide a basis for spliceosomes in TC, and the methods used in this study could provide novel perspectives in other fields of tumor study to help shed light on future oncology research.

Abbreviations

TC: Thyroid cancer; ASEs: Alternative splicing events; PTC: papillary thyroid carcinoma; ATC: anaplastic thyroid carcinoma; FTC: follicular thyroid carcinoma, MTC: medullary thyroid carcinoma; TCGA: cancer genome atlas; SFs: splicing factors; OS: over survival.

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Authors' contributions

W.Z.H. designed and analyzed the research study; W.Z.H. and Z.Y. wrote and revised the manuscript, C.F.C. and W.Z.H. collected the data and all authors contributed to and approved the final version of manuscript.

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

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Not applicable.

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Not applicable.

Competing interests
The authors declare that they have no competing interests.

References

1. Wang TS, Sosa JA. Thyroid surgery for differentiated thyroid cancer-recent advances and future directions. Nat Rev Endocrinol. 2018; 14:670–83.
2. Mackenzie EJ, MortiMer RH. Thyroid nodules and thyroid cancer. Med J Aust 2004; 180: 242-247.
3. Jung CK, Little MP, Lubin JH, Brenner AV, Wells SA Jr, Sigurdson AJ, Nikiforov YE. The increase in thyroid cancer incidence during the last four decades is accompanied by a high frequency of BRAF mutations and a sharp increase in RAS mutations. J Clin Endocrinol Metab. 2014; 99: E276–85.
4. Zhu J, Zhang Q, Jin XY, Cai JB et al. MiR-506 suppresses papillary thyroid carcinoma cell proliferation and metastasis via targeting IL17RD. Eur Rev Med Pharmacol Sci. 2019 Apr;23(7):2856-2862.
5. NiKiForova MN, ChiosEa SI, NiKiForov YE. MicroRNA expression profiles in thyroid tumors. Endocr Pathol 2009; 20: 85-91.
6. Veiga LH, Neta G, Gschebrook-KilfoY B, Ron E, Devesa SS. Thyroid cancer incidence patterns in Sao Paulo, Brazil, and the U.S. SEER program, 1997-2008. Thyroid 2013; 23: 748-757.
7. Frohlich E, Wahl R. The current role of targeted therapies to induce radioiodine uptake in thyroid cancer. Cancer Treat Rev 2014; 40: 665-674.
8. Nilsen TW, Graveley BR. Expansion of the eukaryotic proteome by alternative splicing. Nature (2010) 463:457–63. doi: 10.1038/nature08909
9. Singh B, Eyras E. The role of alternative splicing in cancer. Transcription (2017) 8:91–8.
10. Climente-Gonzalez H, Porta-Pardo E, Godzik A, Eyras E. The functional impact of alternative splicing in cancer. Cell Rep. (2017) 20:2215–26.
11. Oltean S, Bates DO. Hallmarks of alternative splicing in cancer. Oncogene

(2014) 33:5311–8.
12. Frankiw L, Baltimore D, Li G. Alternative mRNA splicing in cancer immunotherapy. Nat Rev Immunol. 2019 Jul 30.
13. Kahles A, Lehmann KV, Toussaint NC et al. Comprehensive Analysis of Alternative Splicing Across Tumors from 8,705 Patients. Cancer Cell. 2018 Aug 13;34(2):211-224.e6.
14. Zhang Y, Yan L, Zeng J et al. Pan-cancer analysis of clinical relevance of alternative splicing events in 31 human cancers. Oncogene. 2019 Aug 7.
15. Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, et al. Alternative isoform regulation in human tissue transcriptomes. Nature. 2008;456:470–6.
16. Liu J, Li H, Shen S, Sun L, Yuan Y and Xing C. Alternative splicing events implicated in carcinogenesis and prognosis of colorectal cancer. J Cancer 2018; 9: 1754-1764.
17. Lin P, He RQ, Ma FC, Liang L, He Y, Yang H, Dang YW and Chen G. Systematic analysis of survival-associated alternative splicing signatures in gastrointestinal pan-adenocarcinomas. EBioMedicine 2018; 34: 46-60.
18. Huang ZG, He RQ and Mo ZN. Prognostic value and potential function of splicing events in prostate adenocarcinoma. Int J Oncol 2018; 53: 2473-2487.
19. He RQ, Zhou XG, Yi QY, Deng CW, Gao JM, Chen G and Wang QY. Prognostic signature of alternative splicing events in bladder urothelial carcinoma based on spliceseq data from 317 cases. Cell Physiol Biochem 2018; 48: 1355- 1368
20. Xie ZC, Wu HY, Ma FC et al. Prognostic alternative splicing signatures and underlying regulatory network in esophageal carcinoma. Am J Transl Res. 2019 Jul 15;11(7):4010-4028. eCollection 2019.
21. Wang C, Zheng M, Wang S et al. Whole Genome Analysis and Prognostic Model Construction Based on Alternative Splicing Events in Endometrial Cancer. Biomed Res Int. 2019 Jul 2;2019:2686875.
22. Chen QF, Li W, Wu P et al. Alternative splicing events are prognostic in hepatocellular carcinoma. Aging (Albany NY). 2019 Jul 13;11(13):4720-4735.
23. Ryan M, Wong WC, Brown R, Akbani R, Su X, Broom B, et al. TCGASpliceSeq a compendium of alternative mRNA splicing in cancer. Nucleic Acids Res. (2015) 44:D1018–22.
24. He RQ, Zhou XG, Yi QY, Deng CW, Gao JM, Chen G and Wang QY. Prognostic signature of alternative splicing events in bladder urothelial carcinoma based on spliceseq data from 317 cases. Cell Physiol Biochem 2018; 48: 1355-1368
25. Conway JR, Lex A, Gehlenborg N. UpSetR: an R package for the visualization of intersecting sets and their properties. Bioinformatics. (2017) 33:2938– 40. Sauerbrei W, Royston P, Binder H. Selection of important variables and determination of functional form for continuous predictors in multivariable model building. Stat Med. 2007; 26:5512–28.
26. Hu X, Martinez-Ledesma E, Zheng S, Kim H, Barthel F, Jiang T, Hess KR,

