Pan-Cancer Analysis of the Carcinogenic Effect of Nuclear Respiratory Factor-1 (NRF1) in Human Tumors

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Abstract

Despite there are many cellular or animal experiments that support a link between NRF1 and cancer, there is currently no pan-cancer analysis available. Therefore, the source of data is TCGA and GEO, we explored the potential role of NRF1 in 33 kinds of tumors. The expression of NRF1 is significantly increased in most tumors, and the expression of NRF1 is correlated to the prognosis of tumor patients. We discovered that the expression level of NRF1 in PAAD was correlated to immune infiltration. The methylation of NRF1 increased significantly in KIRC, PAAD, ACC. In addition, RNA metabolic pathway or cell biology-related functions participate in the functional mechanism of NRF1.Our research provides a comprehensive explanation of the pathogenic effects of NRF1 in related tumors.

Highlights

1. A first pan-cancer analysis of NRF1.
2. NRF1 is differentially associated with the prognosis of different tumor cases.
3. The link between NRF1 and CD8+ T-cell or cancer-associated fibroblast infiltration.
4. An enhanced methylation level in several tumors, such as PAAD.

1. Introduce

The occurrence of cancer is very complicated. It is very significant to carry out pan-cancer analysis of genes and estimate the connection between their clinical prognosis and possible mechanisms. The functional genomics data of different cancers can be found in TCGA project and GEO database [1-4], which provide us with data sources and the methods for pan-cancer analysis

NRF1 (Nuclear respiratory factor 1) is contained in the cap'n'collar (CNC) basic-region leucine zipper (bZIP) transcription factors family, it exists in different species from marine bacteria to humans [5-7]. The structure and physiological functions of NRF1 have been analyzed from a physiological and clinical perspective [8-10]. Some studies have found a link between NRF1 and breast cancer [11] and brain cancer [12] tumors. In this work, the current evidence based on cell or animal experiments that show the connection between NRF1 and diverse cancers was summarized by us. Based on a large amount of clinical data, but no scholars have studied the evidence of pan-cancer to show the connection between NRF1 and different types.

In our study, the TCGA project and GEO database were used for the first time to carry out a pan-cancer analyses of NRF1. To study the conceivable principle of NRF1 in the occurrence or clinical prognosis of diverse tumors from the aspects of gene expression level, survival curve, DNA methylation status, genetic alterations, immune infiltration and related cellular pathways

2. Materials And Methods

2.1 Gene expressions analyses

We entered NRF1 in the "Gene_DE" module of the TIMER2 website (http://timer.cistrome.org/), and become conscious that NRF1 of different tumors between the tumor and normal tissues specific tumor subtypes that express differences of TCGA items. For some tumors with no normal tissues or highly limited normal tissues (such as TCGA-DLBC (Lymphoid Neoplasm Diffuse Large B-cell Lymphoma), TCGA-PAAD (Pancreatic adenocarcinoma), etc.), we use GEPIA2 Web server (http://gepia2.cancer-pku.cn/#analysis) [13] obtains the box plot (genotype tissue expression) database of expression differences between tumor tissues and data were derived from normal tissue expression of GTEx (Genotype-Tissue Expression) data pool, after setting the P cut-off value=0.01, log2FC (fold change) cut-off value=1 and "matching TCGA normal value and GTEx data". In addition, we acquired a violin chart of NRF1 expression level in all TCGA tumors in different disease phases (stage I, stage II, stage III and stage IV) through the "Stage Plot" module of HEPIA2. Apply log2[TPM (per million transcripts) +1] transformed expression data to box plots or violin plots.

The UALCAN website (http://ualcan.path.uab.Edu/Analysis- Port. Html) is an interconnected network resource that can analyze cancer Omics data. It allows us to carry out CPTAC (Clinical Proteomic Tumor Analysis Association) protein expression analyses [14].

2.2 Survival prognosis analyses

The "Survival Analysis" module of GEPIA2 can analyze the survival curve of diverse cancers in the TCGA which was used to get information about the OS (overall survival) and DFS (disease-free survival) map of NRF1. We used the high cutoff value (50%) and the low cutoff value (50%) as separating the expression threshold of high expression population and low expression population.The logarithmic rank test was used to test, and finally GEPIA2 generated the survival prognosis map of all kinds of cancer.

2.3 Genetic information analyses

The cBioPortal website (https://www.cbioportal.org/) [15, 16] can analyze genetic changes. We click "TCGA Pan-Cancer atlas Study" in the quick selection, then search the NRF1 gene and obtain the genetic variation characteristics of NRF1. The genetic variation characteristics of all cancers from TCGA can be found in "Cancer Type Summary" module, including the change frequency, mutation type and CNA (copy number change). The "Comparison" module helps us acquire information on differences in overall, disease-free, progression-free, and disease-free survival whether the gene occurred variation. Kaplan-Meier diagrams with logarithmic rank P value were also generated.

2.4 DNA methylation analyses

The TCGA module of the UALCAN website provides us with the expression of methylation levels of genes in different cancers. The beta value represents the DNA methylation level, ranging from 0 (unmethylated) to 1 (fully methylated). Different lower limits of beta values are considered to be high methylation (beta: 0.7-0.50) or low methylation (beta: 0.3-0.25).

