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Systematic Profiling of Survival-Associated Alternative Splicing Events in Adrenocortical Carcinoma

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Abstract

Background: Aberrant alternative splicing (AS) is involved in many oncogenic processes and systematic analysis of survival-associated aberrant AS events has been reported in many cancers. This study aims to systematic profiling the AS signature in Adrenocortical carcinoma (ACC).

Methods: Data of ACC were downloaded from TCGA and TCGA SpliceSeq. Clinical information and AS events data were integrated with the same TCGA ID. Then, we performed univariate Cox analysis to identify survival-related AS events. Lasso regression and multivariate Cox analysis were used to establish prognostic model. In addition, several bioinformatics analyses were conducted to identify pathways enriched by genes of survival-associated AS events and construct splicing-factor-regulated network.

Results: A total of 77 patients with complete clinical information and PSI values of AS events were included in the present study. We detected 3781 AS events in 2366 genes were associated with overall survival of ACC patients. All the predictive models showed efficiency in distinguishing good and poor outcomes of ACC patients. All the AUCs of predictive models were greater than 0.7. Functional analysis genes with survival-associated AS events suggested that the POLR2H, TCEB2, PSMA1, PSMD11 and SKP2 ranked at the core. The splicing-factor-regulated network revealed the potential regulatory mechanisms of AS events in ACC.

Conclusions: Our systematic profiling of survival-associated AS events in ACC patients provides novel molecular alternations and contributes to decipher the underlying mechanisms of AS in oncogenesis of ACC.

Background

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy that originates in the cortex of the adrenal gland (estimated incidence, 0.7 ~ 2.0 cases per million persons) [1, 2] with rapid progress and poor prognosis. The 5-year survival rate is less than 50% for ACC with locally advanced disease and 15% for patients with distant metastases [3–4]. Complete surgical resection of the tumor is currently the only available curative treatment option for non-metastatic ACC [5] and mitotane [6, 7] plus other chemotherapy is recommended as the treatment for patients with advanced and inoperable ACC [8]. Such poor prognosis and deficient treatments make it is imperative to screen for tumor markers of ACC.

Recently, accumulating evidence has focused on the molecular diagnosis and prognosis of ACC. Systematic analyzing of genetic and epigenetic signatures, such as mutations [9], DNA methylation [10, 11], mRNA [12] and microRNA expression [13], has contributed to the clinical diagnosis and the discovery of potential biomarkers in the patients with ACC. However, these studies, although with promising achievements, mainly focus on alterations at gene expression level while ignoring the RNA isoform diversity from a single gene regulated by alternative splicing (AS).

AS is a process that selective removal or retention of exons and/or introns by different splicing patterns to generate distinct mRNA isoforms from a single gene [14, 15]. It is a pivotal step of post-transcriptional gene expression regulation and plays a vital role in expanding protein diversity in cells [16, 17]. High-throughput sequencing technology estimated that up to 90% of human genes undergo AS [18]. Accordingly, AS can produce multiple mRNA isoforms encoding proteins with structural and/or functional differences that can have profound biological consequences. Therefore, AS is not crucial for normal physiological processes such as hematopoiesis [19], brain development [20] and skeletal muscle function [21], but also for multiple pathological states, including tumorigenesis [22]. Aberrant AS is involved in many oncogenic processes, including uncontrolled cell proliferation, evading growth suppressors, invasion and metastasis, angiogenesis and immune escape [23–25]. More importantly, dysregulation of AS is a fundamental process in cancer and research shows that AS has emerging potential therapeutic targets and biomarkers in cancer therapy [16, 22, 26]. Systematic analysis of survival-associated aberrant AS events has been reported in gastrointestinal pan-adenocarcinomas [27], breast cancer [28], ovarian cancer [29] and lung cancer [30]. However, there is no comprehensive study on the clinical significance and prognostic value of AS in ACC. Here, we performed a systematic analysis of survival-associated aberrant AS in ACC using The Cancer Genome Atlas (TCGA) RNA-seq data and TCGA SpliceSeq PSI data and evaluated the potential functions in tumor biology.

Methods

Data collection and collation

RNA-seq data and matched clinical data of ACC cohort were downloaded from TCGA GDC data portal (https://portal.gdc.cancer.gov/repository). There are seven types of AS events, including alternate acceptor site (AA), alternate donor site (AD), alternate promoter (AP), alternate terminator (AT), exon skip (ES), mutually exclusive exons (ME), and retained intron (RI) [31]. To quantify AS events, percent spliced in (PSI) (ranging from zero to one) values for the AS events of ACC were used [18,32]. Seven splice event types with a PSI value of more than 75% in ACC samples were downloaded from TCGA SpliceSeq (https://bioinformatics.mdanderson.org/TCGASpliceSeq/PSI download.jsp).

When obtained the data of PSI values, we removed the AS events with mean value < 0.05 or standard diversion < 0.01. We integrated clinical information and AS events data with the same TCGA ID.

Survival analysis

A total of 77 ACC patients with overall survival (OS) >90 days were included in this study. UpSet, a visualization technique [33], was used to quantitative analysis of intersecting sets between different types of AS. We performed univariate Cox analysis on all of AS events to calculate the association between the PSI value of each AS events and the OS of ACC patients. Those AS events with P-values < 0.05 were identified as prognosis-related AS events. Then, the selected AS events were screened by the least absolute shrinkage and selection operator (Lasso) regression. Lasso regression is a statistical method that determines the best factors to use for prediction models [34,35] by using the “glmnet” and

"survival" packages in R. We established prediction model for ACC patients by using the multivariate Cox analysis with the "survival" package in R. To further assess the predictive accuracy and sensitivity of the prediction model, we performed the receiver operating characteristic (ROC) curves analysis with the survivalROC package in R. Univariate and multivariate Cox regression were performed to determine the splicing-based prognostic signature as an independent prognostic factor.

Bioinformatics analyses for the genes of survival-associated AS events

To explore the molecular characteristics for the genes with survival-associated AS events, we used "STRING" version 11.0 (http://string-db.org/cgi/input.pl) [36] to conduct bioinformatics analyses, which including protein-protein interaction (PPI), Gene ontology (GO), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

Splicing factors and AS regulatory network

To explore the regulation of splicing factors (SFs) on prognosis-related AS events, we obtained 404 SFs which was previously reported [37]. The level 3 mRNA-seq data of SFs genes were curated from the TCGA dataset. Univariate Cox analysis was performed on the prognosis-related AS events and SFs and then constructed the f SF-AS regulatory network according to the following conditions: P value less than 0.05 and Pearson correlation coefficient more than 0.65. The regulation network was plotted via Cytoscape version 3.7.1.

