

Original Article

Survival associated alternative splicing events in diffuse large B-cell lymphoma

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Abstract: Growing evidence has revealed that the initiation of various malignancies is closely associated with alternative splicing (AS) events in certain key oncogenes. However, in diffuse large B-cell lymphoma (DLBCL), there is still a great deal to learn about AS variants. In this study, 33,724 AS variant profiles were obtained from 16,278 genes in 48 DLBCL cases. A total of 10 AS variants were identified as overall survival (OS)- related events via multivariate Cox regression analysis. Notably, alternative donor (AD) sites in AS events in the low-risk group showed a significantly better outcome in DLBCL patients than in the high-risk group ($P=0.0002$). The area under the curve (AUC) of the receiver-operator characteristic curve (ROC) for ADs in DLBCL was 0.746. Furthermore, 66 related splicing factors were obtained to investigate their potential correlations with AS events. Factors SF1, HNRNPC, HNRNPD, and HNRNPH3 were significantly involved in different OS-related AS variants. Collectively, we constructed valuable prognostic predictors for DLBCL patients and mapped novel splicing networks for further investigation of the underlying mechanisms related to AS variants in DLBCLs.

Keywords: Alternative splicing, diffuse large B-cell lymphoma, prognosis, correlation network

Introduction

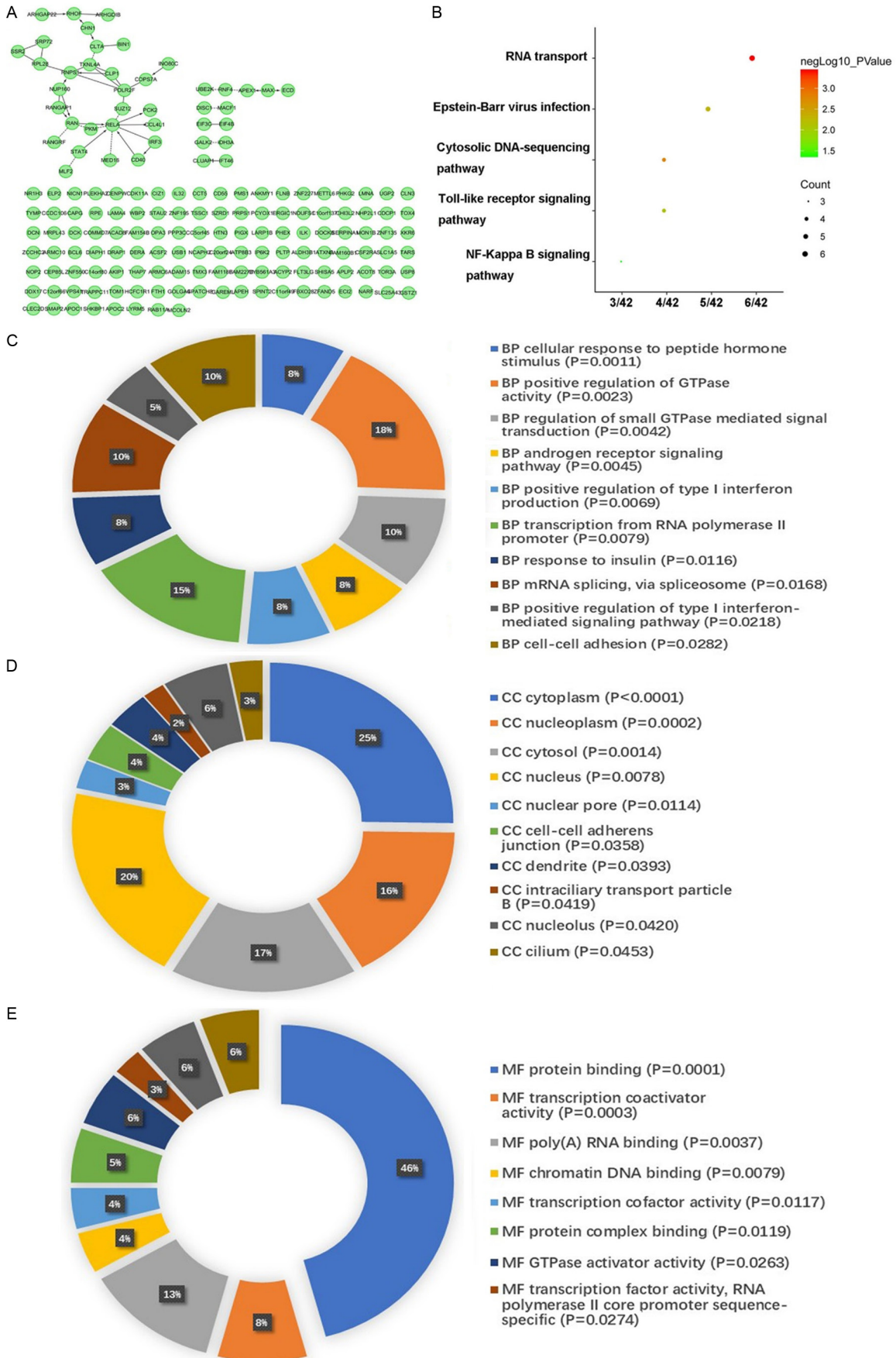
Alternative splicing (AS) is a spatiotemporal and pivotal post-transcriptional process. AS determinatively affects over 90% of multi-exon human genes and is important to such mechanisms as cell functions, cell differentiation, organogenesis [1-3]. A single gene can generate diverse mRNA isoforms through exon skipping, the use of alternative splicing sites, and intron retention [4-6]. The AS process can also enhance the diversity of both proteomes and transcriptomes [7, 8].