2.5 Immune infiltration analyses

The Timer2.0 website provides a module that can be used to analyze the correlation between NRF1 expression levels and immunoinvasion in diverse tumors in the TCGA database. This function is available in the Gene options in Immune Mode. We selected "T cell CD8+" and "cancer associated fibroblast". The immune infiltration was assessed by TIMER, CiberSort, CiberSort-ABS, QuanTISEQ, XCell, MCPCOUNTER and EPIC arithmetics. Through the Spelman rank correlation test after purity adjustment, the P value and some correlated (COR) values were acquired. The datum is visualized in the form of heating maps and scatter maps.

2.6 Enrichment analysis of NRF1 related genes

First, we select human species among the types of organisms on the string website(https://string-db.org/), and use a single protein search to search for NRF1. Then, the hereinafter parameters were set by us: the minimum required interaction score ["low confidence (0.150)"], the meaning of the edge of the network ("evidence"), the maximum number of interaction objects to be displayed ("no more than 50 interaction objects in the first shell"), and the interreaction source ("experiment"). At last, available NRF1 binding proteins were obtained.

The GEPIA2's "Similar Gene Detection" module can analyze the genes associated with the genes of interest. The date source is TCGA, we acquired the top 100 NRF1-related target genes.Then, we used the module "Correlation Analysis" in GEPIA2 to analyze the person correlation between NRF1 and the selected genes.

The interactive Venn diagram viewer Jvenn [17] was used for intersection analysis to compare genes that bind and interact with NFN1. In addition, we united this information for KEGG (Kyoto encyclopedia of genes and genomes) pathway analysis. In summary, we input these genes to David (Database for annotation, visualization, and integrated discovery), the "official_gene_symbol" and "Homo sapiens" were selected, and acquired the information of the function annotation map. Finally, Hiplot (https://hiplot.com.cn/basic) [18] was used for enrichment analysis. We used the "GO/KEGG analysis" in the advanced module to enrich and visualize the obtained genes, selected the latest KEGG database and the human species, and corrected them with BH ($P < 0.05$).

3. Results

3.1 The analysis of gene expression

We aim to investigate the carcinogenicity of anthropic NRF1 in this research. First, we analyze the expression of NRF1 in diverse cells and non-tumor tissues. We searched the HPA (The Human Protein Atlas) date (https://www.proteinatlas.org/) for the expression of NRF1 RNA-seq in various tissues (Figure S1). The results showed that NRF1 was expressed to varying degrees in 27 organs and tissues, among which the expression of the ovary was the highest, followed by lymph node and endometrium.

We used TIMER2 to analyze the expression level of NRF1 in diverse tumors in the TCGA. Presented in Figure 1A, the expression of NRF1 in the tumor of BRCA (Breast invasive carcinoma), CHOL (Cholangiocarcinoma), ESCA (Esophageal carcinoma), HNSC (Head and Neck squamous cell carcinoma), KICH (Kidney Chromophobe), LIHC (Liver hepatocellular carcinoma), STAD(Stomach adenocarcinoma), THCA (Thyroid carcinoma), UCEC (Uterine Corpus Endometrial Carcinoma) ($P<0.001$), PRAD (Prostate adenocarcinoma), SKCM (Skin Cutaneous Melanoma) ($P<0.01$), COAD (Colon adenocarcinoma) and LUAD (Lung Adenocarcinoma) ($P<0.05$) are significantly higher than that in diseaseless organization and cells.

After adding normal tissues and cells from GTEx data as controls, we assessed the variation in the expression of NRF1 between diseaseless and neoplastic organization of DLBC (Bladder Urothelial Carcinoma), THYM (Thymoma) and PAAD (Pancreatic adenocarcinoma). However, for other tumors, for example ACC (Adrenocortical Carcinoma) and LAML (Acute Myeloid Leukemia) or LGG (Brain Lower Grade Glioma), we did not get a significant difference.The results of CPTAC database showed that the total protein of NRF1 in RCC, UCEC, colon cancer has significant difference ($P<0.001$).

Hepia2's "pathological phase" module provides us with a method of observing the connection between the expression of NRF1 and the pathological phase of cancer, including ACC, KICH, LIHC, OV (Ovarian Serous Cystadenocarcinoma), PPAD, and SKCM have statistical difference ($P<0.05$), but others.

3.2 Survival analyses data

According to the expression level of NRF1, tumor cases were separated into high expression group and low expression group, and the relation between NRF1 expression and prognosis of different tumor patients was studied by using TCGA and GEO data sets severally. As take on Figure 2A, in the TCGA project, the high expression of NRF1 mRNA in ACC ($P=0.014$) is closely related to the poor prognosis of MESO ($P=0.025$) tumor. According to the data of disease-free survival analysis (Figure 2B), high expression of NRF1 in TCGA with ACC ($P=0.028$), LICH ($P=0.0092$) and PRAD ($P=0.016$) is related to poor prognosis. In addition, low expression of NRF1 is correlated to cacoethic OS prognosis of KIRC ($P=0.039$) and PAAD ($P=0.0061$) (Figure 2A) and DFS prognosis of KIRC ($P=0.039$) and PAAD ($P=0.042$) (Figure 2B)

