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RESEARCH

SPIN1 across human cancers: mutational, epigenetic, and therapeutic insights

İnsan kanserlerinde SPIN1: mutasyonel, epigenetik ve terapötik bulgular

Dilara Fatma Akın[1], Didem Özkan[2]

[1]Niğde Ömer Halisdemir University, Niğde, Türkiye
[2]Istanbul Okan University, Istanbul, Türkiye

Abstract

Purpose: This study investigates the role of the SPIN1 gene from a pan-cancer perspective.
Materials and Methods: Genomic data from 10,967 TCGA pan-cancer samples were analyzed to evaluate SPIN1's mutation profile, expression patterns, protein interactions, drug sensitivity, and survival associations. The functional impact of mutations was assessed using PolyPhen-2, SIFT, Mutation Assessor, AlphaMissense, and MUpro. Expression and survival data were obtained from TIMER2.0 and GEPIA, drug sensitivity from GSCALite, and protein-protein interactions from STRING and GeneMANIA.
Results: Within the TCGA pan-cancer cohort, a total of 52 somatic mutations were identified in the SPIN1 gene of which 44 were missense mutations, representing 84.6% of all detected variants. The overall somatic mutation frequency of SPIN1 was approximately 0.4%. A substantial proportion of mutations clustered within functionally relevant regions, particularly the intrinsically disordered region (IDR) and the Tudor domains, which are associated with epigenetic regulation and DNA damage response. In silico stability analyses indicated that variants such as p.R158H and p.I253M were associated with a marked reduction in protein stability. Gene expression analyses revealed that SPIN1 was significantly upregulated in AML, breast, gastric, liver, lung, pancreatic and esophageal cancers, with elevated expression showing a trend toward unfavorable prognosis across multiple cancer types. Protein–protein interaction analyses demonstrated strong functional associations between SPIN1 and histone H3 variants as well as members of the SPIN protein family.
Conclusion: These findings suggest that SPIN1 is a key regulator in cancer biology and may serve as a potential target for future cancer therapies.

Keywords:. Epigenetic; histone reader; mutation; pan-cancer analysis; SPIN1

Öz

Amaç: Bu çalışma, SPIN1 geninin pan-kanser perspektifinden rolünü araştırmayı amaçlamaktadır.
Gereç ve Yöntem: 10.967 TCGA pan-kanser örneğinden elde edilen genomik veriler kullanılarak SPIN1'in mutasyon profili, ifade desenleri, protein etkileşimleri, ilaç duyarlılığı ve sağkalım ilişkileri analiz edildi. Mutasyonların işlevsel etkileri PolyPhen-2, SIFT, Mutation Assessor, AlphaMissense ve MUpro araçlarıyla değerlendirildi. Ekspresyon ve sağkalım verileri TIMER2.0 ve GEPIA'dan, ilaç duyarlılığı verileri GSCALite'tan, protein-protein etkileşim verileri ise STRING ve GeneMANIA'dan elde edildi.
Bulgular: TCGA pan-kanser kohortunda SPIN1 geninde toplam 52 somatik mutasyon saptandı; bunların 44'ü missens mutasyon olup tüm varyantların %84,6'sını oluşturdu. Genel somatik mutasyon frekansı yaklaşık %0,4 olarak hesaplandı. Mutasyonların önemli bir kısmı, epigenetik düzenleme ve DNA hasar yanıtı ile ilişkili intrinsik düzensiz bölge (IDR) ve Tudor domenlerinde kümelendi. İn siliko stabilite analizleri, özellikle p.R158H ve p.I253M varyantlarının protein kararlılığında belirgin azalma ile ilişkili olduğunu gösterdi. Ekspresyon analizlerinde SPIN1'in AML, meme, mide, karaciğer, akciğer, pankreas ve özofagus kanserlerinde anlamlı derecede artmış olduğu belirlendi; artmış ekspresyon birçok kanser türünde olumsuz prognoz eğilimi ile ilişkiliydi. Protein-protein etkileşim analizleri, SPIN1'in histon H3 varyantları ve SPIN ailesi proteinleri ile güçlü fonksiyonel bağlantılar sergilediğini ortaya koydu.
Sonuç: Bu bulgular, SPIN1'in kanser biyolojisinde önemli bir düzenleyici olduğunu ve gelecekteki kanser tedavileri için potansiyel bir hedef olabileceğini göstermektedir.

Anahtar kelimeler: Epigenetik; histon okuyucu; mutasyon; pan-kanser analizi; SPIN1

Address for Correspondence: Didem Özkan, Istanbul Okan University, Vocational School of Health Services, İstanbul, Türkiye E-mail: didemtorun@gmail.com
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INTRODUCTION

SPIN1 (Spindlin1) is an oncogenic epigenetic reader protein that recognizes specific histone methylation marks and plays a critical role in chromatin regulation and gene expression. Epigenetic dysregulation, particularly alterations in histone modifications is widely recognized as a fundamental driver of tumorigenesis, influencing transcriptional programs, genome stability and cellular identity (1-3). SPIN1 contains three tandem Tudor-like domains that enable the selective recognition of histone methylation patterns, allowing it to function as a histone code reader. Biochemical and structural studies have demonstrated that SPIN1 binds with high affinity to histone marks such as H3K4me3, H3R8me2a and H4K20me3 thereby modulating chromatin organization and transcriptional activity[1,4,5].

