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IN-SILICO STUDIES ILLUSTRATE THE ONCOGENIC POTENTIAL OF AN ATPASE PROTEIN NSF

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ABSTRACT: Cancer is a complex and life-threatening disease characterized by abnormal cell growth. Several AAA+ ATPases are involved in the oncogenic pathways, however, the role of an ATPase protein *i.e.* N-ethylmaleimaide-Sensitive Factor (NSF) was unclear. Therefore, we performed systematic bioinformatics analysis to predict the prospect of NSF as a cancer biomarker. NSF is found to be upregulated in some cancers whereas down regulated in others. Additionally, this expression was found to be associated with the reduced survival rate of cancer patients. We investigated NFS's mutation and copy number amplification status across the cancers since the genetic alteration is a major cause of cancer. Several hotspot mutation sites were observed at the ATPase domain of NSF indicating that these mutations might have a role in carcinogenesis. Additionally, the copy number amplification study revealed a high amplification percentage of NSF across the cancers. We further focused on the functional characteristics of NSF across cancers. Fascinatingly, we found many interacting protein partners of NSF that are key in carcinogenesis. We further focused on the probable oncogenic pathways related to NSF which indicate that NSF could be crucial for oncogenic function. Therefore, our study unravels the oncogenic potential of NSF and projects it as a potential cancer biomarker and cancer drug target.

INTRODUCTION: AAA+ *ATPases are* a class of mechanoenzymes, found in all biological kingdoms. They generate mechanical force by undergoing conformational changes during cycles of ATP binding and hydrolysis. This mechanical force is used to induce conformational remodeling of a wide range of substrates, including proteins and polynucleotides, thus engaging these ATPases in diverse cellular processes in a large variety of fundamental cellular pathways that govern protein homeostasis, genome stability, cell proliferation, and pathogen infection.

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However, how they coordinate ATP binding/hydrolysis to drive the conformational changes needed to perform mechanical work has been a very critical question. AAA+ (ATPases Associated with various cellular Activities) family represents a molecular machine having diverse cellular functions both in prokaryotes and eukaryotes. The energy for different cellular activities is obtained by the hydrolysis of ATP.

In this way, these proteins convert the stored chemical energy into biological events such as proteolysis, protein disaggregation and refolding, Membrane fusion, and transport, DNA replication, DNA recombination and repair. These proteins are widely spread inside the cell, for example in the transmembrane region, cell organelles, and cytosol [1]. The most important and interesting thing about these proteins is they govern a common mechanism of ATP hydrolysis for divergent biological

functions. Based on the number of domains AAA+ ATPases have been divided into two types, Class I (having two domains) and Class II (having one domain) [2]. Primarily Class I members of this family oligomerize to form hexamers and form a ring-shaped structure with a central cavity [2]. The ATPase domains consist of walker A (GXXXXGKT/S; X: any amino acid) and walker B (VhhhhDE; h: hydrophobic amino acid) motifs. The ATP binding pocket comprises many conserved motifs like Walker A, Walker B, Sensor 1, Sensor 2, and Arginine Fingers.

Many class I ATPases (e.g. VCP/P97, Na+/K+ ATPases, V-ATPases) [3-5] are found to be related to cancer. N-ethylmaleimaide-Sensitive Factor (NSF) is one of this class I ATPase whose role in cancer is still elusive. This protein was discovered in the year 1984 as a vesicular transporter between the Golgi complex and the successive cisternae by Rothman and colleagues [6]. NSF is a hexameric protein with 15nm in diameter and 12nm in height. It consists of six identical subunits with a distinct domain structure [7].

The major function of ATPase domain 1 (NFS-D1; 206-477 a.a.) is ATP hydrolysis and domain 2 (NFS-D2; 478-744 a.a.) is oligomerization [8]. The role of the NFS in vesicular transport is based on the interaction with SNAP-binding receptors i.e. SNAREs [9], and the other one is Soluble NSF Attachment Proteins i.e. SNAPs [10]. NSF may not be consistently active but can be regulated by several different mechanisms such as inactivation by S-nitrosylation and phosphorylation [11-14].

In this study, we analyzed the oncogenic potential of NSF. For this, we undertook a multi-omics approach to investigate the molecular profile of NSF in pan-cancer. We investigated the mRNA expression of NSF, its effect on the patient's survival rate, the causes behind the abnormal expression of NSF, the correlation between NSF and other oncogenes, and its interacting protein partners in various cancers. As per our knowledge, this is the first report detailing the pan-cancer analysis of the NSF. Our results provide novel insights concerning NSF molecular interactions and their potential role in carcinogenic mechanisms. These findings could therefore be explored for a better understanding of underlying cancer mechanisms and to identify novel biological targets for cancer treatment.

MATERIALS AND METHODS:

Sequence Similarity of NSF with other AAA+ ATPase Proteins: Multiple sequence alignments between NSF and other AAA+ ATPases were performed using the Clustal W tool (https://www.genome.jp/tools-bin/clustalw). Further, the alignments of sequences were represented for percentage similarity/identical scores using ESPIRIT 3.0 website (https://espript.ibcp.fr/ESPript/ESPript/).

NSF Transcript Expression Analyses: NSF transcript expression analyses were performed using GEPIA and UALCAN. The GEPIA (http://gepia.cancer-pku.cn/) [4, 15] has enormous gene expression records from TCGA (The Cancer Genome Atlas) and GTEx (the Genotype-Tissue Expression) databases. In this work, we analyzed NSF expression in human cancers. A total of 31 tumor types are available in this database. Further stage-wise transcript analyses were performed using the UALCAN (http://ualcan.path.uab.edu/) [16], a TCGA-based dataset (including normal control tissues from the GTEx database).

Prognostic Significance of NSF Transcripts on Patients' Survival: To find out the correlation between *NSF* expression and patient survival, a KM plotter analysis was performed (KMP, http://kmplot.com/analysis/) [17-20]. In the overall patient survival analyses, stringency was maintained through Log Rank P value < 0.05. Here, the survival rate of patients with high and low *NSF* expression was investigated.

Analysis of Methylation Rate of NSF in Cancer vs Normal Samples: *NSF* methylation levels were investigated using the TCGA-based database 'Wanderer' (http://maplab.imppc.org/wanderer/) [21].