3.3 Genetic variation analyses data

We observe the changes of NRF1 gene in diverse tumor in TCGA queues. As showed in Figure 3A, the highest mutation frequency of Stomach Adenocarcinoma occurs in Stomach Adenocarcinoma patients with "mutation" as the primary, with a mutation frequency of 7.69%. In Ovarian Epithelial Tumor, it is mainly "amplification" type, and the change frequency is about 5%. It is worth mentioning that many cancers relate to gene deletions. Esophageal Squamous Cell Carcinoma is mainly caused by deletions, and the frequency of deletions is about 1%. Figure 3B shows that missense is the main type of genetic change in NRF1 gene. Colon Adenocarcinoma,Signet Ring Cell Carcinoma of the Stomach and Head and Neck Squamous Cell Carcinoma each of them have 2 cases have gene missense in E409k. In addition, we also groped for the potential correlation between NRF1 gene changes and clinical survival in patients with different types of cancer. Figure 3C shows that compared with patients without NRF1 changes, UCEC

patients with NRF1 changes had better progression-free (P =0.0297), but poor overall prognosis (P=0.0163), disease-specific survival (P =0.210), and disease-free survival (P =0.093).

3.4 The analysis of DNA methylation data

We studied the potential relationship between NRF1 methylation and different tumor pathogenesis through TCGA project. The results showed that UCEC (P<0.05), LUSC, ESCA, SARC, KIRC, and PAAD (P<0.001), among these cancers, the methylation degree of DNA was significantly increased except for UCEC (Figure 4), but they all had beta values below 0.3, which is hypomethylated. The effect of methylation on expression of NRF1 proteins needs further analysis to explore the role of NRF1 in tumorigenesis.

3.5 Immune infiltration analysis data

As an important part of tumor microenvironment, tumor infiltrating immune cells is closely correlated to the genesis, progression or metastasis of tumors [19]. It is reported that tumor-associated fibroblasts in tumor microenvironment matrix are involved in regulating the function of a variety of tumors infiltrating immune cells [20]. Here, we use TIMER, CiberSort, CiberSort-ABS, QuanTISEQ, XCell, MCPCOUNTER, and EPIC arithmetics to study the latent correlation between different levels of immune cell infiltration and NRF1 gene expression in different tumor in TCGA. Through the analysis of the date, we discovered a negative relationship between the immune infiltration of CD8+T cells and the expression of NRF1 in LGG and a positive relationship in PAAD, STAD, THYM, and so on. (Figure S2). Moreover, we discovered a positive relationship between NRF1 expression and tumor-associated fibroblast infiltration in most TCGA tumors (Figure 5), such as the ACC, CESC, COAD, ESCA, HNSC, KICH, PAAD and UCEC tumors. The scatter plot data of the above tumors generated by the algorithm are exhibited in Figure 5. For instance, according to the MCPCOUNTER arithmetic, the expression of NRF1 in PAAD is positively related to the invasion of cancer-associated fibroblasts.

3.7 Enrichment analyses of NRF1-related partners

We tried to sort out proteins and genes which is related to NRF1, and carried out pathway enrichment analysis to explore the mechanism of NRF1 gene in tumor genesis.We used the string website to acquired 50 experimental evidence-supported NRF1 binding proteins. The network of interactions between these 50 proteins and NRF1 is shown in Figure 6A. The first 100 genes related to NRF1 were acquired by using GEPIA2 website. As presented in Figure 6B, the expression level of NRF1 was positively related with the expression of EP400 (E1A binding protein p400) (r=0.71), ERCC3 (excision repair cross-complementation group 3) (r=0.71), SAFB (scaffold attachment factor B) (r=0.70), SART3 (squamous cell carcinoma antigen recognized by T cells 3) (r= 0.71) and SP4 (Sp4 transcription factor)). The corresponding heat map data also show that in most detailed cancer types, NRF1 is positively correlated with the above five genes (Figure 6C). A cross-analysis of the above two groups shows that there is a common member, SP4 (Figure 6D). We carried out KEGG and GO enrichment analysis of the screened genes and proteins. GO enrichment analysis exhibited the connection to RNA metabolic pathway or cell biology, such as acting on mRNA processing, RNA splicing, via transesterification reactions with bulged, histone modification and

so on (Figure 6E). The KEGG data of Figure 6F show that the role of NRF1 in tumor pathogenesis may be related to "Wnt signaling pathway " and "Hippo signaling pathway ".

4. Discussion

NRF1 is expressed in many tissues and cells. NRF1 plays an important or even indispensable role in regulating different target gene subsets, which are related to antioxidation, disintoxication, oxidoreanabolism, protesomal degradation, adaptive cytoprotection and other physiological and pathological responses to different cellular stress [21–23]. Some literatures have proved the functional relationship between NRF1 and clinical diseases, particularly cancers. Whether NRF1 participates in the pathogenesis of diverse cancers remains to be proved. We have not found any literature on pan-cancer analysis of NRF1 from the overall tumor point of view. Therefore, data support is provided in TCGA, CPTAC and GEO databases, as well as the molecular characteristics of gene expression, gene change and DNA methylation, we detected the NRF1 gene in 33 diverse cancers.

