Research Article

A Computational Approach to Justifying Stratifin as a Candidate Diagnostic and Prognostic Biomarker for Pancreatic Cancer

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Pancreatic cancer (PC) is considered a silent killer because it does not show specific symptoms at an early stage. Thus, identifying suitable biomarkers is important to avoid the burden of PC. Stratifin (SFN) encodes the 14-3-3σ protein, which is expressed in a tissue-dependent manner and plays a vital role in cell cycle regulation. Thus, SFN could be a promising therapeutic target for several types of cancer. This study was aimed at investigating, using online bioinformatics tools, whether SFN could be used as a diagnostic and prognostic biomarker in PC. SFN expression was explored by utilizing the ONCOMINE, UALCAN, GEPIA2, and GENT2 tools, which revealed that SFN expression is higher in PC than in normal tissues. The clinicopathological analysis using the ULCAN tool showed that the intensity of SFN expression is commensurate with cancer progression. GEPIA2, R2, and OncoLnc revealed a negative correlation between SFN expression and survival probability in PC patients. The ONCOMINE, UCSC Xena, and GEPIA2 tools showed that cofilin 1 is strongly coexpressed with SFN. Moreover, enrichment and network analyses of SFN were performed using the Enrichr and NetworkAnalyst platforms, respectively. Receiver operating characteristic (ROC) curves revealed that tissue-dependent expression of the SFN gene could serve as a diagnostic and prognostic biomarker. However, further wet laboratory studies are necessary to determine the relevance of SFN expression as a biomarker.

1. Introduction

The pancreas is a pear-shaped organ located in the abdomen, and it plays an essential role in converting foods to become fuel for body cells. However, in some cases, the growth of the pancreas becomes uncontrollable due to some reasons and thus becomes cancerous. Pancreatic cancer (PC) is one of the deadliest cancers and is the seventh most common cause of cancer-related deaths in both men and women [1]. According to GLOBOCAN, in 2018, the estimated number of PC cases and deaths were 458,918 and 432,242, respectively, corresponding to 2.5% of all new cancer diagnoses and 4.5% of all cancer deaths [1]. PC has become more common in recent decades, and the number of new cases will reach 355,317 by 2040 [2, 3]. PC incidence is 3–4 times greater in developed countries than in developing and poor countries [4]. In the United States, it is estimated that in 2021, approximately 60,430 individuals (31,950 men and 28,480 women) will be diagnosed with PC and approximately 48,220 individuals (25,270 men and 22,950 women) will die of PC [5]. Furthermore, PC is expected to overtake breast cancer as the third leading cause of cancer-related death in the European Union, as in the United States [3, 6].
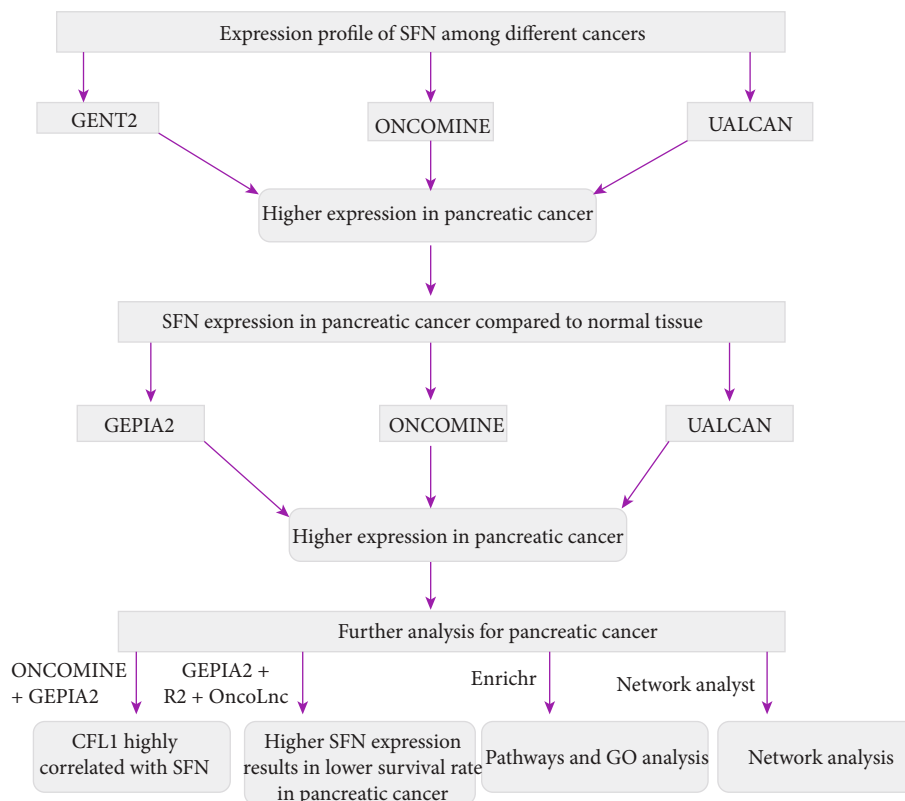
FIGURE 1: Schematic of the overall workflow in this study.

PC is often difficult to diagnose at an early stage; as a result, the majority of PC cases are diagnosed at an advanced stage, and only 10–20% of cases are surgically treatable [7]. This trend is due to the lack of distinct clinical signs and symptoms, due to a lack of accurate biomarkers, and due to the limited resolution of imaging techniques, resulting in a high mortality rate in PC [7, 8]. The 5-year overall survival rate for PC has remained low at 3% in recent years, as more than half of PC patients are diagnosed at an advanced stage [9, 10]. Compared with the screening programs for other cancers, such as lung, breast, colon, and cervical cancers, those for PC are difficult to implement due to the lack of specificity of a particular test [11]. The most common biomarker that has been approved by the US Food and Drug Administration (FDA) for PC diagnosis is the carbohydrate antigen (CA) 19-9. However, CA has not been considered to be the most effective screening tool due to its low sensitivity and specificity and poor predictive value of 0.5–0.9% in asymptomatic patients [12, 13]. Meanwhile, CA 19-9 expression may increase in other medical conditions, such as acute cholangitis, pancreatitis, obstructive jaundice, and liver cirrhosis [11]. Currently, there are no biomarkers with an adequately high accuracy that could be used to screen sporadic PC; therefore, there is an urgent need to identify biomarkers for PC [14].

Stratifin (SFN) encodes the 14-3-3σ protein, which is a member of a highly conserved family of 14-3-3 proteins found in all eukaryotic organisms [15]. SFN was first identified as human mammary epithelial marker 1 before being

rediscovered as a key regulator of cell cycle checkpoints [16, 17]. Decreased SFN expression has been found in various cancers, including breast [18], lung [19], liver [20], endometrium [21], head and neck [22, 23], vulva [24], and prostate cancers [25, 26]. Conversely, upregulation of the SFN gene expression has been observed in other cancers, including pancreatic [27–29], colorectal [30], and esophageal squamous cell carcinoma [31]. The expression of the SFN gene varies in different cancers, and it performs a double-edged function [32]. Therefore, the role of SFN expression is likely context dependent. On the basis of its tissue-dependent expression pattern, SFN can be used as a diagnostic and prognostic biomarker in PC. However, there has been insufficient evidence demonstrating that SFN expression can be used as a biomarker for PC.

This study compared the expression pattern of SFN in PC patients and healthy individuals based on data obtained from online databases. Moreover, clinicopathological features, coexpression, prognostic values, gene ontologies, signaling pathways, and network analysis were performed. The workflow for this study is depicted in Figure 1.

2. Material and Methods

2.1. Investigation of mRNA Expression in Human Cancers.
The ONCOMINE (https://http://www.ONCOMINE.org/) tool was used to examine the mRNA expression levels of SFN in different cancers, wherein the threshold level for P value, gene rank, and fold change were fixed at 1×10^{-4},

10%, and 2, respectively. ONCOMINE is a web-based data-mining platform that is aimed at facilitating the identification of cancer-related genes by analyzing genome-wide expression [33, 34]. The pancancer view for SFN was determined using the UALCAN (http://ualcan.path.uab.edu/) platform. UALCAN is a comprehensive, user-friendly, and interactive web-based resource used to study cancer OMICS data [35]. Then, GENT2 (http://gent2.appex.kr/gent2/) [36] was adopted by using the GPL570 platform (HG_U133_Plus_2) to investigate the SFN expression levels in different types of cancer.

2.2. SFN Expression in PC versus Healthy Tissues. We examined the SFN expression in various datasets collected from the ONCOMINE tool. The SFN expression levels under normal conditions were explored in different PC subtypes, such as pancreatic carcinoma, pancreatic adenocarcinoma, and pancreatic ductal adenocarcinoma. Moreover, the SFN expression in PC obtained from the GEPIA2 (http://gepia2.cancer-pku.cn/#index) platform was compared with that in their normal counterpart. GEPIA2 is a web-based platform used for gene expression analysis involving data for tumor and normal samples retrieved from the TCGA and GTEx databases [37]. UALCAN was utilized to obtain SFN expression data in PC and then compared with those in normal tissues.