The Genetic Alteration Study of NSF: The cBioPortal database (http://www.cbioportal.org/) [22, 23] was used to analyze the genetic alterations in *NSF*. In our study, we analyzed the alteration frequency of *NSF* in different cancers based on the TCGA datasets and summarized the mutated sites in the *NSF* gene. Further, the mutation profiling of the *NSF* gene was performed using the Catalogue

of Somatic Mutations in Cancer (COSMIC) database (www.sanger.ac.uk/cosmic/) [24].

Protein-Protein Interaction Analyses of NSF: To identify the functional protein partners of NSF, the Search Tool for the Retrieval of Interacting Genes/proteins (STRING) database [25] was used. This tool provides critical analyses of protein-protein interaction including direct (physical) as well as indirect (function) protein associations (https://string-db.org/).

At the time of analyses presented herein, the STRING database contained data from a total of 5,090 organisms, 24,584,628 proteins, and 3,123,056,667 protein-protein interactions. The NSF protein network was constructed neighbourhood, gene fusion, co-expression, experiments, and text-mining approaches.

Canonical Pathways of NSF using DAVID Database: Pathways analysis is crucial to understand the role of an oncogene or a tumour suppressor gene in cancer. Therefore, the DAVID database (https://david.ncifcrf.gov/) [26] was used to explore the probable pathways of NSF.

RESULTS:

Sequence Similarity of NSF: The gene *NSF* is located at chromosome 17 which is at 17q21.31 **Fig. 1A**. Fascinatingly, altered chromosome 17 is more frequent in numerous malignancies [27]. Structurally, NSF protein is 744 amino acids long (molecular weight ~ 78kDa). Being an AAA+ ATPase family protein, *NSF* contains two ATPase Domains (D1 and D2) and a N-terminal domain (NTD) **Fig. 1B** and is also proposed to have a hexameric structure **Fig. 1C**. The ATPase domain D1 is highly conserved **Fig. 1D**.

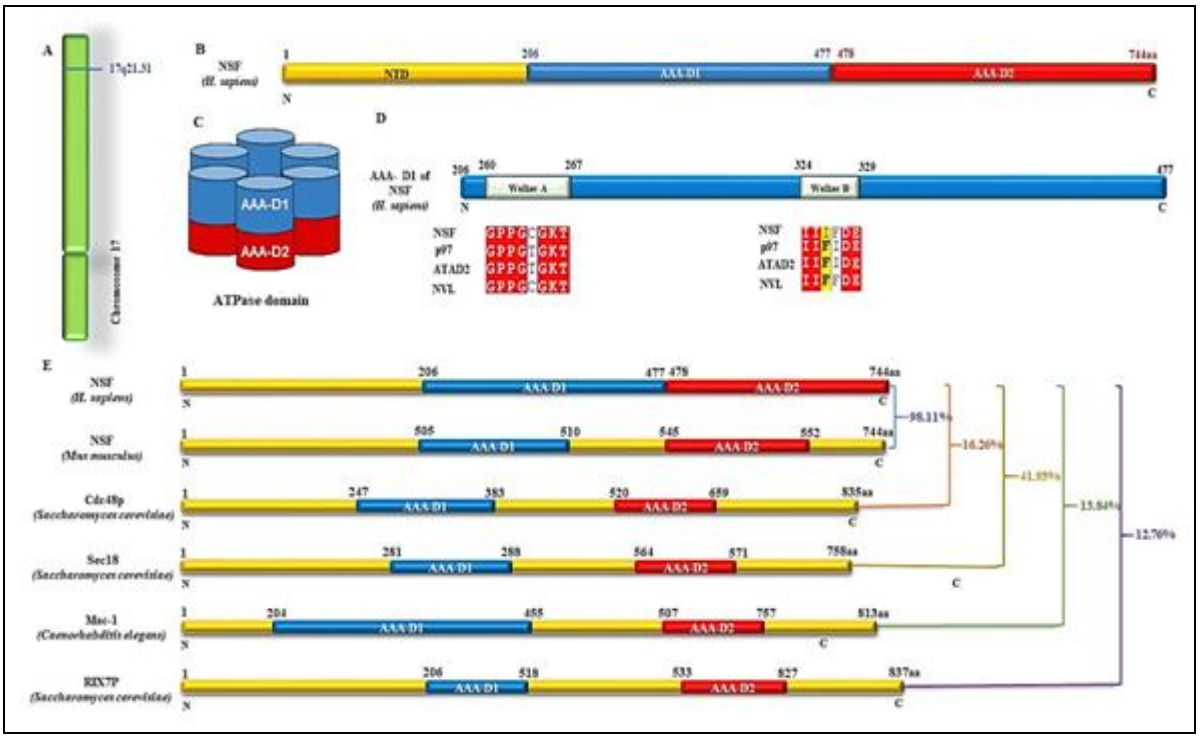

FIG. 1: STRUCTURAL OVERVIEW OF NSF. (A) NSF IS LOCATED EXACTLY AT CHROMOSOME 17Q21.31. (B) DOMAIN STRUCTURE OF HUMAN NSF PROTEIN CONSISTING OF TWO ATPASE DOMAINS AND AN NTD. THE AAA-D1 IS IN BLUE AND AAA-D2 IS IN RED AND THE NTD IS IN YELLOW. (C) THE NSF PROTEIN POSSESSES A HEXAMERIC RING STRUCTURE. HERE DOMAIN 1 (BLUE COLOR) AND DOMAIN 2 (RED COLOR) ARE REPRESENTED ON THE TOP AND BOTTOM. (D) THE REPRESENTATIVE DIAGRAM OF ATPASE DOMAIN 1 (AAA-D1) OF NSF. IT CONSISTS OF TWO CONSERVED MOTIFS LIKE WALKER A AND WALKER B. THE CLUSTAL W ANALYSIS WAS PERFORMED FOR PROTEIN SEQUENCE ALIGNMENT OF BOTH THESE MOTIFS WITH P97, ATAD2, AND NVL-LIKE AAA+ ATPASE PROTEINS. THE ALIGNMENT DATA IS PUT IN ESPRIPT 3.0 FOR REPRESENTATION AS 100% IDENTITY IN RED AND SIMILARITY IN YELLOW RESPECTIVELY. A SCORE > 0.7 IS CONSIDERED FOR THIS ANALYSIS. (E) DOMAIN STRUCTURE OF HUMAN NSF, WITH MOUSE NSF, S. CEREVISIAE CDC48P, S. CEREVISIAE SEC18, *C. ELEGANS* MAC-1, AND SACCHAROMYCES CEREVISIAE RIX7P. THE CLUSTAL W ANALYSIS WAS PERFORMED FOR PROTEIN SEQUENCE ALIGNMENT BETWEEN CONCERNING HUMAN NSF. THE PERCENTAGES OF IDENTITY ARE SPECIFIED FOR THE OVERALL LENGTH OF PROTEIN.