NRF1 is high-expressed in vast majority of cancers. However, NRF1 gene survival and prognosis analysis data suggest different conclusions for different tumors. In the prognosis and survival analysis of TCGA-ACC disease, the survival status of the high-expression group (n=38) was significantly lower than that of the low-expression group (P<0.01, n=37). In the disease-free prognosis, the survival status of the same low-expression group was better (P< 0.0016). In the analysis of disease stages, the expression of NRF1 was increased to different degrees in the different disease stages. Surprisingly, the expression of NRF1 in patients was lower than that in normal control group, but the difference was not statistically significant (P>0.05), which may be due to the lack of data. In the genetic analysis of ACC, "amplification" (2.2%, n=91) was the main mutation type, and the effect of this mutation on ACC and downstream proteins remains to be further studied. Studies have shown that activate Wnt/β-catenin and delete p53 synergically induce adrenal cortical carcinoma in mice [24], and studies have shown that NRF1 has inhibitory effect on Wnt/β-catenin signal transduction in mouse liver [25]. However, the role of NRF1 in regulating of Wnt/β-catenin activation and p53 in adrenal cortical carcinoma remains to be further studied.

In the prognostic survival analysis of TCGA-PAAD disease, the survival status of the high-expression group (n=89) was significantly lower than that of the low-expression group (P<0.0006, n=89). In the disease-free prognosis, the survival status of the same low-expression group was better (P< 0.042). In the TCGA + GTEx data, the expression of NRF1 in PAAD was higher than in normal team (P<0.05). Based on Kaplan-Meier plotter datasets uniting GEO datasets, we found that high expression of NRF1 was bound up with overall prognostic survival (P=0.0024, n=177) and disease-free survival (P=0.037, n=177) (Figure S3) results. This suggests that the expression of NRF1 in PAAD disease may involve in the occurrence and progression of the disease. The expression of NRF1 in patients was different from that in normal team (P<0.05, n=178). In the Cibersort-ABS algorithm, CD8+ immunoinfiltration of T cells showed a positive relationship between NRF1 expression and infiltration (Figure S2). At the same time, the expression of NRF1 was positively related with the immune infiltration of T cells CD4+, B cells, Tregs cells,

NK cells and DCs (Figure S4), which may be because the over expression of NRF1 enhances the expression of TCF7 (Transcriptionfactor7) (Figure S5a). TCF7 is a transcriptional activator necessary for the survival of immature CD4(+), CD8(+) thymocytes [26]. This suggests that the expression of NRF1 is corelated to cellular immunity. The expression of LEF1 (Lymphoid enhancer-binding factor1) interacting with NRF1 also increases (Figure S5b), and LEF1 has a variety of functions in adjusting T cell merisis and differentiation, which is crucial in maintaining the inhibition function of Treg and preventing loss of self-tolerance [27. Meanwhile, LEF1 is a key mediator of Wnt signal transduction [28], indicating that NRF1 enhances Wnt signal transduction by increasing the expression of LEF1. Studies have found that inhibition of Wnt signal can block the proliferation of adenocarcinoma cells in vitro and induce cell apoptosis [29]. These results suggest that the over expression of NRF1 protein to promote cellular immunity, and has a certain therapeutic effect on PAAD. Conversely, the over expression of NRF1 may also enhance Wnt signal transduction and promote the proliferation of PAAD. In general, the high expression of NRF1 can increase the differentiation of immune cells and promote the survival of PAAD patients. On the contrary, it can promote the spread of PAAD through the WNT signaling pathway.

In the prognostic analysis of KIRC, the survival status of the high-expression group (n=258) was significantly higher than that of the low-expression group (P<0.04, n=257). In the disease-free prognosis, the survival status of the same low-expression group was better (P< 0.03,n=258). This suggests that KIRC may be involved in the expression of NRF1. However, the expression of NRF1 was increased in the patients with KIRC which used the normal group as control team, but no marked difference was found (P>0.05). Nrf1 can activate Wnt signal pathway [30]. The function of Wnt signaling pathway in KIRC remains to be further studied.

In conclusion, our first pan-cancer analysis of NRF1 shows that NRF1 expression is statistically associated with clinical prognosis, DNA methylation, immune cell infiltration, and tumor mutation in some tumors. This conduce to comprehend the role of NRF1 in tumorigenesis from the point of view of clinical tumor specimens.

Declarations

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Authors' contributions

YJ, ZW and HK designed the study. YJ, YC, YS, CY and JR conducted the Software, Formal analysis and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of dataand materials

The datasets used and analyzed during the current study are available from the corresponding author upon any reasonable request

Ethics approval and consent to participate

This study was carried out according to the good clinical practice and the Declaration of Helsinki. All patients signed an informed written consent detailing the investigational nature of the trial.

Consent for publication

All authors of the manuscript have read and agreed to its content,and comform that the article is original, has not already been published in a journal, and is not currently under consideration by another journal

Competing interests

We have no conflicts of interest to declare.