Accumulating evidence indicates that SPIN1 is frequently overexpressed in multiple cancer types and contributes to tumor progression, aggressive phenotypes, and unfavorable clinical outcomes. The oncogenic functions of SPIN1 are mediated, at least in part, through its ability to regulate key signaling pathways involved in cell proliferation and survival, including Wnt/β-catenin, PI3K/AKT, and RET signaling cascades[1,5–7]. Dysregulated SPIN1 expression has been linked to enhanced tumor cell proliferation, disruption of cell cycle control and increased genomic instability. Moreover, SPIN1 overexpression has been reported in various malignancies including breast cancer, non-small cell lung cancer (NSCLC), hepatocellular carcinoma and gastric cancer, where elevated SPIN1 levels have been associated with therapy resistance and disease progression[7–10].

Recent mechanistic studies have further expanded the functional landscape of SPIN1 beyond its role as a histone reader. SPIN1 possesses an intrinsically disordered region (IDR) at its N-terminus, which has been implicated in dynamic regulatory processes such as protein protein interactions and phase separation. Emerging evidence suggests that SPIN1 can undergo phase separation via its IDR, facilitating histone methylation readout and transcriptional regulation in a context-dependent manner. This property highlights a previously underappreciated regulatory mechanism by which SPIN1 may influence chromatin-associated processes and tumor biology. In addition to its epigenetic functions, SPIN1 has been shown to participate directly in the DNA damage response. Experimental findings indicate that SPIN1 can interact with poly-ADP-ribose (PAR) and promote homologous recombination (HR) mediated DNA repair by enhancing histone modification dynamics and recruiting DNA repair factors. Through these mechanisms, SPIN1 may contribute to tumor cell survival under genotoxic stress and support resistance to DNA damaging therapies.

Despite increasing recognition of SPIN1 as a multifunctional regulator in cancer biology, its roles across different tumor types remain incompletely understood. Most previous studies have focused on individual cancer contexts or specific molecular mechanisms leaving significant gaps in our understanding of SPIN1's mutational landscape, structural vulnerability, prognostic relevance and therapeutic associations at the pan-cancer level. In particular, a systematic evaluation integrating genomic alterations, functional predictions, expression patterns, survival associations protein interaction networks, and drug response correlations is currently lacking.

In this study, we performed a comprehensive pan-cancer analysis of SPIN1 using TCGA datasets, integrating somatic mutation profiling, in silico pathogenicity and stability predictions, differential expression analyses, survival correlations, protein protein interaction networks and drug sensitivity associations. This integrative framework provides a broader and more systematic perspective on the context-dependent functions of SPIN1 in cancer. We hypothesize that genetic alterations and expression variability of SPIN1 contribute to cancer type spesific biological effects influencing protein stability, epigenetic regulation, clinical outcomes and therapeutic response. The findings from this study aim to clarify the molecular and clinical significance of SPIN1 and to support its potential utility as a prognostic biomarker and therapeutic target.

MATERIALS AND METHODS

Sample

This study utilized publicly available data from The Cancer Genome Atlas (TCGA) project, encompassing a comprehensive pan-cancer dataset. A total of 10,967 samples representing 32 different cancer types were included in the analysis (Table-1).

All data used in this study were retrieved from the cBioPortal for Cancer Genomics.

Table-1. 32 Different cancer types, its abbreviation, and SPIN1 alteration frequency

Cancer type and pan-organ system	Cohort Name	Number of samples	SPIN1 Alteration Frequency (%)
Central Nervous System			
Glioblastoma multiforme	GBM	592	5(0.85)
Brain Lower Grade Glioma	LGG	514	3(0.58)
Pheochromocytoma and paraganglioma	PCPG	184	-
Thoracic			
Lung adenocarcinoma	LUAD	566	7 (1.24)
Lung squamous cell carcinoma	LUSC	487	4 (1.23)
Mesothelioma	MESO	87	-
Gastrointestinal System			
Colorectal adenocarcinoma	COAD	594	10 (1.68)
Rectal adenocarcinoma	READ	155	-
Stomach adenocarcinoma	STAD	165	4 (0.91)
Cholangio carcinoma	CHOL	36	-
Esophageal Adenocarcinoma	ESCA	182	1 (0.55)
Pancreatic adenocarcinoma	PAAD	184	1 (0.54)
Liver hepatocellular carcinoma	LIHC	369	-
Urologic System			
Bladder urothelial carcinoma	BLCA	411	6 (1.46)
Kidney chromophobe	KICH	65	-
Kidney renal clear cell carcinoma	KIRC	512	-
Kidney renal papillary cell carcinoma	KIRP	292	3 (1.06)
Prostate adenocarcinoma	PRAD	494	4 (0.81)
Testicular germ cell tumors	TGCT	149	-
Hematologic and lymphatic malignancies			
Difuse Large B-cell Lymphoma	DLBC	48	-
Acute myeloid leukemia	LAML	200	1 (0.55)
Thymoma	TYHM	123	1 (0.81)
Melanocytic			
Skin cutaneous melanoma	SKCM	448	5 (1.13)
Uveal melanoma	UM	80	-
Head and neck			
Head and neck squamous cell carcinoma	HNSC	523	
Gynecologic System			
Breast invasive carcinoma	BRCA	1084	6 (0.55)
Cervical squamous cell carcinoma	CESC	297	2 (0.67)
Uterine corpus endometrial carcinoma	UCEC	529	18 (3.40)
Uterine carcinosarcoma	UCS	57	2 (3.51)
Ovarian serous cystadenocarcinoma	OV	585	1 (0.17)
Endocrine System			
Adrenocortical carcinoma	ACC	92	1 (1.1)
Thyroid carcinoma	THCA	500	1 (0.2)