2.3. SFN Expression in relation to Clinicopathological Parameters in PC. The UALCAN web tool with default settings was used to assess the mRNA expression of the SFN gene in PC patients based on their clinicopathological features. In this investigation, SFN expression was analyzed based on clinicopathological parameters, such as cancer stages, race, age, nodal metastasis status, and tumor grade. Only the statistically significant results were taken into account in the analysis.

2.4. Association between SFN Expression and Survival Probability in PC Patients. The impact of SFN expression on the survival probability of PC patients was investigated using the GEPIA2, R2 (http://r2platform.com), and OncoLnc (http://www.oncolnc.org/). The R2 genomics platform is a publicly available web-based platform that allows researchers to integrate, analyze, and visualize clinical and genomics data [38]. OncoLnc is an online tool for estimating survival relationships and for accessing clinical data for mRNAs, miRNAs, and lncRNAs (long noncoding RNAs) [39]. The R2 platform was utilized to generate a Kaplan-Meier plot (OS) for the SFN gene against the mixed tumor pancreas Hussain-130-rma-sketch-hugene10t and mixed pancreatic adenocarcinoma Sadanandam-47-MAS5.0-u133p2 datasets by setting the optimum cut-off values. The Kaplan-Meier plot was drawn by splitting the patient population at the median. A $P < 0.05$ was considered significant.

2.5. Coexpression Analysis of the SFN Gene in PC Cancer. The SFN gene's coexpression profile in PC was determined, and the corresponding heat map was obtained from the Collisson Pancreas dataset through the ONCOMINE web tool. From this dataset, the cofilin 1 (CFL1) gene was the most

positively correlated with SFN expression in PC. To confirm the relationship between SFN and CFL1, we used the TCGA (PAAD) dataset from the UCSC Xena server (https://xenabrowser.net/) [40]. Furthermore, correlation data were obtained from the UCSC Xena server, and a scatter plot was drawn by using ggplot2 [41]. The GEPIA2 was utilized to confirm the positive correlation between SFN and CFL1 transcripts in the PC.

2.6. Enrichment Analysis of the SFN Gene. The Enrichr (https://maayanlab.cloud/Enrichr/) web tool was used to extract the gene ontologies and signaling pathways of the SFN gene, as well as the corresponding bar graphs. Enrichr is a user-friendly web-based enrichment analysis tool that graphically presents the collective functions of genes [42, 43]. Gene ontologies were analyzed using GO Biological Process 2018, GO Molecular Process 2018, and GO Cellular Process 2018. Signaling pathways were determined using BioPlanet 2019, Reactome 2016, WikiPathway 2021 Human, KEGG 2021 Human, Biocarta 2016, and Panther 2016.

2.7. Evaluation of the SFN Interaction Network. The STRING (https://string-db.org/) database was employed to investigate the interactions of SFN with other proteins. STRING is a database that contains information on direct (physical) and indirect (functional) connections for over 2000 organisms [44]. We also used the GeneMANIA (https://genemania.org/) web platform to create an interaction network of closely linked genes. GeneMANIA is used to predict the function of a gene or gene lists and to identify the physical interaction, genetic interactions, coexpression, pathway, colocalization, and shared protein domain [45].

2.8. TF and miRNA Network Analyses. TFs are proteins that regulate gene expression by binding to certain DNA sequences [46], and miRNAs are a type of noncoding RNAs that play crucial functions in gene regulation [47]. TF and miRNA networks were constructed based on the ChEA [48] and TarBase [49] repositories, respectively, using the NetworkAnalyst (https://dev.networkanalyst.ca/NetworkAnalyst/uploads/ListUploadView.xhtml) web platform. NetworkAnalyst is a comprehensive web tool used for gene expression analysis, and it generates visual networks [50].

2.9. ROC Curve Analysis of the SFN Gene. For determining the diagnostic and prognostic values of the SFN gene, receiver operating characteristic (ROC) curves were drawn. For this purpose, gene expression data (GSE16515) were retrieved from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds), and survival data were obtained from TCGA-PAAD through the OncoLnc (http://www.oncolnc.org/) platform. The ROC curve was plotted, and the area under the ROC curve (AUC) was calculated by exploiting the Statistical Packages for Social Sciences (SPSS for Windows, version 20, IBM Corp., Armonk, New York, USA) software.

Disease summary of SFN

Analysis type by cancer	Cancer vs. Normal	
Bladder cancer	3	
Brain and CNS Cancer		
Breast cancer		
Cervical cancer		
Colorectal cancer		4
Esophageal cancer		4
Castric cancer		
Head and neck cancer	3	1
Kindney cancer	3	
Leuckemia		
Liver cancer	3	
Lung cancer	7	1
Lymphoma		
Melanoma		1
Myeloma		
Other cancer	3	
Ovarian cancer	4	
Pancreatic cancer	5	
Prostate cancer		
Sarcoma		1
Significant unique analyses	33	12
Total unique analyses	427	

Cell color is determined by the best gene rank percentile for the analyses within the cell. NOTE: An analysis may be counted in more than one cancer type.
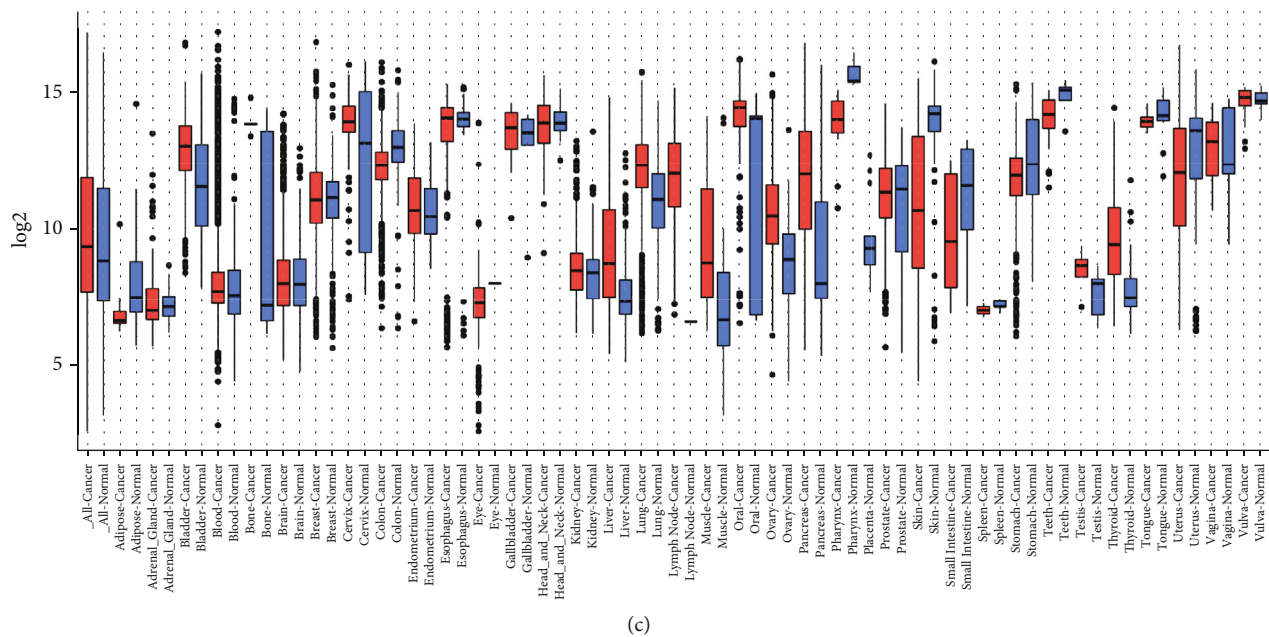
(a)
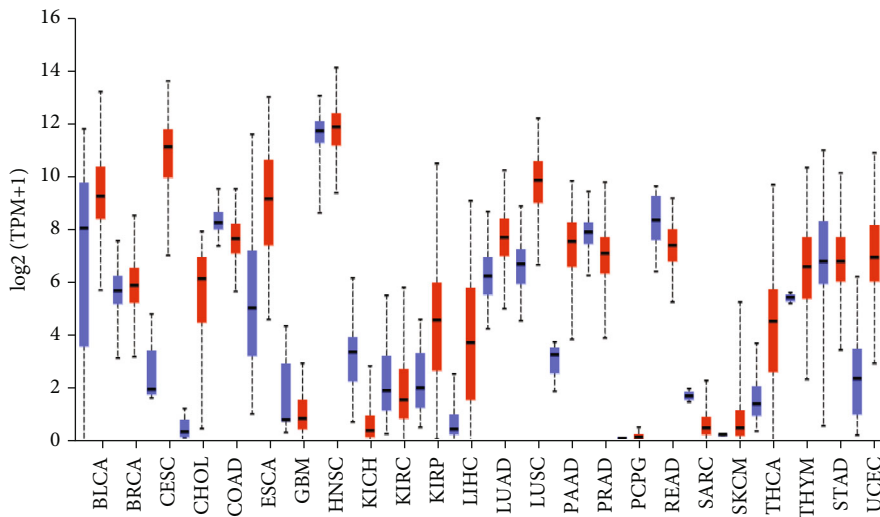
(b)

FIGURE 2: Continued.