Statistical analysis

R version 3.6.1 was used for all statistical analysis. For all statistical methods, p values less than 0.05 were considered significant.

Results

General information of ACC patients in the TCGA cohort

As shown in **Table 1**, 92 tumors with clinical information was downloaded from TCGA. In our study cohort, the median age at diagnosis was 47.16 years old, patient age ranged from 14 year to 83 years. The overall female-to-male ratio was 3:1.6. Most patients were white (84.8%). Stage I disease was found in 9 patients (9.8%), stage II in 44 (47.8%), stage III in 19 (20.7%), stage IV in 18 (19.6%). ACC was the first diagnosed malignancy in 86 patients.

Overview of AS events and survival associated AS events in TCGA ACC cohort

Comprehensive AS events in seven splicing types, including AA, AD, AP, AT, ES, ME, and RI were summarized for ACC. A total of 34,420 AS events in 8994 genes were detected from TCGA SpliceSeq dataset, indicating that one gene may undergo multiple AS events simultaneously. In detail, 2707 AAs in

1960 genes, 2382 ADs in 1688 genes, 6342 APs in 2575 genes, 8201 ATs in 3575 genes, 12,269 ESs in 5337 genes, 124 MEs in 122 genes and 2395 RIs in 1605 genes (**Fig. 1A**).

Univariate Cox analysis revealed that 3781 AS events in 2366 genes were associated with ACC OS rates (P<0.05). In detail, as shown in **Fig. 1B**, 199 AAs in 186 genes, 248 ADs in 221 genes, 679 APs in 423 genes, 1224 ATs in 724 genes, 1184 ESs in 937 genes, 8 MEs in 8 genes and 239 RIs in 209 genes were identified as survival-associated AS events. The UpSet plot (**Fig. 1C**) vividly revealed that one gene could undergo multiple AS events simultaneously.

Molecular characteristics of survival-associated AS events

The distributions of AS events significantly associated with patient survival are shown in **Fig. 2A**. We displayed the top 20 (if available) most significant survival-associated AS events for each AS type in **Fig. 2B-H**. To explore the molecular characteristics of genes with the top 50 most significant survival-associated AS events (if available), we performed several bioinformatics analyses. Firstly, we established a PPI network to reveal the relationships among these genes. As shown in **Fig. 3A-B**, POLR2H, TCEB2, PSMA1, PSMD11 and SKP2 ranked at the core in the network. According to the functional enrichments of these genes, “intracellular membrane-bounded organelle”, “mitochondrion”, “membrane-bounded organelle”, “organelle part” and “intracellular organelle part” were the five most significant cellular component terms (GO) (**Fig. 4A**). For biological process terms (GO), “metabolic process”, “cellular metabolic process”, “nitrogen compound metabolic process”, “intracellular transport” and “organic substance metabolic process” were the five most significant enrichments (**Fig. 4B**). There were no significant pathway enrichments observed in molecular function (GO). Finally, we observed that “Thermogenesis” was the only significant pathway (FDR=0.032) correlated with these genes in KEGG pathway analysis.

Prognostic predictors for ACC patients

We used the Lasso regression and multivariate Cox regression analysis to generate prognostic models (PMs) for seven AS types and for all types: PM-AA, PM-AD, PM-AP, PM-AT, PM-ES, PM-ME, PM-RI, and PM-ALL (**Fig. 5 and Table 2**) following univariate Cox. Then, we divided ACC patients into low and high risk groups based on median values to analyze the efficacy of prognostic models by using Kaplan-Meier (K-M) method. As shown in **Fig. 6A-H**, all the prognostic models could predict good and poor outcomes of ACC patients. ROC curves validated the efficiency of these prognostic models (**Fig. 6I**). To further elucidate the independent prognostic significance of PM-ALL, univariate and multivariate Cox regression analyses were performed. After adjusting for the clinical factors, the PM-ALL remained an independent prognostic factor for ACC patients, with an HR of 1.012 (95%CI: 1.003-1.020, P=0.007) (**Table 3**).

Network of survival-associated AS genes and SFs expression

SFs are RNA-binding proteins that recognize cis-regulatory elements within the pre-mRNA to influence exon selection and splice site choice (38). SF alternations are a hallmark of cancer. Therefore, we

explored the interaction networks of survival-associated AS genes and SFs. Firstly, we found 20 SFs related to survival that could be used as independent prognostic factors by using K-M method and Cox regression analysis (**Table 4**). Next, correlation analyses between the expression of these 20 SFs and the PSI values of survival-associated AS events were performed by using Pearson's correlation analysis (cor > 0.6, P < 0.001). Correlation plots were then generated using Cytoscape 3.7.1. The results showed that the expression of 19 survival-related SFs (triangular nodes) were correlated with 206 survival-associated AS events (**Fig. 7**). Overall, 97 AS events were correlated with favorable OS (rea ovals) and 109 AS events were correlated with poor OS (green ovals).

Discussion

Aberrant AS is involved in the development process of cancer [23–25]. TCGA RNA sequencing data and TCGA SpliceSeq data have enabled investigation of AS patterns in many different kinds of cancers, such as breast cancer (28), ovarian cancer [29]. Growing evidence has shown that AS has emerging potential therapeutic targets and biomarkers in cancer therapy [16, 22, 26]. However, comprehensive information concerning dysregulation AS in ACC, which is an endocrine malignancy with rapid progress and poor prognosis and lacks selective and efficacious treatment options, is lacking.

In this study, we first found that 34,420 AS events in 8994 genes in the TCGA ACC cohort, among which 3781 AS events in 2366 genes were related to survival (P < 0.05). Among these survival-associated AS events, some splice variants may play critical roles in oncogenic processes, such as the AA variant of ZFAND6, the AD variant of ZSCAN18, the AP variant of CMC2, the ES variant of CIRBP, the ME variant of THNSL2 and the RI variant of CIRBP. Many splicing isoforms of these genes have been found to be related to survival in various types of tumors, such as papillary thyroid cancer [39], ovarian cancer [29], esophageal adenocarcinoma and esophageal squamous cell carcinoma [40].

