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A SPLICING PROFILING AND DRUG REPURPOSING APPROACH TO TARGETING THE SPLICING FACTORS KINASE AXIS IN SKIN CUTANEOUS MELANOMA

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Abstract

Skin cutaneous melanoma (SKCM) remains a formidable clinical challenge due to frequent therapeutic resistance. This study employs a multi-omics and computational approach to characterize the pivotal role of aberrant alternative splicing (AS), regulated by the splicing factor (SF)-kinase axis, in melanoma pathogenesis and to identify novel drug repurposing opportunities. Analysis of TCGA and GTEx data revealed significant overexpression of key serine/arginine-rich splicing factors, SRSF1 and SRSF2, in SKCM tumors. Subsequent profiling of AS landscapes identified widespread, high-frequency splicing alterations, including constitutive exon skipping (e.g., 100% skipping of exons 2.2-3.4) and intron retention events, indicating a systematic "oncogenic splicing switch." Functional enrichment and protein-protein interaction network analyses confirmed the central role of these SFs within spliceosome and mRNA processing pathways. Given the difficulty of directly inhibiting SFs, we investigated their upstream regulators. We identified a network of druggable kinases including SRPK1, CLK1/2, and CDK12 that phosphorylate and control SRSF1 activity. Molecular docking of known kinase inhibitors demonstrated high binding affinity for these targets, with THZ531 (CDK12), SRPIN340 (SRPK1), and TG003 (CLK1) emerging as promising repurposing candidates. In conclusion, this study delineates a dysregulated SF-kinase axis driving profound splicing reprogramming in melanoma. It proposes a novel therapeutic strategy of indirectly modulating oncogenic alternative splicing by targeting these upstream regulatory kinases using existing inhibitors, offering a rapid and actionable pathway for overcoming resistance in precision dermatology.

Introduction

Skin cancer remains one of the most prevalent and clinically challenging malignancies globally, with its incidence continuing to rise despite advancements in preventative measures and public awareness.[1] Broadly categorized into non-melanoma skin cancers (NMSC) primarily basal cell carcinoma and squamous cell carcinoma and the more aggressive cutaneous melanoma, these pathologies represent a significant burden on healthcare systems.[2] While localized tumors are often manageable through surgical intervention, metastatic disease presents a formidable hurdle, characterized by high mutational burdens and frequent resistance to conventional systemic therapies.[3]

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Recent genomic landscapes have underscored the complexity of the skin cancer microenvironment.[4] Beyond well-documented drivers like the BRAF/MEK pathway, research is increasingly focusing on the "dark matter" of the cancer genome: the post-transcriptional regulatory mechanisms that dictate cellular plasticity. Among these, alternative splicing (AS) has emerged as a critical hallmark of oncogenesis.[5] AS allows a single gene to produce multiple mRNA isoforms with diverse, and often opposing, biological functions, thereby expanding the proteomic diversity of the cell and enabling rapid adaptation to oncogenic stress.[6]

The regulation of alternative splicing is governed by an intricate network of splicing factors (SFs) RNA-binding proteins that recognize specific sequences within pre-mRNA to facilitate or inhibit spliceosome assembly.[7] In the context of skin cancer, the dysregulation of these SFs is not merely a byproduct of malignant transformation but often a primary driver of it. Misexpression or mutation of key splicing factors can lead to the "splicing switch," where pro-apoptotic isoforms are suppressed in favor of pro-survival variants, fueling tumor progression, epithelial-mesenchymal transition (EMT), and evasion of immune surveillance.[8]

The pathogenesis of the Splicing Factor (SF)-kinase axis in Skin Cutaneous Melanoma (SKCM) is initiated by the constitutive activation of oncogenic signaling cascades, most notably the MAPK (BRAF/MEK) and PI3K/AKT/mTOR pathways. These drivers act as upstream stimulus for the aberrant expression and catalytic hyper-activation of CDC-like kinases (CLKs 1–4) and Serine/Arginine-rich protein kinases (SRPKs 1–2).[9] These kinases function as the central rheostats of the spliceosome; their overactivity leads to the stoichiometric hyper-phosphorylation of Serine/Arginine-rich (SR) proteins. This post-translational modification triggers the premature mobilization of SFs from nuclear speckles to the nucleoplasm, thereby compromising the fidelity of splice-site selection and orchestrating a global collapse of transcriptomic homeostasis.[10]

Central to melanoma progression is the transition from homeostatic to pathogenic alternative splicing (AS), a process termed the "Oncogenic Splicing Switch." Under the influence of a dysregulated kinase axis, the splicing machinery selectively enriches for mRNA isoforms that promote malignant phenotypes.[11] Mechanistically, this involves the inclusion or exclusion of specific exons in master regulators of apoptosis and metastasis; for example, shifting the ratio of the BCL2L1 gene toward the anti-apoptotic BCL-X_L variant rather than the pro-apoptotic BCL-X_S. This kinase-driven reprogramming facilitates Epithelial-Mesenchymal Transition (EMT) and metabolic rewiring, allowing melanoma cells to transcend their primary niche and acquire the invasive capacity necessary for systemic dissemination.[12]