FIGURE 2: SFN mRNA expression in different types of cancer. (a) A graphic constructed based on the data retrieved from the ONCOMINE database; it indicates the number of statistically significant (*P* < 0.01) datasets. mRNA overexpression is represented in red, and downregulation is represented in blue. (b) Expression across TCGA cancer data; tumor (red) and normal (blue) samples are represented by boxplots. (c) The SFN expression patterns in different cancers were determined by utilizing the GENT2 server, where blue represents healthy cells and red represents cancer cells.

3. Results

3.1. mRNA Expression in Human Cancers. We analyzed the expression pattern of SFN in numerous cancer studies by using the ONCOMINE platform. The results showed that SFN was upregulated in seven cancer types, namely, bladder, head and neck, kidney, liver, lung, ovarian, and pancreatic cancers (Figure 2(a)). In the pancancer view based from the ULCAN tool, we found that SFN was upregulated in 16 cancer types, downregulated in 6 cancer types, and equally expressed in 2 cancer types (Figure 2(b)). We also confirmed the upregulation of SFN in different cancers using the GENT2 tool.

3.2. SFN Expression in PC versus Healthy Tissues. SFN was significantly upregulated in different PC types, including pancreatic adenocarcinoma, pancreatic carcinoma, and pancreatic ductal adenocarcinoma, compared with its expression in normal tissues (Figures 3(a)–3(c) and Table 1). Using the GEPIA2 and UALCAN platforms, we further assessed the upregulation of SFN. Our findings indicated that SFN expression was significantly higher in PC tissues than in normal tissues (Figures 3(d) and 3(e)).

3.3. SFN Expression in relation to Clinicopathological Parameters in PC. We looked at variations in SFN gene expression levels in PC patients based on their clinicopathological features. In terms of individual cancer stages, the increase in SFN expression correlated with that in PC progression (Figure 4(a)). In terms of patients' race, SFN expression is increased in Asian patients (Figure 4(b)). In

terms of patient's age, higher SFN expression levels were observed in 41–60- and 81–100-year-old patients, whereas lower SFN expression levels were observed in 21–40-year-old patients (Figure 4(c)). As regards nodal metastasis status, a positive nodal status revealed a high SFN expression in PC (Figure 4(d)). Analysis based on tumor grade showed increased SFN expression in grade 3 PC (Figure 4(e)).

3.4. Association between SFN Expression and Survival Probability in PC Patients. To evaluate the prognostic value of the SFN gene, we determined the survival probability of PC patients using GEPIA2, R2, and OncoLnc. The results obtained from these tools revealed a negative correlation between survival probability and SFN expression (i.e., high SFN expression results in low survival probability). GEPI A2 provided data on the overall and disease-free survival probability of PC patients (Figures 5(a) and 5(b)), whereas R2 and OncoLnc provided information on overall survival probability (Figures 5(c)–5(e)). The analysis results underscored the prognostic relevance of a high SFN expression in PC patients.

3.5. Coexpression Analysis of the SFN Gene in PC Cancer. We determined the genes that are positively associated with SFN expression to identify the coexpressed genes associated with PC development. A heat map (Figure 6(a)) involving 13 genes coexpressed with SFN was obtained from ONCO-MINE. Among these genes, CFL1 was strongly (*R* = 0.92) coexpressed with SFN. Moreover, we observed a positive association between SFN and CFL1 using the TCGA data from the UCSC Xena tool, with Pearson's and Spearman's

Legend
1. Pancreas (5)
2. Pancreatic adenocarcinoma(10)

(a)

Legend
1. Pancreas (16)
2. Pancreatic carcinoma (36)

(b)

Legend
1. Pancreas (39)
2. Pancreatic ductal adenocarcinoma (39)

(c)

PAAD
(num(T) = 179; num(N) = 171)

(d)

(e)
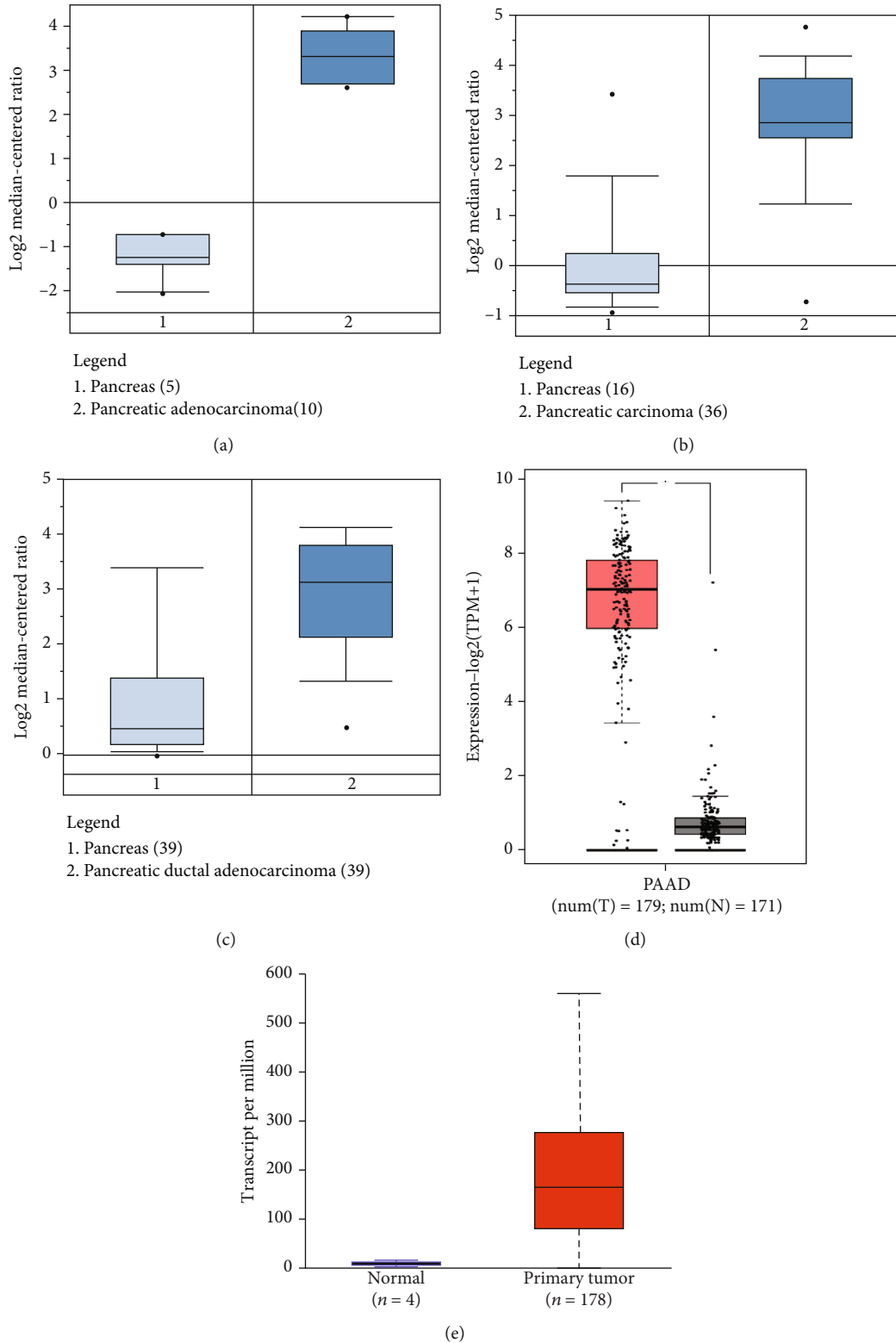
FIGURE 3: Comparison of SFN expression in PC and normal tissues. (a–c) Boxplot comparing the specific SFN expression in normal (left) and cancer (right) tissues; this boxplot was retrieved from the ONCOMINE tool. (d) Boxplot showing the SFN expression in normal tissue (right) and PC (left) (∗ indicates $P \leq 0.05$). (e) SFN expression based on the TCGA dataset obtained from UALCAN, where red represents primary tumor and blue represents normal tissues.

TABLE 1: SFN expression in different PC subtypes.