The walker A (GXXXXGKT/S) and walker B (hhhhDE) motifs of D1 share sequence similarity with most of the AAA+ ATPase family proteins **Fig. 1D**. Further, when the sequence of Human NSF was compared with NSF of Mouse, Cdc48p, Sec18, and RIX7P of *S. cerevisiae*, Mac-1 of *C. elegans*, and Saccharomyces cerevisiae RIX7P, it is found that all of them share conserved ATPase domain features **Fig. 1E.**

mRNA Expression of *NSF* in Cancer: To investigate *NSF* mRNA expression patterns, data from the TCGA database were analyzed across 31 cancer types compared to matched normal tissues **Table 1, Fig. 2A**.

We observed that NSF transcription was significantly higher in Cholangiocarcinoma (CHOL), Diffuse Large B Cell Lymphoma (DLBC), Pheochromocytoma and Paraganglioma (PCPG), Thymic Carcinoma (THYM), and Stomach Adenocarcinoma (STAD) (Figure 2B). NSF transcription was significantly lower in Glioblastoma Multiforme (GBM), Low-Grade Gliomas (LGG), Kidney Chromophobe (KICH), Head and Neck Squamous Cell Carcinoma (HNSC) and Adrenocortical carcinoma (ACC) (Figure C). Boxplots presented in Figure B and Figure C were generated only for those TCGA tumours which exhibited significantly high and low differential expression of *NSF*as compared to controls.

We further investigated *NSF* expression patterns concerning patients' tumour size in 10 tumours(which showed significant differential expression of NSF i.e., CHOL, DLBC, PCPG, STAD, THYM, LGG, HNSC, GBM, ACC and KICH). Among them, hepatobiliary tumours and CHOL showed significantly increased NSF expression in all tumour stages **Fig. D**. However, KICH and HNSC showed significant downregulation of *NSF* in all stages of cancer.

TABLE 1: *NSF* EXPRESSION IN VARIOUS CANCERS WITH FOLD CHANGE

Sl. no.	Full Name	Fc=T/N
1	Human Skin Cutaneous Melanoma	0.13
2	Glioblastoma Multiforme	0.29
3	Low-Grade Gliomas	0.33
4	Kidney Chromophobe	0.63
5	Adenoid Cystic Carcinoma	0.64
6	Tenosynovial Giant Cell Tumor	0.83
7	Head And Neck Squamous Cell Carcinoma	0.85
8	Kidney Renal Cell Carcinoma	0.91
9	Thyroid Cancer	1.04
10	Bladder Cancer	1.09
11	Uterine Corpus Endometrial Carcinoma	1.11
12	Cervical Squamous Cell Carcinoma And Endocervical Adenocarcinoma	1.12
13	Acute Myeloid Leukemia	1.14
14	Uterine Carcinosarcoma	1.23
15	Kidney Renal Cell Carcinoma	1.37
16	Lung Squamous Cell Carcinoma	1.4
17	Ovarian Cancer	1.5
18	Esophageal Carcinoma	1.55
19	Sarcoma	1.55
20	Colon Adenocarcinoma	1.62
21	Rectum Adenocarcinoma	1.76
22	Lung Adenocarcinoma	1.78
23	Cancer Genome Atlas Prostate Adenocarcinoma	1.81
24	Breast Cancer Gene	2.03
25	Liver Hepatocellular Carcinoma	2.04
26	Pancreatic Ductal Adenocarcinoma	2.08
27	Stomach Adenocarcinoma	2.26
28	Thymic Carcinoma	2.78
29	Pheochromocytoma And Paraganglioma	3.18
30	Diffuse Large B Cell Lymphoma	4.05
31	Cholangiocarcinoma	4.17