In 2018, the estimated new cases and deaths from non-Hodgkin lymphoma ranked 7th and 9th, respectively, in both sexes [9]. As the most common subtype, diffuse large B-cell lymphoma (DLBCL) accounts for 30%-40% of non-Hodgkin lymphomas in adults [10-14]. Currently, prognoses for DLBCL patients can be slightly improved through the use of rituximab and a

regimen consisting of a combination of cyclophosphamide, doxorubicin (hydroxydaunomycin), vincristine (Oncovin[®]), and prednisolone (a steroid) (CHOP) [15, 16]. However, approximately 30% of advanced stage DLBCL patients are still refractory to standard chemotherapy. Almost 50% of relapsed cases fail to respond to high-dose chemotherapy and a great majority of relapsed patients die of lymphoma [17-19]. Thus, to improve prognoses, there is an urgent need for novel treatments that go beyond simple chemotherapy.

The International Prognostic Index developed a prognostic model of DLBCL based on several clinical parameters without molecular insights. However, in DLBCL patients, a growing body of evidence has identified various biological molecular markers and gene signatures that are correlated with prognostic significance. Studies have demonstrated that ICT1, Mad2, and decreased LMR could be unfavorable prog-

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Alternative splicing variants in DLBCL

Figure 2. Correlation network and enrichment analysis of OS-correlated genes. A. Interacting network of OS-related genes. B. KEGG enrichment analysis of OS-related genes. C. Biological process enrichment analysis of OS-related genes. D. Cellular component enrichment analysis of OS-related genes. E. Molecular function enrichment analysis of OS-related genes.

Table 1. The ten AS events in the final prognostic model

| Splice type | Gene symbol | AS ID | Exons | From exon | To exon | Hazard ratio | P-value |
|-------------|-------------|-------|-------|-----------|---------|--------------|---------|
| AA | EIF3C | 35828 | 5.1 | 4 | 5.2 | 1.08E-13 | 0.0014 |
| | TYMP | 62854 | 2.2 | 1 | 2.3 | 1.2554 | 0.0031 |
| AD | TOX4 | 26588 | 3.2 | 3.1 | 5.1 | 15.1828 | 0.0044 |
| | USB1 | 36628 | 3.2 | 3.1 | 4 | 0.0093 | 0.0043 |
| AP | NHP2L1 | 62447 | 1 | Null | Null | 7.2020 | 0.0213 |
| | MLF2 | 19962 | 2 | Null | Null | 0.5920 | 0.0016 |
| AT | STAT4 | 56602 | 26 | Null | Null | 0.7468 | 0.0032 |
| | POLR2F | 62182 | 11 | Null | Null | 15.5894 | 0.0004 |
| ME | COX16 | 28177 | 2 3 | 1 | 4 | 1.51E-09 | 0.0410 |
| | SS18 | 94799 | 5 6 | 3 | 9 | 1.0407 | 0.0235 |

using ImageGP (<http://www.ehbio.com/ImageGP/>).

Survival analysis

The clinical parameters of 48 DLBCL patients were obtained from TCGA database. A total of 44 DLBCL patients with an overall survival (OS) time of more than 90 days were finally included in the study. To assess the association between AS events and OS, univariate Cox regression was carried out. Multivariate Cox regression was performed to select the independent prognostic factors (SPSS, v 22.0). The survival ROC package in R (v 3.2.4) was applied to estimate the significance of the prognostic predictors in DLBCL via generating receiver-operator characteristic curves (ROC) with censored data.

Correlations between splicing factor-related events and prognosis-related AS events

We obtained splicing factors from SpliceAid 2 (http://193.206.120.249/splicing_tissue.html). Expression data (level 3) for the splicing factors associated with AS were downloaded from TCGA. Correlations between splicing factor-related events and prognosis-related AS events were evaluated using Spearman's rank-order correlation. The correlation diagrams and survival curves were generated with GraphPad (v 5.01). *P*-values of ≤ 0.05 were considered significant.