This study was conducted using only publicly available and fully anonymized genomic and transcriptomic datasets. The analyses did not involve direct human participation, patient recruitment, or

access to identifiable personal information. Therefore, ethical committee approval and informed consent were not required. All data were obtained from databases operating under established ethical and regulatory frameworks. The study was performed in accordance with the ethical principles governing biomedical research and complied with the data usage policies of the respective platforms. This study was conducted by the authors affiliated with Niğde Ömer Halisdemir University and Istanbul Okan University. All computational analyses, data processing and bioinformatics evaluations were performed by the study authors. The study did not involve laboratory experiments or clinical procedures, as it was based entirely on publicly available datasets. The design of the study, data analysis, interpretation of results and preparation of the manuscript were carried out collaboratively by the authors.

Data acquisition

The TCGA pan-cancer dataset was downloaded from the cBioPortal online platform (http://cbioportal.org), an open-access resource that provides access to multidimensional cancer genomics data (11). The dataset, which includes mutation data, copy number alterations, mRNA expression data from microarray and RNA sequencing, DNA methylation profiles, as well as protein and phosphoprotein levels, was accessed and downloaded on May 2, 2025.

Mutation profiling

For a comprehensive analysis of SPIN1-related genetic alterations, the gene symbol "SPIN1" was queried across the TCGA pan-cancer dataset using cBioPortal. The platform integrates multiple data types and offers several visualization and analysis tools. Specifically, the OncoPrint, Cancer Types Summary, and Mutations modules were utilized to assess mutation frequency, mutation types, and the distribution of SPIN1 alterations across different cancer types. These analyses allowed for an in-depth exploration of SPIN1's mutational landscape within the TCGA pan-cancer cohort.

In silico characterization of SPIN1 missense mutations in terms of pathogenicity and stability

To assess the potential pathogenicity of missense mutations in the SPIN1 gene, several bioinformatics tools were employed, including PolyPhen-2, SIFT, Mutation Assessor, and AlphaMissense. PolyPhen-2 (https://genetics.bwh.harvard.edu/pph2/) evaluates the structural and functional impact of amino acid changes and classifies variants as "probably damaging," "possibly damaging," "benign," or "unknown"12. SIFT (https://sift.bii.a-star.edu.sg/) predicts whether substitutions affect protein function based on sequence conservation and amino acid properties, categorizing them as "tolerated" or "deleterious."[13] Mutation Assessor (http://mutationassessor.org/r3/) estimates functional impact using evolutionary conservation, while AlphaMissense(https://alphamissense.hegelab.org/) applies deep learning to assign a pathogenicity score between 0 and 1, grouping variants as "likely pathogenic," "uncertain significance," or "likely benign" [14,15] . Using these complementary tools improves the reliability of functional predictions. The full amino acid sequence of human SPIN1 (UniProt ID: Q9Y657) was retrieved from UniProt. The MUpro tool (http://mupro.proteomics.ics.uci.edu) was used to predict how each missense mutation might affect protein stability. MUpro provides a confidence score from -1 to +1, where negative values indicate decreased stability and positive values suggest increased stability[16]

Differential gene expression and survival correlates of SPIN1 across cancer types

The TNMplot database (https://tnmplot.com/analysis/) was utilized to evaluate and visualize the differential mRNA expression levels of SPIN1 between tumor and normal tissues across various cancer types. This tool integrates gene expression data from multiple sources, including TCGA, Gene Expression Omnibus (GEO), The Genotype-Tissue Expression (GTEx) project, and Therapeutically Applicable Research to Generate Effective Treatments (TARGET) database. The statistical significance of expression differences was assessed using the Mann–Whitney U test [17]. To further investigate the prognostic value of SPIN1 expression, survival analysis was conducted using the GEPIA database (http://gepia.cancer-pku.cn/) [18] Kaplan–Meier survival curves were plotted to assess the impact of high versus low SPIN1 mRNA expression levels on patient survival. Patients were stratified into high- and low-expression groups based on the median

SPIN1 mRNA expression value. The analysis revealed that patients with higher SPIN1 expression had significantly reduced recurrence-free survival and overall worse prognosis compared to those with lower expression levels.

Drug sensitivity analysis

GSCALite (https://guolab.wchscu.cn/GSCA/#/drug_/) is an online platform for analyzing the correlation between gene expression and drug sensitivity[19]

Protein-protein interaction (PPI) network construction

The STRING database (http://string-db.org) is a widely used online platform for constructing protein-protein interaction (PPI) networks, which evaluates each interaction between target proteins based on a confidence score[20]. In this study, the predicted interaction network of the SPIN1 protein was generated using the STRING database to reveal both direct (physical) and indirect (functional) relationships between proteins. Additionally, GeneMANIA (http://www.genemania.org) is a web-based tool that integrates existing genomic and proteomic data to provide predictions about the functional characteristics of genes[21]. GeneMANIA was also utilized to identify potential genes related to SPIN1 and to visualize the interactions among these genes.