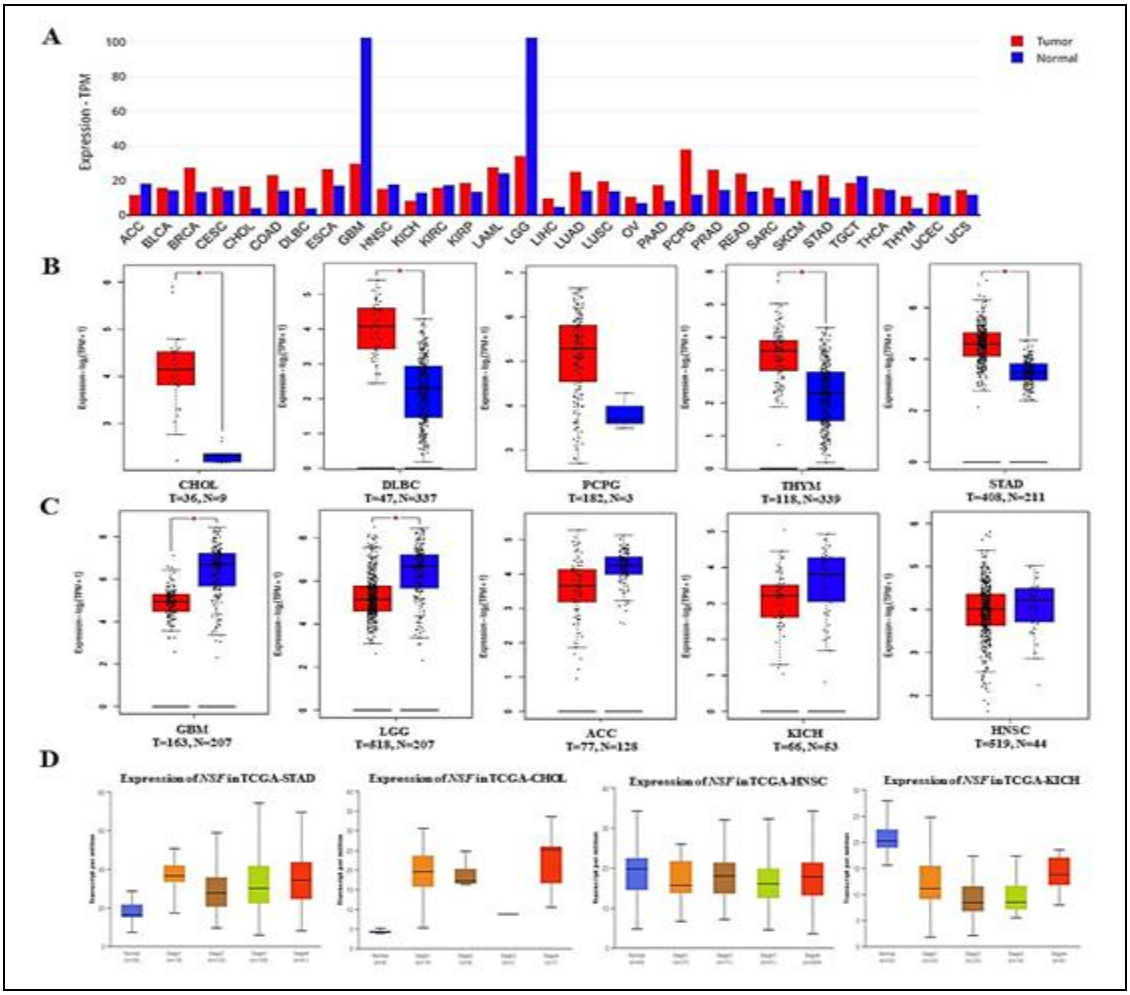

FIG. 2: PAN-CANCER ANALYSIS OF *NSF* GENE EXPRESSION. (A) THE DIFFERENTIAL EXPRESSION PATTERN OF *NSF*IN 31 TCGA CANCER TYPES. (B) TUMOR TYPES EXHIBITING SIGNIFICANT OVEREXPRESSION OF *NSF*. (C) TUMOR TYPES EXHIBITING SIGNIFICANT DOWNREGULATION OF *NSF* EXPRESSION. (D) STAGE-SPECIFIC EXPRESSION ANALYSIS OF *NSF*.

Role of *NSF* mRNA Levels in Prognosis/Patient Survival: Kaplan-Mayer Plotter curves were generated for pan-cancer using tumour data sets from the TCGA database to investigate the potential role of *NSF* mRNA levels in tumourprognosis and patient survival. Interestingly, patients with higher *NSF* mRNA levels showed a significantly shorter overall survival in BRCA, KIRC, and SARC **Fig. 3**. This result showed that *NSF* Expression is a critical prognostic factor in various tumours.

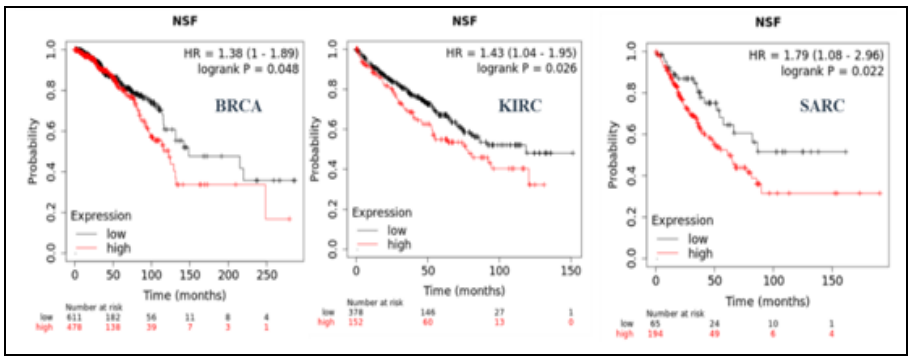

FIG. 3: NSF EXPRESSION AS A PROGNOSTIC FACTOR IN VARIOUS TUMOURS. PATIENTS WITH HIGH NSF EXPRESSION SHOWING LOW SURVIVAL RATE. THE RED LINE REPRESENTS TUMORS EXPRESSING HIGH LEVELS OF NSF TRANSCRIPTS WHILE THE BLACK LINES REPRESENT TUMORS WITH LOW-LEVEL NSF TRANSCRIPT EXPRESSION. THE DATA IS VERY STRINGENT SINCE THE P VALUE IS < 0.05.

Genetic Alteration Analysis of NSF Mutations in the TCGA Pan-Cancer: Next, we analyzed, the genetic alteration status of NSF in different tumour samples of the TCGA cohorts. Among all the studied cancer types, Prostate Adenocarcinoma (Pan-Cancer Atlas) was found to have the highest frequency of NSF alteration i.e. 3.04% **Fig. 4A**. The 3D structure of NSF is shown with mutation in green colour in **Fig. 4B**. Further, our study found that missense mutation of NSF is the main type of genetic alteration. Excitingly, an overall of 73 mutation sites with a missense of 54 sites, truncating of 5 sites, inframe of 1, splice of 6, and fusion of 7 sites were identified in NSF **Fig. 4C**. The mutation frequency and amplification frequency are shown in **Table 2** and **Table 3**. Further analysis with the COSMIC database also indicated that the frequency of missense substitution was the highest (22.55%). There were 33.96% G > A and 19.50% C > T mutations found in *NSF* shown in **Fig. 5.**

Methylation and NSF Mutation Profile in Pan-Cancer: Since both genetic and epigenetic alterations play significant role in cancer, we focused on the role of epigenetic modifications in NSF. We analyzed the methylation rate of *NSF* in both cancer and normal samples **Fig. 6**. Interestingly, no significant changes in NSF methylation rate was observed between the cancer and normal samples.

NSF Protein Network and Co-expression Analysis: We used the STRING database to investigate potential partners of NSF protein. Proteins with the strongest NSF interaction scores included NAPA, GRIA2, SCFD1, STX5, YKT6, SEC22B, NAPB, GRIP1, NAPG, and GABARAP **Fig. 7A, Table 4**. Most important thing is that all of them showed positive correlation with NSF expression in most of the TCGA tumour types suggesting their potential role in carcinogenesis **Fig. 7B.**