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Figures

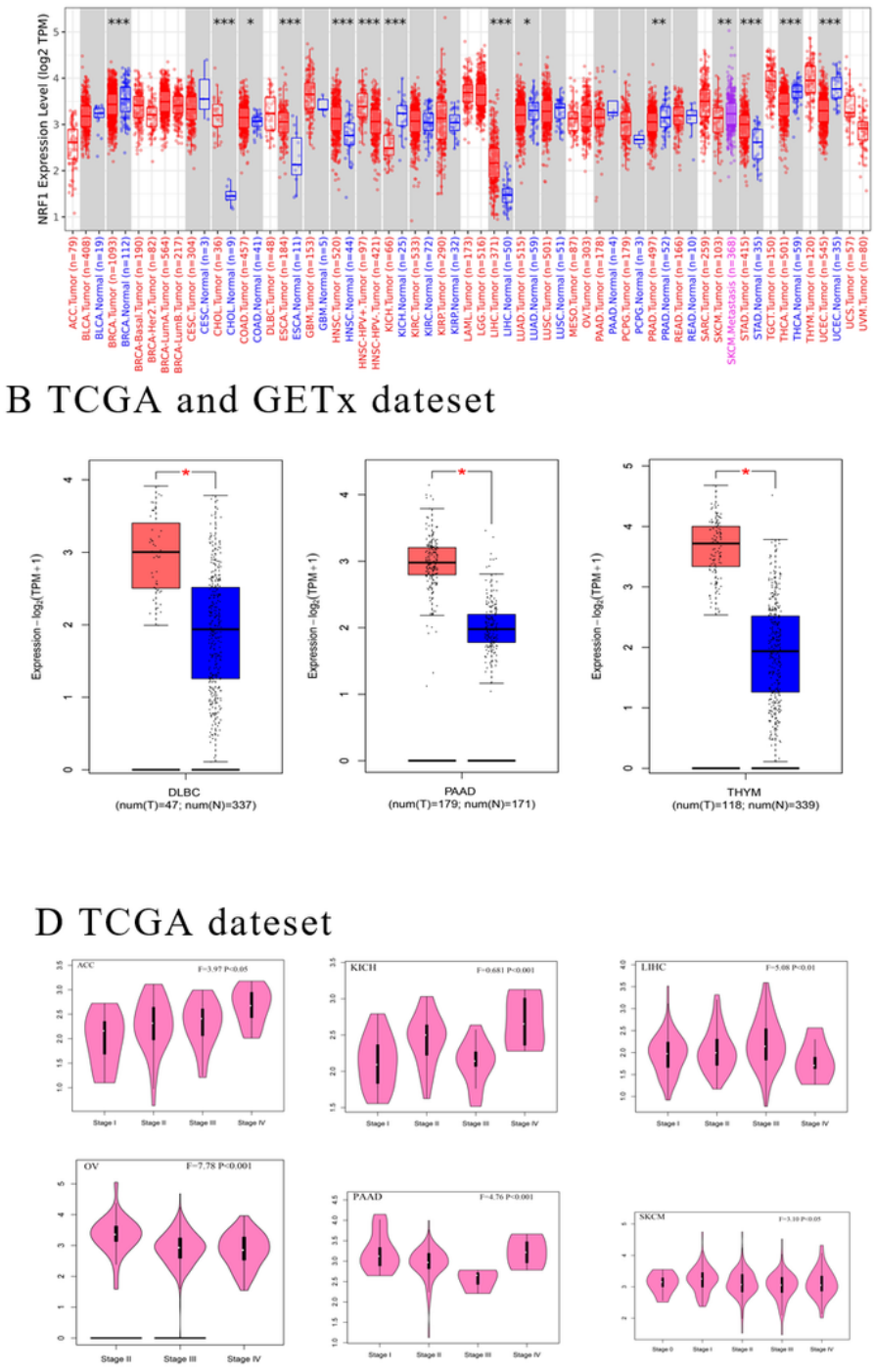

Figure 1

Expression of NRF1 gene in diverse cancers and morbid phases. (A) TIMER2 was used to analyze the expression of NRF1 gene in diverse cancers. * P<0.05; ** P<0.01; *** P<0.001. (B) The DLBC, PAAD and THYM in the TCGA are compared with the homologous normal tissues in the GTEx dataset, and a block diagram is drawn. ** P <0.01. (C) The expression of NRF1 protein in RCC, colon cancer and UCEC were compared with normal tissues using CPTAC datasets. *** P<0.001. (D) We used the TCGA dateset to

comparing NRF1's expression in morbid phases（phase I、phase II、phase III、phase IIV） of ACC、KICH、LICH、OV、PAAD and SKCM. Adopt Log2(TPM+1) as log-scale.

