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Combinatorial Pan-cancer analysis of NF1 in Multiple Cancers

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Abstract: The GTPase-activating protein, NF1, negatively regulates the RAS/MAPK signaling pathway. Higher mutation rates of NF1 negatively impact cancer cell signaling and progression by altering cellular pathways. The expression pattern of the NF1 gene in both normal and cancer cells of different types as well as their mutations and gene networks need to be evaluated for better cancer management and enhancing therapeutic drug development pipelines. Herein, the expression pattern of the NF1 gene was explored via the GEPIA2, GENT2, and UALCAN tools. Lower expression and methylation rates were found in most cancer types, but variability was observed based on race, gender, and cancer stage. The corresponding protein levels in normal and cancer tissues were also investigated, revealing medium staining and moderate intensity in normal lung tissues but high staining and strong intensity in lung cancer tissues as well as in other cancer types. Additionally, 15 missense mutations, 351 truncations, no inframe mutations, 97 splices and 48 fusions were found in the NF1 gene through cBioportal, where amplifications, deep deletions, structural variants, and multiple alterations were observed. The SUZ12 gene was found among the most correlated and co-expressed genes of NF1 in BRCA, KIRC, LIHC, and LUAD. This study suggests the variable role of NF1 in normal tissues and cancer tissues in terms of cancer progression, gene expression, and methylation level and should be extended for therapeutics development against cancer.

Keywords: NF1, Pan-cancer, BRCA, KIRC, LIHC, and LUAD

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1. Introduction

Cancer is recognized as the second leading cause of death following cardiovascular diseases according to global mortality rates and is a prime reason for reduced human life expectancy in almost all regions of the world [1], [2], [3]. Somatic changes, in tandem with inherited mutations of specific genes, lead to unusual cell growth signals and form a tumor that subsequently destroys the organism through cancer progression [4]. In the last year, the approximate number of cancer cases worldwide was about 10 million, and remarkably, the United States (US) has reported 608,570 cancer deaths in the current year, supporting the predictions that the cancer mortality rate increase to more than 28% by 2060 [2], [3], [5]. A number of cancer treatments, including chemotherapy, radiation therapy, immunotherapy, surgery, hormonal therapy, and adjuvant therapy, are suggested to combat cancer [1]. However, disseminated cancer caused by metastasis is resistant to treatment because of the limitations and complexity of the available cancer treatments [1], [6], [7].

The neurofibromatosis 1 (NF1) gene maps to chromosome 17qll.2 and comprises 350 kb genomic DNA along with 60 exons that encode a giant protein named neurofibromin; this protein comprises over 2800 amino acids and has a molecular mass of 327 kDa [8], [9], [10], [11]. As a negative switch that regulates the RAS oncogene, neurofibromin is considered a tumor suppressor with various functional domains, including the GTPase-activating protein-related domain (GAP), whose function concerns the control of cell growth through RAS interaction [10], [11], [12]. NF1 is involved in numerous pathways, including Akt/mammalian target of rapamycin (Akt/mTOR), RAS/mitogen-activated protein kinase (MAPK), cAMP/PKA, and ROCK/LIMK/cofilin, among which the RAS/MAPK pathway regulating cell growth, senescence, and differentiation and the phosphoinositide 3-kinase (PI3K)/mTOR-related signaling pathways are chiefly analyzed [10], [12]. However, recognized somatic NF1 mutations, including deletions, translocations, point mutations, and insertions, inactivate the genes causing lung cancer, ovarian cancer, breast cancer, prostate cancer, brain cancer, liver cancer, kidney cancer, skin cancer, leukemia, uterine adenocarcinoma, and gastrointestinal stromal tumor [9], [13], [14], [15], [16], [17].

RAS activation is triggered when RAS-GDP is converted into RAS-GTP; in addition, the conversion is negatively regulated by NF1-encoded neurofibromin 1 through the conversion of kinetic RAS-GTP into inactive RAS-GDP. Several downstream mediators, including the RAF-1 proto-oncogene, interact with activated RAS, causing homo and heterodimerization and RAF activation that subsequently stimulates the MAP kinases, including MAP2K1 and MAP2K2, through phosphorylation, ultimately activating MAPK3 or ERK1 and/or MAPK1 or ERK2 and controlling cell cycle progression as well as cell growth and differentiation. However, dysregulation in the RAS/MAPK pathway in which NF1 acts as a key mutated gene facilitates oncogenesis and cancer progression [18]. Likewise, the role of the PI3K/mTOR pathway is confirmed by the stimulation of the pathway in malignant peripheral nerve sheath tumor (MPNST) cell lines, NF1-mutant neurofibromas, in tandem with primary tumors [19]. Moreover, the cAMP pathway is dysregulated throughout NF1-deficient tumors, and the cAMP level is altered in human NF1-associated tumors [20]. Somatic NF1 mutations are reported to induce MPNST, acute lymphoblastic leukemia, glioblastoma, lung squamous cell carcinoma, non-small cell lung cancer (NSCLC), lung adenocarcinoma, uterine carcinosarcoma, bladder urothelial carcinoma, uterine endometrial carcinoma, pancreatic carcinoma, ovarian serous cystadenocarcinoma, metastatic cutaneous squamous cell carcinoma, and gastric adenocarcinoma in humans. Additionally, the probability of cancer development has been noted to be increased in several syndromes like Noonan syndrome, Costello syndrome, cardio-facio-cutaneous syndrome, and Legius syndrome through the activation of mutations in the PTPN11, HRAS (BRAF, MAP2K1, and MAP2K2), and SPRED1 genes, particularly where the somatic mutations in the genes co-occur with NF1 mutations [10], [21], [22].

Being located on the same chromosome (chromosome 17; long arm), a concurrence of NF1 and inherited breast cancer indicates a combination of mutations in the NF1 and BRCA1 genes concerning breast cancer progression due to the co-segregation of individual mutations at the two loci, supporting the possible interaction of BRCA1 with NF1 during breast cancer progression [23], [24], [25]. Additionally, the well-distinguished function of NF1 in the regulation of RAS signaling to utilize the MAPK–ERK pathway that is related to NSCLC either individually (maximum cases) or via concurrent oncogenic alteration with KRAS (predominant), HRAS, or NRAS mutations, in tandem with ERBB2 or BRAF mutations, refers to the prognostication of lung cancer progression through the NF1 gene [26].

Furthermore, lack of the NF1 gene causes RAS signaling pathway activation, leading to the anomalous growth of hematopoietic cells. Notably, promoter hypermethylation in tandem with LOH in the NF1 and DAB2IP genes was correlated with NF1 and DAB2IP gene downregulation, implying that molecular-level mechanisms are responsible for NF1 and DAB2IP gene silencing during human hepatocarcinogenesis and the emergence of liver cancer through the RAS signaling pathway [27], [28]. Moreover, VHL gene inactivation through mutation leads to the inability to target HIF1a for degeneration, which is regulated by an mTOR signaling pathway related to the NF1 gene, thereby causing HIF1 accumulation. Hence, it can result in the enhanced transcription of VEGF, PDGF, GLUT-1, TGF, and erythropoietin, causing the emergence of clear cell renal carcinoma and kidney cancer [29], [30], [31], [32], [33].

Systematic computational investigations combining large experimental datasets regarding the NF1 gene have not been reported thus far. In the current study, we utilized abundant data collected from various online databases and web servers and evaluated the role of expression patterns of the NF1 gene in the evolution of several cancers. The expression pattern of the NF1 gene was assessed in the study to identify its association with diverse clinicopathological properties and evaluate its function in the emergence of distinct cancers. Additionally, we analyzed the interconnected genes and predicted

datasets library to estimate the activity in tandem with predictions associated with the NF1 gene. Furthermore, the obtained systematic data may assist researchers in determining the biomarker ability and mechanisms of the NF1 gene in-depth, which will serve in additional studies to shed light on the explicit function of this gene in cancer progression.

2. Materials and Methods

2.1. Gene Expression

Gene expression profiling was conducted using the GEPIA2 tool [34], which consists of 9736 tumors and 8587 normal samples, to analyze cancer types, different pathological stages of cancer, survival, and normal and cancer cell differential expression (http://gepia2.cancer-pku.cn). This tool functions through TCGA and GTEx data with comprehensive interactive analysis of gene expression. Furthermore, the Gene Expression database of Normal and Tumor tissues 2 (GENT2) tool [35] enabled gene expression analysis of normal and cancer tissues from public databases (http://gent2.appex.kr/gent2/). Currently this tool contains more than 68,000 samples and gene expression data for 72 different tissues. The UALCAN tool [36] was also used to gain further wide-ranging insights into gene expression of the NF1 gene family (http://ualcan.path.uab.edu/index.html). This web tool compiles TCGA-RNA sequencing data from patients with 33 different types of cancer, with onco-pathological features and the impact of certain genes in the expression patterning in normal and cancer tissues.

2.2. Methylation

The methylation of the NF1 gene was also extracted from the UALCAN tool (http://ualcan.path.uab.edu/index.html) [36], which combines the transcriptomic data from cancer patients with 793 cancer samples and 97 normal samples. This platform reveals the promoter DNA methylation of the NF1 gene in the BRCA, KIRC, LUAD, and LIHC cancer types compared to normal tissues. The DNA methylations of the NF1 gene were classified by beta values of 0 for unmethylated, 1 for fully methylated, 0.5 to 0.7 for hypermethylated, and 0.3 to 0.25 for hypomethylated DNA.

2.3. Immunohistochemistry

The Human Protein Atlas gives information on the expression of genes at the protein and tissue level, combining data on 44 normal tissue types derived from antibody-based protein profiling by immunohistochemical methods (https://www.proteinatlas.org/). Currently, this web tool compiles data from 15,320 genes derived by immunohistochemical analysis [37].

2.4. Mutation

The mutation profiling of the NF1 protein was explored using cBioportals [38], [39], [40], which consists of 715 datasets along with more than 87,000 samples (http://www.cbioportal.org/). Multiple mutational descriptors were evaluated for the NF1 gene, including DNA copy number, mutation, mRNA expression, protein and phosphoprotein, and DNA methylation. The location and mutation number of NF1 genes were also determined through cBioportal, wherein mutations, amplifications, deep deletions, and alternations were investigated.