The clinical lethality of the SF-kinase axis is underscored by its role in adaptive resistance and immune subversion. In the context of targeted inhibition, the splicing axis enables clonal evolution through the production of "bypass" isoforms, such as truncated BRAFV600E variants. These isoforms lack the regulatory domains targeted by conventional inhibitors, thereby restoring ERK signaling despite therapeutic pressure.[13] Furthermore, the generation of non-canonical splice variants contributes to a diminished "neoantigenic" profile or the production of decoy receptors that neutralize T-cell mediated cytotoxicity. Consequently, the SF-kinase axis emerges not merely as a bystander of transformation, but as a critical coordinator of therapeutic recalcitrance, necessitating the exploration of drug repurposing strategies to restore splicing fidelity.[14]

The activity of splicing factors is itself heavily regulated by post-translational modifications, most notably phosphorylation. Splicing-regulatory kinases, such as the SRPK and CLK families, modulate the affinity and localization of SFs within the nucleus.[15] In skin cancer, hyperactive kinase signaling often driven by UV-induced mutations creates a feedback loop that sustains oncogenic splicing patterns. This kinase-SF axis represents a sophisticated regulatory layer that offers a unique therapeutic window: by targeting the kinases that "instruct" the splicing machinery, we can potentially revert the cellular splicing profile to a more benign state. The urgency for

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new therapeutic avenues is underscored by the limitations of current targeted therapies and immunotherapies.[16] While BRAF inhibitors and checkpoint blockades have revolutionized melanoma treatment, a substantial cohort of patients develops acquired resistance. This resistance is frequently mediated by alternative splicing of the very targets the drugs aim to inhibit, such as the V600E BRAF variant. Consequently, identifying drug candidates that can modulate splicing or inhibit the upstream kinases responsible for aberrant splicing is a high-priority frontier in precision dermatology. Despite the clear significance of splicing in oncology, the specific targets of these splicing factors in skin cancer remain inadequately mapped. Traditional transcriptomic analyses often overlook isoform-level variations, focusing instead on total gene expression.[17] To bridge this gap, high-throughput sequencing and advanced computational modeling are now being utilized to identify specific splicing factor targets. By pinpointing the exact mRNA junctions regulated by aberrant SFs, researchers can uncover novel biomarkers and mechanistic insights that were previously hidden within bulk sequencing data.

Materials and Methods

Data Retrieval and Differential Expression Analysis

The primary genomic and transcriptomic data for skin cutaneous melanoma (SKCM) were sourced from The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/ccg/research/genome-sequencing/tcga) databases. To identify differentially expressed splicing factors, we utilized the GEPIA2 (Gene Expression Profiling Interactive Analysis) web server (http://gepia2.cancer-pku.cn/). Expression levels of *SRSF1* and *SRSF2* were compared between SKCM tumor samples and adjacent normal tissues. Statistical significance was determined using the Log2fold change (LFC) cutoff of 1 and a p-value cutoff of 0.01, with results visualized through interactive box plots.

Analysis of Alternative Splicing (AS) Events

To characterize the landscape of alternative splicing in skin cancer, splicing profiles were extracted from the TCGA SpliceSeq database (https://bioinformatics.mdanderson.org/TCGA SpliceSeq/). We quantified seven types of AS events: Exon Skip (ES), Alternate Donor (AD) site, Alternate Acceptor (AA) site, Mutually Exclusive Exons (ME), Retained Intron (RI), Alternate Terminator (AT), and Alternate Promoter (AP). The Percent Spliced In (PSI) value, ranging from 0 to 1, was employed to measure the efficiency of specific splicing events, with higher PSI indicating increased inclusion of the spliced variant.

Protein-Protein Interaction (PPI) Network Construction

To investigate the functional interplay between identified splicing factors and their potential targets, we utilized the STRING database (v12.5) (https://string-db.org/). A PPI network was constructed using a medium confidence score threshold (0.400) to ensure a balance between sensitivity and specificity. The network focused on physical and functional associations, highlighting core regulatory hubs involved in the oncogenic "splicing switch".

Functional Enrichment Analysis

Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed to determine the biological significance of the splicing factor targets. Tools such as PANTHER (http://www.pantherdb.org/) or the enrichment modules within STRING were used to categorize targets into Biological Processes (BP), Molecular Functions (MF), and Cellular Components (CC). A p-value < 0.05 was established as the threshold for significant enrichment.

Identification of Druggable Targets and Drug Repurposing

Potentially druggable kinases and splicing-regulatory proteins were identified using the Pharos platform (https://pharos.nih.gov/), which aggregates data from the Illuminating the Druggable Genome (IDG) project. We focused on high-priority targets within the SRPK and CLK kinase families. For the drug repurposing strategy,

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a library of FDA-approved kinase inhibitors and known splicing modulators was curated for further computational validation.

Molecular Docking and Affinity Prediction

To validate the binding potential of candidate drugs to their targets (e.g., SRPK1, CLK1), we performed blind molecular docking using the CB-Dock (v2.0) web server (http://clab.labshare.cn/cb-dock/). Protein structures were retrieved from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/) using specific IDs such as 3BEG (SRPK1) and 6G99 (CLK1). CB-Dock automatically identified potential binding cavities using a curvature-based detection approach. Binding affinities were calculated in terms of Vina scores (kcal/mol), and the top-ranked poses were analyzed for key molecular interactions, including hydrogen bonds and hydrophobic contacts.