Results

Number of AS events in the DLBCL cohort from TCGA

In the present study, out of 48 DLBCL cases, 7 AS events were classified, including alternate acceptor (AA) sites, alternate donor (AD) sites, alternate promoter (AP), alternate terminator (AT), exon skip (ES), mutually exclusive exon (ME) and retained intron (RI). As shown in **Figure 1A**, a total of 33,724 AS events from 16,098 genes were curated. Out of 5,086 genes, the

maximum number of ES events was 12,101, and out of 150 genes, the minimum number of ME events was 156. Furthermore, via univariate Cox regression analysis, 1,262 AS events in 971 genes were considered significantly associated with OS in cases of DLBCL ($P < 0.05$) (**Figure 1B**). We calculated 116 AAs in 110 genes, 84 ADs in 81 genes, 225 APs in 136 genes, 288 ATs in 165 genes, 475 ESs in 411 genes, 9 MEs in 9 genes, and 65 RIs in 59 genes. The intersecting sets in various AS events were performed by UpSet plot. As shown in **Figure 1C**, most genes that related to OS were collected from ES events. In addition, one single survival-related gene could appear in up to three types of AS events simultaneously. For example, gene SF1 significantly correlated to OS in AP, ES, and RI.

Underlying OS-related gene pathways

Two hundred and four (204) genes that significantly relate to OS were certified utilizing univariate Cox regression ($P < 0.01$). As shown in **Figure 2A**, 42 of these selected genes interacted with different genes. For additional study, the Kyoto Encyclopedia of Genes and Genomes (KEGG) was used and gene ontology (GO) enrichment analysis was performed (**Figure 2B-E**). KEGG pathways indicated that 6 genes including EIF3C, EIF4B, NUP160, RAN, RANGAP1, and RNPS1 co-functioned in the most significant pathway, "RNA transport". Meanwhile, the GO