Datasets	Pancreatic cancer subtype	P value	t test	Fold change
Logsdon pancreas	Pancreatic adenocarcinoma (10)	7.02E-9	16.428	24.921
Iacobuzio-Donahue pancreas 2	Pancreatic adenocarcinoma (12)	1.14E-6	10.684	20.186
Segara pancreas	Pancreatic carcinoma (11)	3.31E-6	7.810	9.735
Pei pancreas	Pancreatic carcinoma (36)	2.27E-9	8.043	7.017
Badea pancreas	Pancreatic ductal adenocarcinoma (39)	1.82E-11	7.746	6.660
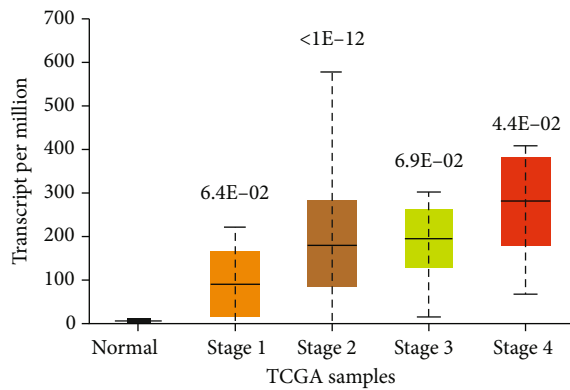
(a) Expression of SFN in PAAD based on individual cancer stages
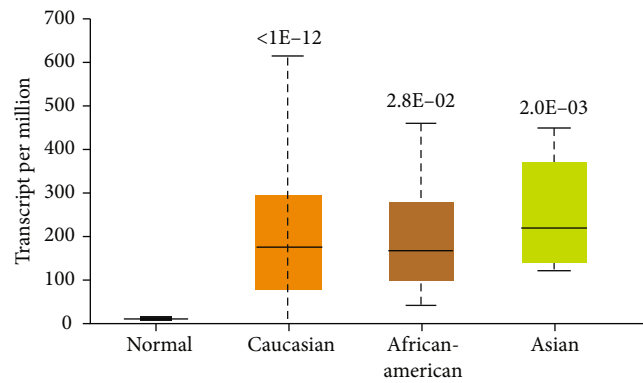
(b) Expression of SFN in PAAD based on patient's race
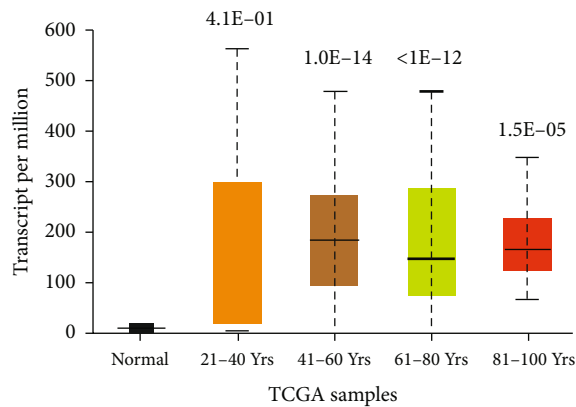
(c) Expression of SFN in PAAD based on patient's age
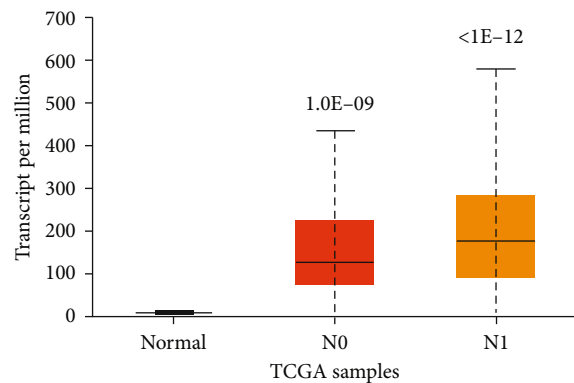
(d) Expression of SFN in PAAD based on nodal metastasis status
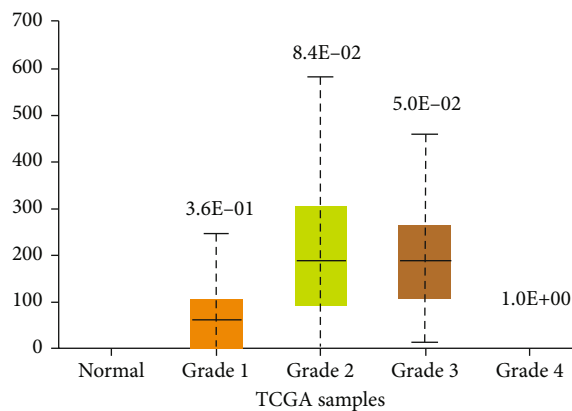
(e) Expression of SFN in PAAD based on tumor grade

FIGURE 4: Clinicopathological analysis of SFN in PC: (a) individual cancer stages; (b) patient's race; (c) patient's age; (d) nodal metastasis status; (e) tumor grade.
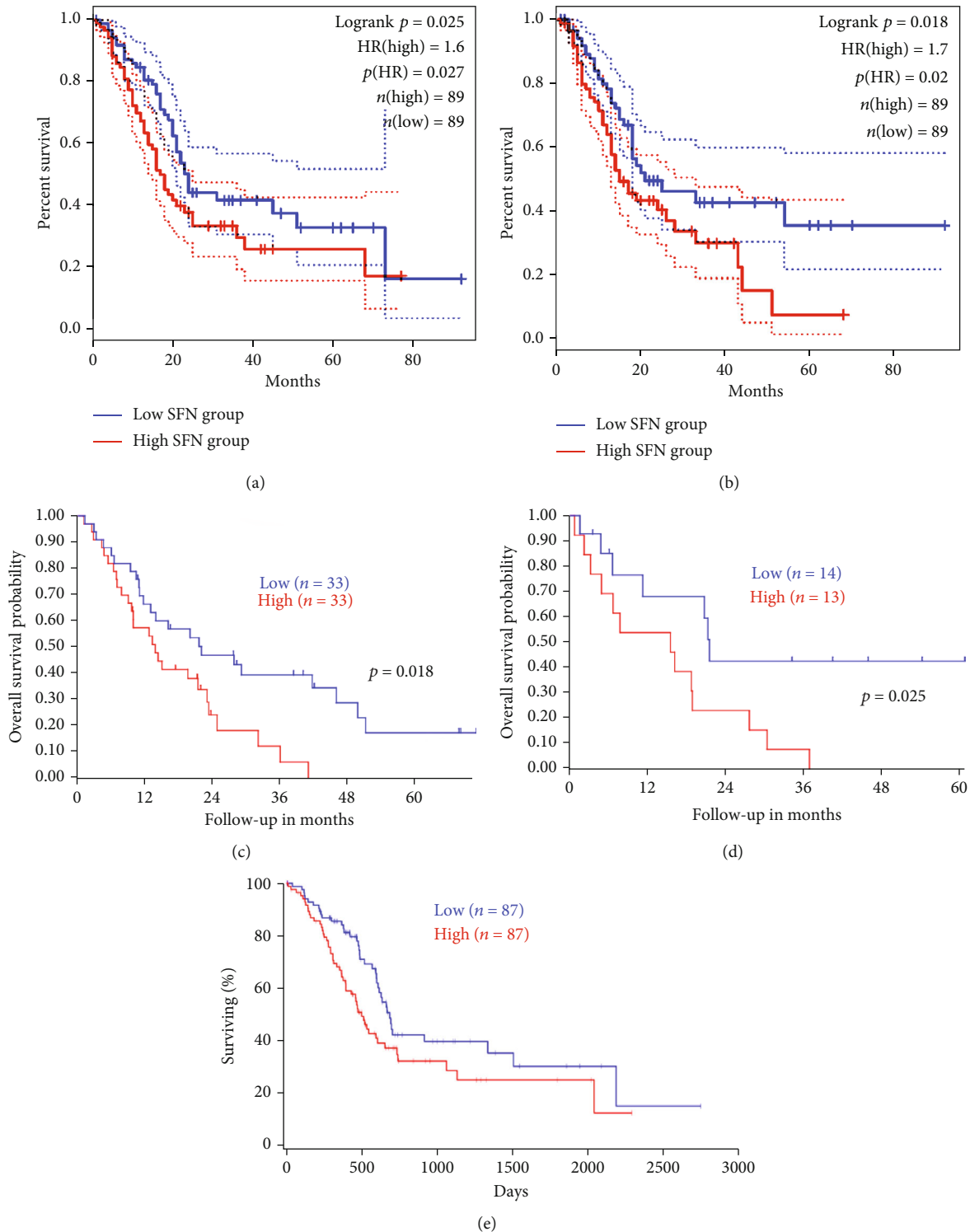
Figure 5: Correlation between SFN expression and prognosis in cancer patients. Red lines indicate SFN overexpression, and blue lines indicate low SFN expression. (a) Overall survival (OS) data retrieved from GEPIA2. (b) Disease-free survival (DFS) data retrieved from GEPIA2. (c) OS from the R2 platform (mixed tumor pancreas Hussain-130-rma-sketch-hugene10t SFN (7899265)). (d) OS from the R2 platform (mixed pancreatic adenocarcinoma Sadanandam-47-MAS5.0-u133p2 SFN (33323-r-at)). (e) OS data collected from the OncoLnc server.

values of 0.67 and 0.60, respectively (Figure 6(b)). The GEPI A2 tool validated the positive correlation between SFN and CFL1, with a Pearson value of 0.54 (Figure 6(c)).