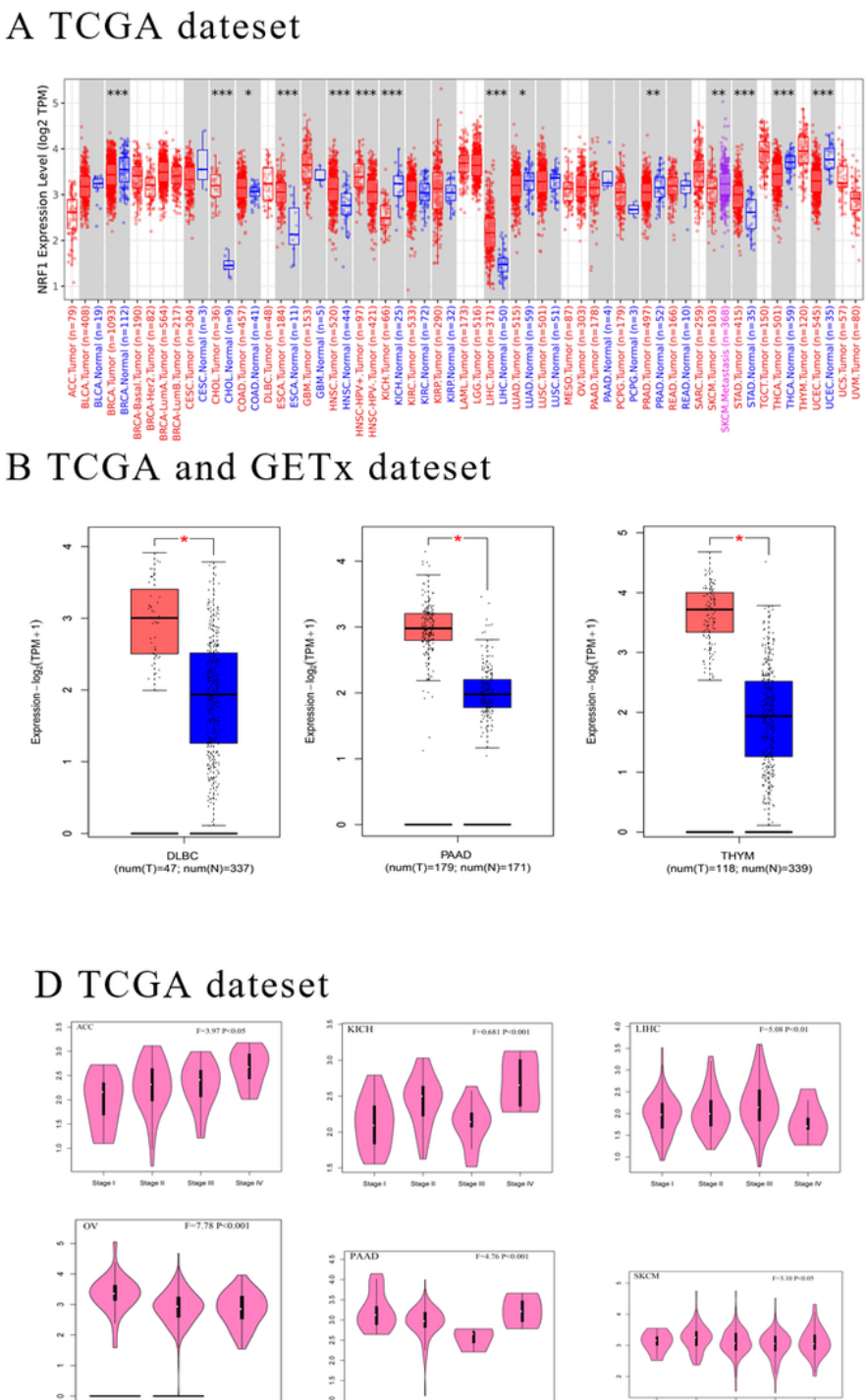

Figure 2

Rrelationship between NRF1 expression and survival prognosis of tumors in TCGA. The GEPIA2 website was used to analyze overall survival (A) and disease-free survival (B) of NRF1 expression in diverse cancers in TCGA.