Next, given the molecular function of AS events is partly described by the downstream functional impact, we conducted PPI network analysis. We found POLR2H, TCEB2, PSMA1, PSMD11 and SKP2 ranked at the core in the network. As THE HUAMAN GENE DATABASE GeneCards displays that POLR2H encodes an essential and highly conserved subunit of RNA polymerase II that is shared by the other two eukaryotic DNA-directed RNA polymerases, I and III and alternative splicing of POLR2H generates multiple transcript variants. To our knowledge the related study of POLR2H was rare, but it has been shown to be associated with the occurrence and progression of prostate cancer [41]. TCEB2 (also known Elongin B, ELOB) encodes the protein elongin B, which is a subunit of the transcription factor B (SIII) [42, 43]. Deng et al. reported that TCEB2 Confers Resistance to VEGF-targeted Therapy in ovarian cancer [44]. As many problems of ACC are still unresolved, such as disease prevention and earlier detection, these new discovered core genes may provide new insights for us.

In addition, we proposed the predictive model for each splice type and all available splice types of AS events using Lasso regression and multivariate Cox regression. All the predictive models showed efficiency in distinguishing good and poor outcomes of ACC patients. All the AUCs of predictive models

were greater than 0.7, suggesting good power and potential in application of prognosis prediction for ACC patients. However, the efficiency should be verified by another independent cohort.

Alterations in activity of regulatory SFs are an important mechanism of aberrant AS in cancer [45]. Therefore, we identified 20 survival-associated SFs in ACC patients, such as KHSRP, SRSF7, SRSF2 and HNRNPA2B1. Serine/arginine (SR) proteins and heterogenous ribonuclear proteins (hnRNPs) are the two key families of SFs. Many studies have confirmed that SR and hnRNPs involve in tumorigenesis, such as isoforms PKM2 or TP53β of SRSF3 alters cell metabolism and induces cellular senescence [46, 47]. SRSF7 is upregulated in lung cancer, and its knockdown impacts cell proliferation [48]. In glioblastoma, hnRNPA2/B1 mediates its tumorigenic effect through alternative splicing of key oncogenes and tumor suppressors [49]. Moreover, splicing correlation network revealed that multiple AS events were correlated with SFs expression in ACC. These findings might provide new insight into the mechanisms underlying the development and progression of ACC.

Conclusions

The current study conducted a comprehensive analysis base of survival-associated AS events in ACC patients, which might contribute to uncover the function of AS events in ACC. Moreover, the identification of prognostic SFs and construction of the correlation networks between SFs and AS events will pave the way for in-depth exploration of splicing-related mechanisms in the oncogenic process of ACC. This comprehensive analysis AS events and SFs also provided valuable therapeutic targets that require further validation.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of the Affiliated Hospital of Southwest Medical University, and was performed following the TCGA publication guidelines.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YX, XF and QW conceived and designed the study. XF, WQ, FT, MG and ZJ collected the data. XF, QW, YL and XT performed the analysis and graphics. XF, QW, YL, FT, XT, MG and ZJ wrote the manuscript. YX revised the manuscript. All authors have read and approved the final manuscript.

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Abbreviations

AS, Alternative splicing; ACC, Adrenocortical carcinoma; PSI, Percent spliced in; AA, Alternate acceptor; AD, Alternate donor; AP, Alternate promoter; AT, Alternate terminator; ES, Exon skip; ME, Exclusive exons; RI, Retained intron; ROC, receiver operating characteristic; PPI, protein-protein interaction; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; SFs, splicing factors; PM, Prognostic models.

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Tables

Table 1. General characteristics of ACC patients in TCGA Cohort

Clinical characteristics		Total (92)	Percentage (%)
Age at diagnosis (y)		47.16 (14-83)	
Gender	Female	60	65.2
	Male	32	34.8
Race	White	78	84.8
	Asian	2	2.2
	Black or AA	1	1.1
	Unknow	11	12
T	T1	9	9.8
	T2	49	53.3
	T3	11	12
	T4	21	22.8
	Unknow	2	2.2
N	N0	80	87
	N1	10	10.9
	Unknow	2	2.2
M	M0	72	78.3
	M1	18	19.6
	Unknow	2	2.2
Stage	I	9	9.8
	II	44	47.8
	III	19	20.7
	IV	18	19.6
	Unknow	2	2.2
Prior_malignancy	Yes	6	6.5
	No	86	93.5
Synchronous_malignancy	No	86	93.5
	Unknow	6	6.5
Treatment_type	Chemotherapy	53	57.6
	Radiotherapy	39	42.4

AA, African American; ACC, adrenocortical carcinoma.

Table 2. Prognostic models for Adrenocortical Carcinoma

Type	Gene	Exon	Coef	HR	Lower	Upper	P-value
AA	MED11	3.1	13.07661	477637.8	222.0287	1.03E+09	0.000838
	ZNF692	3.1:3.2:3.3	-2.08471	0.124343	0.015923	0.971023	0.046811
	WASH4P	3.1	-14.8861	3.43E-07	1.10E-09	0.000107	3.82E-07
	STRADA	11.1	-30.2268	7.46E-14	8.27E-24	0.000673	0.009753
	RHOC	2.2	-26.7194	2.49E-12	4.02E-22	0.015405	0.020194
	CIRBP	9.5:9.6:9.7	-9.07659	0.000114	4.86E-07	0.026889	0.001123
	CERS5	6.1	-24.9959	1.39E-11	8.70E-19	0.000224	0.003147
	HMGA2	8.1	-12.4732	3.83E-06	6.14E-10	0.023871	0.005146
AD	TRAFD1	1.2	8.258912	3861.89	9.012561	1654823	0.007562
	BEX2	1.2	-19.1183	4.98E-09	5.85E-18	4.233694	0.068393
	NOP2	1.2	2.479085	11.93034	0.469043	303.4542	0.133239
	PRKAG1	1.2	6.649182	772.1521	1.865698	319568.8	0.030555
	RPS6	1.2:1.3:1.4	17.27027	31650541	4060.882	2.47E+11	0.000159
	SLC35F5	1.2	10.7926	48659.2	151.0658	15673415	0.000249
	ARMC6	1.2	13.20319	542091.2	625.385	4.7E+08	0.000131
	ABCC5	7.2:7.3:7.4	-2.42907	0.088119	0.013187	0.588852	0.012197
ALL	CIRBP	7.2:7.3	13.73552	923122.2	0.053504	1.59E+13	0.106187
	BLOC1S1	1	-25.1966	1.14E-11	4.99E-20	0.002607	0.010293
	TRAFD1	1.2	6.282554	535.1535	1.979789	144656.5	0.027876
	METTL15	12.3	-22.7632	1.30E-10	1.31E-18	0.012921	0.015399
	HM13	11:12.1:12.2	-2.0453	0.129341	0.010183	1.642906	0.114764
AP	DUT	2.1	-5.52697	0.003978	8.55E-05	0.18519	0.004794
	PGRMC2	2.1	6.215016	500.2041	30.45824	8214.66	1.35E-05
	PSMG3	3.1	11.17208	71116.75	597.7077	8461648	4.61E-06
	PSMA1	3.1	-21.7079	3.74E-10	8.66E-18	0.016109	0.01551
AT	METTL15	12.3	-29.3332	1.82E-13	1.85E-20	1.79E-06	0.000356
	DNAJC12	5.2	9.264801	10559.71	30.50774	3655053	0.001898
	USP4	7	16.0561	9398832	334.0949	2.64E+11	0.002128
	KLHL3	11	2.259391	9.577251	0.548639	167.1842	0.121496
ES	STOML1	8.2	-9.32077	8.95E-05	4.10E-07	0.019558	0.000695
	C1RL	2	-28.7786	3.17E-13	4.26E-19	2.37E-07	3.03E-05
	USMG5	2.2	35.87414	3.8E+15	2778089	5.20E+24	0.000831
	GGCX	2	-9.15251	0.000106	4.89E-07	0.022977	0.000854
	MMAA	5	-21.7097	3.73E-10	5.03E-14	2.76E-06	1.80E-06
	PSEN2	11	-23.5099	6.16E-11	1.92E-20	0.198095	0.035298
ME	THNSL2	9|10					