2.5. Correlation and Co-expression

Correlation and co-expression analysis was performed via the UALCAN tool [36] for different types of cancer, namely BRCA, KIRC, LUAD, and LIHC (http://ualcan.path.uab.edu/index.html). A P-value less than 0.05 was considered statistically significant. Furthermore, correlation analysis was performed in TIMER (TIMER [shinyapps.io]) to analyze immune filtration in multiple cancer cells [41]. Currently, this database contains 10,897 samples from 32 different types of cancer from the TCGA or Cancer Genome Atlas to explore the immune infiltration pattern. The NF1 gene was analyzed in the TIMER database to explore the correlation of immune infiltrating cells, including T-cells, B cells, macrophages, neutrophils, and DCs.

2.6. Network Pharmacology

The GeneMANIA tool [42], [43], [44] was utilized for the protein-protein expression of the NF1 protein with co-expression gene family annotations (https://genemania.org/). The protein and genetic interactions, pathways, co-expressions, colocalizations, similar protein domains, and physical interactions were analyzed for the NF1 protein in this tool.

2.7. Pathway and Gene Ontologies

The pathways and ontologies of the Enricher tool [45], which combines information from a large number of mammalian gene sets, were used (https://maayanlab.cloud/Enrichr/).

3. Results

3.1. NF1 Gene Expression in Cancer

The expression pattern of the NF1 gene was explored using the GEPIA2 tool in different cancer types, adjusting the P-value to 0.05 in ANOVA testing. **Figure 1A** shows that this gene is upregulated in ESCA, LGG, PAAD, STAD, and THYM, whereas downregulation was observed in ACC, BLCA, BRCA, CESC, CHOL, COD, DLBC, GBM, HNSC, KICH, KIRC, LAML, LIHC, LUAD, LUSC, MESO, OV, PCPG, PRAD, READ, SARC, SKCM, THCA, UCEC, UCS and UVM. The gene expression was also analyzed via the GENT2 tool, and low expression was found in multiple cancer types including BRCA, KIRC, LUAD, and LIHC.

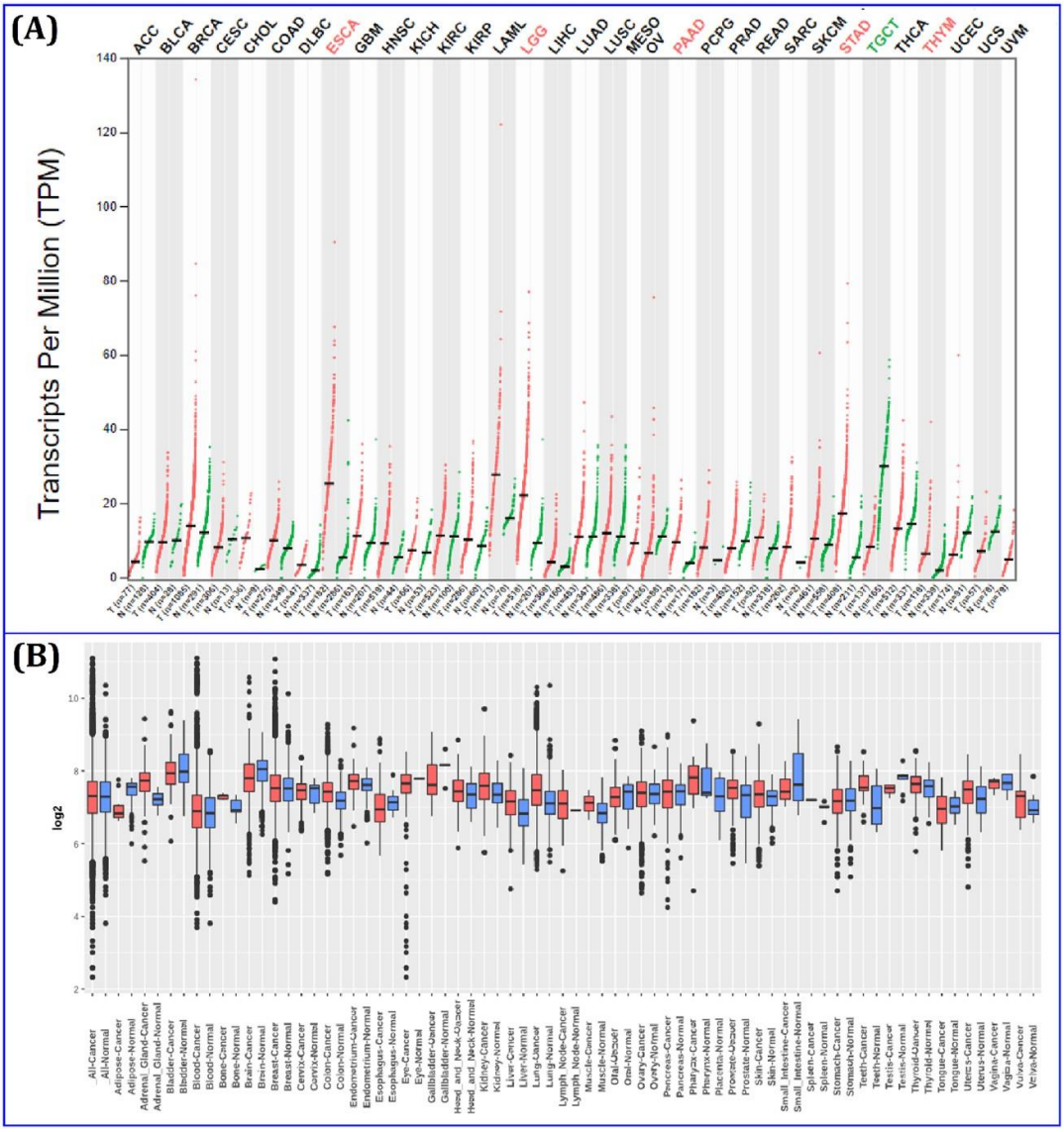

Figure 1: Gene Expression profile of NF1 gene in multiple cancer types; (A) the transcription profile of mRNA from NF1 gene in multiple types of cancer from GEPIA tools, (B) NF1 gene expression in different cancer types from GENT2 tool.

Moreover, the expression pattern was also analyzed through the GEPIA2 and UALCAN tools for cross-validation of the data. **Figure 2** indicates that the expression profile of the NF1 gene is higher in BRCA and LIHC than in normal tissues on the $\log_2$ scale. Higher expression was also observed for KIRC and LUAD for both normal and cancer tissues **(Figure 2A, C, E, and G)**. Moreover, the NF1 gene was also significantly upregulated in LIHC (1.11E-16) and LUAD (P=1.62-12), whereas downregulation was observed for BRCA (P=1.21E-02) and KIRC (1.99E-04).

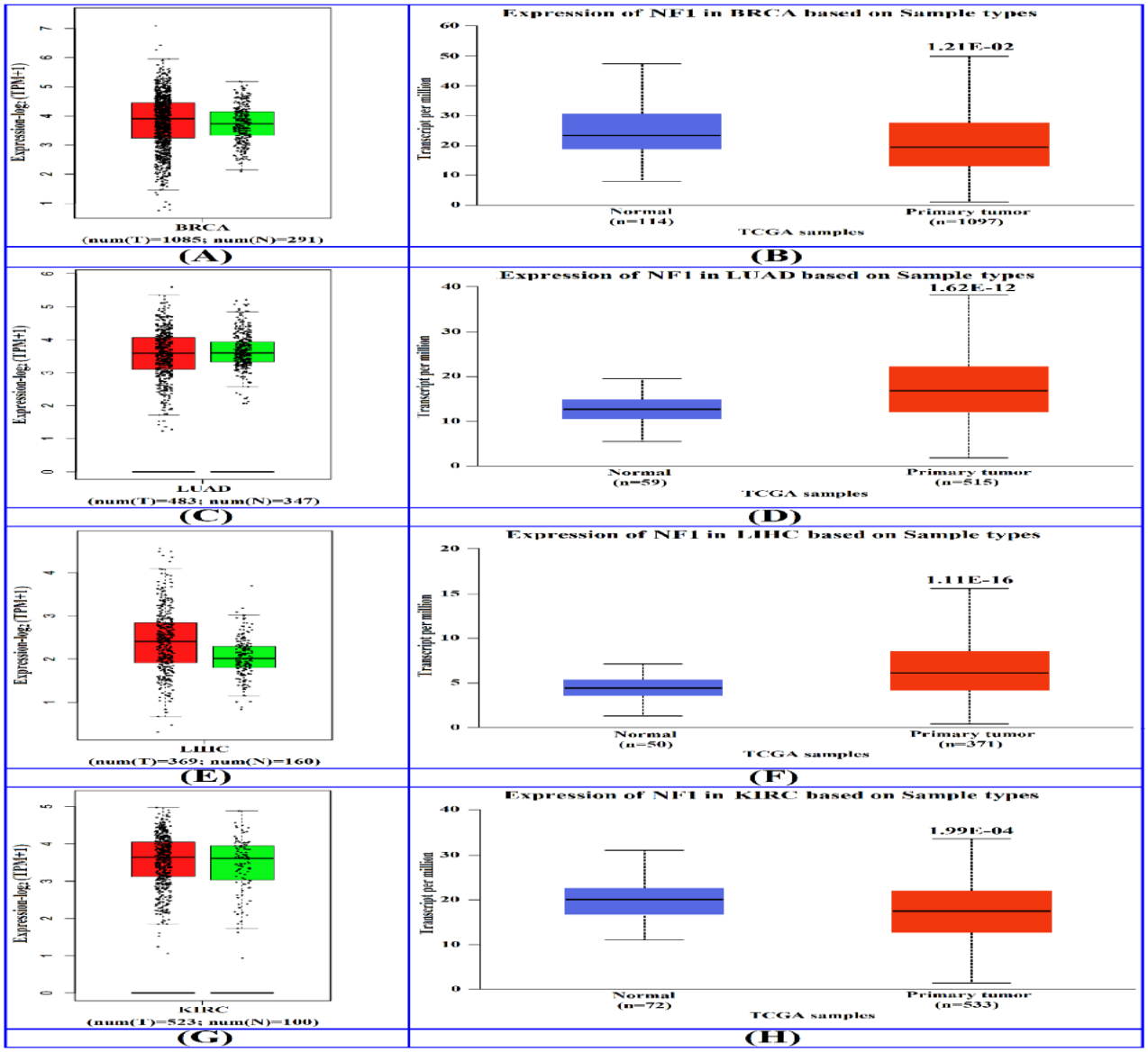

Figure 2: The GEPIA2 and UALCAN tools utilized for expression pattern analysis of NF! Gene in BRCA, KIRC, LUAD, and LIHC cancer types.