Statistical Analysis

All analyses were conducted using publicly available pan-cancer genomic datasets. Differences in SPIN1 expression between cancer patients and healthy controls were assessed using the Mann–Whitney test. The prognostic value of SPIN1 mRNA levels was evaluated via Kaplan–Meier survival analysis on the GEPIA platform, with patients split into high and low expression groups based on the median. Survival differences were tested using the log-rank test, with p-values < 0.05 considered significant. Drug sensitivity analysis was performed using GSCALite, which correlates SPIN1 expression with response to 265 anticancer drugs. Spearman correlation coefficients were calculated, and results were visualized through bar plots and heatmaps. Protein–protein interaction (PPI) networks were generated using STRING and GeneMANIA, incorporating experimentally supported, predicted, and co-expression data. Functional enrichment and network topology analyses were also conducted to explore the biological relevance of SPIN1. Statistical significance was defined as $p < 0.05$.

RESULTS

Mutation profiling analysis

Analysis of TCGA cohorts revealed the presence of various somatic alterations in the SPIN1 gene. These alterations included missense mutations, nonsense mutations, frameshift mutations, splice site mutations, gene fusions, deep deletions, and gene amplifications (Figure-1A). A detailed examination identified a total of 52 somatic mutations, of which 44 were missense, 4 nonsense, 2 gene fusions, 1 frameshift deletion, and 1 splice site mutation. Additionally, gene amplification anomalies and deep deletions resulting in homozygous loss were observed. The detailed characteristics of these mutations are presented in Table-2 (Supplemental material). The somatic mutation frequency of the SPIN1 gene was calculated as 0.4%, and no putative driver mutations were identified. The cancer type with the highest mutation frequency was endometrial carcinoma, whereas no mutations in the SPIN1 gene were detected in cholangiocarcinoma, diffuse large B-cell lymphoma, chromophobe renal cell carcinoma, clear cell renal cell carcinoma, hepatocellular carcinoma, mesothelioma, pheochromocytoma and paraganglioma, testicular germ cell tumors, and uveal melanoma samples. Figure 1B and 1C illustrates the frequency distribution of SPIN1 mutations across TCGA cohorts, while Figure 1D shows the localization of these mutations within the functional domains of the SPIN1 protein. The SPIN1 protein is a polypeptide composed of 262 amino acids and contains three tandem Tudor-like domains. These domains enable SPIN1 to bind double-stranded DNA with high affinity and recognize post-translational histone modifications following histone methylation. The three Tudor-like domains form a three-dimensional structure that allows SPIN1 to act as a histone code reader, capable of recognizing various post-translational histone modifications (PTMs). Each domain is approximately 50 amino acids long and contains a characteristic Spin/Ssty repeat sequence[9,22]. Additionally, these domains facilitate the homodimerization ability of the SPIN1 protein. Structural localization of the mutations revealed that the p.1M* nonsense mutation is located at the start codon, potentially preventing translation

initiation. The p.G93* and p.E101* nonsense mutations are positioned within the first Spin/Ssty domain and may disrupt the functional integrity of the protein. The p.E138Dfs*19 frameshift mutation resides within the second Spin/Ssty domain, causing a frameshift that introduces a premature stop codon. The p.X197 splice region mutation is located at the boundary of exon 4 and intron 5, within an evolutionarily conserved splicing site with 100% conservation. Consequently, this mutation is highly likely to disrupt proper mRNA splicing, leading to the formation of nonfunctional, truncated transcripts.

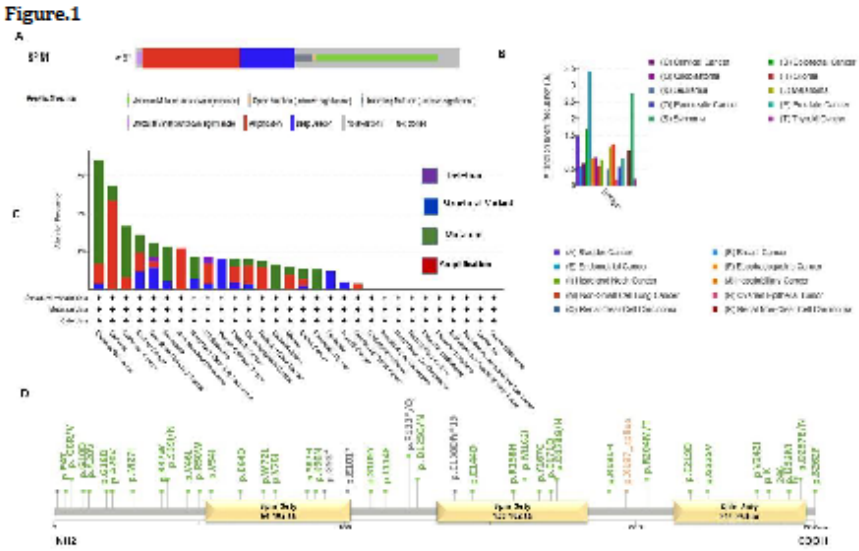

Figure-1. Genetic alteration landscape of the SPIN1 gene across TCGA pan-cancer cohorts.