Alternative splicing variants in DLBCL

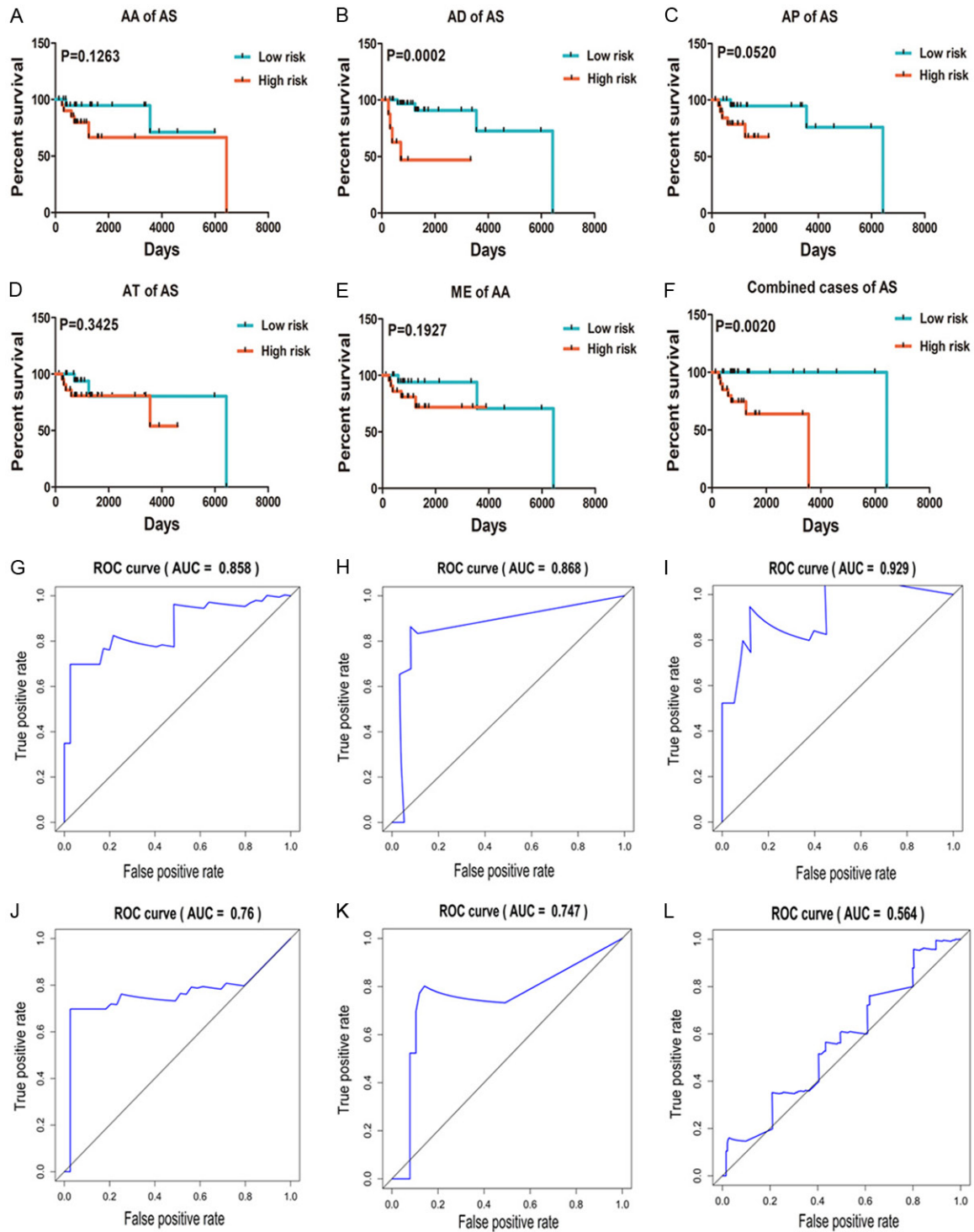


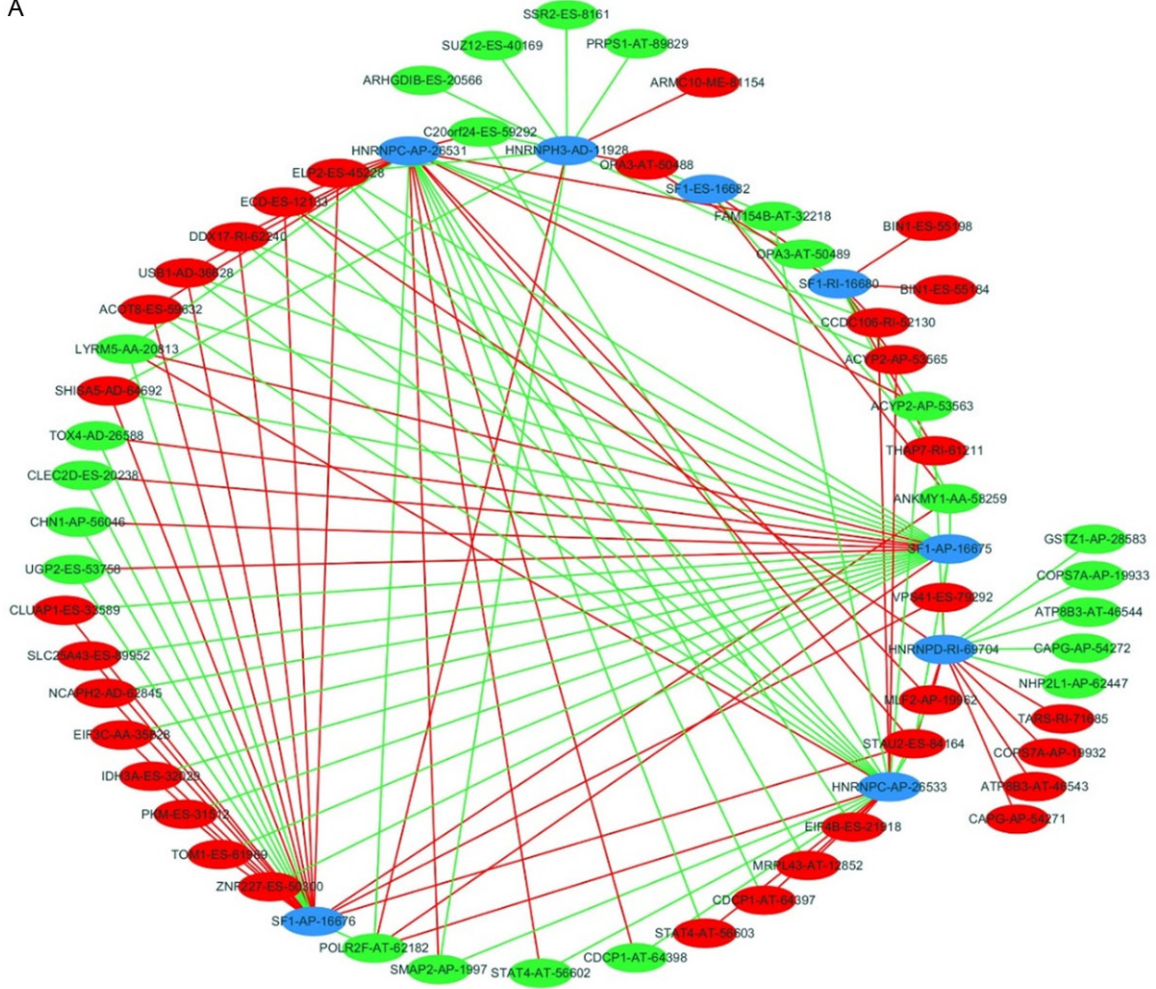
Figure 3. Kaplan-Meier and ROC curves for prognostic predictors of DLBCLs. A-E. Kaplan-Meier curves for AA, AD, AP, AT, and ME for DLBCL. The red line represents the high-risk group, and the green line represents the low-risk group. F. Kaplan-Meier curves for all five types of AS variants for DLBCL. The red line represents the high-risk group, and the green line represents the low-risk group. G-K. ROC curves with AUC for AA, AD, AP, AT, and ME in DLBCL. L. ROC curves for all five types of AS events.

enrichment annotations showed that these 42 interacting genes were enriched in various cru-

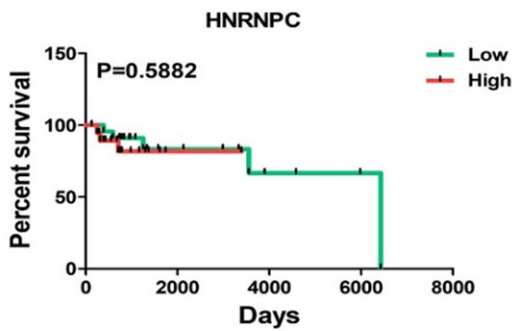
cial pathways, such as mRNA splicing, poly (A) RNA binding, and protein complex binding.