3.2. Expression of NF1 Regarding Clinical Features

The expression of NF1 genes regarding clinical features was also explored in the UALCAN tool; overexpression was observed in patients with stage 2 (P=4.86e-02) and stage 3 (P=3.97e-02) BRCA cancer (Figure 3A); additionally, Caucasians (P=2.01e-01; Figure 3B) and female patients (P=4.47e-01) had higher expression of the NF1 gene (Figure 3C), whereas lower expression was observed in male patients (P=1.01e-02) and African Americans (P=2.07e-13). Furthermore, patients with stage 4 LUAD cancer (P=1.64e-04) had higher expression levels than patients in other stages, although other cancer stages, namely stage 1 (P=2.32e-14), stage 2 (P=2.10e-09), and stage 3 (P=1.57e-08) had a significantly higher expression level compared to normal tissues (Figure 3D). More expression was also observed in Caucasians (P=1.62e-12) and Asians (1.51e-02), and the least expression was observed in African Americans (2.73e-03; Figure 3E). Male (P=4.18e-14) and female (P=2.66e-15) patients had similar expression levels (Figure 3F) in LUAD. A higher expression level was observed in stage 3 patients (P=1.89e-08), whereas the least expression was found in stage 1 patients (3.09e-10; Figure 3G). African American men had higher expression levels of the NF1 gene, and similar expression profiles were found in Caucasians (P=1.01e-13) and Asians (P=7.25e-11) in LIHC (Figure 3H). Female patients (P=2.94e-13) with LIHC had higher expression levels (Figure 3I), whereas male patients (2.14e-11) had lower expression. Moreover, higher expression was found in Asians with KIRC (P=6.68e-01), and patients with stage 1, stage 2, stage 3, and stage 4 KIRC exhibited lower expression of NF1 compared to the normal tissues (Figure 3 J–L).

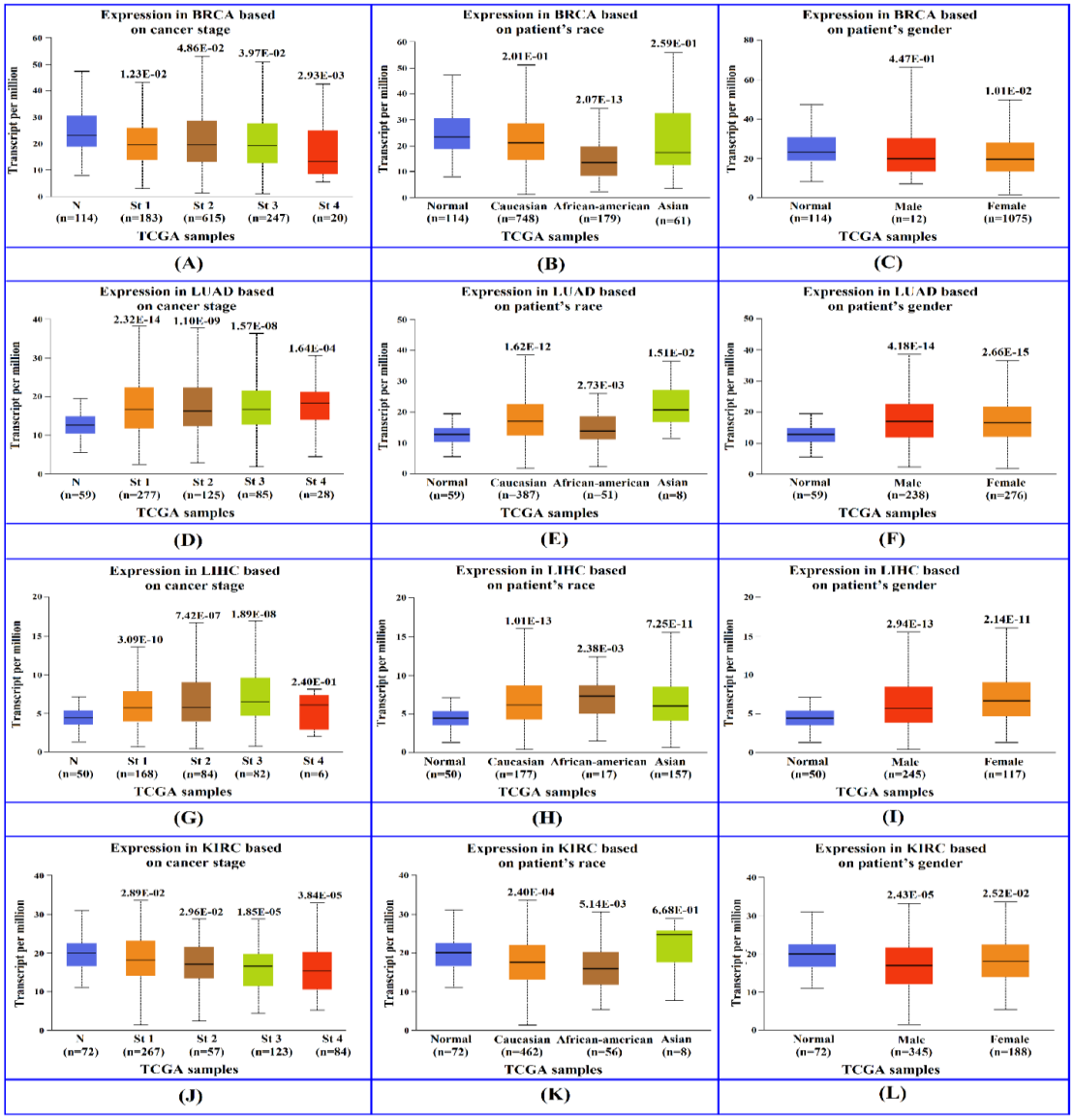

Figure 3: The expression profiling of NF1 gene BRCA cancer types based on different cancer stage, race, and gender(A-C), expression in LUAD based on cancer stage, race, and gender(D-F), expression in LIHC based on cancer stage, race, and gender (G-I), expression in KIRC based on cancer stage, race, and gender (J-L).

3.3. Methylation of the NF1 Gene in Cancer

The methylation of NF1 was also explored for multiple cancer stages, patient race, gender, and tissue types in BRCA, LUAD, LIHC, and KIRC. The methylation level was higher in normal tissues compared to the cancer tissues (P=1.62e-12) for all types of cancer tissues investigated, namely BRCA, LUAD, LIHC, and KIRC **(Figure 4A)**. The stage 4 (P=2.40e-05) cancer patients had the least number of methylations but stages 1 (1.11e-16), 2 (p≤1E-12), and 3 (p≤1E-12) had similar methylation levels **(Figure 4B)**. Asian populations (P=2.40-05) exhibited the least expression, and Caucasians (P≤1e-12) and African Americans (p≤1.62e-12) exhibited similar types of methylation **(Figure 4C)** of the NF1 gene in BRCA. Female patients (P=1.62e-12) had higher methylation **(Figure 4D)** compared to male patients in BRCA. A superior fold of methylation was also observed in normal tissues compared to cancer tissues (P=2.97e-04) in LUAD **(Figure 4E)**, greater methylation was observed for stage 4 patients (6.31e-01) **(Figure 4F)**, and the Caucasian population exhibited higher levels of methylation (P=3.67e-03; **Figure 4G**). Male and female patients had parallel methylation levels in LUAD **(Figure 4H)**. The methylation pattern was similar in normal tissue and cancer tissue in LIHC **(Figure 4I)**, and stage 4 patients (P=9.41e--01) had greater methylation **(Figure 4J)**, the Asian population (P=1.22e-01) displayed the least methylation, and Caucasians (P=1.70e-01) and African Americans (P=5.47e-01) had similar methylation levels **(Figure 4K)**. Male and female patients with

LIHC had parallel methylation **(Figure 4L)**. Moreover, male and female patients exhibited identical methylation in KIRC stages 1 and 2, and Caucasians had higher methylation compared to other populations **(Figure 4I–L)**.