(A) Overview of the types and distribution of SPIN1 genetic alterations. Somatic alterations include missense, truncating (nonsense), splice site mutations, structural variants, gene amplifications, and deep deletions. (B) Frequency of SPIN1 genetic alterations across different cancer types. Endometrial cancer exhibited the highest frequency of SPIN1 alterations. (C) Alteration frequency of SPIN1 across individual cancer types, stratified by mutation type (mutation, amplification, deletion, and structural variant). CNA: copy number alteration. Data were obtained from the cBioPortal platform, and only cancer types with complete structural variant, mutation, and CNA data are shown. (D) Schematic representation of the domain architecture of the human SPIN1 protein and the localization of somatic mutations identified in TCGA pan-cancer cohorts. SPIN1 is composed of 262 amino acids and contains three tandem Tudor-like domains. Mutations detected in cancer samples are mapped onto their respective positions across the protein structure.

In silico characterization of SPIN1 missense mutations in terms of pathogenicity and stability

Using PolyPhen-2, SIFT, Mutation Assessor, and AlphaMissense tools for pathogenic/onco-genic prediction analyses, 18 out of the 44 missense mutations identified in our study were classified as pathogenic or oncogenic by at least two different tools. Detailed information regarding these mutations is provided in Table 2. Subsequently, the potential structural impacts of the SPIN1 missense mutations were analyzed. Protein stability prediction performed with MUpro revealed that all mutations except p.S262F, p.V76I, p.V46L, p.A20V, and p.R12Q led to varying degrees of decreased protein stability. MUpro predicts the effects of single amino acid mutations on protein stability based solely on sequence information, using Support Vector Machines and Neural Networks. Negative ΔΔG values indicate a decrease in protein stability.

Accordingly, mutations such as p.R158H, p.I253M, p.Y87H, p.D173G, p.Y167C, p.E144D, and p.G18D are predicted to significantly reduce protein stability, whereas p.S262F, p.V76I, p.V46L, p.A20V, and p.R12Q mutations are likely to increase protein stability.

Protein-protein interaction network construction

In the STRING database analysis, SPIN1 protein was observed to establish strong interactions primarily with various histone proteins such as H3-3A, H3-3B, H3-5, H3C10, H3C12, H3C13, and H3C14. These interactions are supported both by experimentally determined data and predictive methods including text mining, gene co-expression, and protein homology. This suggests that SPIN1 may play an active role in chromatin structure and nucleosomal organization (Figure 2A). Analysis using the GeneMANIA database revealed the relationships of SPIN1 with other members of the SPIN gene family, including SPIN2A, SPIN2B, SPIN3, SPIN4, and SPINDOC. Additionally, it was found to be connected to genes such as EIF4A3, MAGOH, AGO3, TCF7L2, PAX3, and YRDC in terms of genetic and physical interactions. These genes are mostly involved in mRNA processing, translational control, and genetic programming. The high density of physical interactions and shared protein domains further supports the involvement of SPIN1 in these processes (Figure 2B).

Figure.2

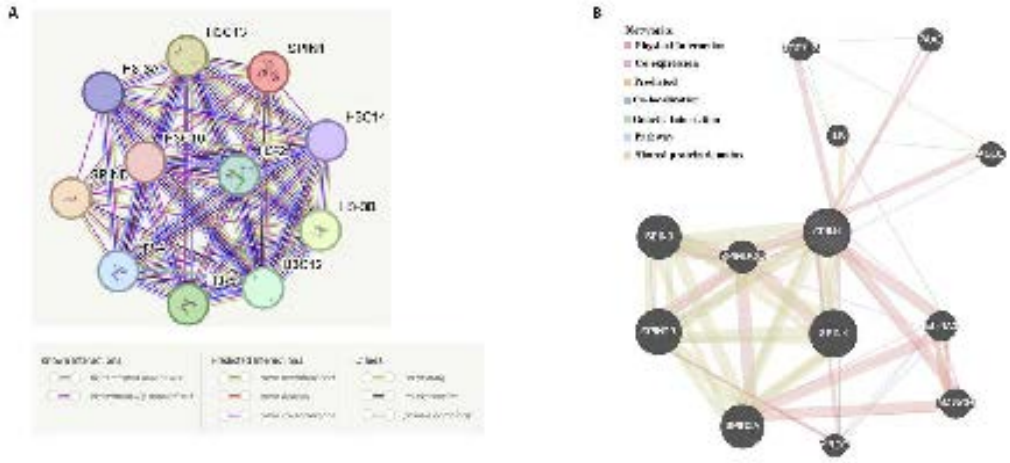

Figure-2 (A)Schematic representation of known and predicted protein-protein interactions with the SPIN1 gene. Each line has features. [Red line-indicates the presence of fusion evidence; Green line- neighborhood evidence; Blue line- co-occurrence evidence; Purple line-experimental evidence; Yellow line- text mining evidence; Light blue line database evidence; Black line co-expression evidence.]. (B) The PPI network of four genes constructed by GeneMANIA. PPI network for four hub genes was constructed in GeneMANIA website. The interconnections between proteins were explored in term of physical interaction, co-expression, predicted, co-localization, common pathway, genetic interaction and shared protein domains.