RI	EIF6	2.3	17.87569	57984965	83.73439	4.02E+13	0.00918
	TST	1.2	20.13454	5.55E+08	8768.056	3.51E+13	0.000358
	PILRB	8.4	-4.27109	0.013966	0.000379	0.514864	0.020305

AA, Alternate acceptor site; AD, Alternate donor site; AP, Alternate promoter; AT, Alternate terminator; ES, Exon skip; ME, Mutually exclusive exons; RI, Retained intron.

Table 3. Univariate and multivariate Cox analysis for PM-ALL

	Univariate			P-value	Multivariate			P-value
Clinical factors	HR	95%CI			HR	95%CI		
		Lower	Upper			Lower	Upper	
Age	1.368	0.618	3.026	0.440	1.513	0.566	4.047	0.409
Gender	0.963	0.425	2.180	0.928	0.815	0.313	2.124	0.675
Stage	2.886	1.819	4.579	0.000	0.778	0.154	3.927	0.762
T	3.349	2.075	5.403	0.000	3.911	1.422	10.754	0.008
M	6.338	2.741	14.657	0.000	1.237	0.185	8.273	0.827
N	2.088	0.781	5.578	0.142	2.388	0.639	8.931	0.196
Treatment_type	1.229	0.557	2.715	0.609	0.863	0.359	2.076	0.743
RiskScore	1.014	1.007	1.021	0.000	1.012	1.003	1.020	0.007

PM, Prognostic model.

Table 4. Survival-associated SFs in ACC

Gene Name	HR	HR.95L	HR.95H	P-value
KHSRP	1.106488	1.059608	1.155441	4.62E-06
SRSF7	1.251807	1.124576	1.393432	4.01E-05
SRSF3	1.178449	1.084695	1.280308	0.000104
ILF3	1.134072	1.060237	1.213049	0.000249
HNRNPA2B1	1.058154	1.024804	1.092589	0.000541
HNRNPH1	1.161704	1.063532	1.268938	0.000877
PNN	1.320241	1.118295	1.558656	0.001038
KHDRBS1	1.082557	1.02699	1.141131	0.003172
DDX39A	1.061124	1.019533	1.104411	0.003635
CLASRP	1.348143	1.101165	1.650515	0.003812
HNRNPR	1.232519	1.069703	1.420117	0.003827
HNRNPA1	1.02519	1.007966	1.042708	0.004005
DDX50	1.242495	1.070727	1.441819	0.004234
SRRT	1.150545	1.0451	1.266629	0.004244
DHX9	1.175994	1.047023	1.32085	0.006233
SRSF2	1.078669	1.020899	1.139708	0.007008
SNRPD1	1.197308	1.049665	1.365718	0.007322
SNRPE	1.056598	1.014811	1.100106	0.007493
ZC3H11A	1.27433	1.062298	1.528683	0.009032
ILF2	1.021163	1.005041	1.037543	0.009899