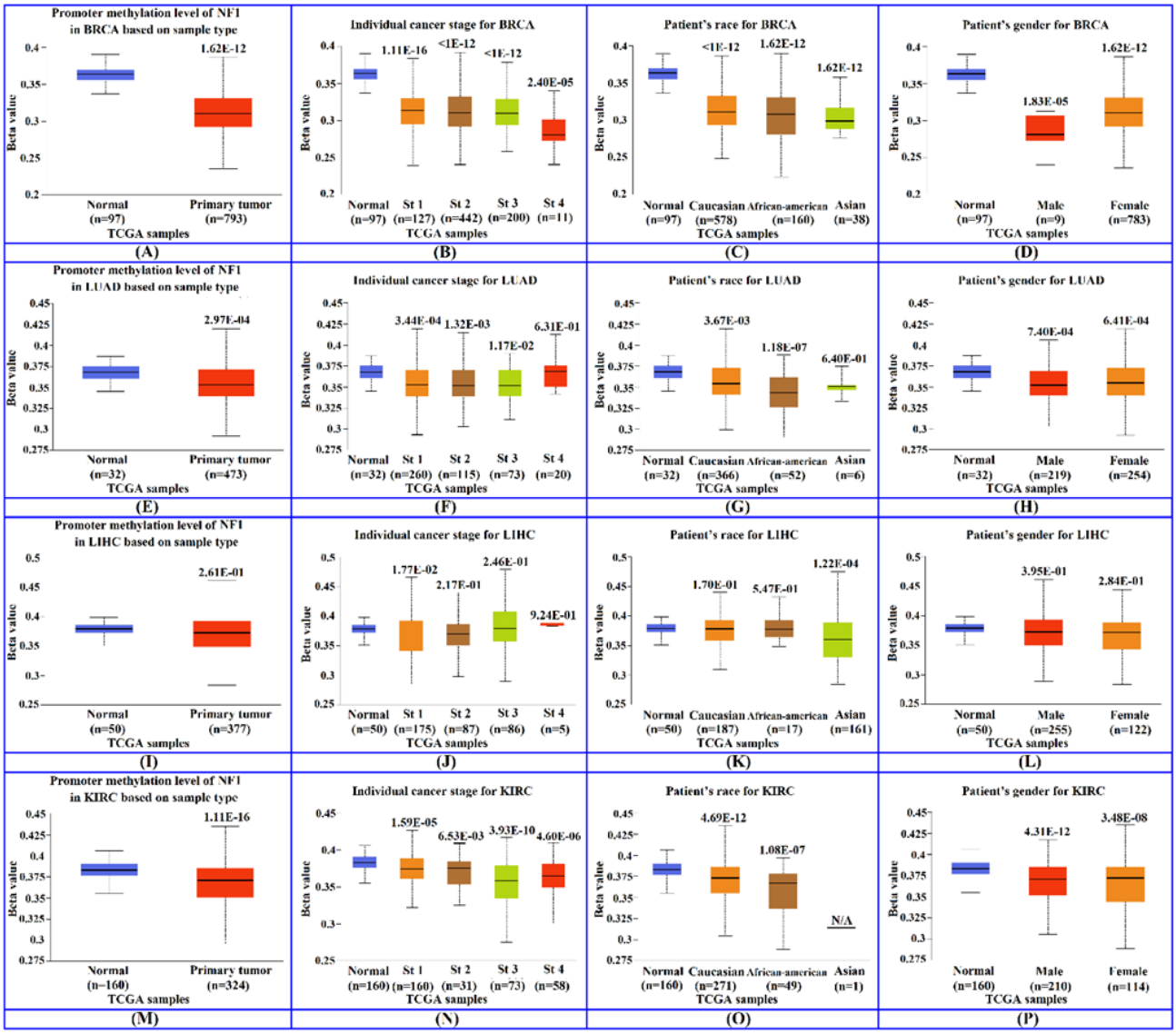

Figure 4: Promoter methylation of NF1 gene in BRCA cancer with multiple clinical characteristics; A-D shows methylations in cancer stage, race and gender. E-H shows promoter methylation in LUAD by cancer stage, race and gender. I-L depicts the promoter methylation in LIHC by cancer stage, race and gender. M-P shows the promoter methylation in KIRC by cancer stage, race and gender.

3.4. Immunohistochemistry

NF1 gene expression was explored through antibody profiling based on immunohistochemistry for normal tissues and multiple types of cancer tissues. The BRCA cancer type revealed high staining and strong intensity for both cancer and normal tissue types **(Figure 5A)**, whereas medium staining and moderate intensity was found in normal lung tissues, although high staining and strong intensity was revealed for lung adenocarcinoma **(Figure 5B)**. LIHC and KIRC cancer tissues as well as normal liver and kidney tissues also exhibited strong staining and high intensity **(Figure 5C and D)**.

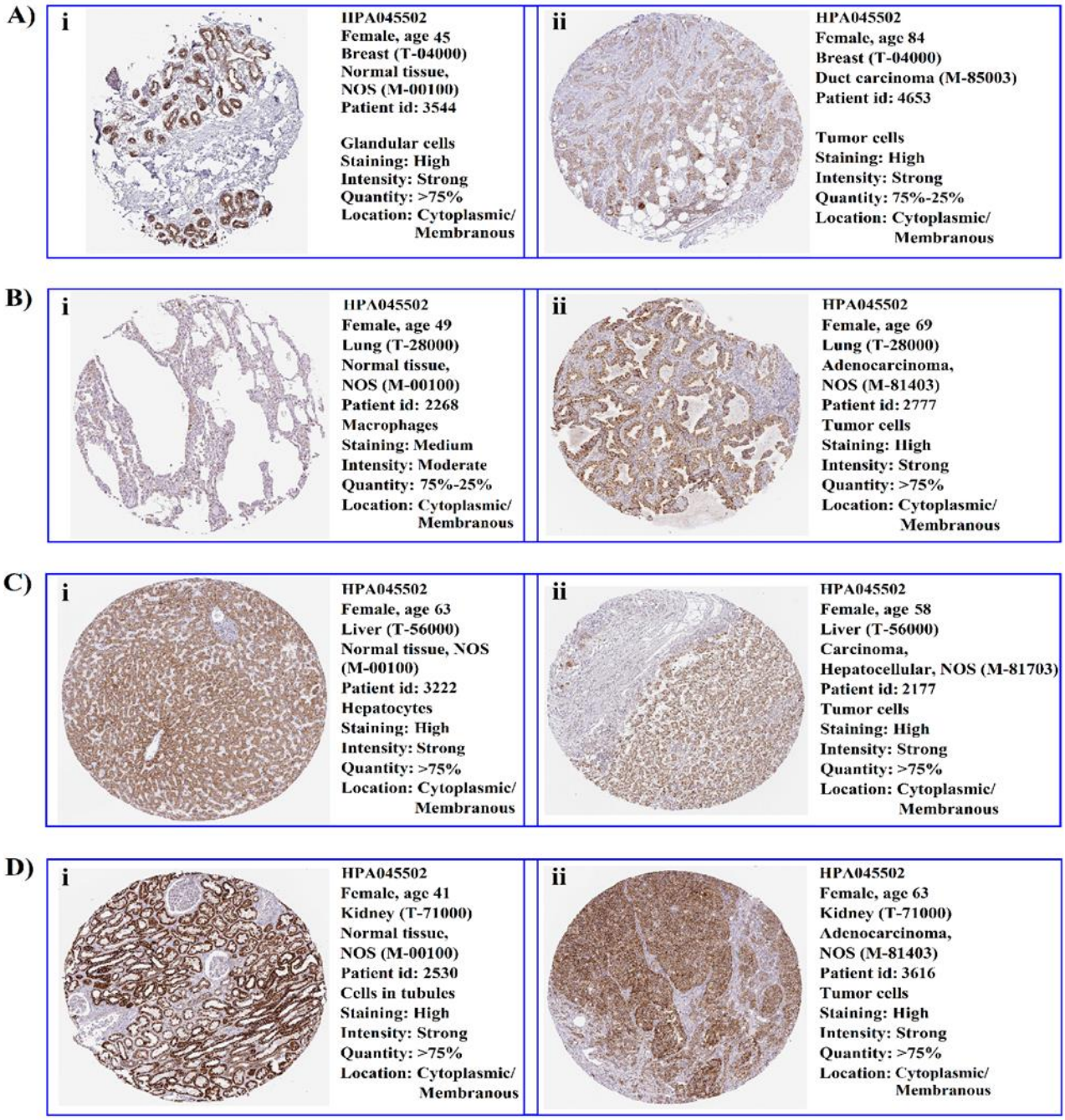

Figure 5: Expression of NF1 gene in normal tissues and compared to breast duct carcinoma (A), lung normal tissue to lung adenocarcinoma (B), liver normal tissue to liver adenocarcinoma (C), kidney normal tissue to kidney adenocarcinoma (D).

3.5. Mutations

The mutation profile of the NF1 gene was explored through cBioPortal, searching for over 10,967 samples from 10,953 patients in 32 studies. This tool identified 15 missense mutations, 351 truncations, no inframe mutations, 97 splices, and 48 fusions in the NF1 gene **(Figure 6A)**. Therefore, the impact of mutation types on multiple cancer types, namely BRCA, KIRC, LIHC, and LUAD, was observed, where amplifications, deep deletions, structural variants, and multiple alterations were seen **(Figure 6B)**. The copy number alterations revealed that amplification plays a driving role in the upregulation in the mRNA sequence **(Figure 6C)**.