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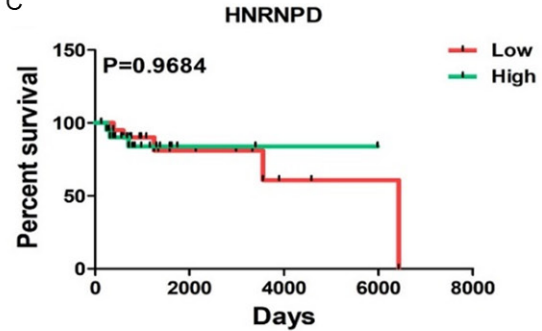
A



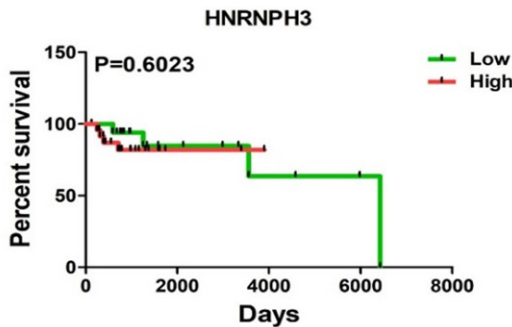
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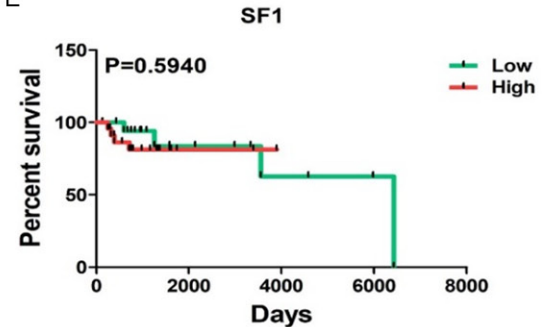
C



D



E



Alternative splicing variants in DLBCL

Figure 4. Correlation splicing network and Kaplan-Meier curves for OS splicing factors. A. Splicing network of OS-related AS variants. The blue dots represent the AS events from OS-related splicing factors. The red/green dots represent the AS events that are positively (red lines) or negatively (green lines) associated with AS events from splicing factors. B-E. Kaplan-Meier curves for four splicing factors in DLBCL. The red line represents the high level group and the green line represents the low level group.

Potential prognostic roles in DLBCL

In order to achieve some reliable prognostic predictors in DLBCL patients, we chose the five most significant univariate AS events among all seven types. Through the use of multivariate Cox regression analysis, 10 AS events, including EIF3C and TYMP in AAs, TOX4 and USB1 in ADs, NHP2L1 and MLF2 in APs, STAT4 and POLR2F in ATs, and COX16 and SS18 in MEs (**Table 1**), were finally selected from AS events in 35 candidates. Unfortunately, no significant results were calculated in ES or RI events. Meanwhile, a prognostic index (PI) model was built to estimate the outcomes for DLBCL patients. Median PI values divided the patients into high and low-risk groups. As shown in **Figure 3A-E**, there was a trend toward longer OS for five different AS events in the low-risk group. The median OS days for AAs in the low-risk group were 1,972, and in the high-risk group, it was 651. In the AD low-risk and high-risk groups, the median OS was 4,989 and 352 days, respectively. For ATs, 1,252 days and 391 days represented the median OS in the low-risk and high-risk groups, respectively. Meanwhile, the median OS for both APs and MEs were calculated as 3,553 days and 391 days in the low-risk and high-risk groups, respectively. Moreover, ADs of AS events in the low-risk group indicated a significantly better outcome than in the high-risk group among DLBCL patients ($P=0.0002$). A combination of 10 events also verified considerable power in predicting good prognostic conditions in the low-risk group ($P=0.002$) (**Figure 3F**). The median survival days for the low-risk and high-risk groups were 6,425 and 595 days, respectively. Furthermore, ROC curves were used to compare the efficiencies of these 10 events. Areas under the curve (AUCs) of AAs, ADs, APs, ATs, MEs, and all five types of AS events were estimated (**Figure 3G-L**); APs showed high prognosis predicting efficiency with AUCs over 0.9.