Figures

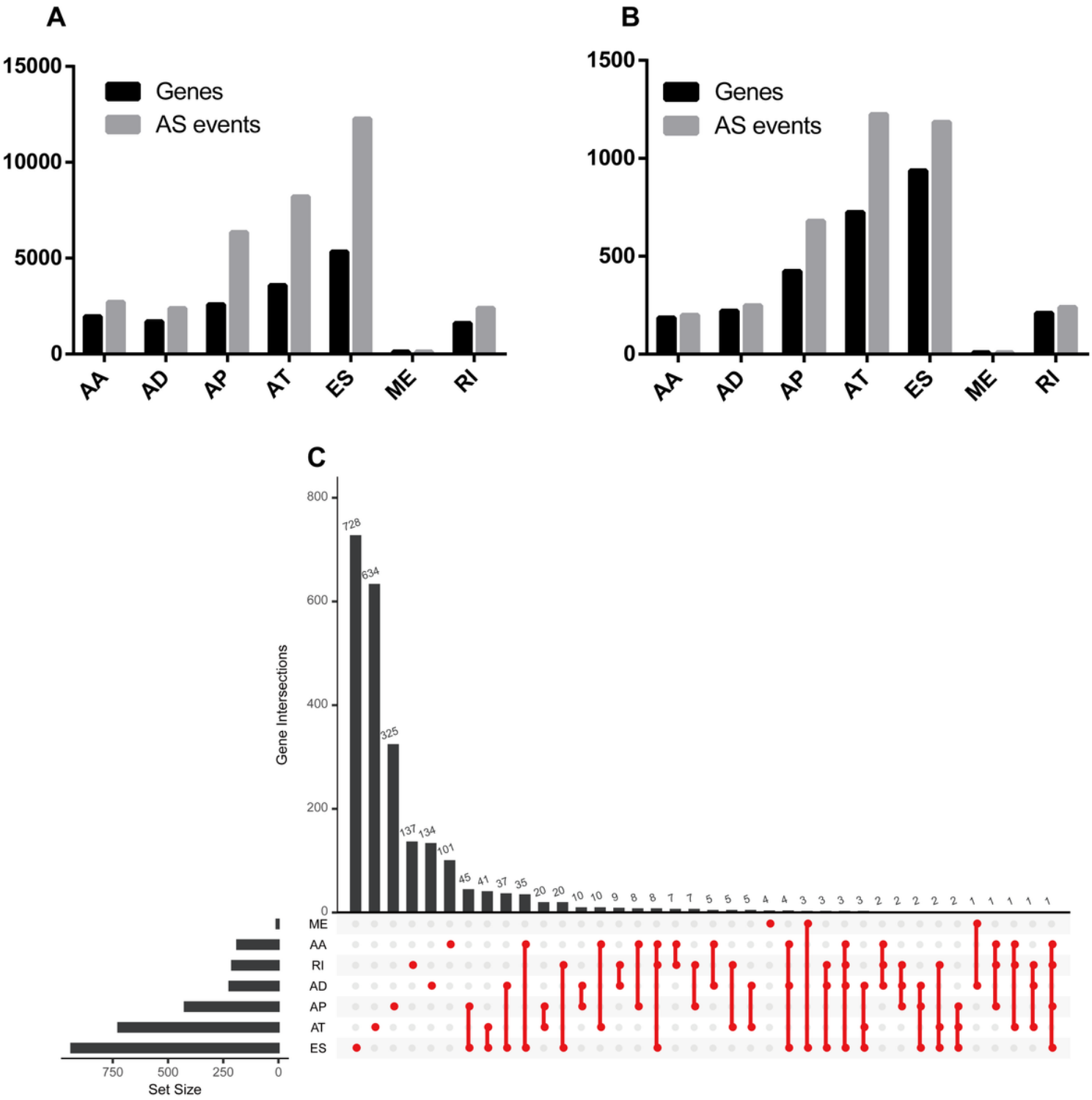

Figure 1

Overview of AS events in ACC. A: Number of AS events and corresponding genes; B: Number of survival-associated AS events and involved genes; C: UpSet plot in ACC, showing the interactions among the seven types of survival-associated AS events. One gene could undergo more than one type of AS events. AS: Alternative splicing; ACC, Adrenocortical carcinoma; AA, Alternate acceptor site; AD, Alternate donor site; AP, Alternate promoter; AT, Alternate terminator; ES, Exon skip; ME, Mutually exclusive exons; RI, Retained intron.

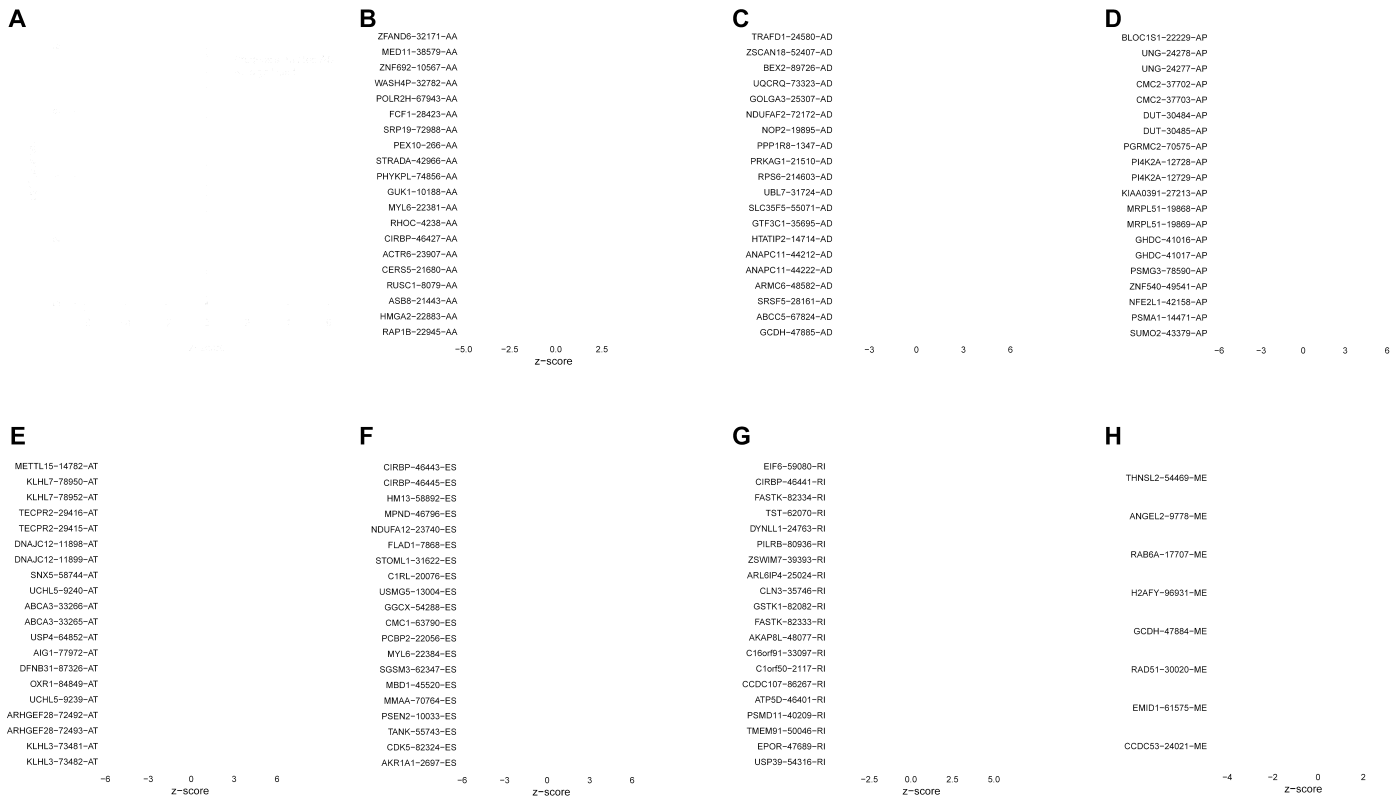

Figure 2

Top 20 (if available) most significant survival associated AS events in ACC. A: Volcano map of AS events associated with patient survival. B-G: The top 20 survival-associated AS events for AA, AD, AP, AT, ES and RI splicing types. H: The whole survival-related AS events for ME splicing type.