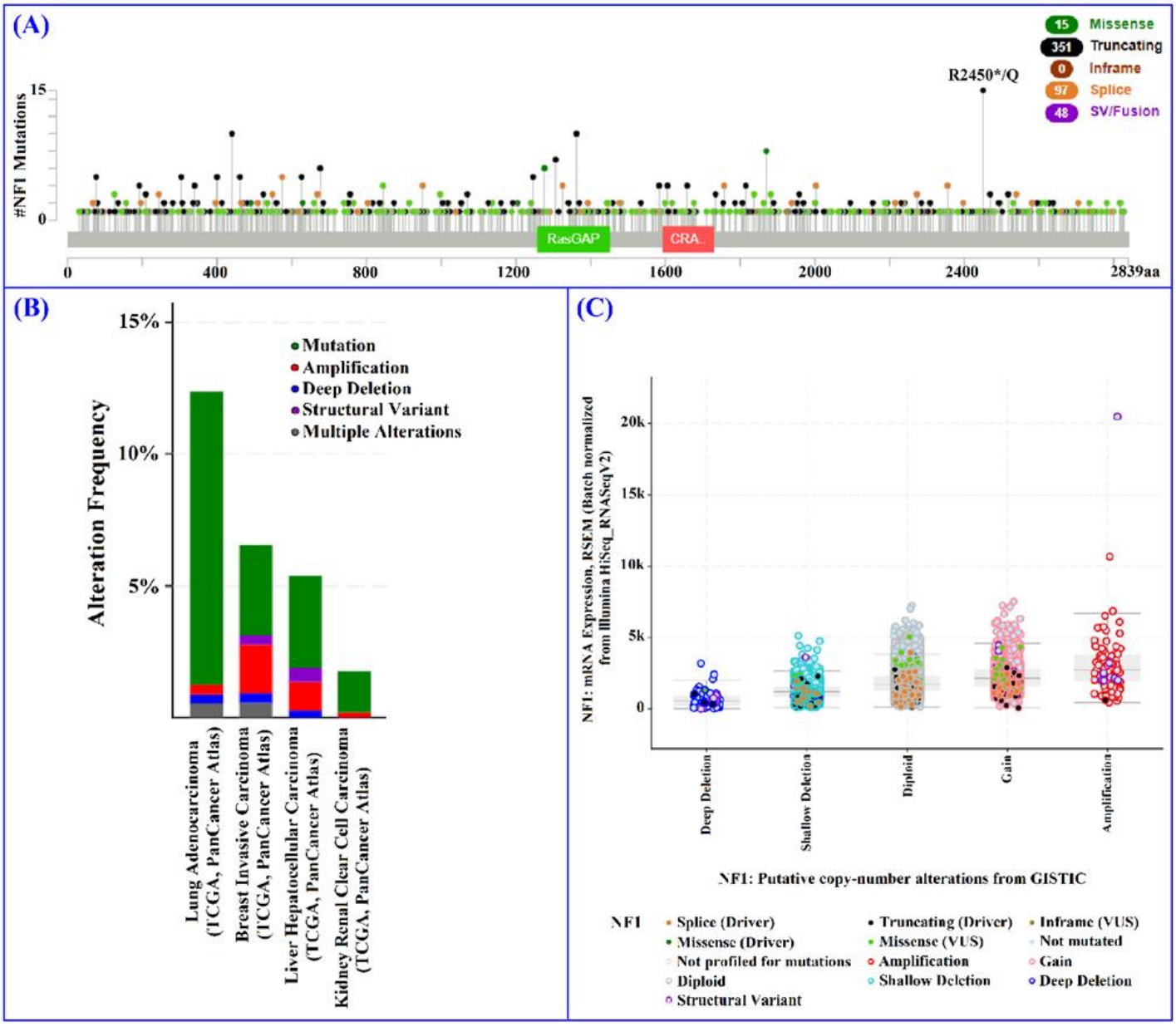

Figure 6: The mutation profiling of NF1 gene (A) Mutation in protein sequence regions in NF1, (B) Frequency of mutations where green, red, blue, violet, gray indicates mutation, amplification, deep deletion, structural variant, and multiple alternation respectively.

3.6. Correlation and Co-expression

Cancer progression and co-expression of the NF1 gene was explored in UALCAN, and SUZ12 was found to be mostly correlated with NF1 (Pearson's r=0.71), LUAD (Pearson's r=0.74), LIHC (Pearson's r=0.84), and KIRC (Pearson's r=0.88) **(Figure 7A, 7B, 7C, and 7D)**. The correlation and immune infiltration were also examined using the TIMER tool for multiple cancer types. The NF1 gene positively correlated in B cells (partial correlation=0.02, P=9.59e-01), CD8+ T cells (partial correlation=0.282, P=2.56e-19), CD4+ T cells (partial correlation=0.65, P=4.45e-02), macrophages (partial correlation=0.281, P=3.06e-19), neutrophils (partial correlation=0.166, P=2.66e-07), and dendritic cells (partial correlation=0.082, P=1.18e-02) for BRCA **(Figure 8)**. A similar positive immune infiltrating pattern was observed in KIRC for B cells (partial correlation=0.226, P=9.55e-07), CD8+ T cells (partial correlation=0.136, P=4.37e-03), CD4+ T cells (partial correlation=0.375, P=7.62e-17), macrophages (partial correlation=0.441, P=9.88e-23), neutrophils (partial correlation=0.41, P=5.31e-20), and dendritic cells (partial correlation=0.288, P=4.06e-10). Moreover, a higher correlation was observed in CD4+ T cells (partial correlation=0.441, P=8.09e-18), macrophages (partial correlation=0.424, P=2.48e-16), and neutrophils (partial

correlation=0.466, P=5.53e-20) **(Figure 8)**. For LIHC, slightly higher and moderate levels of correlation were observed for all types of immune cells.

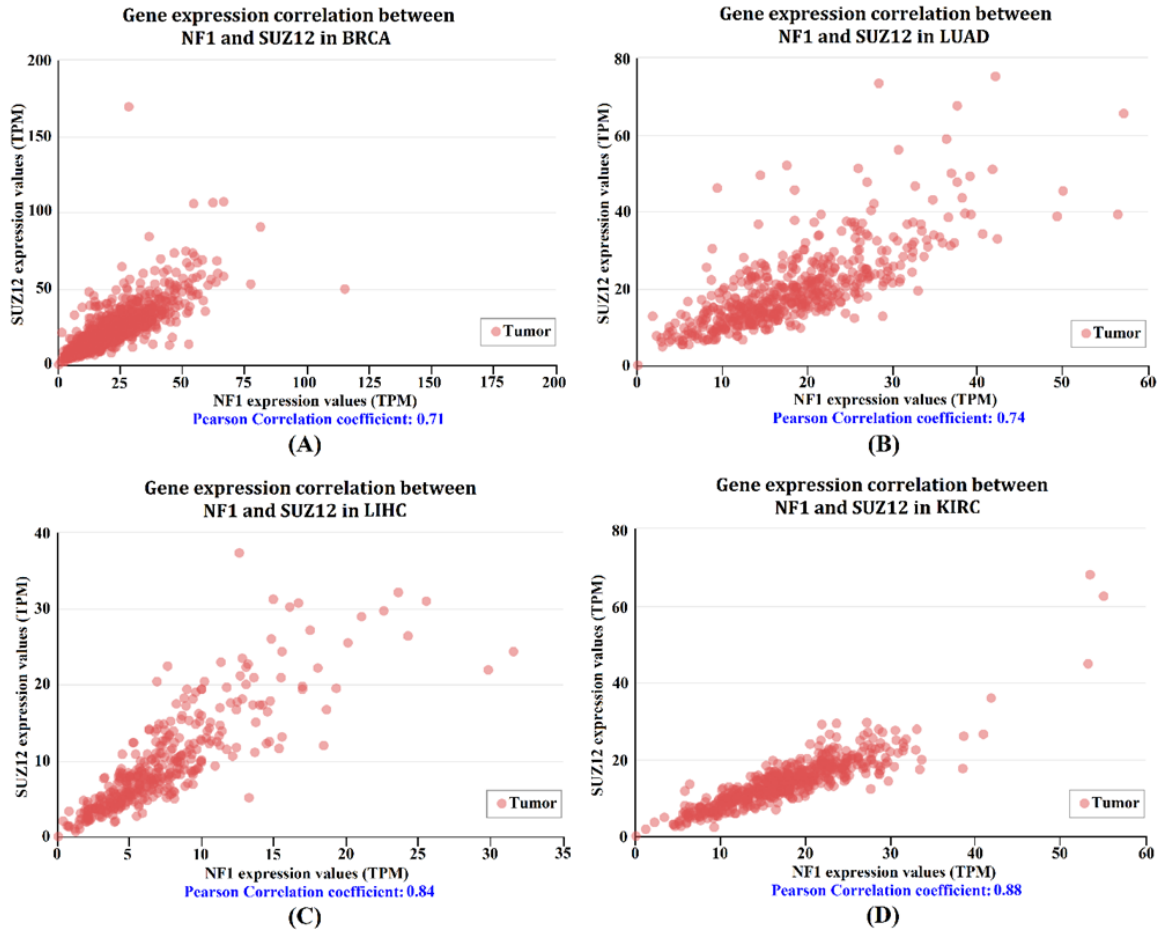

Figure 7: The correlation between co-expression profiling of NF1 in (A) BRCA, (B) LUAD, (C) LIHC, (D) KIRC.

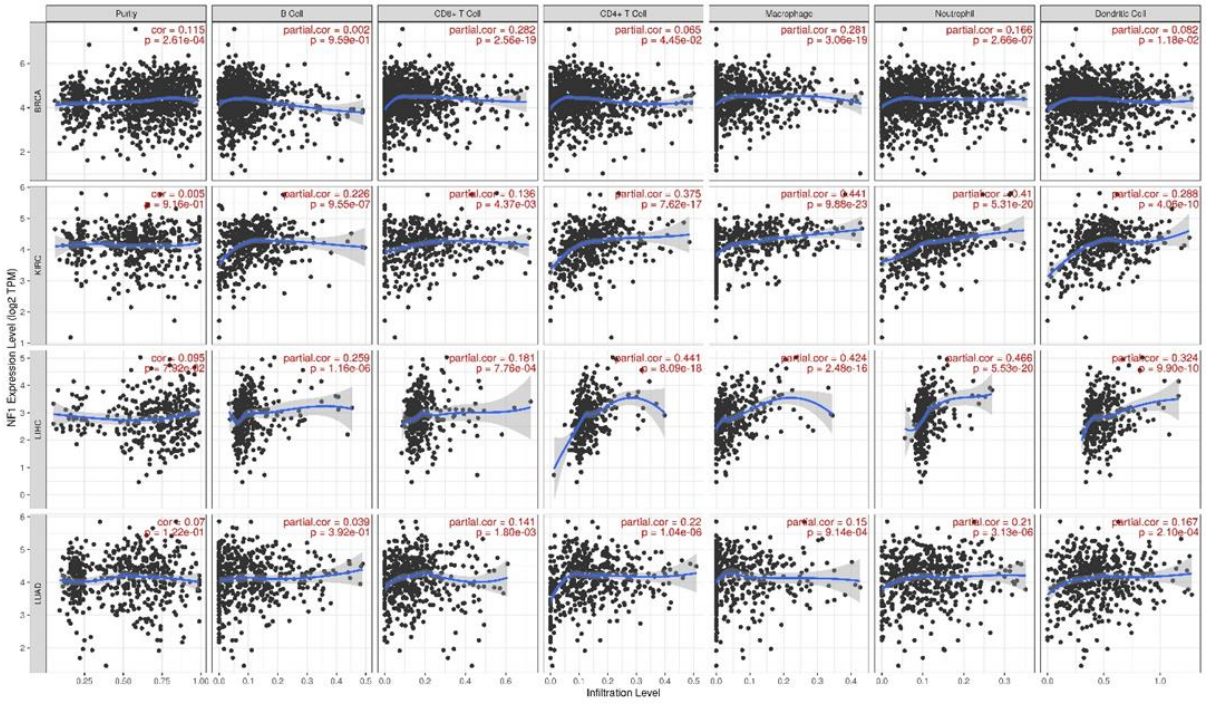

Figure 8: Correlation of the NF1 gene family with immune infiltration levels in different types of cancer cells. Significant infiltration levels were observed for B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells.