Differential gene expression and survival correlations of SPIN1 across cancer types

Analysis of SPIN1 gene expression in TCGA cohorts revealed significant upregulation of SPIN1 in seven tumor types, including acute myeloid leukemia (AML), breast, esophageal, liver, lung, pancreatic, and stomach cancers, compared to matched normal tissues ($p<0.05$). In contrast, SPIN1 expression was significantly downregulated in colon, ovarian,

prostate, rectal, skin, thyroid, and uterine cancers (Figure 3A). Kaplan–Meier survival analysis demonstrated that patients with high SPIN1 mRNA expression in Kidney Renal Clear Cell Carcinoma (KIRC) (p=0.00000078) and Sarcoma (SARC) (p=0.0083) exhibited significantly longer overall survival compared to patients with low expression levels. Conversely, in Adrenocortical Carcinoma (ACC) (p= 0.015) patients with low SPIN1 expression had a significantly improved overall survival relative to those with high expression (Figure 3B).

Drug sensitivity analysis

Analyses performed using the GSCALite platform indicated that SPIN1 expression may be associated with sensitivity and resistance to various anticancer agents. Specifically, negative correlations were observed between SPIN1 expression and drugs such as Afatinib, AG-014699, Bleomycin, Docetaxel, Elesclomol, Midostaurin, Olaparib, RO-3306, and Talazoparib, suggesting that higher SPIN1 expression could be linked to increased sensitivity to these agents. Conversely, SPIN1 expression exhibited positive correlations with 21 different drugs, implying a potential association with resistance development to these treatments (Figure 3C). These findings underscore the potential clinical significance of SPIN1 in guiding drug target identification, drug screening efforts, and the development of personalized therapeutic strategies.

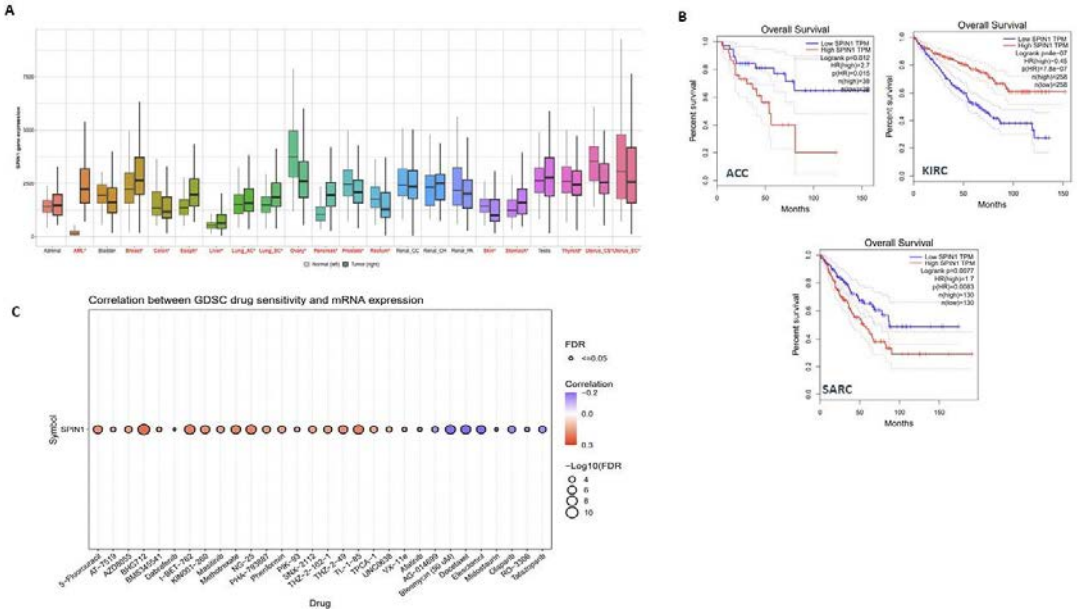

Figure 3. (A) SPIN1 gene expression across cancer types and its association with overall survival. Boxplot analysis of SPIN1 gene expression between tumor and adjacent normal tissues across TCGA cancer types. Asterisks (*) indicate cancer types with statistically significant differences in SPIN1 expression between tumor and normal tissues (p < 0.05). SPIN1 expression is notably upregulated in cancers such as AML, breast, stomach, liver, lung, pancreas, and uterus. (B) Kaplan–Meier overall survival analysis based on SPIN1 expression in adrenocortical carcinoma (ACC), kidney renal clear cell carcinoma (KIRC), and sarcoma (SARC). Patients were stratified into high and low SPIN1 expression groups. High SPIN1 expression was associated with poorer survival in ACC and SARC, whereas in KIRC, high expression correlated with improved prognosis. Log-rank test p-values and hazard ratios (HR) are indicated in each panel. (C) Correlation between SPIN1 mRNA expression and drug sensitivity across 265 anticancer drugs from the GDSC database.

Each dot represents a drug, with the size indicating false discovery rate (FDR) and the color showing Spearman correlation strength (blue = resistance, red = sensitivity).

DISCUSSION

In this study, we performed the first integrated pan-cancer analysis of SPIN1 by combining genomic alterations, structural and functional mutation predictions, expression patterns, prognostic associations, protein interaction networks, and drug sensitivity profiles. Unlike previous reports that examined SPIN1 in specific tumor types or focused on individual mechanisms, our analysis reveals cancer type spesific and context dependent roles of SPIN1, highlighting both oncogenic and prognostically favorable associations depending on the tumor context. Multifaceted approaches including mutation profiling, expression level assessment, protein-protein interactions, drug sensitivity, and survival analyses were employed to investigate the functions of SPIN1 in cancer biology in detail. Mutation profiling of TCGA cohorts revealed a total of 52 somatic mutations in the SPIN1 gene, including 44 missense, 4 nonsense, 2 gene fusions, 1 frameshift deletion, and 1 splice site mutation.