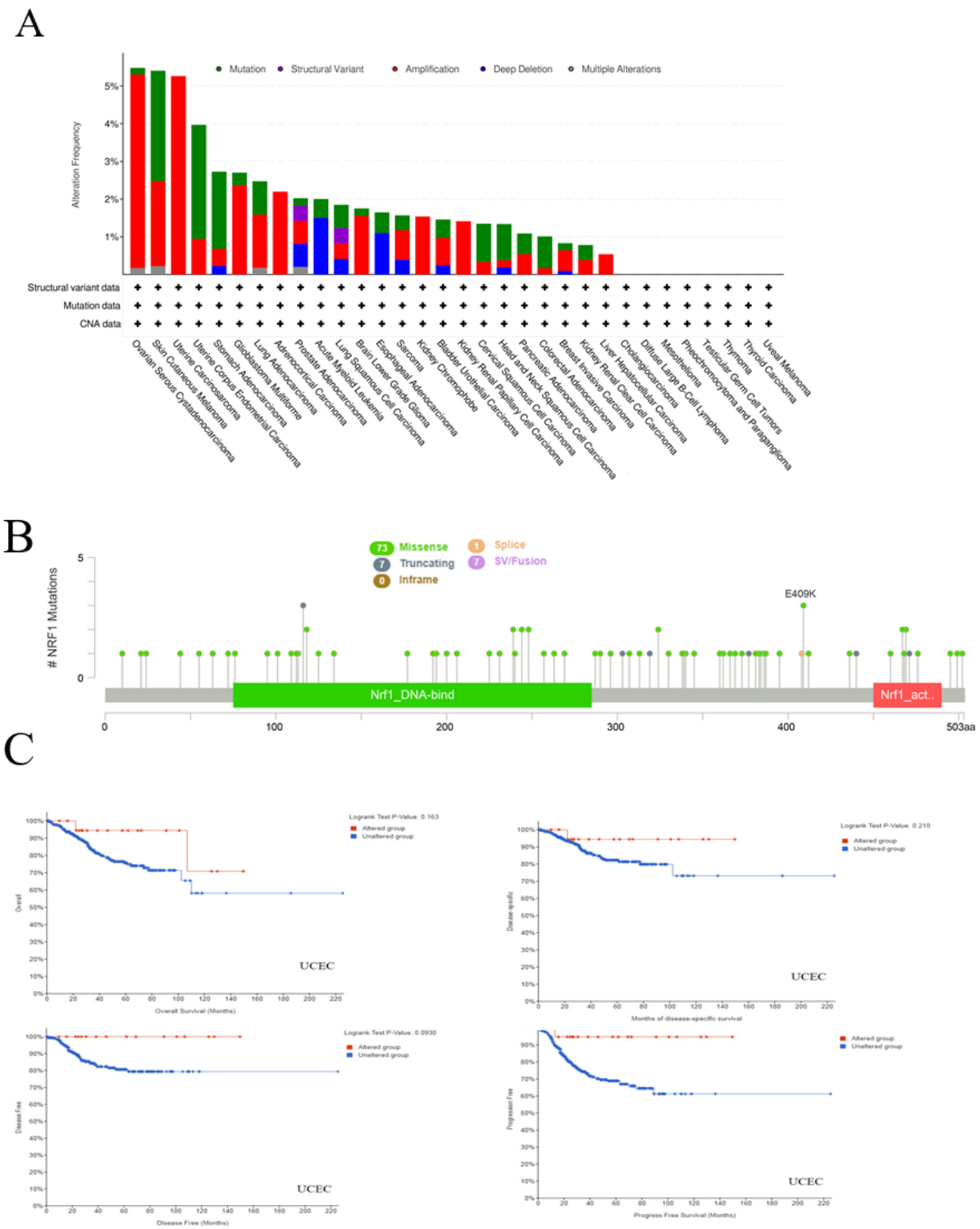

Figure 3

Mutation characteristic of NRF1 in diverse cancers of TCGA. The cBioPortal website was used to analyze the mutation characteristic of NRF1 in TCGA cancers . The frequency of change in mutation type (A) and mutation site (B) was shown. The Cbioportal site was used by us to analyze the relationship between mutations and survival status, among which UCEC had some significant differences(C).