3.7. Network Pharmacology

Protein-protein networks were explored through the GENEMANIA tool. SOX4, SDC3, SDC2, DYNC1H1, TOP3A, POUF1, CAV1, FOXA2, AKT1, NRAS, HRAS, NOSIP, RAF1, CALM1, TIRP, GADD45A, EPHA2, MAP2K3, ALAS1, and HTR6 contributed to forming the protein networks of NF1. The protein networks were built through physical interactions, co-expression, prediction, co-localization, genetic interactions, pathways, and shared protein domains, which contributed 77.64%, 8.01%, 5.37%, 3.63%, 2.87%, 1.88%, and 0.60% of the total network, respectively **(Figure 9)**.

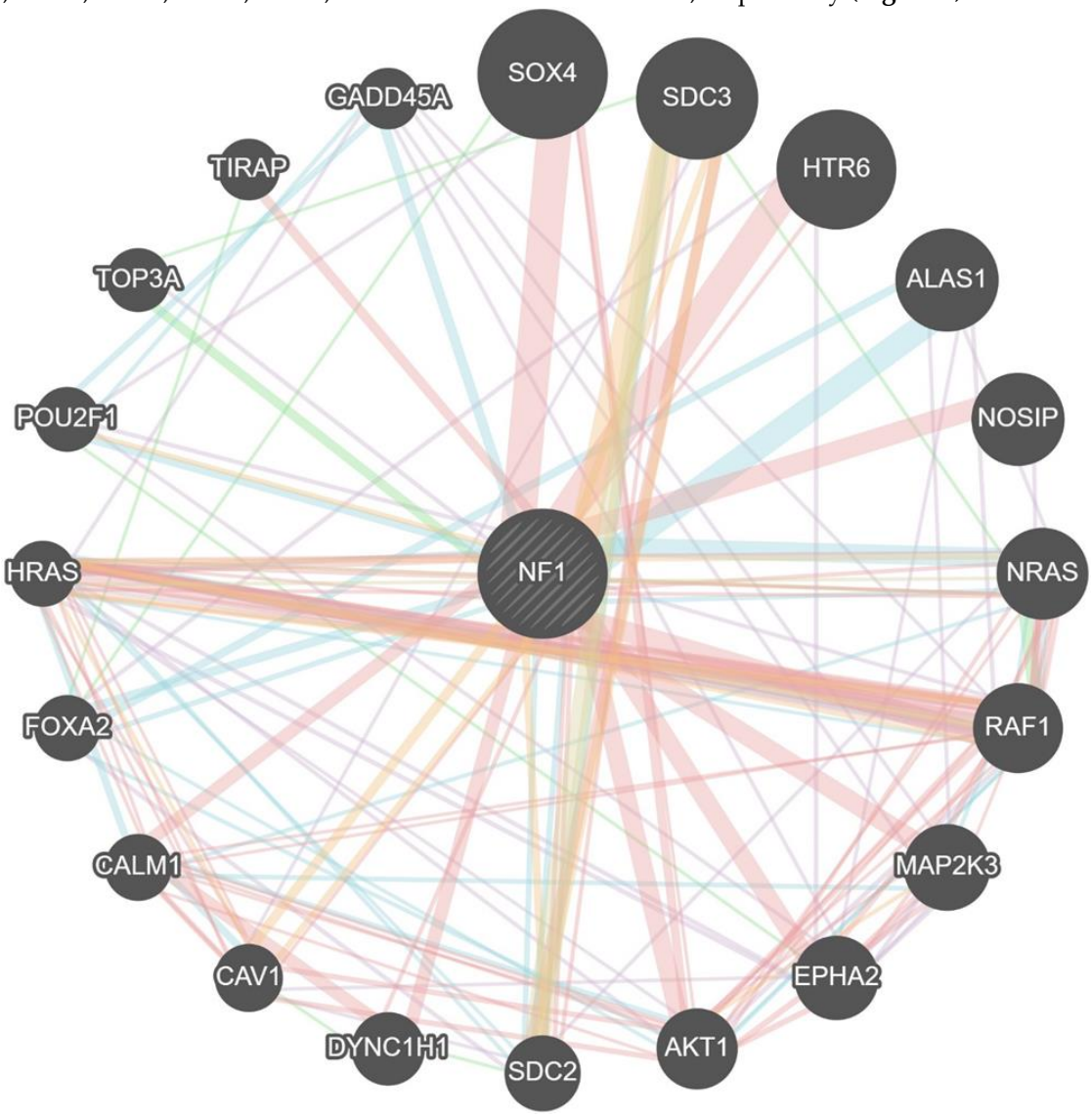

Figure 9: The protein-protein interaction analysis of NF1 protein with other family based on co-expression, genetic interactions, pathways, physical interactions, colocalization, predicted shared protein.

3.8. Pathway and gene ontologies of NF1

Pathway analysis was conducted in Enricher to understand the molecular role of the NF1 gene in the human body. The KEGG pathways revealed that the NF1 gene primarily associated with lysine degradation, ubiquitin-mediated proteolysis, axon guidance, leukocyte transendothelial migration, and signaling pathways regulating pluripotency **(Figure 10A)**. The Panther database **(Figure 10B)** revealed a more significant role in Hedgehog signaling, insulin pathways, and protein kinase B signaling for NF1. The GO biological process **(Figure 10C)** revealed the role of NF1 in the regulation of microtube-based process, negative regulation of microtube depolymerization, and negative regulation of protein depolymerization. Any abnormal changes of these cellular pathways might be responsible for tumorigenesis. The GO molecular function **(Figure 10D)** revealed microtubule plus-end binding, tau protein kinase activity and ubiquitin protein transferase activity. The GO cellular component analysis **(Figure 10E)** revealed the role of NF1 in the microtubule cytoskeleton, polymeric cytoskeleton fiber, and nucleus.

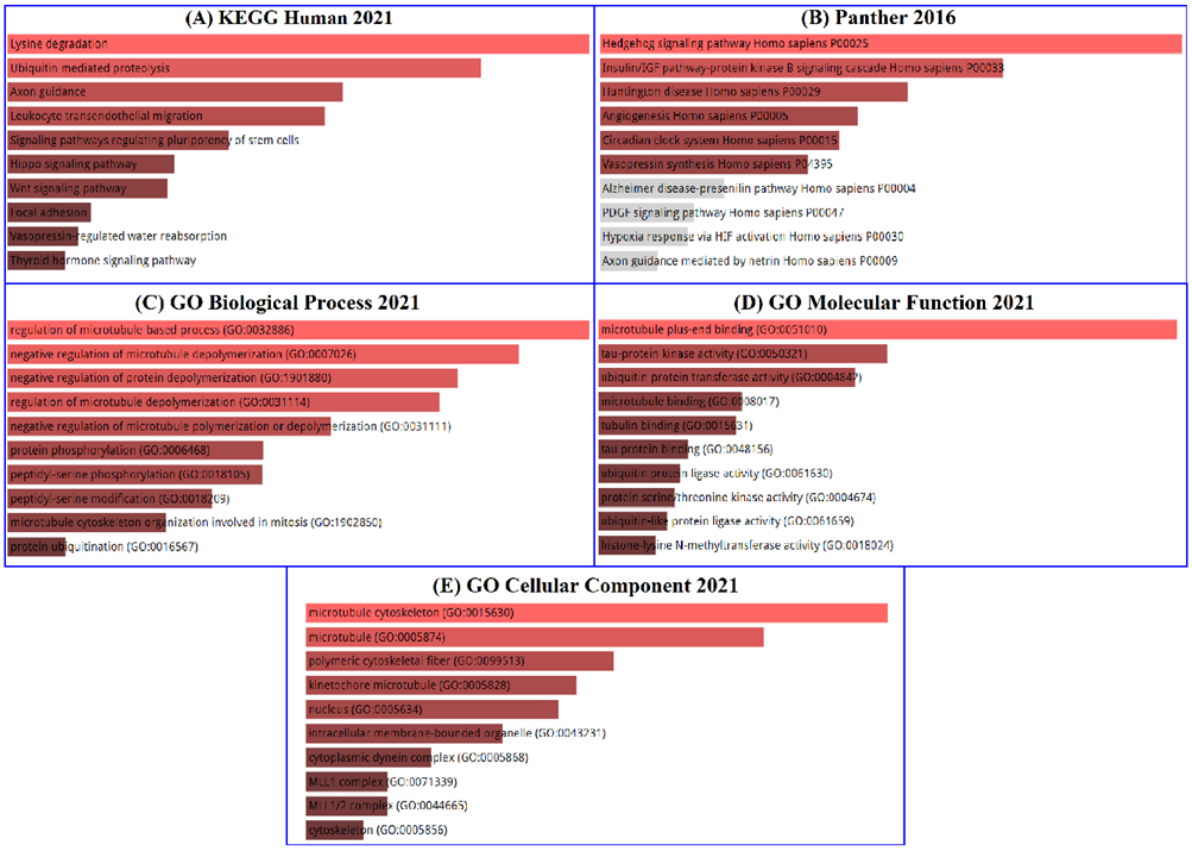

Figure 10: Signaling analysis and pathways of NF1 gene with positive correlation of BRCA, LUAd, KIRC, LIHC in (A) KEGG human pathways, (B) Panther 2016, (C) Go biological process 2021, (D) go molecular function 2021, (E) go cellular component 2021.