Network of splicing factor-related events

Splicing factors control different genes by affecting the transcriptome. Meanwhile, mRNA

stability is regulated by interactions between trans-splicing and basal splicing factors [28]. In this study, we obtained 66 splicing factors, and 65 of these factors functioned in different types of the 193 AS events. Eventually, eight splicing factor related AS events were selected after univariate regression analysis. For deeper insight into correlations between splicing factor-related AS events and significant univariate AS events, PSI values of 71 significant univariate AS events ($P<0.005$) and eight splicing factor-related AS events were calculated. As shown in **Figure 4A**, eight splicing factor-related AS events (blue dots) positively correlated to 63 univariate events (red dots) and negatively correlated to 65 univariate events (green dots) (**Table 2**). Furthermore, survival analysis of four splicing factors involved in eight AS events were estimated (**Figure 4B-E**); however, no significant results were obtained.

Discussion

Profound modulatory points in gene expression have significant effects on the development of cancers. As one of the regulatory factors in gene control, AS variants are involved in the reorganization of proteomes and can modulate the levels of a number of oncogenes and tumor inhibitor isoforms [29]. As an identified splicing factor oncoprotein, SF2/ASF amplifies in various cancers and generates the activation of proximal 5' splicing sites. Up-regulated SF2/ASF also blocks aberrant exon-skipping and manages AS events in the tumor-suppressor BIN1 [30, 31]. Meanwhile, accurate FOX2 binding sites have been correlated to alternative exons at silencer or enhancer proteins [32]. Furthermore, the level of splicing factors could be influenced by various cancer pathways; for example, hnRNPA1 and hnRNPA2 are up-regulated by c-Myc [29].

In DLBCL patients, a relatively small number of studies have reported pre-mRNA AS variants to be predictive biomarkers and AS events have also been associated with diagnosis and prognosis. Dysregulated CD44 levels can alter lymph node draining [33]. As an isoform of

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Table 2. Involvement of four splicing factors in various AS events

| Splicing factor-related AS events | Negatively correlated events | Correlation value | P-value | Positively correlated events | Correlation value | P-value |
|-----------------------------------|------------------------------|-------------------|---------|------------------------------|-------------------|---------|
| HNRNPH3-AD-11928 | C20orf24-ES-59292 | -0.513 | 0.0004 | OPA3-AT-50488 | 0.344 | 0.0222 |
| | ARHGDI3-ES-20566 | -0.454 | 0.0020 | ARMC10-ME-81154 | 0.344 | 0.0223 |
| | ELP2-ES-45228 | -0.403 | 0.0110 | POLR2F-AT-62182 | 0.302 | 0.0463 |
| | SHISA5-AD-64692 | -0.375 | 0.0170 | | | |
| | OPA3-AT-50489 | -0.344 | 0.0222 | | | |
| | SMAP2-AP-1997 | -0.339 | 0.0245 | | | |
| | SUZ12-ES-40169 | -0.331 | 0.0300 | | | |
| | SSR2-ES-8161 | -0.318 | 0.0356 | | | |
| HNRNPC-AP-26531 | PRPS1-AT-89829 | -0.297 | 0.0499 | | | |
| | POLR2F-AT-62182 | -0.452 | 0.0021 | SMAP2-AP-1997 | 0.519 | 0.0003 |
| | EIF4B-ES-21918 | -0.395 | 0.0080 | MLF2-AP-19962 | 0.488 | 0.0008 |
| | CCDC106-RI-52130 | -0.395 | 0.0087 | ACOT8-ES-59632 | 0.429 | 0.0041 |
| | MRPL43-AT-12852 | -0.363 | 0.0153 | ELP2-ES-45228 | 0.416 | 0.0084 |
| | CDCP1-AT-64397 | -0.358 | 0.0169 | CDCP1-AT-64398 | 0.358 | 0.0169 |
| | ACYP2-AP-53565 | -0.336 | 0.0259 | USB1-AD-36628 | 0.357 | 0.0187 |
| | STAT4-AT-56603 | -0.324 | 0.0338 | ACYP2-AP-53563 | 0.346 | 0.0216 |
| | LYRM5-AA-20813 | -0.363 | 0.0351 | FAM154B-AT-32218 | 0.335 | 0.0279 |
| | | | | STAT4-AT-56602 | 0.324 | 0.0338 |
| | | | | DDX17-RI-62240 | 0.315 | 0.0375 |
| | | | | C20orf24-ES-59292 | 0.309 | 0.0416 |
| | | | | ECD-ES-12133 | 0.316 | 0.0442 |
| | | | | STAU2-ES-84164 | 0.316 | 0.0471 |
| HNRNPC-AP-26533 | SMAP2-AP-1997 | -0.519 | 0.0003 | POLR2F-AT-62182 | 0.452 | 0.0021 |
| | MLF2-AP-19962 | -0.488 | 0.0008 | EIF4B-ES-21918 | 0.395 | 0.0080 |
| | ACOT8-ES-59632 | -0.429 | 0.0041 | CCDC106-RI-52130 | 0.395 | 0.0087 |
| | ELP2-ES-45228 | -0.416 | 0.0084 | MRPL43-AT-12852 | 0.363 | 0.0153 |
| | CDCP1-AT-64398 | -0.358 | 0.0169 | CDCP1-AT-64397 | 0.358 | 0.0169 |
| | USB1-AD-36628 | -0.357 | 0.0187 | ACYP2-AP-53565 | 0.336 | 0.0259 |
| | ACYP2-AP-53563 | -0.346 | 0.0216 | STAT4-AT-56603 | 0.324 | 0.0338 |
| | FAM154B-AT-32218 | -0.335 | 0.0279 | LYRM5-AA-20813 | 0.363 | 0.0351 |
| | STAT4-AT-56602 | -0.324 | 0.0338 | | | |
| | DDX17-RI-62240 | -0.315 | 0.0375 | | | |
| | C20orf24-ES-59292 | -0.309 | 0.0416 | | | |
| | ECD-ES-12133 | -0.316 | 0.0442 | | | |
| | STAU2-ES-84164 | -0.316 | 0.0471 | | | |
| | SF1-AP-16675 | ELP2-ES-45228 | -0.546 | 0.0003 | UGP2-ES-53758 | 0.416 |
| ZNF227-ES-50300 | | -0.508 | 0.0013 | CHN1-AP-56046 | 0.384 | 0.0131 |
| USB1-AD-36628 | | -0.442 | 0.0030 | POLR2F-AT-62182 | 0.338 | 0.0268 |
| TOM1-ES-61969 | | -0.431 | 0.0039 | CLEC2D-ES-20238 | 0.337 | 0.0270 |
| PKM-ES-31512 | | -0.429 | 0.0041 | LYRM5-AA-20813 | 0.349 | 0.0430 |
| ECD-ES-12133 | | -0.435 | 0.0045 | TOX4-AD-26588 | 0.337 | 0.0445 |
| ANKMY1-AA-58259 | | -0.433 | 0.0047 | | | |
| IDH3A-ES-32029 | | -0.374 | 0.0135 | | | |
| EIF3C-AA-35828 | | -0.372 | 0.0141 | | | |
| NCAPH2-AD-62845 | | -0.375 | 0.0144 | | | |
| SLC25A43-ES-89952 | | -0.395 | 0.0154 | | | |
| DDX17-RI-62240 | | -0.342 | 0.0247 | | | |