3.6. *Enrichment Analysis of the SFN Gene.* Significantly enriched pathways involving the SFN gene were determined from six databases depicted in Figures 7(a)–7(f). For

Correlation	Gene		Reporter	Gene
1.000	SFN		33322_i_at	SFN
0.996	SFN		33322_r_at	SFN
0.920	CFL1		200021_at	CFL1
0.881	SUMO2		208838_x_at	SUMO2
0.881	SUMO2		213881_x_at	SUMO2
0.863	–		226052_at	–
0.824	TNPO1		209225_x_at	TNPO1
0.824	TNPO1		221829_s_at	TNPO1
0.798	–		1558678_s_at	–
0.798	MALAT1		224567_x_at	MALAT1
0.798	CSNK1G2		202573_at	CSNK1G2
0.798	EEF1D		214394_x_at	EEF1D
0.798	ATP6V0E1		214150_x_at	ATP6V0E1
0.798	–		AFFX-hum_alu_at	–
0.798	LAMP1		201553_s_at	LAMP1
0.798	RPL37A		201429_s_at	RPL37A
0.798	RPL39		208695_s_at	RPL39
0.735	WSB1		201296_s_at	WSB1
0.735	–		242235_x_at	–
0.735	TCF4		212386_at	TCF4
0.735	ZMIZ2		54970_at	ZMIZ2

Least expressed   Most expressed   ☐ Not measured

Note: colors are z-score normalized to depict relative values within rows. They cannot be used to compare values between rows.
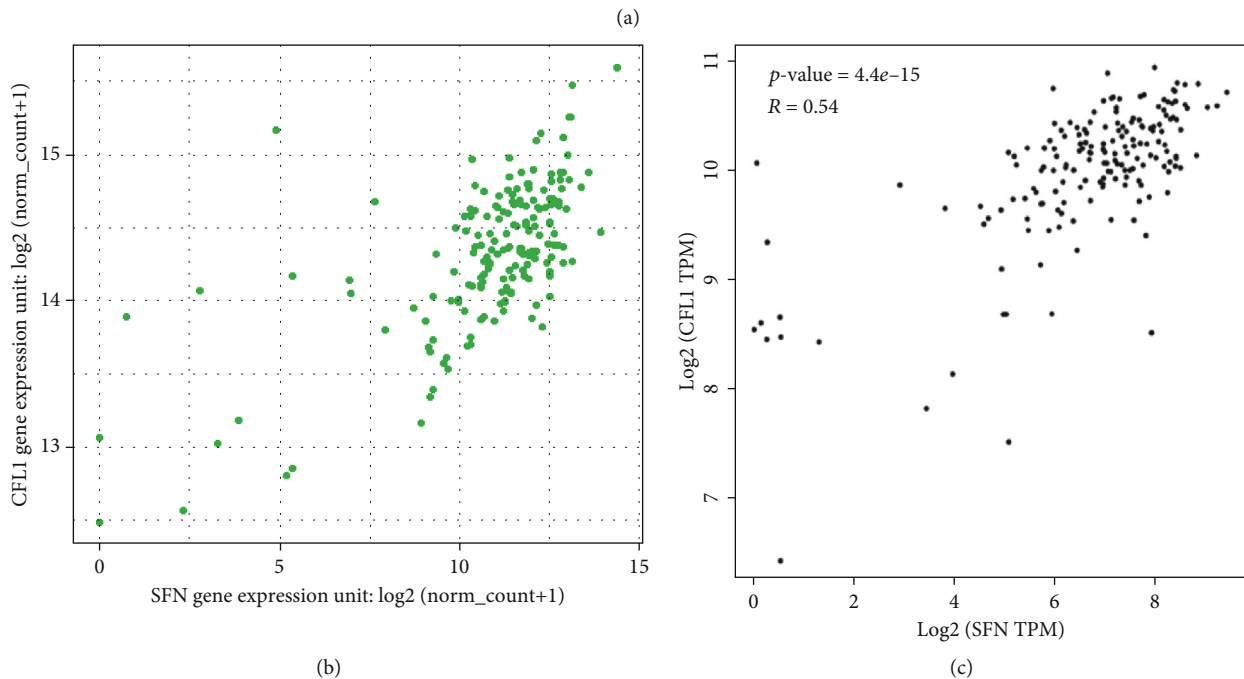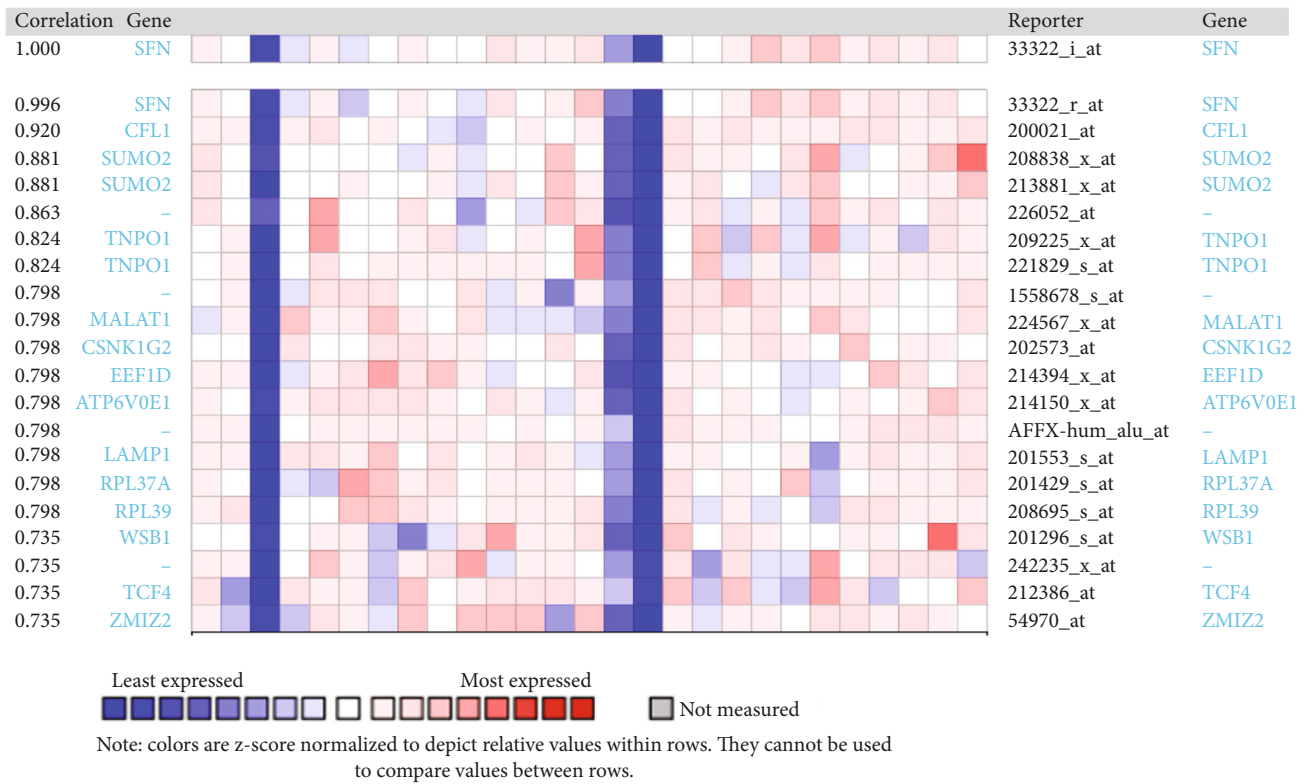
(a)

(b)

(c)

FIGURE 6: Coexpression analysis of the SFN gene in PC. (a) Heat map presenting the genes that are positively correlated with SFN based on the data retrieved from ONCOMINE. (b) Correlation analysis between SFN and CFL1 using the UCSC Xena web tool. (c) Coexpression of the SFN and CFL1 transcript levels in PC tissue is illustrated using the GEPIA2 web tool.

BioPlanet 2019, we observed significantly enriched pathways, namely, cell cycle control pathway, p38 MK2 pathway, G2/M checkpoint control pathway, insulin regulation of blood glucose, PICK3C/AKT pathway, and PI3K/PLC/TRK pathway (Figure 7(a)). Similarly, Reactome 2016 revealed the significantly enriched pathways related to Chk1/Chk2-mediated inactivation of cyclin B, BAD activation and its translocation to the mitochondria, TP53-regulated G2 cell cycle arrest genes, activation of BH3-only proteins, intrinsic pathway of apoptosis, and TP53-regulated cell genes (Figure 7(b)). In WikiPathway 2021 (Figure 7(c)), KEGG 2021 (Figure 7(d)), Biocarta 2016 (Figure 7(e)), and Panther

Control of cell cycle and breast tumor growth by estrogen-responsive protein Efp

p38 MK2 pathway

Cell cycle: G2/M checkpoint

Insulin regulation of blood glucose

PIK3C1/AK pathway

PI3K/PLC/TRK pathway

Aldosterone-regulated sodium reabsorption

Alpha-6 beta-1 and alpha-6 beta-4 integrin signaling

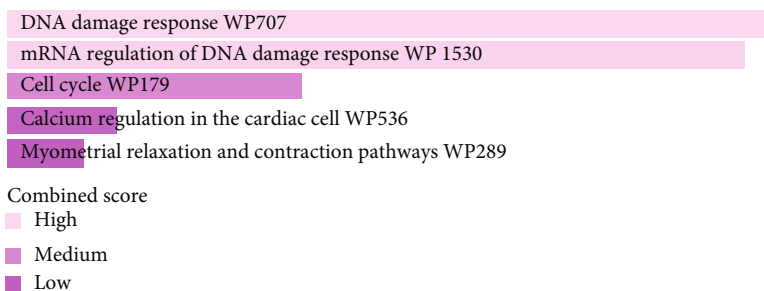LKB1 signaling events

Delta Np63 pathway

Combined score
- High
- Medium
- Low

(a)