4. Discussion

It is reported specifically that the GTPase-activating protein-related domain negatively regulates RAS by generating inactive RAS-GDP from active RAS-GTP to inhibit downstream RAS signaling, referring to the complex role of NF1 gene-encoded neurofibromin 1 in NF1 pathogenesis. Additionally, upstream regulation of neurofibromin 1 incorporates the tyrosine kinase cKIT receptor, the tyrosine kinase anaplastic lymphoma kinase (ALK) receptor, endothelin receptor B (EDNRB), and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor, whereas downstream regulation incorporates several signaling pathways including the RAS/MAPK signaling pathway, PI3K/mTOR signaling pathway, and cAMP signaling pathway, related to cell cycle succession. Therefore, somatic mutations in the NF1 gene favoring dysregulation of the mentioned signals increase the risk of sporadic cancers in humans [10], [19], [46], [47].

Several usually expressed genetic modifications regarding NSCLC interact with the MAPK signaling network. The MAPK signal is detected in ALK, ROS proto-oncogene receptor tyrosine kinase 1 (ROS1), NF1 in tandem with epidermal growth factor receptor (EGFR), Kiras 2 Kirsten rat sarcoma viral oncogene homolog (KRAS), B-Raf proto-oncogene (BRAF), and serine/threonine kinase, confirming their apparent connection in the MAPK-oriented (EGFR/RAS/BRAF/MEK/ERK) pathway for presumable lung cancer [26], [48], [49], [50], [51], [52], [53]. Likewise, NF1-encoded neurofibromin functions as the GTPase-activating protein stimulating RAS oncogene inactivation, and LOH in the NF1 gene recognized in tumor tissue confirms the role of LOH in NF1 gene-related breast cancer progression along with BRCA1 gene interaction [54], [55], [56]. Additionally, as an onco-suppressor gene, NF1 negatively regulates the RAS signaling pathway, resulting in aberrant activation; hence, the gene is omnipresent regarding human hepatocarcinogenesis. Upregulation of NF1 expression in several tumors with a reduction in a meager HCC subset in conjunction with the downregulation of DAB2IP as well as the ubiquitous occurrence of RASAL1 mRNAs indicates that the lack of NF1, in tandem with RASAL1 and DAB2IP inactivation, induces unconstrained activation concerning wild-type RAS in human HCC, with 12.5%, 21.6%, and 28.4% LOH at NF1, DAB2IP, in tandem with RASAL1 loci sequentially, which induces liver cancer through promoter hypermethylation or

LOH [27], [28], [57]. In addition, the VHL gene targets hypoxia-inducible factor 1a (HIF1a) that controls the transcription of vascular endothelial growth factor (VEGF), glucose transporter 1 (GLUT1), platelet-derived growth factor (PDGF), transforming growth factor (TGF), and erythropoietin, which is probably significant regarding cancer cells. Therefore, the collapse of either the mentioned VHL gene or NF1-induced mTOR pathway to regulate HIF1a can occur via the enhanced transcription of cancer-related genes, causing clear cell renal carcinoma [29], [58], [59].

The pan cancer analysis of the NF1 gene has been performed along with the expression pattern of this gene in different cancer types, namely BRCA, LUAD, LIHC, and KIRC, as well as progession and development of cancer due to upregulation or downregulation of the targeted gene. ULCAN, GEPIA2, and GENT2 were utilized to explore the gene expression level along with cross validation with multiple sources. Our study suggests that lower expression and promoter methylation are found in BRCA, LUAD, LIHC, and KIRC through multiple tools compared to normal tissue, which aligns with the literature regarding the signaling role NF1 gene as both copies of NF1 were found to be inactivated in malignant tumors of patients [60], [61], [62].

We also analyzed the differences in the protein expression level of NF1 in different cancer cells from the Human Protein Atlas project database. NF1 protein levels were high and strong in intensity in normal tissue and breast, liver, and kidney cancer types but moderate and medium intensity in lung tissues. Therefore, network pharmacology, pathways, and gene ontology analysis suggest the involvement of the NF1 gene in cellular signaling mechanisms, where any disruptions lead to cancer development [10].

5. Conclusion

To understand the plausible mRNA expression, prognosis, and mutational effects on the development of different types of cancer regarding NF1, we combined multiple bioinformatics approaches to explore the role of NF1 in cancer development. This study revealed that lower expressions of NF1 were observed specially in BRCA and KIRC. The lower expression of NF1 may be responsible for the higher mutation trend in NF1. These expression patterns of the NF1 gene in cancer cells impact cancer progression across different ethnicities, gender, and cancer stages along with methylation patterns. Additionally, network pharmacology and NF1-related pathway analysis demonstrates its signaling role as well as tumor progression due to the abnormal signaling of NF1. Therefore, the immune infiltrations in BRCA, LUAD, KIRC, and LIHC were also explored for six different cell types. Overall, the findings of this study will further assist researchers to assess invasive mechanisms, tumor development, and therapeutic drug discovery for cancer patients.

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References

[1] A. Sudhakar, "History of Cancer, Ancient and Modern Treatment Methods," *J. Cancer Sci. Ther.*, vol. 01, no. 02, pp. i–iv, 2009, doi: 10.4172/1948-5956.100000e2.

[2] R. L. Siegel, K. D. Miller, H. E. Fuchs, and A. Jemal, "Cancer Statistics, 2021," *CA. Cancer J. Clin.*, vol. 71, no. 1, pp. 7–33, 2021, doi: 10.3322/caac.21654.

[3] H. Sung *et al.*, "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries," *CA. Cancer J. Clin.*, vol. 71, no. 3, pp. 209–249, 2021, doi: 10.3322/caac.21660.

[4] M. Shipitsin and K. Polyak, "The cancer stem cell hypothesis: In search of definitions, markers, and relevance," *Lab. Investig.*, vol. 88, no. 5, pp. 459–463, 2008, doi: 10.1038/labinvest.2008.14.

[5] C. Mattiuzzi and G. Lippi, "Current cancer epidemiology," *J. Epidemiol. Glob. Health*, vol. 9, no. 4, pp. 217–222, 2019, doi: 10.2991/jegh.k.191008.001.

[6] W. E. Gutteridge, "Existing chemotherapy and its limitations," *Br. Med. Bull.*, vol. 41, no. 2, pp. 162–168, 1985, doi: 10.1093/oxfordjournals.bmb.a072044.

[7] M. M. Gottesman, "Mechanisms of cancer drug resistance," *Annu. Rev. Med.*, vol. 53, pp. 615–627, 2002, doi: 10.1146/annurev.med.53.082901.103929.

[8] R. M. Cawthon *et al.*, "A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations," *Cell*, vol. 62, no. 1, pp. 193–201, 1990, doi: 10.1016/0092-8674(90)90253-B.

[9] Y. Li *et al.*, "Genomic organization of the neurofibromatosis 1 gene (NF1)," *Genomics*, vol. 25, no. 1, pp. 9–18, 1995, doi: 10.1016/0888-7543(95)80104-T.
[10] M. Kiuru and K. J. Busam, "The NF1 gene in tumor syndromes and melanoma," *Lab. Investig.*, vol. 97, no. 2, pp. 146–157, 2017, doi: 10.1038/labinvest.2016.142.
[11] G. Xu *et al.*, "The catalytic domain of the neurofibromatosis type 1 gene product stimulates ras GTPase and complements ira mutants of S. cerevisiae," *Cell*, vol. 63, no. 4, pp. 835–841, 1990, doi: 10.1016/0092-8674(90)90149-9.
[12] M. Bergoug, M. Doudeau, F. Godin, C. Mosrin, B. Vallée, and H. Bénédetti, "Neurofibromin Structure, Functions and Regulation," *Cells*, vol. 9, no. 11, pp. 1–33, 2020, doi: 10.3390/cells9112365.
[13] N. Ratner and S. J. Miller, "A RASopathy gene commonly mutated in cancer: The neurofibromatosis type 1 tumour suppressor," *Nat. Rev. Cancer*, vol. 15, no. 5, pp. 290–301, 2015, doi: 10.1038/nrc3911.
[14] L. Ding *et al.*, "Somatic mutations affect key pathways in lung adenocarcinoma," *Nature*, vol. 455, no. 7216, pp. 1069–1075, 2008, doi: 10.1038/nature07423.
[15] T. Cancer and G. Atlas, "Integrated Genomic Analyses of Ovarian Carcinoma The Cancer Genome Atlas Research Network," *Nature*, vol. 474, no. 7353, pp. 609–615, 2011, doi: 10.1038/nature10166.Integrated.
[16] F. Meric-Bernstam *et al.*, "Concordance of genomic alterations between primary and recurrent breast cancer," *Mol. Cancer Ther.*, vol. 13, no. 5, pp. 1382–1389, 2014, doi: 10.1158/1535-7163.MCT-13-0482.
[17] J. P. Landry *et al.*, "Comparison of Cancer Prevalence in Patients with Neurofibromatosis Type 1 at an Academic Cancer Center vs in the General Population from 1985 to 2020," *JAMA Netw. Open*, vol. 4, no. 3, pp. 1–15, 2021, doi: 10.1001/jamanetworkopen.2021.0945.
[18] R. J. Sullivan, "The role of mitogen-activated protein targeting in melanoma beyond BRAFV600.," *Curr. Opin. Oncol.*, vol. 28, no. 2, pp. 185–191, Mar. 2016, doi: 10.1097/CCO.0000000000000271.
[19] L. Larribère and J. Utikal, "Multiple roles of NF1 in the melanocyte lineage," *Pigment Cell Melanoma Res.*, vol. 29, no. 4, pp. 417–425, 2016, doi: 10.1111/pcmr.12488.
[20] N. M. Warrington *et al.*, "The cyclic AMP pathway is a sex-specific modifier of glioma risk in type I neurofibromatosis patients," *Cancer Res.*, vol. 75, no. 1, pp. 16–21, 2015, doi: 10.1158/0008-5472.CAN-14-1891.
[21] E. Pasmant *et al.*, "SPRED1 disorder and predisposition to leukemia in children," *Blood*, vol. 114, no. 5, p. 1131, 2009, doi: 10.1182/blood-2009-04-218503.
[22] E. Cerami *et al.*, "The cBio Cancer Genomics Portal: An open platform for exploring multidimensional cancer genomics data," *Cancer Discov.*, vol. 2, no. 5, pp. 401–404, 2012, doi: 10.1158/2159-8290.CD-12-0095.
[23] B. Campos *et al.*, "Germline mutations in NF1 and BRCA1 in a family with neurofibromatosis type 1 and early-onset breast cancer," *Breast Cancer Res. Treat.*, vol. 139, no. 2, pp. 597–602, 2013, doi: 10.1007/s10549-013-2538-6.
[24] Y. Murayama *et al.*, "T1 breast cancer associated with von Recklinghausen's neurofibromatosis," *Breast Cancer*, vol. 6, no. 3, pp. 227–230, 1999, doi: 10.1007/BF02967173.
[25] M. Nakamura, A. Tangoku, H. Kusanagi, M. Oka, and T. Suzuki, "Breast cancer associated with Recklinghausen's disease: report of a case.," *Nihon Geka Hokan.*, vol. 67, no. 1, pp. 3–9, Mar. 1998.
[26] A. J. Redig *et al.*, "Clinical and molecular characteristics of NF1-mutant lung cancer," *Clin. Cancer Res.*, vol. 22, no. 13, pp. 3148–3156, 2016, doi: 10.1158/1078-0432.CCR-15-2377.
[27] G. Bollag *et al.*, "Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells," *Nat. Genet.*, vol. 12, no. 2, pp. 144–148, 1996, doi: 10.1038/ng0296-144.
[28] D. F. Calvisi *et al.*, "Inactivation of Ras GTPase-activating proteins promotes unrestrained activity of wild-type Ras in human liver cancer," *J. Hepatol.*, vol. 54, no. 2, pp. 311–319, 2011, doi: 10.1016/j.jhep.2010.06.036.
[29] W. M. Linehan, M. M. Walther, and B. Zbar, "The genetic basis of cancer of the kidney," *J. Urol.*, vol. 170, no. 6 I, pp. 2163–2172, 2003, doi: 10.1097/01.ju.0000096060.92397.ed.
[30] C. M. Johannessen *et al.*, "TORC1 Is Essential for NF1-Associated Malignancies," *Curr. Biol.*, vol. 18, no. 1, pp. 56–62, 2008, doi: 10.1016/j.cub.2007.11.066.
[31] M. Tyers and R. Rottapel, "VHL: A very hip ligase," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 96, no. 22, pp. 12230–12232, 1999, doi: 10.1073/pnas.96.22.12230.
[32] M. Ohh *et al.*, "Ubiquitination of HIF requires direct binding to the beta-domain of the vHL protein," *Nat Cell Biol*, vol. 2, no. 7, pp. 423–427, 2000.
[33] C. C. Hudson *et al.*, "Regulation of Hypoxia-Inducible Factor 1α Expression and Function by the Mammalian Target of Rapamycin," *Mol. Cell. Biol.*, vol. 22, no. 20, pp. 7004–7014, 2002, doi: 10.1128/mcb.22.20.7004-7014.2002.