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| | | | | | | |
|-----------------|------------------|--------|--------|-------------------|-------|--------|
| | SHISA5-AD-64692 | -0.338 | 0.0329 | | | |
| | ACOT8-ES-59632 | -0.321 | 0.0381 | | | |
| | CLUAP1-ES-33589 | -0.318 | 0.0404 | | | |
| | VPS41-ES-79292 | -0.338 | 0.0469 | | | |
| | STAU2-ES-84164 | -0.314 | 0.0483 | | | |
| SF1-AP-16676 | UGP2-ES-53758 | -0.416 | 0.0062 | ELP2-ES-45228 | 0.546 | 0.0003 |
| | CHN1-AP-56046 | -0.384 | 0.0131 | ZNF227-ES-50300 | 0.508 | 0.0013 |
| | POLR2F-AT-62182 | -0.338 | 0.0268 | USB1-AD-36628 | 0.442 | 0.0030 |
| | CLEC2D-ES-20238 | -0.337 | 0.0270 | TOM1-ES-61969 | 0.431 | 0.0039 |
| | LYRM5-AA-20813 | -0.349 | 0.0430 | PKM-ES-31512 | 0.429 | 0.0041 |
| | TOX4-AD-26588 | -0.337 | 0.0445 | ECD-ES-12133 | 0.435 | 0.0045 |
| | | | | ANKMY1-AA-58259 | 0.433 | 0.0047 |
| | | | | IDH3A-ES-32029 | 0.374 | 0.0135 |
| | | | | EIF3C-AA-35828 | 0.372 | 0.0141 |
| | | | | NCAPH2-AD-62845 | 0.375 | 0.0144 |
| | | | | SLC25A43-ES-89952 | 0.395 | 0.0154 |
| | | | | DDX17-RI-62240 | 0.342 | 0.0247 |
| | | | | SHISA5-AD-64692 | 0.338 | 0.0329 |
| | | | | ACOT8-ES-59632 | 0.321 | 0.0381 |
| | | | | CLUAP1-ES-33589 | 0.318 | 0.0404 |
| | | | | VPS41-ES-79292 | 0.338 | 0.0469 |
| | | | | STAU2-ES-84164 | 0.314 | 0.0483 |
| SF1-ES-16682 | OPA3-AT-50488 | -0.454 | 0.0020 | OPA3-AT-50489 | 0.454 | 0.0020 |
| | FAM154B-AT-32218 | -0.311 | 0.0424 | ANKMY1-AA-58259 | 0.356 | 0.0225 |
| | THAP7-RI-61211 | -0.299 | 0.0490 | | | |
| HNRNPD-RI-69704 | GSTZ1-AP-28583 | -0.407 | 0.0067 | TARS-RI-71685 | 0.528 | 0.0008 |
| | COPS7A-AP-19933 | -0.344 | 0.0240 | COPS7A-AP-19932 | 0.344 | 0.0240 |
| | ATP8B3-AT-46544 | -0.332 | 0.0296 | ATP8B3-AT-46543 | 0.332 | 0.0296 |
| | THAP7-RI-61211 | -0.330 | 0.0306 | MLF2-AP-19962 | 0.323 | 0.0346 |
| | CAPG-AP-54272 | -0.322 | 0.0355 | CAPG-AP-54271 | 0.322 | 0.0355 |
| | NHP2L1-AP-62447 | -0.303 | 0.0480 | STAU2-ES-84164 | 0.325 | 0.0410 |
| | | | | ECD-ES-12133 | 0.319 | 0.0419 |
| | | | | VPS41-ES-79292 | 0.34 | 0.0455 |
| SF1-RI-16680 | ANKMY1-AA-58259 | -0.448 | 0.0033 | CCDC106-RI-52130 | 0.442 | 0.0030 |
| | ACYP2-AP-53563 | -0.342 | 0.0229 | BIN1-ES-55198 | 0.372 | 0.0129 |
| | OPA3-AT-50489 | -0.330 | 0.0289 | THAP7-RI-61211 | 0.358 | 0.0169 |
| | | | | ACYP2-AP-53565 | 0.348 | 0.0207 |
| | | | | BIN1-ES-55184 | 0.366 | 0.0240 |
| | | | | OPA3-AT-50488 | 0.33 | 0.0289 |