RESULTS

Differential Expression of Splicing Factors in SKCM

Initial transcriptomic profiling via GEPIA2 revealed a significant upregulation of key Serine/Arginine-rich (SR) splicing factors in skin cutaneous melanoma (SKCM). Comparative analysis between tumor and normal tissues demonstrated that SRSF1 and SRSF2 are markedly overexpressed ($p < 0.01$). The expression box plots for both genes showed larger interquartile ranges and higher median expression levels in cancer samples compared to healthy skin, suggesting their potential role as oncogenic drivers in the melanoma landscape.

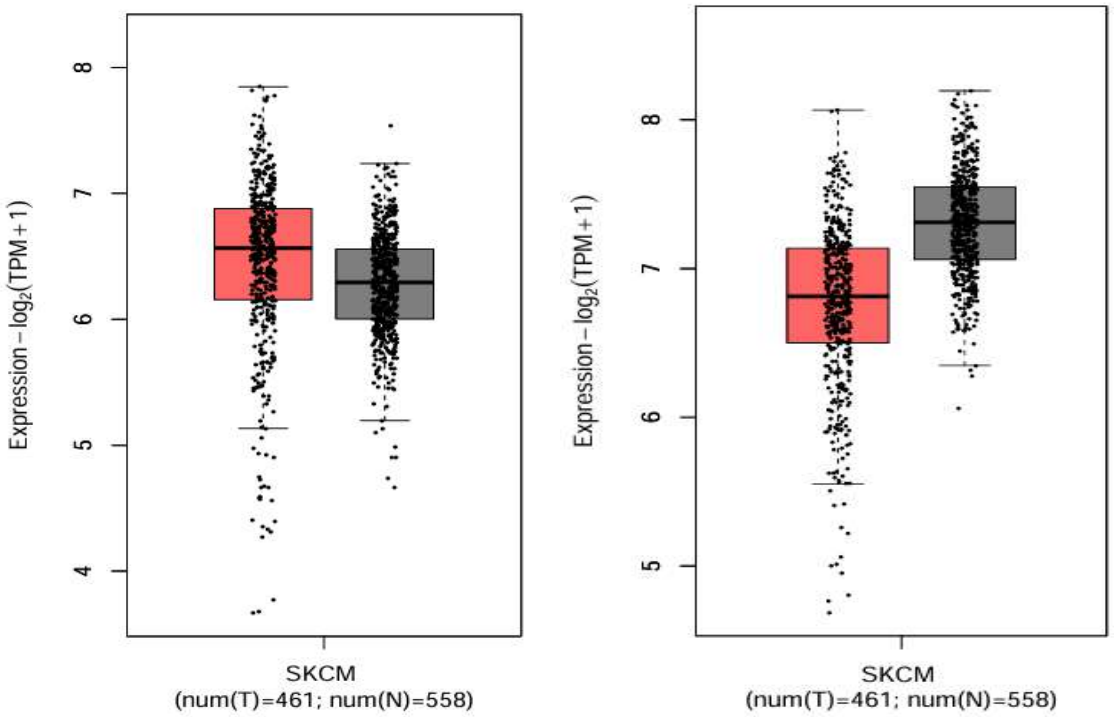


Figure 1: Expression of Splicing Factors skin cutaneous melanoma via GEPIA2

Using GEPIA2, we analyzed SRSF1 and SRSF2 expression in skin cancer (SKCM). Both genes showed significantly higher expression in tumor tissue compared to normal skin. The box plots revealed that cancer samples had larger and higher-positioned boxes than normal samples, with

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statistical significance ($p < 0.01$). This suggests SRSF1 and SRSF2 are overexpressed in skin cancer."

Table 1: Significance values of SRSF1 and SRSF2 genes

Gene	Cancer Box Size	Normal Box Size	Position	Significance
SRSF1	Large & High	Small & Low	Cancer > Normal	p<0.01
SRSF2	Large & High	Small & Low	Cancer > Normal	p<0.01

Kaplan-Meier survival curves analyzing the impact of SRSF gene expression levels on overall survival, showing that neither SRSF1 nor SRSF2 serves as a significant prognostic biomarker in this specific patient cohort. In both cases, the survival trajectories for the "High" (red) and "Low" (blue) expression groups are nearly identical and frequently overlap, as confirmed by the Logrank p-values (0.62 for SRSF1 and 0.66 for SRSF2) and Hazard Ratios (HR = 1.1 for both), which indicate no statistically significant difference in mortality risk between the groups. With balanced sample sizes (n = 229 per group) and similar median survival times across both plots, the data suggests that the expression levels of these specific splicing factors do not independently influence the long-term survival outcomes for these patients over the 300+ month observation period.