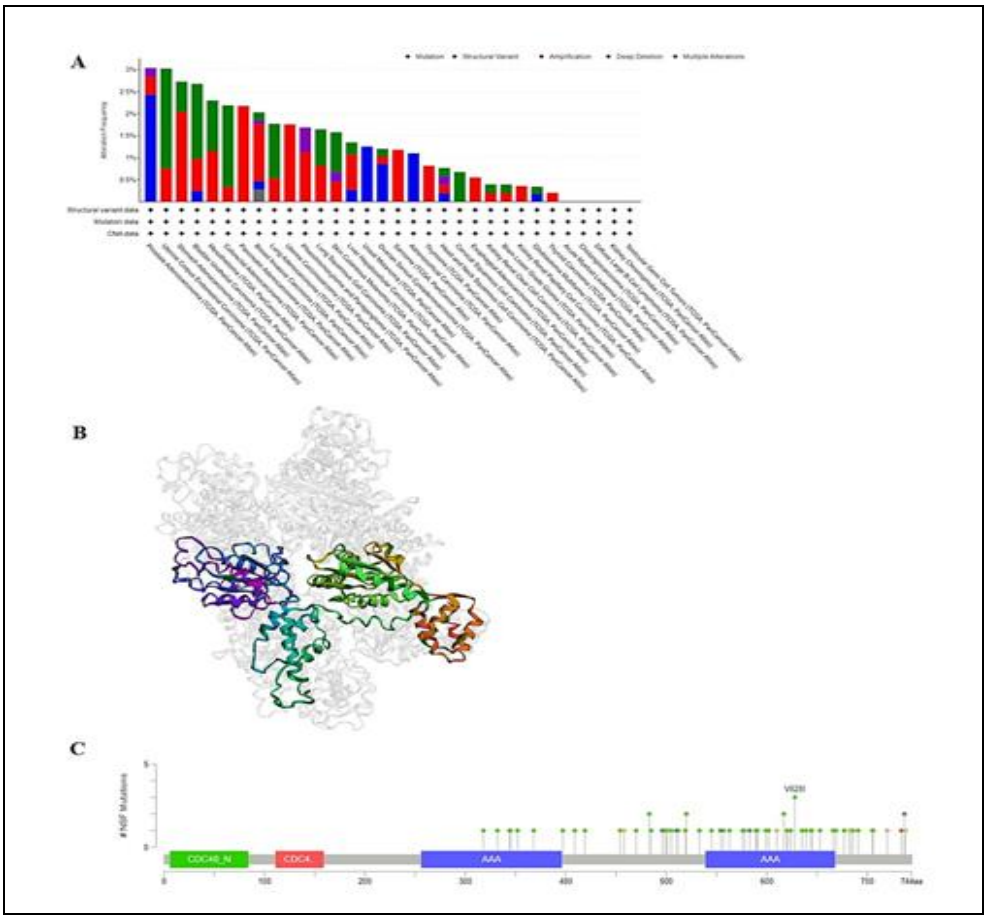

FIG. 4: GENETIC ALTERATION STATUS OF NSF IN DIFFERENT TUMORS. (A) THE AMPLIFICATION FREQUENCY OF NSF IN CANCER (DISPLAYED IN RED). (B) THE MUTATION FREQUENCY OF NSF IN CANCER (DISPLAYED IN GREEN). (C) THE 3D STRUCTURE OF NSF WITH MUTATIONAL POINTS IN GREEN. (D) POTENTIAL MUTATION SITES ACROSS THE NSF PROTEIN IN VARIOUS CANCERS. THE DIFFERENT MUTATION SITES ARE REPRESENTED WITH GREEN AND BLACK DOTS RESPECTIVELY.

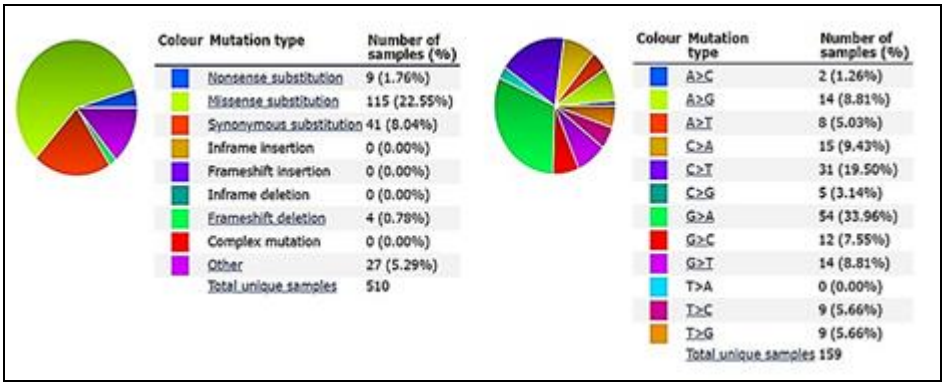

FIG. 5: THE COSMIC DATABASE REPRESENTS MUTATIONAL FREQUENCIES OF *NSF*

TABLE 2: GENETIC ALTERATION SUMMARY FOR NSF IN TOP 5 CANCERS

Cancer (TCGA, Firehose)	Data source	N	Frequency (%)	Amplification (%)	Deletion (%)
Prostate Adenocarcinoma	TCGA, PanCancer Atlas	489 cases	2.86%	0.41%	2.45%
Pancreatic Adenocarcinoma	TCGA, PanCancer Atlas	184 cases	2.17%	2.17%	-
Stomach Adenocarcinoma	TCGA, PanCancer Atlas	438 cases	2.05%	2.05%	-
Uterine Carcinosarcoma	TCGA, PanCancer Atlas	56 cases	1.79%	1.79%	-
Breast Invasive Carcinoma	TCGA, PanCancer Atlas	1071 cases	1.77%	1.59%	0.19%
Uveal Melanoma	TCGA, PanCancer Atlas	80 cases	1.25%	-	1.25%
Pheochromocytoma and Paraganglioma	TCGA, PanCancer Atlas	162 cases	1.23%	1.23%	-
Sarcoma	TCGA, PanCancer Atlas	253 cases	1.19%	1.19%	-
Mesothelioma	TCGA, PanCancer Atlas	87 cases	1.15%	1.15%	-
Adrenocortical Carcinoma	TCGA, PanCancer Atlas	89 cases	1.12%	-	1.12%
Liver Hepatocellular Carcinoma	TCGA, PanCancer Atlas	367 cases	1.09%	0.82%	0.27%
Ovarian Serous Cystadenocarcinoma	TCGA, PanCancer Atlas	572 cases	1.05%	0.17%	0.87%
Bladder Urothelial Carcinoma	TCGA, PanCancer Atlas	408 cases	0.98%	0.74%	0.25%
Lung Squamous Cell Carcinoma	TCGA, PanCancer Atlas	487 cases	0.82%	0.82%	-
Thymoma	TCGA, PanCancer Atlas	123 cases	0.81%	0.81%	-
Uterine Corpus Endometrial Carcinoma	TCGA, PanCancer Atlas	523 cases	0.76%	0.76%	-
Lung Adenocarcinoma	TCGA, PanCancer Atlas	511 cases	0.59%	0.59%	-
Esophageal Adenocarcinoma	TCGA, PanCancer Atlas	182 cases	0.55%	0.55%	-
Skin Cutaneous Melanoma	TCGA, PanCancer Atlas	367 cases	0.54%	0.54%	-
Head and Neck Squamous Cell Carcinoma	TCGA, PanCancer Atlas	517 cases	0.39%	0.19%	0.19%
Kidney Renal Papillary Cell Carcinoma	TCGA, PanCancer Atlas	283 cases	0.35%	0.35%	-
Colorectal Adenocarcinoma	TCGA, PanCancer Atlas	592 cases	0.34%	0.34%	
Thyroid Carcinoma	TCGA, PanCancer Atlas	497 cases	0.2%	0.2%	
Kidney Renal Clear Cell Carcinoma	TCGA, PanCancer Atlas	509 cases	0.2%	0.2%	-
Brain Lower Grade Glioma	TCGA, PanCancer Atlas	511 cases	0.2%	0.2%	-
Brain Lower Grade Glioma	TCGA, PanCancer Atlas	575 cases	0.17%	-	0.17%