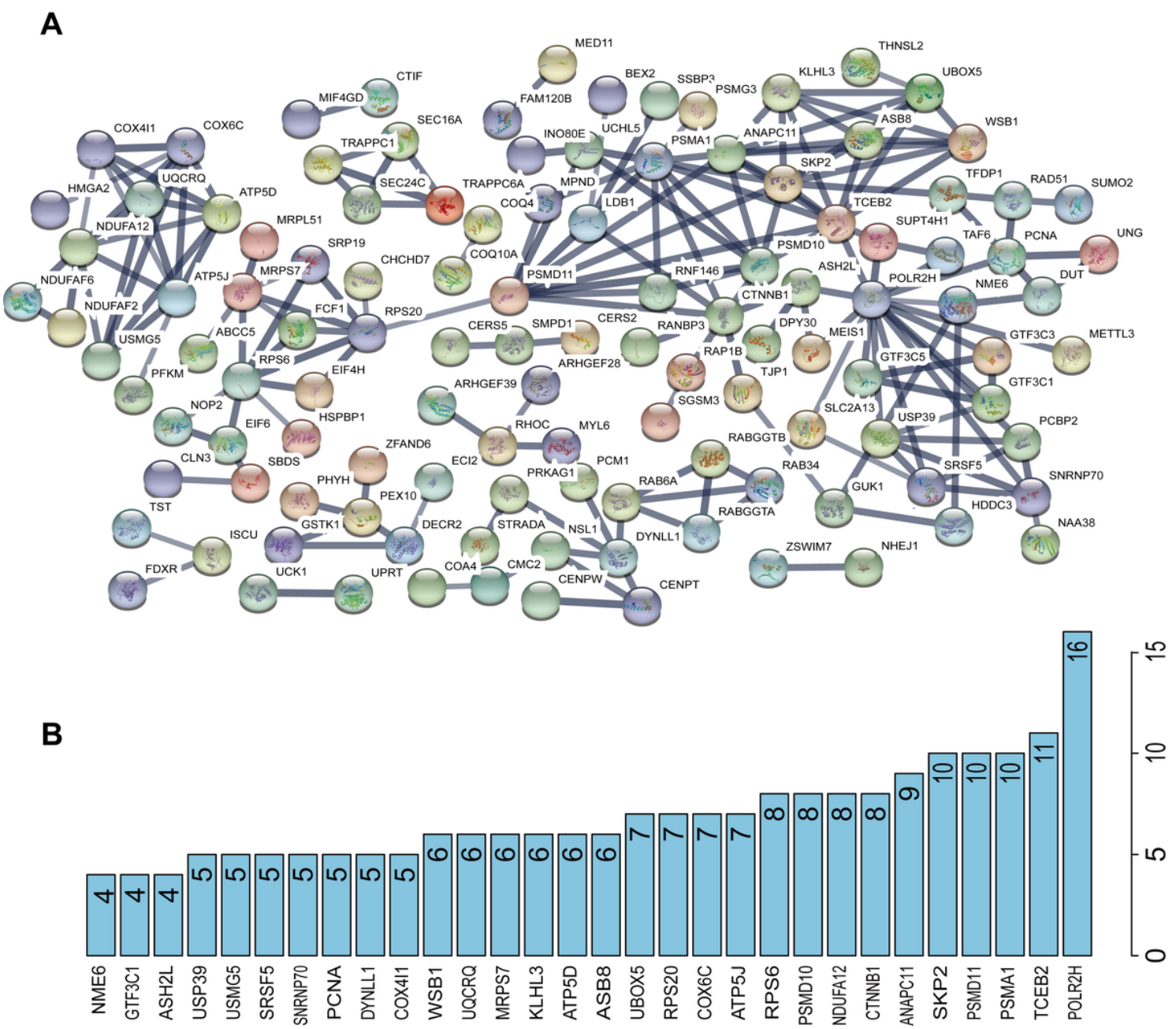

Figure 3

The PPI network analysis of genes with survival-related AS events in ACC. A: The PPI network; B: The hub nodes in the PPI network. The top 30 hub nodes are displayed. PPI: Protein-protein interaction.

A

term ID	Cellular component	FDR	Gene count
GO:0043231	intracellular membrane-bounded organelle	2.89E-08	178
GO:0005739	mitochondrion	9.49E-08	50
GO:0043227	membrane-bounded organelle	1.47E-06	182
GO:0044422	organelle part	1.47E-06	157
GO:0044446	intracellular organelle part	1.47E-06	154
GO:0044428	nuclear part	2.04E-06	92
GO:0070013	intracellular organelle lumen	2.04E-06	104
GO:0005634	nucleus	4.07E-06	126
GO:0044429	mitochondrial part	9.87E-06	34
GO:0005654	nucleoplasm	1.50E-05	75
GO:0032991	protein-containing complex	1.50E-05	95
GO:0031981	nuclear lumen	2.44E-05	83
GO:0043229	intracellular organelle	4.09E-05	186
GO:0044424	intracellular part	4.27E-05	205
GO:0005730	nucleolus	6.93E-05	30
GO:0043226	organelle	0.00011	187
GO:0005759	mitochondrial matrix	0.00074	18
GO:0044444	cytoplasmic part	0.00074	148
GO:0031967	organelle envelope	0.0011	31
GO:0005740	mitochondrial envelope	0.0029	22
GO:0000127	transcription factor TFIIIC complex	0.0031	3
GO:0005829	cytosol	0.0038	87
GO:0044451	nucleoplasm part	0.0088	27
GO:0005737	cytoplasm	0.0127	164
GO:0005743	mitochondrial inner membrane	0.0132	15
GO:1902494	catalytic complex	0.0144	30
GO:1990234	transferase complex	0.0153	20
GO:0044798	nuclear transcription factor complex	0.0229	8
GO:0044439	peroxisomal part	0.0256	6
GO:0005968	Rab-protein geranylgeranyltransferase complex	0.0329	2
GO:0031966	mitochondrial membrane	0.0343	18
GO:0044464	cell part	0.0364	217
GO:0005753	mitochondrial proton-transporting ATP synthase complex	0.0414	3

B

term ID	Biological process	FDR	Gene count
GO:0008152	metabolic process	0.00039	159
GO:0044237	cellular metabolic process	0.00039	149
GO:0006807	nitrogen compound metabolic process	0.0033	139
GO:0046907	intracellular transport	0.0037	38
GO:0071704	organic substance metabolic process	0.0037	148
GO:0044238	primary metabolic process	0.0083	142
GO:0044248	cellular catabolic process	0.0083	41
GO:0034641	cellular nitrogen compound metabolic process	0.0169	92
GO:1901564	organonitrogen compound metabolic process	0.0169	94
GO:0034622	cellular protein-containing complex assembly	0.0172	25
GO:0065003	protein-containing complex assembly	0.0194	37
GO:0007031	peroxisome organization	0.0237	7
GO:0042791	5S class rRNA transcription by RNA polymerase III	0.0331	3
GO:0042797	tRNA transcription by RNA polymerase III	0.0331	3
GO:0051726	regulation of cell cycle	0.0395	29
GO:1901575	organic substance catabolic process	0.0395	37
GO:0006625	protein targeting to peroxisome	0.0467	6
GO:0006886	intracellular protein transport	0.0467	23
GO:0022618	ribonucleoprotein complex assembly	0.0467	10
GO:0043933	protein-containing complex subunit organization	0.0467	39
GO:0070647	protein modification by small protein conjugation or removal	0.0467	25

Figure 4

GO analysis of genes with survival-related AS events in ACC. A: Cellular component; B: Biological process. GO: Gene ontology.