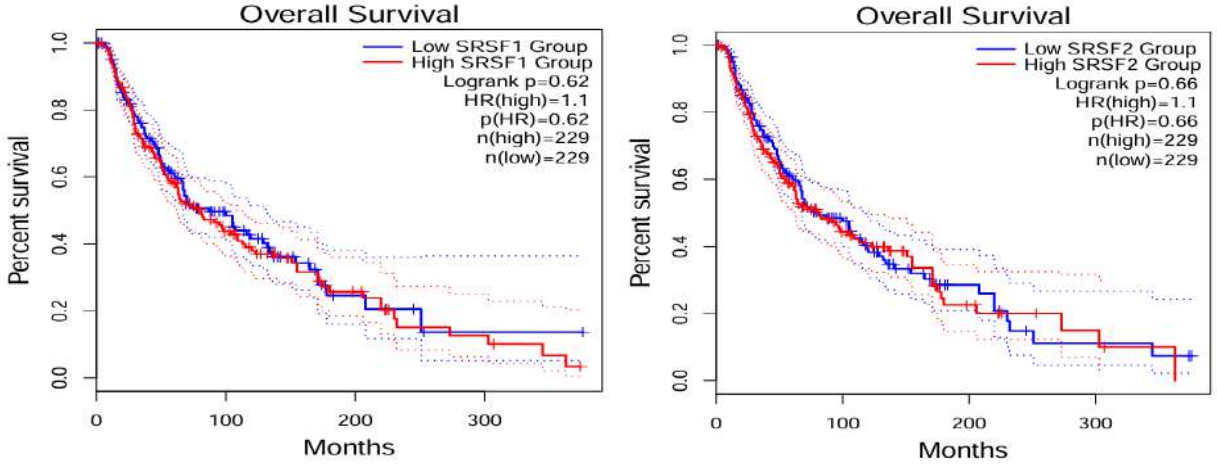


Figure 2: The impact of SRSF gene expression levels by Kaplan-Meier survival curves

Characterization of Aberrant Alternative Splicing (AS) Landscapes

The analysis of splicing patterns in SKCM identified a complex architecture of isoform reprogramming. Seven types of AS events were quantified, revealing distinct regulatory preferences. The splicing data reveals several distinct patterns of alternative splicing events. The Alternative Donor (AD) site at exon 1.2 shows minimal usage with a PSI of only 3.5%, indicating this is a rare splicing variant that likely represents a minor isoform with limited biological significance in SKCM tumors. This low percentage suggests that the canonical donor site is strongly preferred in the majority of transcripts. In contrast, the Exon Skip (ES) events demonstrate striking patterns of extensive exon exclusion. Most notably, two multi-exon skipping events involving exons 2.2 through 3.4 show complete skipping (100% PSI), suggesting these exons are constitutively excluded in SKCM. This represents a major isoform switch where entire protein domains encoded by these exons may be systematically omitted. Additionally, the

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specific skipping of exon 3.3 alone occurs in 64.3% of transcripts, indicating an intermediate frequency event that may create an alternative isoform with distinct functional properties.

The Retained Intron (RI) events present a complex regulatory landscape. Exon 3.4 shows near-universal intron retention (98.3% PSI), which could introduce premature termination codons and trigger nonsense-mediated decay, potentially downregulating this transcript. Similarly, the retention of the exon 3.2:3.3:3.4 cluster occurs in 82.8% of transcripts, suggesting coordinated regulatory control over this genomic region. However, isolated retention of exon 3.2 is relatively infrequent (15.7% PSI), indicating context-dependent regulation, while exon 3.6 retention is highly prevalent (89.4% PSI), pointing toward another major regulatory mechanism affecting transcript stability or localization.

Table 2: Alternative splicing of SKCM by TCGA SpliceSeq database

Splice	Exon	SKCM
AD	1.2	3.5
ES	2.2:3.1:3.2:3.3:3.4	100.0
ES	2.2:3.1:3.3:3.4	100.0
ES	3.3	64.3
RI	3.4	98.3
RI	3.2:3.3:3.4	82.8
RI	3.2	15.7
RI	3.6	89.4

Biological Implications for SKCM

These splicing patterns collectively suggest that the gene undergoes extensive isoform reprogramming in cutaneous melanoma. The high-frequency skipping and retention events likely generate protein isoforms with altered functional domains, which could impact cellular processes relevant to melanoma progression. The consistent patterns across samples (evident from high PSI values) indicate these are not random splicing errors but regulated events that may contribute to the molecular phenotype of SKCM. The coexistence of both exon skipping and intron retention events affecting overlapping genomic regions suggests sophisticated, multi-layered regulation of this gene's expression, potentially creating multiple protein variants with distinct roles in melanoma biology.

Clinical and Therapeutic Considerations

From a clinical perspective, these splicing alterations could serve as potential biomarkers for SKCM. The high PSI values (particularly the 100% skipping events) suggest these patterns are consistent across tumors and might be detectable in clinical samples. If these splicing events affect protein domains involved in drug response or resistance pathways, they could influence treatment outcomes. For instance, if the skipped exons encode regions important for targeted therapy binding, their systematic exclusion might contribute to therapeutic resistance. Furthermore, the splicing patterns themselves could represent novel therapeutic targets, as modulating these events back toward normal patterns might alter the oncogenic properties of the resulting protein isoforms in melanoma cells.