Previous studies have established that epigenetic mechanisms, including histone modifications and chromatin remodeling, play a significant role in the pathogenesis of endometrial carcinoma. For example, histone modification and chromatin structure alterations have been implicated in gene expression changes during endometrial carcinogenesis[23]. Epigenetic dysregulation through DNA methylation and histone modification has also been characterized as contributing to tumor progression and prognosis in endometrial cancer[24]. Furthermore, sarcomas are known to exhibit extensive chromatin pathway alterations, with epigenomic dysregulation contributing to malignant behavior across subtypes[25]. This context supports our finding of relatively high SPIN1 mutation frequencies in these tumor types and suggests that SPIN1 alterations may be associated with broader epigenetic dysregulation in cancers with permissive mutational landscapes.

SPIN1 protein is a histone code reader containing three Tudor-like domains, known to bind with high affinity to H3K4me3, typically occurring in transcriptionally active promoter regions[26,27]. These Tudor-like domains constitute the biochemical basis for SPIN1's role in regulating cellular events. It has been demonstrated that Thr95 within the first Spin/Ssty repeat domain forms strong hydrogen bonds with two phosphate ions, stabilizing the long loop structure between the first and second domains of SPIN1[4,28,29]. This structural stability is associated with SPIN1's cell cycle regulatory activity. In our study, a p.G93* nonsense mutation located in the first Spin/Ssty domain was identified, potentially causing premature transcript termination and disrupting this binding site. This mutation was found in a 60-year-old female patient with metastatic skin cutaneous melanoma.

SPIN1 is known to harbor multiple potential phosphorylation sites. [1,29] Missense mutations p.S39I/N and p.S109Y detected in endometrial cancer and skin cutaneous melanoma patients in this study are located within these phosphorylation regions. Pathogenic mutations predicted in the C-terminal region, including p.K246N, p.I253M, p.D257E, and p.S262F, suggest critical roles in nuclear localization. SPIN1 dynamically localizes during mitosis and contributes to maintaining the proper structure of the mitotic spindle, thereby regulating cell division[29].

The intrinsically disordered region (IDR) of SPIN1, spanning approximately the first 50 amino acids at the N-terminus, lacks defined secondary structure and is flexible. This region has been implicated in vital processes such as cellular stress response and DNA damage repair[22,26] Particularly, SPIN1-IDR directly interacts with poly-ADP-ribose (PAR), facilitating homologous recombination (HR)-mediated repair of DNA double-strand breaks. This interaction directs SPIN1 to DNA damage sites, promotes accumulation of H3K9me3, and facilitates Tip60 binding, which activates ATM kinase[4,5,27-29]. Consequently, SPIN1 plays a crucial role in chemotherapy resistance and tumor cell survival. In our study, pathogenic mutations including p.M1* nonsense mutation at the start codon, as well as p.G6V, p.S39I, p.R12Q, p.P4T, and p.A20V, were identified within the IDR. Notably, all six patients harboring these mutations exhibited metastatic and recurrent disease, suggesting a strong association between structural disruption in this region and aggressive tumor phenotype. Given the IDR's role in PAR interaction and DNA repair, these mutations may impair SPIN1's participation in DNA damage response, reduce HR efficiency, and increase genomic instability in tumor cells.

SPIN1's epigenetic regulatory capacity is related to its ability to recognize histone H3K4me3 and H3R8me2a methylations. H3K4me3 binds with high affinity to an aromatic pocket in SPIN1's second

Tudor-like domain[27,29]. Mutations in residues F141 or Y170 within this domain disrupt binding to both H3K4me3 and combined H3K4me3-H3R8me2a marks.

In our dataset, frame-shift mutation p.E138Dfs19 and nonsense mutations p.G93*, p.E101*, and p.R122*/Q were detected in this domain. These mutations likely abolish the second Tudor-like domain, impairing SPIN1's binding to histone modifications and weakening its epigenetic regulatory function, potentially contributing to the inactivation of tumor suppressor pathways.

Analyses of TCGA datasets showed significant upregulation of SPIN1 expression in seven cancer types including AML, breast, esophageal, liver, lung, pancreatic and stomach cancers supporting its oncogenic potential in these tumors. These findings align with literature reporting SPIN1's contribution to tumor progression through various mechanisms in both solid tumors and hematological malignancies[26,30]. For instance, SPIN1 has been reported to activate the MDM2-p21-E2F1 feedback loop to promote tumor proliferation in gastric cancer, and to regulate the FOXO3a/FOXM1 axis enhancing radioresistance in non-small cell lung cancer [6,31] Increased SPIN1 expression in liposarcomas compared to normal adipose tissue correlates with tumor aggressiveness and involves RET signaling pathways promoting tumor growth. Additionally, SPIN1 is negatively regulated by miR-148/152 clusters in breast cancer with this regulation affecting drug metabolism enzyme expression and contributing to Adriamycin resistance[7,32]. The observation that SPIN1 exhibits unfavorable prognostic effects in some cancers (e.g., ACC, SARC) while showing favorable prognostic associations in others (e.g., KIRC) may be explained by its context-dependent interaction with tumor-specific epigenetic landscapes and signaling networks. In several solid tumors, SPIN1 is known to activate proliferative pathways such as Wnt/TCF4, PI3K/AKT, and RET signaling, which may account for the poor outcomes associated with high SPIN1 expression in aggressive cancer types like ACC and SARC [9,26,30]. In contrast, the unique metabolic and epigenetic characteristics of KIRC may allow SPIN1 to support the activation of cell-cycle–regulatory or differentiation-related gene programs, potentially mediated by its ability to read specific histone methylation patterns such as H3K4me3 and H4K20me3 or reflect weaker oncogenic signaling dependencies in this tumor type [27,29]. Moreover, tissue-specific regulation of SPIN1 by microRNAs, including miR-489 and miR-148/152 as well as interactions with regulatory partners such as SPINDOC may further modulate its biological impact across cancer types [32,33]. Together, these findings suggest that the prognostic heterogeneity of SPIN1 arises from its integration into tumor-type–specific epigenetic and signaling contexts.