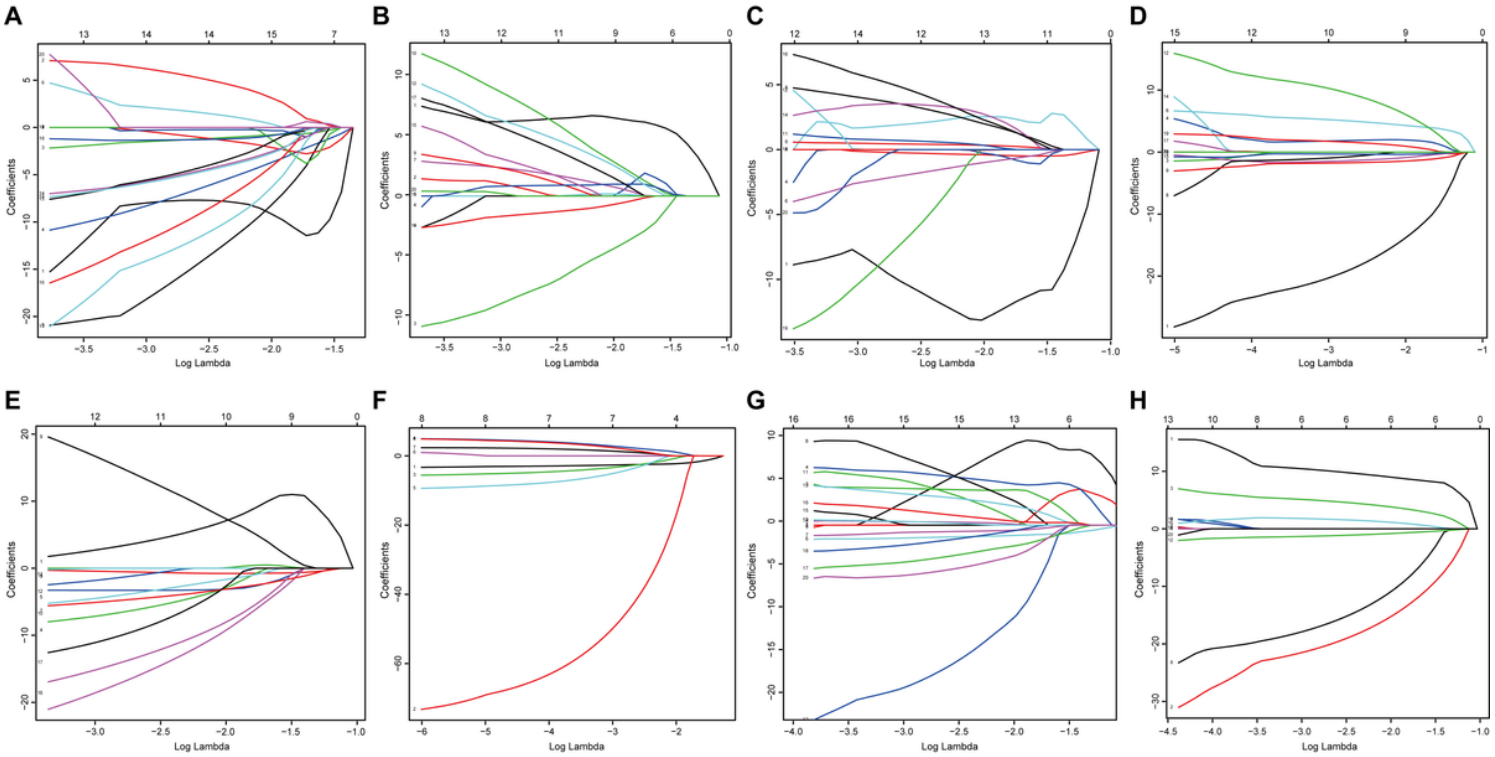

Figure 5

Lasso regression analysis for seven splicing types and all splicing types. A-H: AA, AD, AP, AT, ES, ME, RI, and all splicing types.