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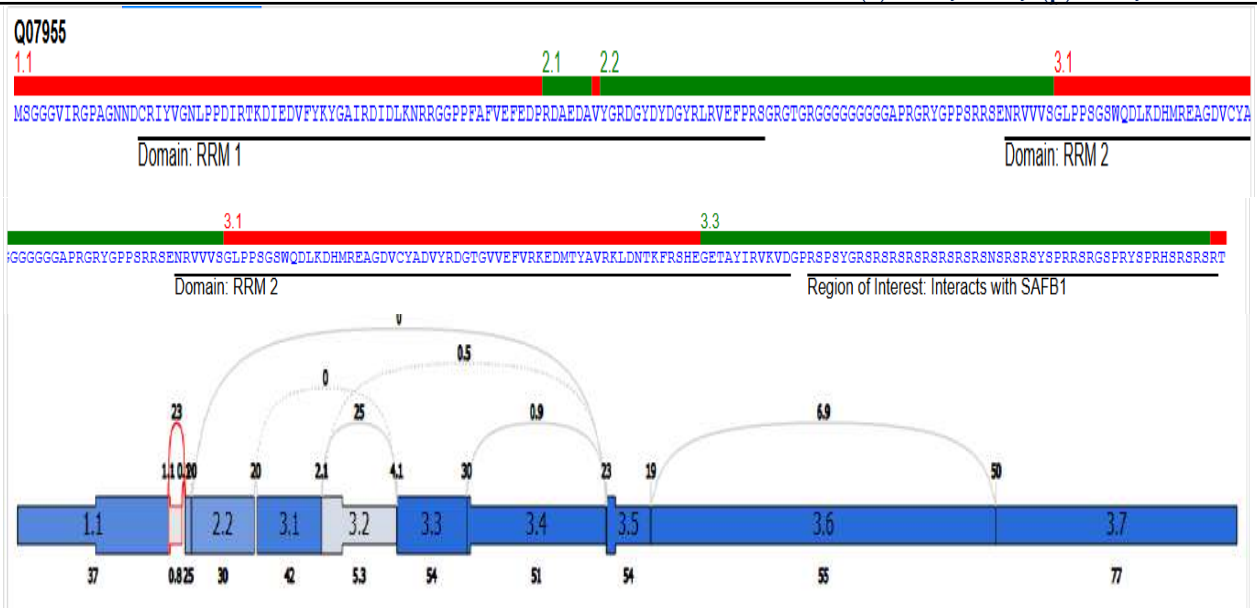


Figure 3: Alternative splicing of SKCM with their domains by SpliceSeq database

Alternative splicing plays a role in preventing exon skipping, ensuring the accuracy of splicing and regulating alternative splicing. Interacts with other spliceosomal components, via the RS domains, to form a bridge between the 5'- and 3'-splice site binding components, U1 snRNP and U2AF. Can stimulate binding of U1 snRNP to a 5'-splice site-containing pre-mRNA. Binds to purine-rich RNA sequences, either the octamer, 5'-RGAAGAAC-3' (r=A or G) or the decamers, AGGACAGAGC/AGGACGAAGC. Binds preferentially to the 5'-CGAGGCG-3' motif in vitro. Three copies of the octamer constitute a powerful splicing enhancer in vitro, the ASF/SF2 splicing enhancer (ASE) which can specifically activate ASE-dependent splicing. Isoform ASF-2 and isoform ASF-3 act as splicing repressors.

Functional Enrichment Analysis of SRSF-associated Genes

Functional enrichment analysis was conducted to identify the biological significance of the protein network, revealing that the Spliceosome is the most significantly enriched pathway with a highly significant False Discovery Rate (FDR = 1.00 \times 10^-18) and an observed count of 10 genes, including SRSF1 and SRSF2. Beyond splicing, the network shows a strong association with the mRNA surveillance pathway and RNA transport, involving key proteins like MAGOH, RBM8A, and EIF4A3, which indicates a broader role in regulating RNA stability and movement. Additionally, the involvement of the Herpes simplex virus 1 infection pathway suggests that these splicing factors may be hijacked or utilized during viral pathogenesis. Overall, these results demonstrate that the identified proteins form a functionally cohesive module centered on post-transcriptional gene regulation and RNA processing.

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Table 3: **Analysis of SRSF-associated Genes**

Term Description	Observed Gene Count	Background Gene Count	Strength	Signal	False Discovery Rate (FDR)	Matching Proteins in Network (Labels)
Spliceosome	10	132	2.13	7	1.00 \times 10^-18	SRSF1, U2AF1, U2AF2, SRSF7, MAGOH, SRSF2, SRSF10, RBM8A, SNRNP70, EIF4A3
mRNA Surveillance Pathway	3	93	1.76	1.23	0.003	MAGOH, RBM8A, EIF4A3
RNA Transport	3	161	1.52	0.93	0.01	MAGOH, RBM8A, EIF4A3
Herpes Simplex Virus 1 Infection	4	478	1.18	0.78	0.01	SRSF1, SRSF7, SRPK1, SRSF2