Chk1/Chk2(Cds1) mediated inactivation of cyclin B:Cdk1 complex homo sapiens

Activation of BAD and translocation to mitochondria homo sapiens

TP53 regulates transcription of genes involved in G2 cell cycle arrest homo sapiens

Activation of BH3-only proteins homo sapiens

Intrinsic pathway for apoptosis homo sapiens

TP53 regulates transcription of cell cycle genes homo sapiens

Translocation of GLUT4 to the plasma membrane homo sapiens

RHO GTPasea activate PKNS homo sapiens

G2/M DNA damage checkpoint homo sapiens

TP53 regulates metabolic genes homo sapiens

Combined score
- High
- Medium
- Low

(b)

DNA damage response WP707

mRNA regulation of DNA damage response WP 1530

Cell cycle WP179

Calcium regulation in the cardiac cell WP536

Myometrial relaxation and contraction pathways WP289

Combined score
- High
- Medium
- Low

(c)

Aldosterone-regulated sodium reabsorption

p53 signaling pathway

Cell cycle

Combined score
- High
- Medium
- Low

(d)

FIGURE 7: Continued.

Estrogen-responsive protein Efp controls cell cycle and breast tumors growth homo spiens h efp pathway

Cell cycle: G2/M check point homo sapiens h g2pathway

Combined score

■ High

■ Medium

■ Low

(e)

p53 pathway homo sapiens P00059

Parkinson disease homo sapiens P00049

FGF signaling pathway homo sapiens P00021

EGF receptor signaling pathway homo sapiens P00018

Combined score

■ High

■ Medium

■ Low

(f)

Go Biological Process 2021

Positive regulation of epidermis development (GO:0045684)

Release of cytochrome c from mitochondria (GO:0001836)

Regulation of water loss via skin (GO:0033561)

Establishment of skin barrier (GO:0061436)

Positive regulation of epidermal cell differentiation (GO:0045606)

Positive regulation of epithelial cell differentiation (GO:0030858)

Regulation of epidermal cell differentiation (GO:0045604)

Regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway (GO:1900739)

Positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway (GO:1900740)

Apoptotic mitochondrial changes (GO:0008637)

Combined score

■ High

■ Medium

■ Low

(g)

Protein serine/threonine kinase inhibitor activity (GC:0030291)

Cadherin binding (GO:0045296)

Combined score

■ High

■ Medium

■ Low
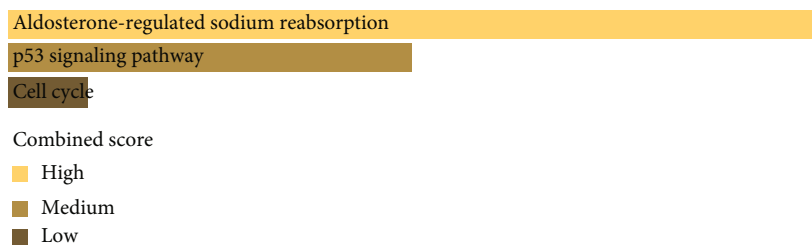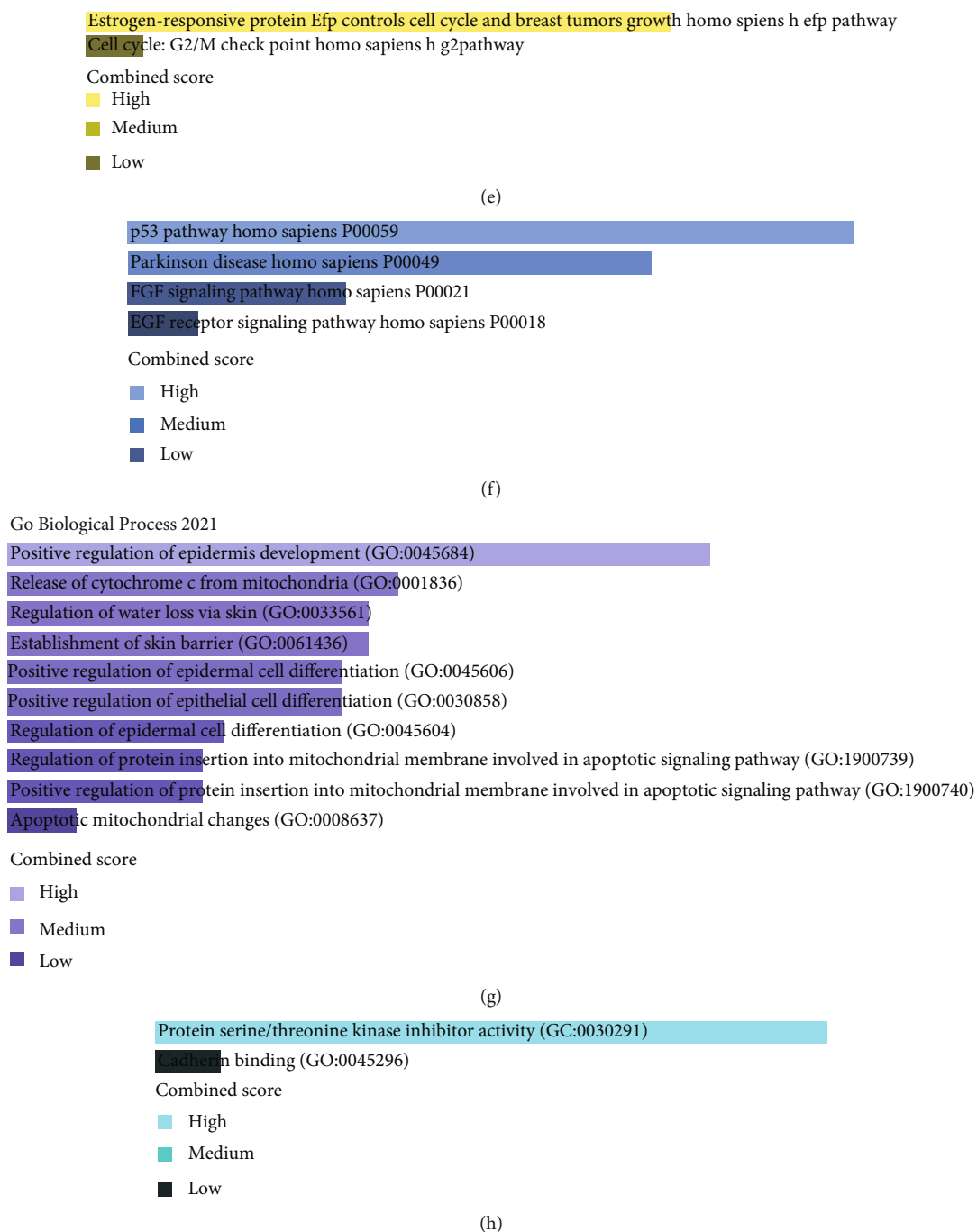
(h)

FIGURE 7: Enrichment analysis of the SFN gene: (a) BioPlanet 2019 pathway; (b) Reactome 2016 pathway; (c) WikiPathway 2021 Human; (d) KEGG 2021 Human pathway; (e) Biocarta 2016 pathway; (f) Panther 2016 pathway; (g) GO Biological Process 2021. (H) GO Molecular Function 2021.

2016 (Figure 7(f)), the most prominent pathways were DNA damage response, miRNA regulation of DNA damage response, cell cycle regulation, p53 signaling pathway, and FGF signaling pathway. Furthermore, we investigated the gene ontologies for the SFN gene. The GO Biological Process 2021 determined the predominant biological processes, such as positive regulation of epidermal development, release of cytochrome c from mitochondria, regulation of water loss via the skin, positive regulation of epidermal and epithelial cell differentiation, and apoptotic mitochondrial change (Figure 7(g)). In GO Molecular Function 2021, the most significantly enriched function was the protein serine/threonine kinase inhibitory activity (Figure 7(h)).