Verhaak RG. Multigene signature for predicting prognosis of patients with 1p19q codeletion diffuse glioma. Neuro Oncol. 2017; 19:786–95.
27. Kozlovski I, Siegfried Z, Amar-Schwartz A, et al. The role of RNA alternative splicing in regulating cancer metabolism[J]. Human Genetics, 2017.
28. Wojtuszkiewicz A, Assaraf Y G, Maas M J, et al. Pre-mRNA splicing in cancer: the relevance in oncogenesis, treatment and drug resistance[J]. Expert Opinion on Drug Metabolism & Toxicology, 2015, 11(5):673-689.
29. Wang BD, Lee NH. Aberrant RNA Splicing in Cancer and Drug Resistance. Cancers (Basel). 2018 Nov 20;10(11). pii: E458.
30. Ghigna, C.; Giordano, S.; Shen, H.; Benvenuto, F.; Castiglioni, F.; Comoglio, P.M.; Green, M.R.; Riva, S.; Biamonti, G. Cell motility is controlled by SF2/ASF through alternative splicing of the Ron protooncogene. Mol. Cell 2005, 20, 881–890.
31. Karni, R.; de Stanchina, E.; Lowe, S.W.; Sinha, R.; Mu, D.; Krainer, A.R. The gene encoding the splicing factor SF2/ASF is a proto-oncogene. Nat. Struct. Mol. Biol. 2007, 14, 185–193.
32. Piqué L, Martinez de Paz A, Piñeyro D et al. Epigenetic inactivation of the splicing RNA-binding protein CELF2 in human breast cancer. Oncogene. 2019 Aug 13.
33. Duan Y, Zhang D. Identification of novel prognostic alternative splicing signature in papillary renal cell carcinoma. J Cell Biochem. 2019 Aug 12.
34. Takehara, T.; Liu, X.; Fujimoto, J.; Friedman, S.L.; Takahashi, H. Expression and role of Bcl-xL in human hepatocellular carcinomas. Hepatology 2001, 34, 55–61.
35. Mercatante, D.R.; Mohler, J.L.; Kole, R. Cellular response to an antisense-mediated shift of Bcl-x pre-mRNA splicing and antineoplastic agents. J. Biol. Chem. 2002, 277, 49374–49382.
36. Kutluay SB, Emery A, Penumutchu S et al. Genome wide analysis of hnRNP binding to HIV-1 RNA reveals a key role for hnRNP H1 in alternative viral mRNA splicing. J Virol. 2019 Aug 14. pii: JVI.01048-19.
37. Oka Y, Varmark H, Vitting-Seerup K, et al. UBL5 is essential for pre-mRNA splicing and sister chromatid cohesion in human cells[J]. EMBO reports, 2014, 15(9):956-964.
38. Xu F, Ackerley C, Maj MC et al. Disruption of a mitochondrial RNA-binding protein gene results in decreased cytochrome b expression and a marked reduction in ubiquinol-cytochrome c reductase activity in mouse heart mitochondria. Biochem J. 2008 Nov 15;416(1):15-26.

Figure legends:

Figure 1. a. Upset plots for the intersection of seven types of ASEs. The dark bar on the right of each drawing represents the amount of each type of ASEs. The dark dots in the matrix at bottom part of each drawing represent the intersections of AS events, while the dark bar on the top represents the gene number involving in AS. B, A subset of overlapping survival associated ASEs

among the seven types of AS in TC were illustrated by UpSet plot diagram.

Figure 2. Bubble plots for subgroup analyses of survival associated ASEs in TC cohort. (A–G) Forest plots of HRs for top 20 survival associated AA, AD, AP, AT, ES, ME and RI events in TC, respectively. (H) The vol plot survival associated AS events.

Figure 3. Cross validation with the LASSO Cox regression algorithm associated ASEs in TC cohort. (A–H) Cross validation plots of seven AS events AA, AD, AP, AT, ES, ME, RI and ALL events in TC.

Figure 4. Kaplan-Meier curves of prognostic predictors in TC cohort. (A–G) Kaplan-Meier plot depicting the survival probability over time for prognostic predictor of seven types of AS events with high (red) and low (blue) risk group, respectively. (H) Kaplan-Meier plot depicting the survival probability over time for the final prognostic predictor with high (red) and low (blue) risk group.

Figure 5: Construction of eight ASEs models for TC. (a–h) Risk scores for AA, AD, AP, AT, ES, ME, RI and ALL models in TC, respectively. Each individual plot (Top) represents the distribution of survival time and survival status of high- and low-risk groups. (middle) represents the distribution of patients in the high- and low-risk groups, (bottom) represents the PSI value heat map of the alternative splicing genes in the constructed model.

Figure 6. Cox regression analysis of OS-associated clinical features ALL ASEs. (A) Univariate analysis; (B) Multivariate analysis. (Gender, F vs M; Stage, I vs II, III, IV; T, T1 vs T2, T3, T4; M, M0 vs M1, Mx; N, N0 vs N1)

Figure 7. Correlation analysis between splicing factor expression and TC-ASEs. Triangles represent the splicing factors and oval nodes represent the TC-ASEs. Red ovals represent the TC-ASEs that displayed a positive correlation with OS while the green ovals represent OS-ASEs that exhibited a negative correlation with OS. The blue and red lines indicate negative and positive correlations, respectively.