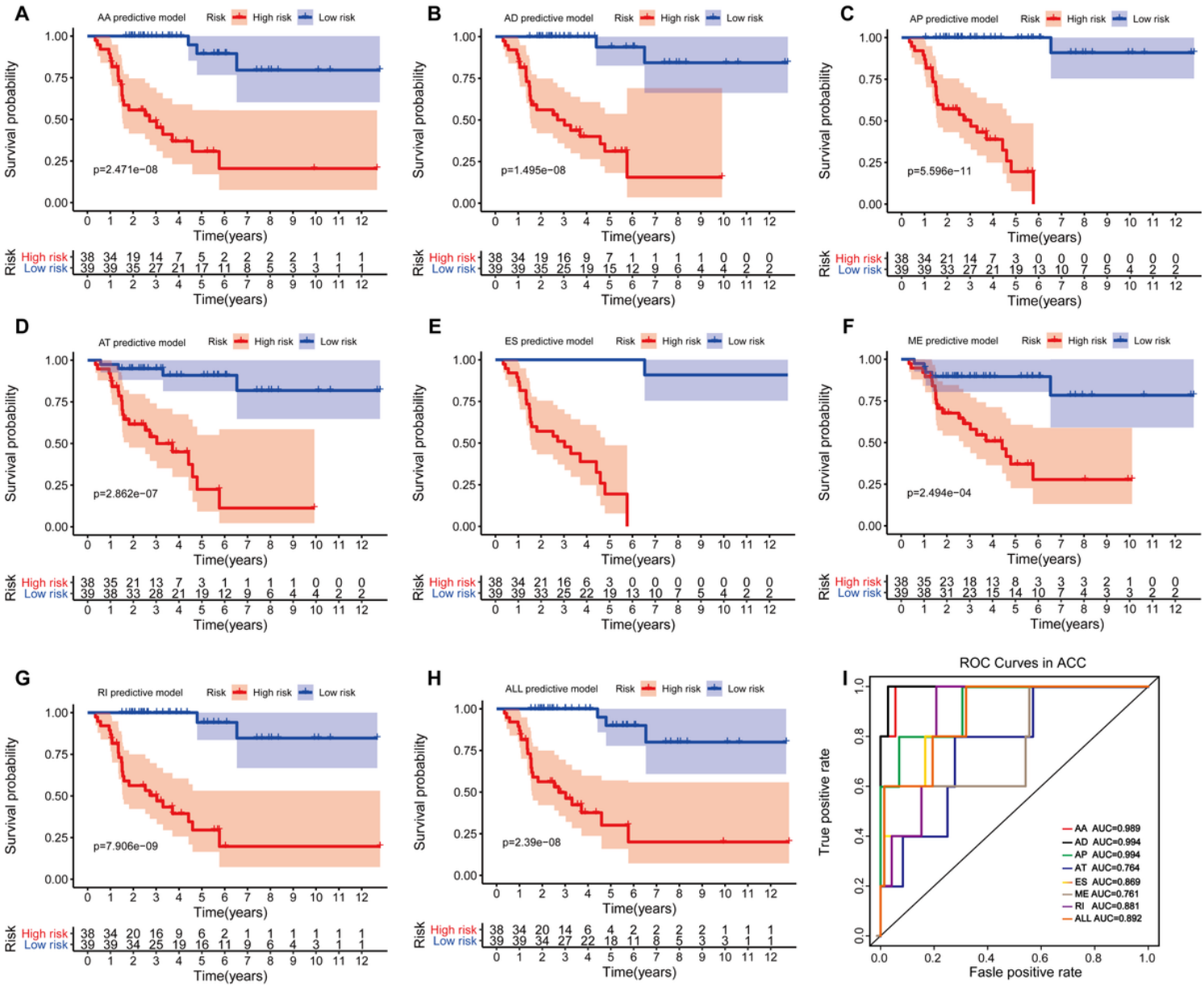

Figure 6

Kaplan-Meier and ROC curves of the eight prognostic models for ACC. A-H: Kaplan-Meier curves for each prognostic model. Red line indicates high-risk group whereas green line indicates low-risk group. I: The ROC curves of each predictive model.

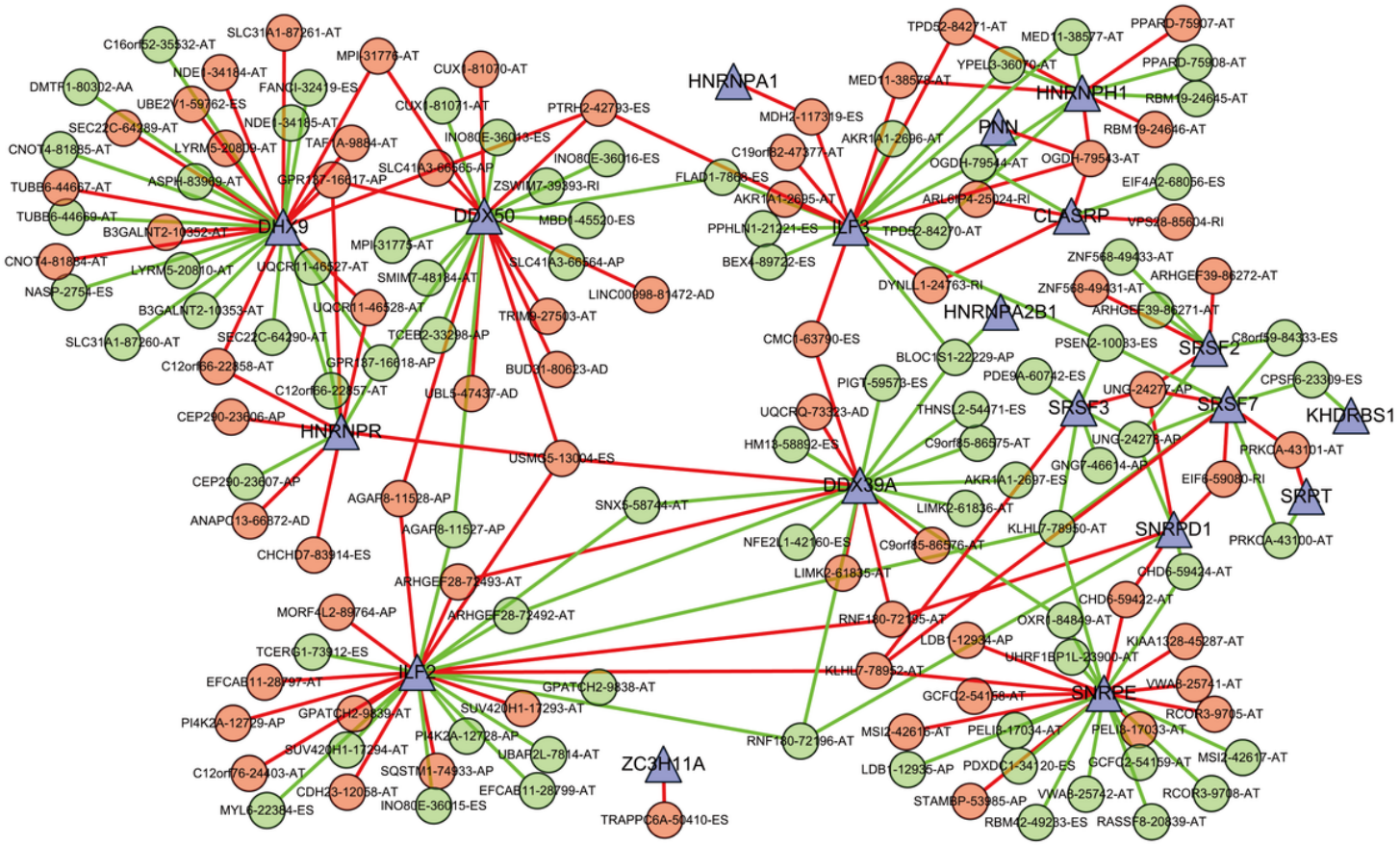

Figure 7

Correlation network of survival-associated SFs and AS events in ACC. Survival-associated SFs (Triangles) were positively (red lines) or negatively (green lines) associated with the PSI value of adverse prognosis AS events (red dots) or favorable prognosis AS events (green dots).