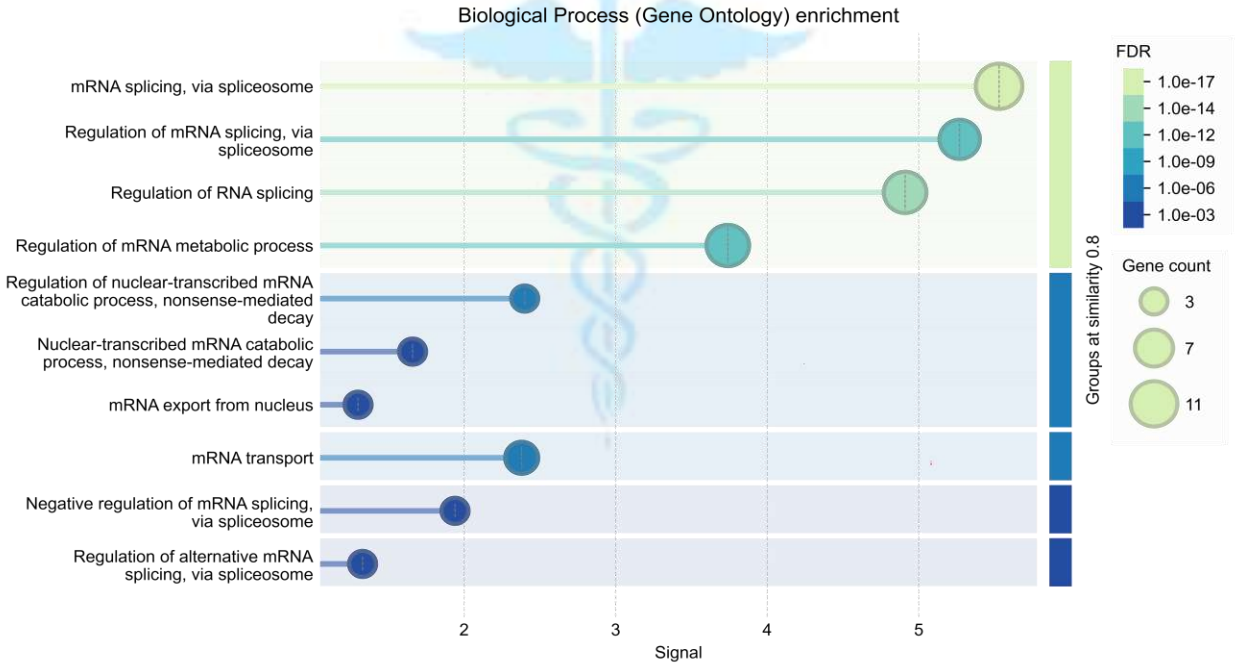


Figure 4: Gene ontology of SKCM

Functional Interaction Network and Pathway Enrichment Analysis of Splicing Regulators

Functional enrichment and network analysis demonstrate that the identified proteins form a highly interconnected Protein-Protein Interaction (PPI) network centered on the Spliceosome and mRNA splicing pathways. This molecular machine is driven by core splicing factors such as SRSF1 and SRSF2, which interact with essential assembly proteins like U2AF1, U2AF2, and SNRNP70 to regulate gene expression. While clinical data indicates that the total expression of SRSF1 (p=0.62) and SRSF2 (p=0.66) does not independently predict overall survival in the SKCM cohort, the high frequency of specific Alternative Splicing events such as Exon Skipping

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(ES) occurring in 100% of cases suggests that the functional diversity of these proteins is instead managed through precise isoform variations. These splicing changes intersect with critical RNA Recognition Motifs (RRM) and regions interacting with SAFB1, highlighting a complex regulatory mechanism that extends beyond simple gene expression to include mRNA transport and surveillance pathways.