3.7. Evaluation of the SFN Interaction Network. We utilized GeneMANIA and STRING, two different web-based network analysis tools, to explore the SFN interaction network. Protein–protein interactions (PPIs) play important roles in
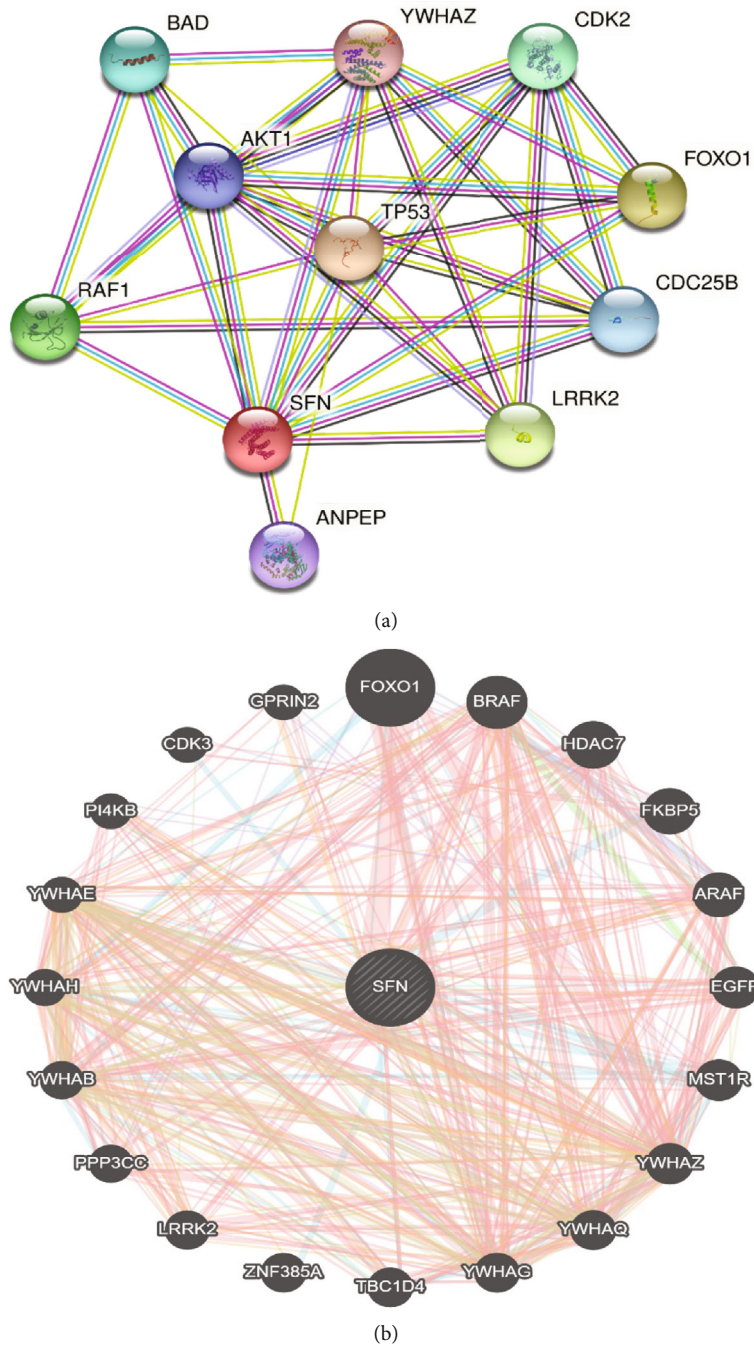
(a)

(b)

FIGURE 8: SFN interaction network. SFN-interacting proteins play a role in cell cycle regulation, apoptosis, and cancer. (a) PPI interaction network obtained from the STRING database. (b) SFN interaction network retrieved from GeneMANIA.

cellular activities and biological signaling in all animals, and this information helps researchers to gain a better understanding of various connections and pathways [51]. The PPI network from the STRING database showed the interactions of SFN with TP53, FOXO1, LRRK2, RAF1, CDK2, BAD, CDC25B, AKT1, ANPEP, and YWHAZ (Figure 8(a)). Analysis of the network provided information about the number of nodes (i.e., 11), number of edges (i.e., 49), average node degree (i.e., 8.91), average local clustering coefficient (i.e., 0.922), and PPI enrichment P value (i.e., $6.49e$-10). GeneMANIA revealed the interaction of SNF with

FOXO1, BRAF, HDAC7, FKBP5, ARAF, EGFR, MST1R, YWHAZ, YWHAQ, YWHAG, TPC1D4, ZNF385A, LRRK2, PPP3CC, YWHAB, YWHAH, YWHAE, PI4KB, CDK3, and GPRIN2 (Figure 8(b)).

3.8. TF and miRNA Network Analyses. The TF network constructed using the NetworkAnalyst platform revealed the direct interaction of 21 transcription factors (TFs) with SFN. The TFs for SFN were ASH2L, E2F4, CNOT3, SRY, ZNF281, NANOG, TFCP2L1, HSF1, POU5F1, SMAD3, KLF4, XRN2, MITF, TCF4, SMAD4, TP63, SMAD2, REST,
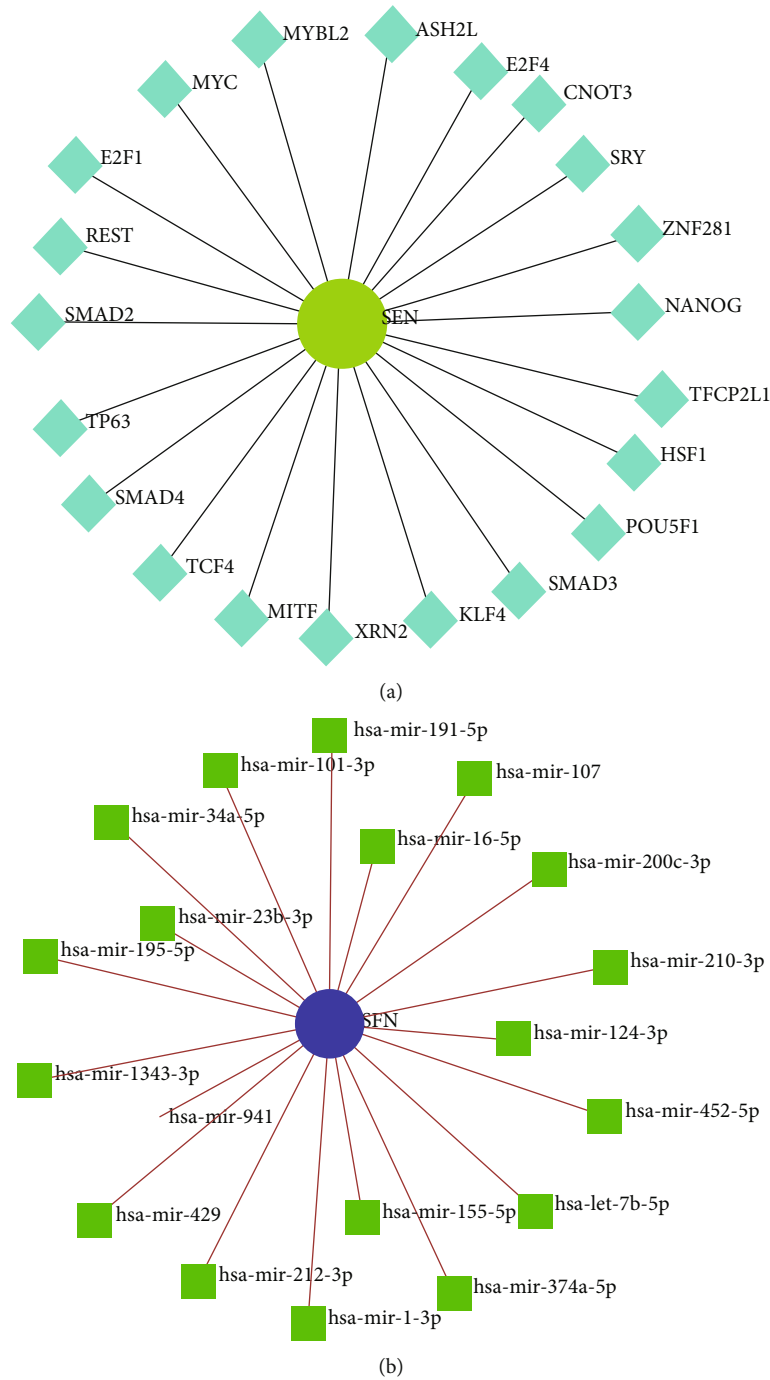
(a)

(b)

FIGURE 9: Interaction network of SFN with TFs and miRNAs. (a) TF network constructed from the ChEA database through the NetworkAnalyst platform. (b) miRNA network constructed from the TarBase database using the NetworkAnalyst platform.

E2F1, MYC, and MYBL2 (Figure 9(a)). Modification of these TFs might play a significant role in altering the SFN gene expression in PC. In the miRNA analysis, we obtained a network showing the direct interaction of 19 miRNAs with SFN (Figure 9(b)). These miRNAs can modify the SFN expression at the posttranscriptional stage.

3.9. ROC Curve Analysis of the SFN Gene. In the ROC curve, the area under the curve (AUC) is used to discriminate between classes. In the GSE16515 dataset, the AUC for the

SFN gene expression was 0.965 (Figure 10(a)) and the AUC for the survival of patients was 0.637 (Figure 10(b)). These AUC results indicate that the SFN gene might be used as a diagnostic and prognostic marker.