TABLE 3: GENETIC MUTATIONAL FREQUENCY SUMMARY FOR *NSF* IN TOP 5 CANCERS

Cancer (TCGA, Firehose)	Data source	N	Frequency (%)	Mutation (%)
Uterine Corpus Endometrial Carcinoma	TCGA, PanCancer Atlas	517 cases	2.32%	2.32%
Colorectal Adenocarcinoma	TCGA, PanCancer Atlas	534 cases	2.06%	2.06%
Bladder Urothelial Carcinoma	TCGA, PanCancer Atlas	410 cases	1.71%	1.71%
Lung Adenocarcinoma	TCGA, PanCancer Atlas	566 cases	1.24%	1.24%
Mesothelioma	TCGA, PanCancer Atlas	86 cases	1.16%	1.16%
Skin Cutaneous Melanoma	TCGA, PanCancer Atlas	440 cases	0.91%	0.91%
Lung Squamous Cell Carcinoma	TCGA, PanCancer Atlas	484 cases	0.83%	0.83%

Stomach Adenocarcinoma	TCGA, PanCancer Atlas	436 cases	0.69%	0.69%
Cervical Squamous Cell Carcinoma	TCGA, PanCancer Atlas	291 cases	0.69%	0.69%
Breast Invasive Carcinoma	TCGA, PanCancer Atlas	1066 cases	0.28%	0.28%
Liver Hepatocellular Carcinoma	TCGA, PanCancer Atlas	366 cases	0.27%	0.27%
Glioblastoma Multiforme	TCGA, PanCancer Atlas	397 cases	0.25%	0.25%
Kidney Renal Clear Cell Carcinoma	TCGA, PanCancer Atlas	402 cases	0.25%	0.25%
Brain Lower Grade Glioma	TCGA, PanCancer Atlas	514 cases	0.19%	0.19%
Head and Neck Squamous Cell Carcinoma	TCGA, PanCancer Atlas	515 cases	0.19%	0.19%
Ovarian Serous Cystadenocarcinoma	TCGA, PanCancer Atlas	523 cases	0.19%	0.19%