[34] T. Zefang, K. Boxi, L. Chenwei, C. Tianxiang, and Z. Zemin, "GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis," *Nucleic Acids Res.*, no. W1, p. W1, 2019.
[35] S. J. Park, B. H. Yoon, S. K. Kim, and S. Y. Kim, "GENT2: An updated gene expression database for normal and tumor tissues," *BMC Med. Genomics*, 2019, doi: 10.1186/s12920-019-0514-7.
[36] D. S. Chandrashekar *et al.*, "UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses," *Neoplasia (United States)*, vol. 19, no. 8, pp. 649–658, 2017, doi: 10.1016/j.neo.2017.05.002.
[37] A. Digre and C. Lindskog, "The Human Protein Atlas—Spatial localization of the human proteome in health and disease," *Protein Sci.*, 2021, doi: 10.1002/pro.3987.
[38] K. Klonowska *et al.*, "Oncogenomic portals for the visualization and analysis of genome-wide cancer data," *Oncotarget*, 2016, doi: 10.18632/ONCOTARGET.6128.
[39] P. Wu *et al.*, "Integration and analysis of CPTAC proteomics data in the context of cancer genomics in the CBIOPortal," *Mol. Cell. Proteomics*, 2019, doi: 10.1074/mcp.TIR119.001673.
[40] J. Gao *et al.*, "Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal," *Sci. Signal.*, vol. 6, no. 269, 2013, doi: 10.1126/scisignal.2004088.
[41] T. Li *et al.*, "TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells," *Cancer Res.*, vol. 77, no. 21, pp. e108–e110, 2017, doi: 10.1158/0008-5472.CAN-17-0307.
[42] D. Warde-Farley *et al.*, "The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function," *Nucleic Acids Res.*, 2010, doi: 10.1093/nar/gkq537.
[43] K. Zuberi *et al.*, "GeneMANIA prediction server 2013 update.," *Nucleic Acids Res.*, 2013, doi: 10.1093/nar/gkt533.
[44] J. Montojo *et al.*, "GeneMANIA cytoscape plugin: Fast gene function predictions on the desktop," *Bioinformatics*, 2010, doi: 10.1093/bioinformatics/btq562.
[45] E. Y. Chen *et al.*, "Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool," *BMC Bioinformatics*, vol. 14, 2013, doi: 10.1186/1471-2105-14-128.
[46] A. Klose *et al.*, "Selective disactivation of neurofibromin GAP activity in neurofibromatosis type 1 (NF1)," *Hum. Mol. Genet.*, vol. 7, no. 8, pp. 1261–1268, 1998, doi: 10.1093/hmg/7.8.1261.
[47] C. P. Kratz, S. Rapisuwon, H. Reed, H. Hasle, and P. S. Rosenberg, "Cancer in Noonan, Costello, cardiofaciocutaneous and LEOPARD syndromes," *Am. J. Med. Genet. Part C Semin. Med. Genet.*, vol. 157, no. 2, pp. 83–89, 2011, doi: 10.1002/ajmg.c.30300.
[48] L. C. Young *et al.*, "Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer," *Cancer Res.*, vol. 68, no. 13, pp. 4971–4976, 2008, doi: 10.1158/0008-5472.CAN-07-6158.
[49] K. Rikova *et al.*, "Global Survey of Phosphotyrosine Signaling Identifies Oncogenic Kinases in Lung Cancer," *Cell*, vol. 131, no. 6, pp. 1190–1203, 2007, doi: 10.1016/j.cell.2007.11.025.
[50] G. V Scagliotti, G. Selvaggi, S. Novello, and F. R. Hirsch, "The biology of epidermal growth factor receptor in lung cancer.," *Clin. cancer Res. an Off. J. Am. Assoc. Cancer Res.*, vol. 10, no. 12 Pt 2, pp. 4227s-4232s, Jun. 2004, doi: 10.1158/1078-0432.CCR-040007.
[51] P. H. . J. B. M. . S. C. T. et al Gary, "The New England Journal of Medicine Downloaded from nejm.org on April 1, 2015. For personal use only. No other uses without permission. Copyright © 1990 Massachusetts Medical Society. All rights reserved.," *New English J. Med.*, vol. 323, no. 16, pp. 1120–1123, 1990.
[52] A. Leonetti *et al.*, *BRAF in non-small cell lung cancer (NSCLC): Pickaxing another brick in the wall*, vol. 66. Elsevier Ltd, 2018. doi: 10.1016/j.ctrv.2018.04.006.
[53] L. Bowman *et al.*, "Clinical Characteristics, Treatments, and Concurrent Mutations in Non–Small Cell Lung Cancer Patients With NF1 Mutations," *Clin. Lung Cancer*, vol. 22, no. 1, pp. 32-41.e1, 2021, doi: 10.1016/j.cllc.2020.09.011.
[54] Ş. Güran and M. Safali, "A case of neurofibromatosis and breast cancer: Loss of heterozygosity of NF1 in breast cancer," *Cancer Genet. Cytogenet.*, vol. 156, no. 1, pp. 86–88, 2005, doi: 10.1016/j.cancergencyto.2004.04.019.
[55] H. Ogata, H. Sato, J. Takatsuka, and L. M. De Luca, "Human breast cancer MDA-MB-231 cells fail to express the neurofibromin protein, lack its type I mRNA isoform and show accumulation of P-MAPK and activated Ras," *Cancer Lett.*, vol. 172, no. 2, pp. 159–164, 2001, doi: 10.1016/S0304-3835(01)00648-6.
[56] K. M. Shannon *et al.*, "Loss of the normal NF1 allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders.," *N. Engl. J. Med.*, vol. 330, no. 9, pp. 597–601, Mar. 1994, doi: 10.1056/NEJM199403033300903.

[57] A. Bernards and J. Settleman, "GAPs in growth factor signalling," *Growth Factors*, vol. 23, no. 2, pp. 143–149, 2005, doi: 10.1080/08977190500130480.
[58] F. Latif *et al.*, "Identification of the von Hippel-Lindau disease tumor suppressor gene," *Science (80-.).*, vol. 260, no. 5112, pp. 1317–1320, 1993, doi: 10.1126/science.8493574.
[59] J. R. Gnarra *et al.*, "Mutations of the VHL tumour suppressor gene in renal carcinoma," *Nat. Genet.*, vol. 7, no. 1, pp. 85–90, 1994, doi: 10.1038/ng0594-85.
[60] I. Eisenbarth, K. Beyer, W. Krone, and G. Assum, "Toward a survey of somatic mutation of the NF1 gene in benign neurofibromas of patients with neurofibromatosis type 1," *Am. J. Hum. Genet.*, vol. 66, no. 2, pp. 393–401, 2000, doi: 10.1086/302747.
[61] S. A. Rasmussen *et al.*, "Chromosome 17 loss-of-heterozygosity studies in benign and malignant tumors in neurofibromatosis type 1," *Genes, Chromosom. Cancer*, vol. 28, no. 4, pp. 425–431, 2000, doi: 10.1002/1098-2264(200008)28:4<425::aid-gcc8>3.3.co;2-5.
[62] A. M. John, M. Ruggieri, R. Ferner, and M. Upadhyaya, "A search for evidence of somatic mutations in the NF1 gene," *J. Med. Genet.*, vol. 37, no. 1, pp. 44–49, 2000, doi: 10.1136/jmg.37.1.44.