CD44, CD44H is strongly related to poor survival of DLBCL cohorts, and rituximab could decrease the significance of its prognosis [34]. Meanwhile, four likely splice variants of the STAT3 gene have all been represented in DLBCL, including the $\Delta S/S$ and α/β splicing variants [35]. Co-worked terms for S and ΔS could sustain activated B-cell-like DLBCL cell survival and modulate STAT3 targets, such as

NFKBIA and NFKBIZ [36]. In addition, p63 directly impact cell apoptosis and differentiation. Isoform TAp63, which is a product of an upstream intronic promoter, has been found to be highly expressed in DLBCL cell lines [37]. Collectively, these results outline the critical role of AS variants in DLBCL. A wide variety of isoforms might be valuable candidates for further prospective research on DLBCL.

In the current study, the functional categories of interacting AS genes were analyzed using KEGG and GO. The KEGG enrichment results revealed that “RNA transport” was the most significant term at the $P=0.0004$ level. We found several consistent pathways, such as “transcription factor activity, RNA polymerase II core promoter sequence-specific”, “mRNA splicing, via spliceosome”, and “protein complex binding” related to AS events from GO annotations. Hence, in the future, gaining insight into these significant interacting AS-related genes in terms of DLBCL mechanisms will be of interest.

Out of 66 splicing factors, genes SF1, HNRNPC, HNRNPD, and HNRNPH3 were identified as the factors involved in OS correlated AS events. To date, it had been well established that protein SF1 was able to recognize the 3' splice site by binding the branchpoints of certain introns and repressing transcription. Meanwhile, SF1 played a critical role in the retention of nuclear pre-mRNA and SF1 silencing closely related to AS variants of endogenous transcription [38-40]. In recent years, there has been an increase in the literature on SF1 in various cancers, such as in epithelial ovarian cancer [41] and testicular germ cell tumors [42]. In this study, we discovered that protein SF1 was simultaneously engaged in AP, ES, and RI events in DLBCL. However, several studies have indicated that SF1 was identified in just one type of malignancy and rarely seen in lymphoid malignancies [43, 44]. Thus, the role of SF1 requires further investigation; our study offers a novel aspect of SF1 in DLBCL. Furthermore, the remaining factors, HNRNPC, HNRNPD, and HNRNPH3, are members of the heterogeneous nuclear ribonucleoproteins (hnRNPs). AS transcript variants of these three factors were previously revealed. Fawal et al. reported that AUF1/hnRNP is the partner of anaplastic lymphoma kinase (ALK) [45]. Compared to conventional DLBCL patients, ALK positive DLBCL patients displayed unique clinical features and histological types [46]. Collectively, the aforementioned results imply that these four splicing factors might perform multiple functions and offer an orientation from which we might investigate the underlying mechanisms of AS variants in DLBCL.

Conclusion

Taken together, the results of this study revealed that OS-related AD of AS variants is a

potential valuable predictor of DLBCL prognosis. In addition, correlation networks and enrichment analysis were performed to uncover the potential regulatory roles of key splicing factors. Considering this evidence, in DLBCL, oncoproteins spliced by certain AS events or the aberrant transcription of splicing factors would be provided. However, the mechanisms of OS-related AS variants and splicing factors are still not clearly understood. Further investigation needs to be conducted with regard to AS in DLBCL patients.

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Disclosure of conflict of interest

None.

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