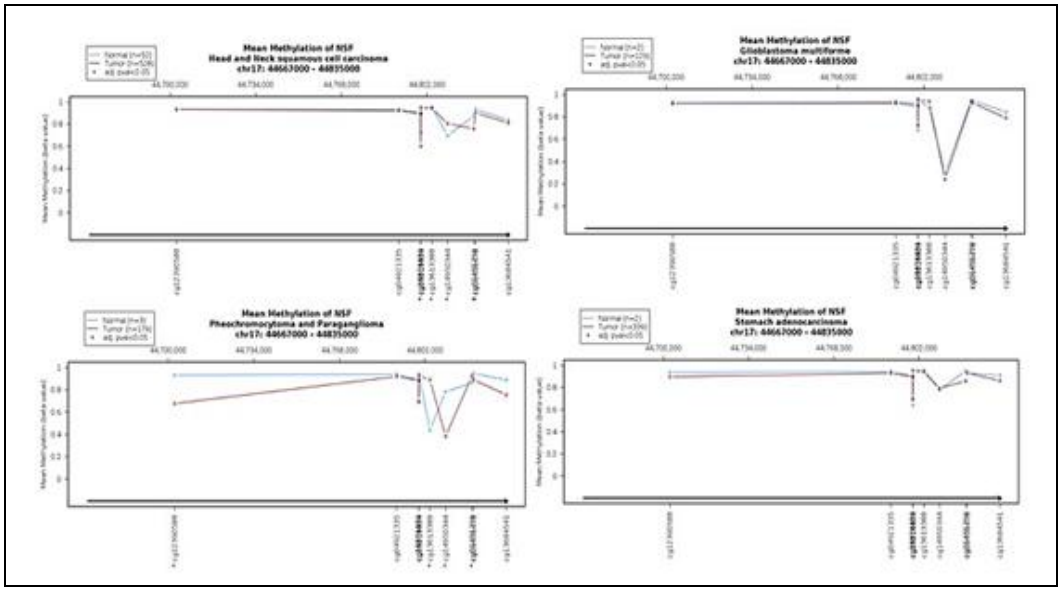

FIG. 6: MEAN METHYLATION OF NSF BETWEEN CANCER AND NORMAL SAMPLES

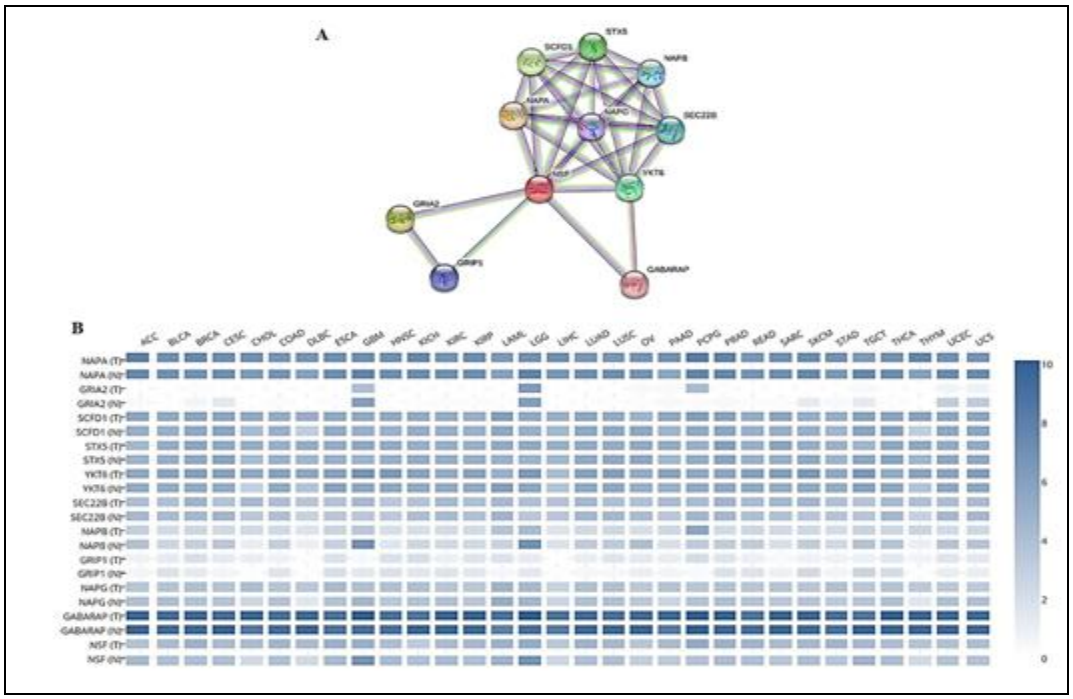

FIG. 7: (A) PROTEIN-PROTEIN INTERACTION NETWORK OF NSF WAS IDENTIFIED USING THE STRING DATABASE. PROTEINS WITH THE STRONGEST INTERACTION SCORES AND DIRECT INTERACTION PREDICTION ARE INCLUDED IN THE FIGURE. (B) THE DIFFERENTIAL EXPRESSION OF COEXPRESSED GENES OF NSF IN CANCER VS. NORMAL SAMPLES ARE REPRESENTED IN A MATRIX PLOT FORM. THE EXPRESSIONS ARE CALCULATED IN LOG_2 (TPM/ TRANSCRIPTS PER MILLION+1). THE COLOR INTENSITY INDICATES THE LEVEL OF EXPRESSION; A MORE INTENSE COLOR MEANS A BETTER EXPRESSION OF THE RESPECTIVE GENE.

TABLE 4: TOP 10 INTERACTING PARTNERS OF NSF

Interactor-Uniprot ID	Full Name Of The Proteins	Interactor-Entez Gene Id	Interactor-Gene Symbol	Score
NAPA_HUMAN	Alpha-soluble NSF attachment protein;	8775	NAPA	0.999
GRIA2_HUMAN	Glutamate ionotropic receptor ampa type subunit 2	2891	GRIA2	0.989
SCFD1_HUMAN	Sec1 family domain-containing protein 1	23256	SCFD1	0.986
STX5_HUMAN	Syntaxin-5	6811	STX5	0.973
YKT6_HUMAN	Synaptobrevin homolog YKT6	10652	YKT6	0.967
SEC22B_HUMAN	Vesicle-trafficking protein SEC22b	9554	SEC22B	0.962
NAPB_HUMAN	Beta-soluble NSF attachment protein	63908	NAPB	0.962
GRIP1_HUMAN	Glutamate receptor-interacting protein 1;	23426	GRIP1	0.957
NAPG_HUMAN	Gamma-soluble NSF attachment protein;	8774	NAPG	0.951
GABARAP_HUMAN	Gamma-aminobutyric acid receptor-associated protein	11337	GABARAP	0.945

Canonical Pathway Analysis of NSF: Finally, the gene *NSF* was analyzed for the biological pathways enrichment analysis using the DAVID tool which has highly curated canonical pathways. A cut-off of P-value <0.001 was used for the enrichment analysis. The top biological pathways are shown in **Table 5**. As expected, all top enriched pathways of *NSF* are mostly associated with cancer development and progression.

TABLE 5: SIGNALING PATHWAYS RELATED TO NSF

Serial no.	Term	Count	% (Involved genes/total genes)	P-value
1.	COPII-mediated vesicle transport	8	72.7	3.10E-14
2.	ER to Golgi Anterograde Transport	8	72.7	1.10E-11
3.	Transport to the Golgi and subsequent modification	8	72.7	4.00E-11
4.	Intra-Golgi traffic	6	54.5	2.10E-10
5.	Asparagine N-linked glycosylation	8	72.7	1.30E-09
6.	Membrane Trafficking	9	81.8	4.90E-09
7.	Intra-Golgi and retrograde Golgi-to-ER traffic	7	63.6	7.20E-09
8.	Vesicle-mediated transport	9	81.8	7.90E-09
9.	COPI-mediated anterograde transport	6	54.5	1.50E-08
10.	COPI-dependent Golgi-to-ER retrograde traffic	5	45.5	1.30E-06
11.	Golgi-to-ER retrograde transport	5	45.5	4.10E-06
12.	Retrograde transport at the Trans-Golgi-Network	4	36.4	9.90E-06
13.	Post-translational protein modification	8	72.7	5.60E-05
14.	Trafficking of GluR2-containing AMPA receptors	3	27.3	1.00E-04
15.	Trafficking of AMPA receptors	3	27.3	3.40E-04
16.	Glutamate binding, activation of AMPA receptors and synaptic plasticity	3	27.3	3.40E-04
17.	Metabolism of proteins	8	72.7	4.40E-04
18.	RHOA GTPase cycle	3	27.3	7.50E-03
19.	Neurotransmitter receptors and postsynaptic signal transmission	3	27.3	1.40E-02
20.	Transmission across Chemical Synapses	3	27.3	2.40E-02
21.	Cargo concentration in the ER	2	18.2	3.00E-02
22.	Neuronal System	3	27.3	5.20E-02
23.	RHO GTPase cycle	3	27.3	6.10E-02
24.	RHOG GTPase cycle	2	27.3	6.60E-02