Differential expression analyses were performed using TNMplot, which integrates data from multiple repositories (TCGA, GTEx, GEO). Because these datasets originate from different platforms and processing pipelines, potential batch effects and cross-dataset heterogeneity may influence tumor–normal comparisons. We did not perform formal batch-correction or independent cross-validation of TNMplot results in this study; therefore, these tumor–normal expression differences should be interpreted with caution.

Protein-protein interaction analyses revealed that SPIN1 is involved in key cellular processes such as nucleosomal regulation, epigenetic modification, and gene expression control. STRING database analysis showed strong physical and functional interactions between SPIN1 and various histone H3 variants, supporting its role as a transcriptional co-activator. GeneMANIA analysis highlighted close relationships with SPIN family members and other genes implicated in mRNA processing and translational control. Notably, the interaction with SPINDOC suggests a dual regulatory role of SPIN1 in transcription, acting as both an activator and repressor[33]. Drug sensitivity analyses via the GSCALite platform indicated that SPIN1 expression may serve as a biomarker for response to anticancer agents. Negative correlations with drugs such as Afatinib, Rucaparib (AG-014699), Bleomycin, Docetaxel, Elesclomol, Midostaurin, Olaparib, RO-3306 and Talazoparib suggest increased sensitivity with high SPIN1 expression. Especially, the negative correlation with PARP inhibitors Olaparib and Talazoparib aligns biologically with SPIN1's role in DNA repair pathways.

STRING and GeneMANIA based protein protein interaction analyses, together with SPINDOC associated networks support the functional relevance of SPIN1 in cancer-related pathways. Several SPIN1-interacting partners have been implicated in transcriptional regulation and therapeutic response in different cancer types. In this context, the observed associations between SPIN1 expression and drug

sensitivity may reflect tumor type spesific dependencies on epigenetic and chromatin-related mechanisms. Although cancer type specific subgroup analyses were not performed, these findings provide a biologically meaningful framework that may guide future stratified and experimental studies.

A limitation of this study is the absence of an independent validation cohort. All analyses, including mutation profiling, differential expression, prognostic associations, and drug sensitivity correlations, were performed using TCGA-derived datasets and related bioinformatics platforms, without validation in external patient cohorts or experimental models. In addition, the functional consequences of SPIN1 mutations showed pronounced heterogeneity across different cancer types. Given the context-dependent involvement of SPIN1 in distinct signaling pathways, identical genetic alterations may result in divergent biological effects depending on tumor-specific epigenetic landscapes and pathway dependencies. Consequently pathway specific functional effects of SPIN1 mutations could not be fully resolved within the scope of this pan-cancer analysis and future studies incorporating independent clinical cohorts and experimental validation will be essential to substantiate these findings.

While the pan-cancer approach used in this study provides a comprehensive overview of SPIN1-related alterations across multiple tumor types, it may also mask cancer-specific biological features. Aggregating data across diverse cancers can obscure tissue-specific regulatory mechanisms and pathway dependencies that are critical for understanding contextdependent gene function. Therefore, the pan-cancer nature of this analysis represents an additional limitation and future cancer type focused studies will be necessary to refine and validate these findings.

SPIN1 emerges as a critical regulator in cancer development and therapeutic response with accumulating genetic and functional evidence supporting its potential as both a prognostic biomarker and a therapeutic target. Taken together, the findings of this study underscore the multifaceted and context-dependent role of SPIN1 in cancer biology. The observed mutation patterns, expression variability, prognostic heterogeneity and drug sensitivity associations collectively suggest that SPIN1 may exert tumor type specific biological effects and may serve as a clinically relevant molecular indicator. Further functional and translational studies are warranted to validate these in silico observations, with particular emphasis on experimental characterization of SPIN1 mutations affecting the intrinsically disordered region and Tudor domains to clarify their structural and functional consequences. In addition, mechanistic investigations exploring SPIN1-mediated regulatory pathways will be essential to better understand its contribution to tumor-specific epigenetic control and therapy response. The integration of functional assays, independent clinical cohorts, and prospective studies may provide deeper biological insight and facilitate the development of SPIN1-guided precision oncology strategies.

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Ethical Approval: Our study analyzes the mutation profile, expression patterns, protein interactions, drug sensitivity, and survival associations of SPIN1 using genomic data obtained from 10,967 TCGA pan-cancer samples. As this study is a bioinformatics-based analysis using publicly available data, ethics committee approval is not required."
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