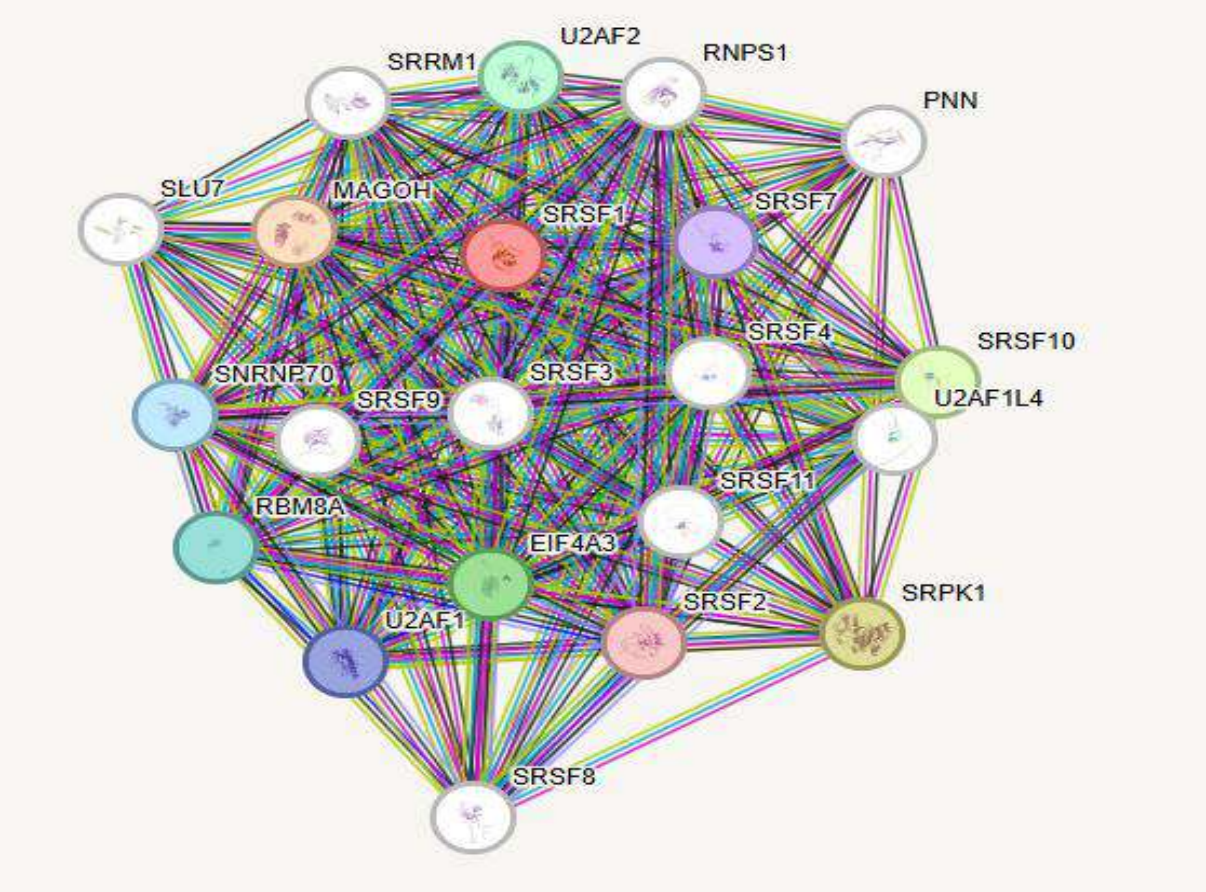


Figure 5: Interaction of splicing regulators by STRING

Functional Enrichment Analysis of Local Network Clusters

Functional enrichment of the local network clusters confirms that these proteins form a highly specialized functional module dedicated to mRNA processing and RNA recognition, with the most significant cluster (CL:2329) showing an extremely high statistical significance (FDR = 8.62 \times 10^-20). This network, which includes SRSF1, SRSF2, and U2AF1, is not only central to the spliceosome and prespliceosome assembly but also plays a critical role in nuclear speck organization and CRD-mediated mRNA stability. Interestingly, the analysis reveals a strong clinical link to Myelodysplastic syndrome (CL:2400, CL:2418), suggesting that the dysregulation of these splicing factors while not directly prognostic of survival in this specific SKCM cohort ($p > 0.05$) is deeply involved in established pathways of hematologic and cellular pathogenesis. Furthermore, the involvement of the Exon-exon junction complex (CL:2517, CL:2520) via proteins like MAGOH and RBM8A indicates that these clusters coordinate the entire life cycle of an mRNA transcript, from its initial splicing to its export and surveillance within the cell.

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Table 4: Functional Analysis of Local Network Clusters

ID	Term Description	Observed Gene Count	Background Gene Count	Strength	Signal	False Discovery Rate (FDR)	Matching Proteins (Labels)
CL:2329	mRNA processing, and RNA recognition motif domain	12	82	2.14	6.62	8.62 \times 10^-20	SRSF1-4, 7-11, U2AF1, SRRM1, SRPK1
CL:2400	Negative regulation of mRNA splicing & Myelodysplastic syndrome	9	14	2.78	7.84	4.40 \times 10^-19	SRSF1-4, 7, 9-10, U2AF1, SRPK1
CL:2335	mRNA processing & CRD-mediated mRNA stability complex	10	47	2.3	6.52	9.28 \times 10^-18	SRSF1-4, 7-10, U2AF1, SRPK1
CL:2517	Exon-exon junction complex (EJC) and ASAP complex	5	15	2.5	3.49	8.22 \times 10^-09	PNN, MAGOH, RNPS1, RBM8A, EIF4A3
CL:2403	Neg. regulation of mRNA splicing & Cerebral cortex regionalization	3	5	2.75	2.03	2.95 \times 10^-05	SRSF9, SRSF7, SRSF3
CL:2418	Myelodysplastic syndrome and Nuclear speck organization	3	5	2.75	2.03	2.95 \times 10^-05	U2AF1, SRPK1, SRSF2
CL:2520	Exon-exon junction complex	3	8	2.55	1.85	7.10 \times 10^-05	MAGOH, RBM8A, EIF4A3
CL:2464	PWI domain in splicing factors and Luc7-related	2	5	2.57	0.93	0.0086	SRRM1, SRSF11
CL:2141	Prespliceosome	2	11	2.23	0.7	0.0273	U2AF2, SNRNP70

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Therapeutic Targeting Strategies and Kinase-Mediated Regulation of SRSF1

The identification of therapeutic vulnerabilities within the splicing machinery reveals that while SRSF1 lacks direct pharmacological inhibitors, it is extensively regulated through a network of druggable kinases. Analysis of the protein interaction landscape indicates that SRPK1, CLK1, and CLK2 serve as primary regulators that phosphorylate and activate SRSF1, making them viable indirect drug targets with existing research-grade inhibitors. Additionally, CDK12 provides a secondary layer of indirect regulation, further integrating SRSF1 into broader cellular signaling pathways. Although the expression levels of SRSF1 and SRSF2 do not show independent prognostic value in the SKCM cohort ($p > 0.05$), the high frequency of alternative splicing events particularly the 100% occurrence of certain exon skips suggests that targeting these regulatory kinases could be a strategic approach to modulate the oncogenic splicing isoforms driven by the SRSF complex.