4. Discussion

PC is one of the most aggressive cancers affecting human health, and it is considered the "silent disease," as it does not show noticeable symptoms at an early stage [52]. Given
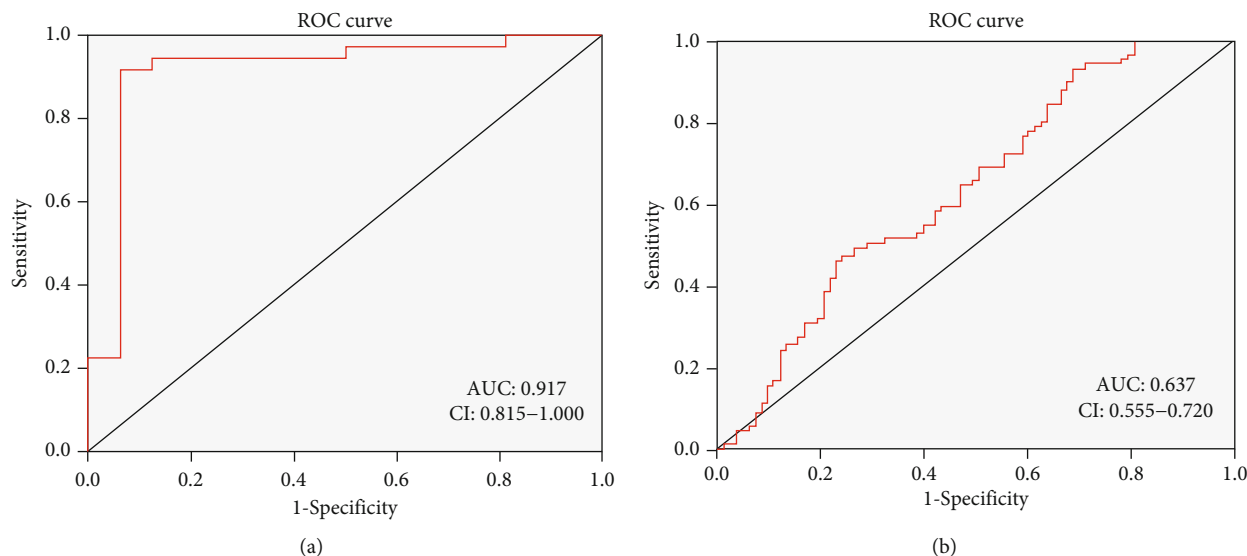
FIGURE 10: Evaluation of the diagnostic and prognostic values of the SFN gene. (a) ROC curve for SFN expression in pancreatic cancer. (b) ROC curve for patients' survival. AUC: area under the curve, CI: confidence interval.

that it displays characteristics similar to those of other diseases, such as ulcer, gastritis, and pancreatitis, it is mostly diagnosed at an advanced stage [53]. As early detection remains difficult, finding a potential novel biomarker that aids in early detection is desired. In this study, we utilized bioinformatics approaches to assess the importance of the SFN gene as a biomarker in PC prediction.

Upregulated SFN gene expression in PC and other cancer types was observed in ONCOMINE, UALCAN, and GENT2. The upregulated SFN expression in PC cells was compared with that in normal pancreatic cells using the data from ONCOMINE, GEPIA2, and UALCAN. A study on the molecular profiling of stroma in pancreatic ductal adenocarcinoma has revealed the upregulated expression of SFN, along with other genes [54]. This upregulated SFN expression in PC is supported by other studies [27, 55–57]. Gene expression levels in cancers can vary under different clinicopathological conditions, as cancer is a heterogeneous and complex disease. We analyzed the SFN expression based on patients' age, race, tumor grade, tumor stage, and nodal status. The results showed that SFN was highly upregulated among Asians, among 41–60-year-old individuals, among those with a positive nodal status, and among grade 3 tumor patients. Interestingly, in the case of cancer stages, SFN expression increased proportionally with cancer stage progression. Then, the prognostic value of SFN in PC was evaluated using the GEPIA2, OncoLnc, and R2 platforms. High SFN expression significantly ($P < 0.05$) correlated with low overall and disease-free survival. Our current findings agreed with those of another study in which SFN was considered an independent prognostic biomarker in pancreatic ductal adenocarcinoma [54]. Moreover, it has been demonstrated that the elevated 14-3-3σ protein levels likely contribute to the poor prognostic outcome of human pancreatic tumors, as they promote resistance to radiation and anticancer treatments [15].

Gene coexpression provides information that aids in the identification of functionally linked genes. Coexpression analysis using the ONCOMINE platform revealed 13 genes, among which CFL1 was highly coexpressed with SFN. Furthermore, CFL1 coexpression in PC was confirmed by GEPIA2 and UCSC Xena. CFL1 is a small, ubiquitous, actin-binding protein that plays important roles in cytokinesis, endocytosis, apoptosis, cell proliferation, and migration, as well as in tumor development, infiltration, and metastasis [58, 59]. Moreover, it has been reported that this protein is necessary for the invasion and spread of numerous human malignant solid tumors [60, 61]. Recent studies have found a positive association between high CFL1 gene expression and PC progression [59, 62].

Enrichment analysis for the SFN gene was performed by utilizing the Enrichr web platform. The most prominent pathways, including cell cycle control, Chk1/Chk2-mediated inactivation of cyclin B, DNA damage response, aldosterone-regulated sodium reabsorption, estrogen-responsive protein efp control cell cycle, and p53 pathway, were obtained from BioPlanet 2019, Reactome 2016, WikiPathway 2021, KEGG 2021, Biocarta 2016, and Panther 2016, respectively. SFN was initially found to be a p53-inducible gene that responds to DNA-damaging agents [63]. A study has reported that SFN inhibits the initiation of mitosis by sequestering the mitotic initiation complex (cdc2-cyclin B1) and preventing it from entering the nucleus [64]. In this manner, SFN causes G2 arrest, allowing damaged DNA to be repaired. It has been demonstrated that SFN directly controls the G2/M checkpoint of the cell cycle by protecting p53 against MDM2-mediated ubiquitination and degradation [65–67]. These findings indicate that SFN acts as a negative regulator of cell cycle progression and might be considered a tumor suppressor. However, SFN plays a double-edged function in human cancers, and its function may vary among organs and tissues [32, 68]. Meanwhile, accumulation of 14-3-3σ has been

observed in PC, but it cannot perform its major ascribed functions, such as sustaining a G2 checkpoint and performing an antiapoptotic action, due to multiple alterations in its interaction with downstream partners [69].

Network analysis based from the STRING database revealed the functional interaction partners of SFN, namely, TP53, FOXO1, LRRK2, RAF1, CDK2, BAD, CDC25B, AKT1, ANPEP, and YWHAZ. It has been reported that overexpression of CDC25B is associated with pancreatic ductal adenocarcinoma and that its inhibitor prevents PC cell growth by blocking the G2/M phase transition via the inhibition of cdc2 dephosphorylation [70]. According to the NCBI, defects in the ANPEP gene enhances angiogenesis, tumor growth, and metastasis [71]. Meanwhile, overexpression of the YWHAZ gene has been demonstrated to be a prognostic and therapeutic target in gastric cancer [72, 73]. In GeneMANIA, SFN shares consolidated pathways with MST1R and YWHAG. MST1R expression has been shown to play an oncogenic function in human pancreatic intraepithelial neoplasia, as well as in primary human and animal metastatic cell lines [74]. In PC, the overexpression of the YWHAG gene is associated with poor overall survival compared with low YWHAG expression [75]. Furthermore, our network analysis revealed some TFs and miRNAs that might play important roles in determining how SFN gene expression is regulated at the transcriptional and posttranscriptional levels.

In ROC analysis, SFN expression showed excellent (AUC = 0.917) diagnostic value of pancreatic cancer. A meta-analysis study showed that the sensitivity and specificity of CA 19-9 were 78.2% and 82.8%, respectively [76]. However, the CA 19-9 level may be augmented in other medical conditions, such as acute cholangitis, pancreatitis, obstructive jaundice, and liver cirrhosis [11]. In our study, SFN also exhibited as a good (AUC = 0.637) prognostic marker in pancreatic cancer. In these aspects, SFN might be considered as an auxiliary biomarker of CA 19-9 in PC. Of course, there are some limitations in our study. First, due to the lack of enough datasets, the sample size for analysis was relatively small. Second, the absence of in vivo and in vitro experiments is another flaw of our study. Third, this study cannot explain how the tissue-specific upregulation SFN gene is related to pancreatic cancer. Therefore, further wet laboratory molecular studies are needed.

5. Conclusion

Data from the online bioinformatics platforms utilized in this study showed that SFN expression in PC was upregulated relative to that in normal tissues. Moreover, a negative correlation between SFN expression and survival probability was found in PC. In our network analysis, SFN-associated proteins, TFs, and miRNAs were identified. Based on these findings, we can conclude that the high tissue-dependent SFN expression might be used as a biomarker for diagnosis, prognosis, and therapeutic purposes. However, further wet laboratory-based studies are needed to bolster the significance of SFN overexpression in PC.

Data Availability

Any data or information used in this current study is available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they do not have conflicts of interest.

Acknowledgments

The authors express their gratitude to the Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, for providing technical support.

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