CONCLUSION: Cancer is a complex disease. Even if the disease originates in different organs, may share similarities at the molecular level. For example, p53 mutations drive high-grade serous ovarian, serous endometrial, and basal-like breast carcinomas; all of them share a global transcriptional signature triggering similar carcinogenic pathways [28, 29]. Therefore, potential molecular drivers that are expressed in different cancers demand an in-depth cross-cancer analysis. We show that mRNA expression of NSF is significantly increased in all studied cancer types

and elevated NSF expression level leads to poor survival of the cancer patients. The low expression of NSF in certain cancer reflects its altered roles in different cancer types. To our knowledge, this is the first study where systematic bioinformatics analysis is applied to address the prognostic importance of NSF in multiple cancers. The analysis reported here establishes NSF as a promising prognostic marker for several cancers. Such analyses illustrate the importance of developing a comprehensive perspective across tumors. Cancer arises due to deleterious aberrations in the genome and its consequences. Therefore, fundamentally it is a genomic disease. Copy-number alteration (CNA) and point mutation are the two most important types of mutational events that impact the development and progression of the disease. In cancer, high alteration frequency is observed in NSF with73 potential mutational sites where missense mutations dominate the distribution. V628I, a mutational hotspot found in the conserved AAA+ domain. Moreover, the gene *NSF* makes direct interactionswith many oncogenes in a dense network whose components are associated with critical cellular processes. In conclusion, our analysis predicts a high prognostic value of NSF and considers NSF as an important therapeutic target, particularly for cancer.

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REFERENCES:

1. Ogura T and Wilkinson AJ: AAA+ super family ATPases: common structure–diverse function. Genes to Cells 2001; 6: 575-597.
2. White SR and Lauring B: AAA+ ATPases: achieving diversity of function with conserved machinery. Traffic 2007; 8: 1657-1667.
3. Nayak A, Dutta M and Roychowdhury A: Emerging oncogene ATAD2: Signaling cascades and therapeutic initiatives. Life Sciences 2021; 119322.
4. Nayak A, Kumar S, Singh SP, Bhattacharyya A, Dixit A and Roychowdhury A: Oncogenic potential of ATAD2 in stomach cancer and insights into the protein-protein interactions at its AAA+ ATPase domain and bromodomain. J of Biomolec Struc and Dyna 2021; 1-17.
5. Nayak A, Roy AD, Rout N, Singh SP, Bhattacharyya A and Roychowdhury A: HIF1α-dependent upregulation of ATAD2 promotes proliferation and migration of stomach cancer cells in response to hypoxia. Biochemical and Biophysical Research Communications 2020; 523: 916-923.
6. Block MR, Glick BS, Wilcox CA, Wieland FT and Rothman JE: Purification of an N-ethylmaleimide-sensitive protein catalyzing vesicular transport. Proceedings of the National Academy of Sciences 1988; 85: 7852-7856.
7. Hanson PI, Roth R, Morisaki H, Jahn R and Heuser JE: Structure and conformational changes in NSF and its membrane receptor complexes visualized by quick-freeze/deep-etch electron microscopy. Cell 1997; 90: 523-535.
8. Nagiec EE, Bernstein A and Whiteheart SW: Each domain of the N-ethylmaleimide-sensitive fusion protein contributes to its transport activity. Journal of Biological Chemistry 1995; 270: 29182-29188.
9. Söllner T, Whiteheart SW and Brunner M: SNAP receptors implicated in vesicle targeting and fusion. Nature 1993; 362: 318-324.
10. Clary DO, Griff IC and Rothman JE: SNAPs, a family of NSF attachment proteins involved in intracellular membrane fusion in animals and yeast. Cell 1990; 61: 709-721.
11. Matsushita K, Morrell CN and Cambien B: Nitric oxide regulates exocytosis by S-nitrosylation of N-ethylmaleimide-sensitive factor. Cell 2003; 115: 139-150.
12. Huynh H, Bottini N and Williams S: Control of vesicle fusion by a tyrosine phosphatase. Nature Cell Biology 2004;6: 831-839.
13. Liu Y, Cheng K, Gong K, Fu AK and Ip NY: Pctaire1 phosphorylates N-ethylmaleimide-sensitive fusion protein: implications in the regulation of its hexamerization and exocytosis. J of Biological Chemistry 2006; 281: 9852-58.
14. Matveeva EA, Whiteheart SW, Vanaman TC and Slevin JT: Phosphorylation of the N-ethylmaleimide-sensitive factor is associated with depolarization-dependent neurotransmitter release from synaptosomes. Journal of Biological Chemistry 2001; 276: 12174-12181.
15. Tang Z, Kang B, Li C, Chen T and Zhang Z: GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Research 2019; 47: 556-560.
16. Chandrashekar DS, Bashel B and Balasubramanya SAH: UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 2017; 19: 649-658.
17. Győrffy B, Lanczky A and Eklund AC: An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients 2010; 123: 725-731.
18. Győrffy B, Surowiak P, Budczies J and Lánczky AJPo: Online survival analysis software to assess the prognostic

value of biomarkers using transcriptomic data in non-small-cell lung cancer 2013; 8: 82241.
19. Gyorffy B, Lanczky A and Szallasi ZJE-rc: Implementing an online tool for genome-wide validation of survival-associated biomarkers in ovarian-cancer using microarray data from 1287 patients 2012; 11-0329.
20. Szász AM, Lánczky A and Nagy Á: Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients 2016; 7: 49322.
21. Díez-Villanueva A, Mallona I and Peinado MA: Wanderer, an interactive viewer to explore DNA methylation and gene expression data in human cancer. Epigenetics & Chromatin 2015; 8: 1-8.
22. Gao J, Aksoy BA and Dogrusoz U: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal 2013; 6: l1-l1.
23. Cerami E, Gao J and Dogrusoz U: The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data: AACR 2012.
24. Bamford S, Dawson E and Forbes S: The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website 2004; 91: 355.
25. Szklarczyk D, Franceschini A and Wyder S: STRING v10: protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Research 2015; 43: 447-452.
26. Sherman BT, Hao M and Qiu J: DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Research 2022; 50: 216-221.
27. Zhang W and Yu Y: The important molecular markers on chromosome 17 and their clinical impact in breast cancer. International Journal of Molecular Sciences 2011; 12: 5672-5683.
28. Nature CGANJ: Comprehensive molecular portraits of human breast tumours 2012; 490: 61.
29. Levine DA: Nature CGARNJ. Integrated genomic characterization of endometrial carcinoma 2013; 497: 67.

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