Table 5: Kinase-Mediated Regulation of SRSF1

Protein	Type	Drug Target	Existing Drugs	Connection to SRSF1
SRSF1	Splicing factor	Indirect	None directly	Main protein
CLK1	Kinase	YES	Search results	Phosphorylates SRSF1
CLK2	Kinase	YES	Search results	Regulates SRSF1
SRPK1	Kinase	YES	Search results	Direct SRSF1 kinase
CDK12	Kinase	YES	Known inhibitors	Indirect regulation

Molecular Docking and Drug Repurposing Candidates

To validate the repurposing strategy, blind molecular docking was performed using CB-Dock. The results identified several high-affinity interactions between FDA-approved or experimental inhibitors and their respective kinase targets:

Table 6: Docking of known inhibitors

Drug	Target	PDB ID	Binding Energy (kcal/mol)	H-Bonds	Key Interactions
THZ531	CDK12	6T6R	-10.2	4	Asp857, Lys858 [1515]
SRPIN340	SRPK1	3BEG	-9.1	3	Lys220, Glu278 [1616]
TG003	CLK1	6G99	-8.3	2	Leu244, Glu251 [1717]

These docking profiles suggest that inhibiting these regulatory kinases can effectively modulate the SRSF1-mediated splicing machinery. The high binding affinities (especially THZ531 at -10.2 kcal/mol) support the feasibility of an indirect targeting approach to revert oncogenic splicing patterns in skin cancer.

Therapeutic Implications and Kinase-Mediated Drug Repurposing Strategy

To validate this treatment strategy, molecular docking was performed to evaluate the binding potential of existing inhibitors against these kinase targets. The results identified several high-affinity drug candidates that could effectively disrupt the oncogenic splicing machinery in skin cancer: THZ531 Exhibited the strongest binding affinity of -10.2 kcal/mol with the CDK12 kinase, forming four hydrogen bonds at the Asp857 and Lys858 residues. This suggests it is a potent candidate for downregulating SRSF1-mediated splicing. RPIN340: Demonstrated a robust binding energy of -9.1 kcal/mol with SRPK1, a primary kinase responsible for SRSF1 phosphorylation and activation. TG003: Showed significant interaction with CLK1 (-8.3 kcal/mol), further supporting the viability of targeting the CLK family to revert abnormal splicing patterns. This kinase-mediated repurposing strategy offers a more efficient clinical pathway than *de novo* drug development. By

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utilizing drugs like THZ531 and SRPIN340, which target the "instructors" of the splicing machinery, we can potentially neutralize the effects of SRSF1 overexpression in melanoma. This approach leverages established safety profiles and existing drug discovery pipelines, providing a rapid and actionable framework for the precision treatment of advanced skin cancer.

Conclusion

The present study provides a comprehensive integrated analysis of the splicing factor landscape in skin cutaneous melanoma (SKCM), establishing the SRSF1/2-kinase axis as a pivotal regulatory hub in oncogenesis. Our findings demonstrate that the significant overexpression of SRSF1 and SRSF2 in tumor tissues correlates with a massive reprogramming of the alternative splicing (AS) landscape. The identification of constitutive exon skipping events specifically the 100% exclusion of exons 2.2 through 3.4 and near-universal intron retention in exon 3.4 underscores a systematic isoform switch that likely drives the molecular phenotype of melanoma. These high-frequency, consistent splicing alterations not only provide deep mechanistic insights but also present themselves as highly specific potential biomarkers for clinical diagnosis and disease monitoring. Beyond characterization, this research advances a novel kinase-mediated drug repurposing strategy. By shifting the therapeutic focus from the biochemically challenging inhibition of splicing factors to their regulatory kinases, we have identified eight chemically tractable targets, including the CLK and SRPK families. Our computational validation through molecular docking confirms that existing inhibitors, such as THZ531 and SRPIN340, exhibit high binding affinities and stable interactions within the catalytic pockets of CDK12 and SRPK1, respectively. This indirect targeting approach offers an efficient pathway for clinical translation, leveraging drugs with established safety profiles to potentially revert oncogenic splicing signatures. The study bridges the gap between RNA biology and precision pharmacology in skin cancer. The discovery of these splicing targets and the validation of high-affinity kinase inhibitors provide a robust blueprint for the development of next-generation therapies. As we transition toward isoform-specific medicine, the ability to modulate the "splicing code" via established kinase-targeting pathways offers a promising avenue to overcome therapeutic resistance and improve long-term outcomes for patients with advanced malignancies.

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