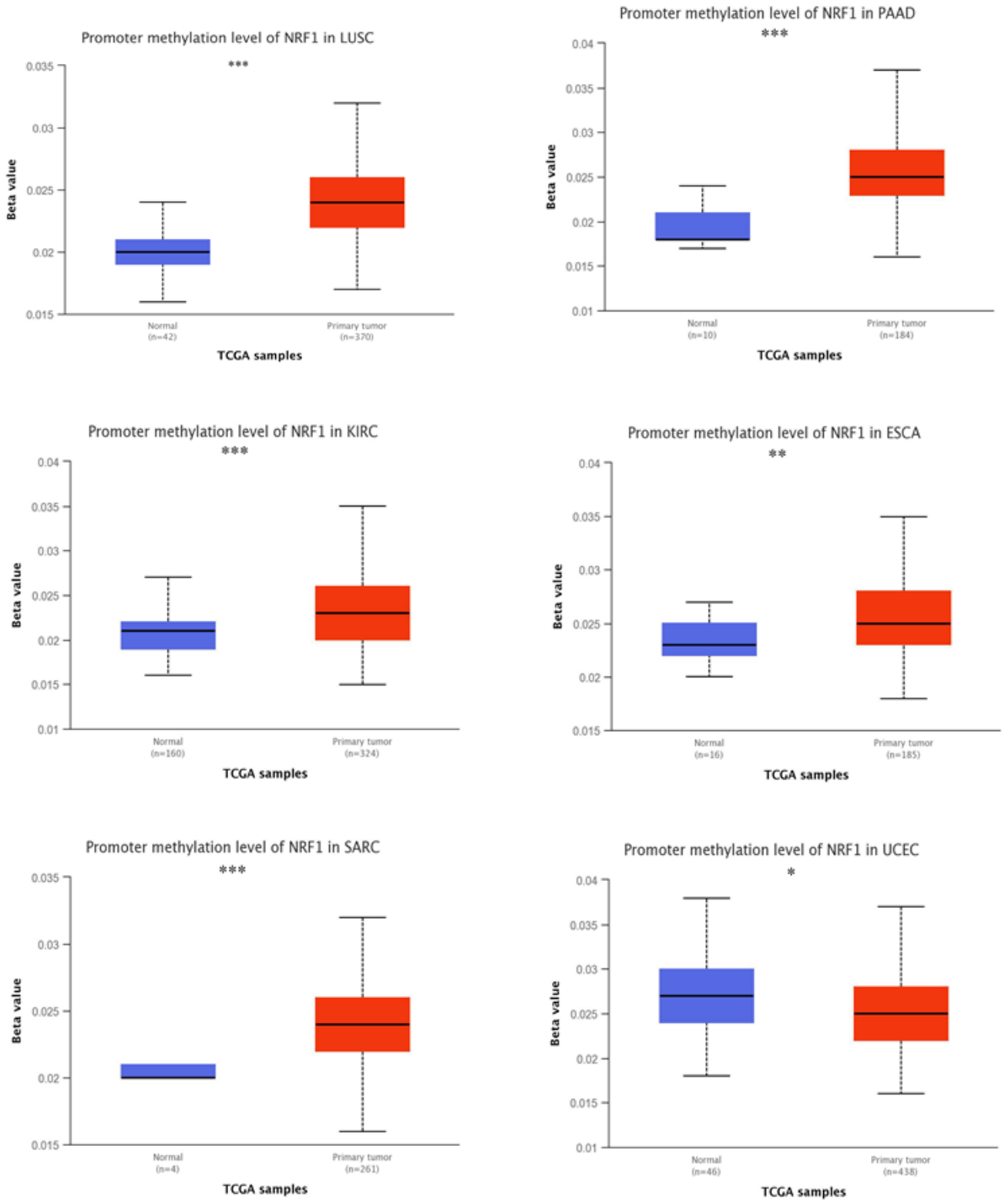

Figure 4

The expression status of the NRF1 methylation in different cancers were analyzed by UALCAN portal. * P<0.05; ** P<0.01; *** P<0.001.

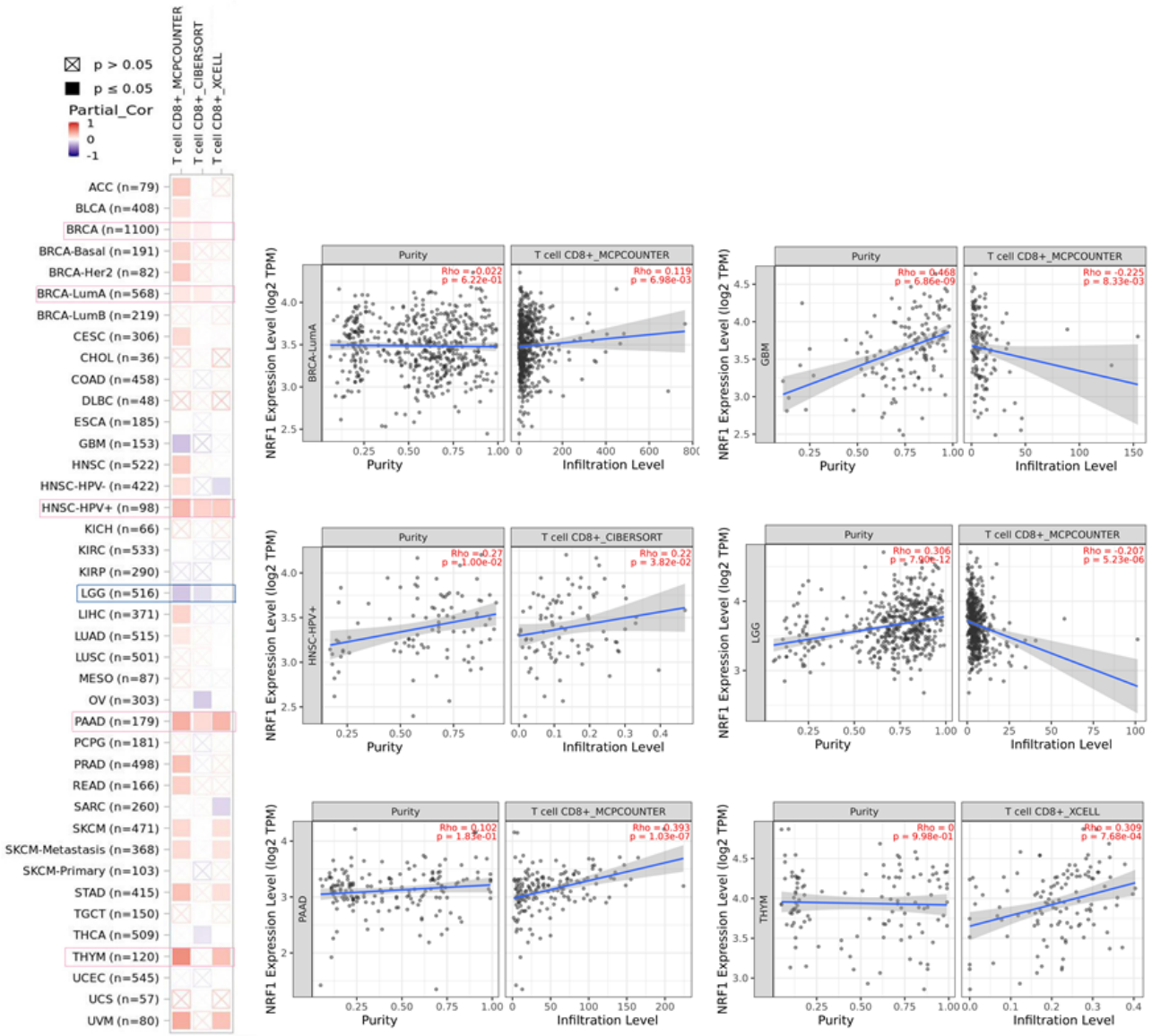

Figure 5

The annysis of the relationship between NRF1 expression and tumor associated fibroblast immune infiltration. The relationship between NRF1 gene expression and cancer-related fibroblast infiltration in TCGA's cancers was assayed by diverse arithmetics

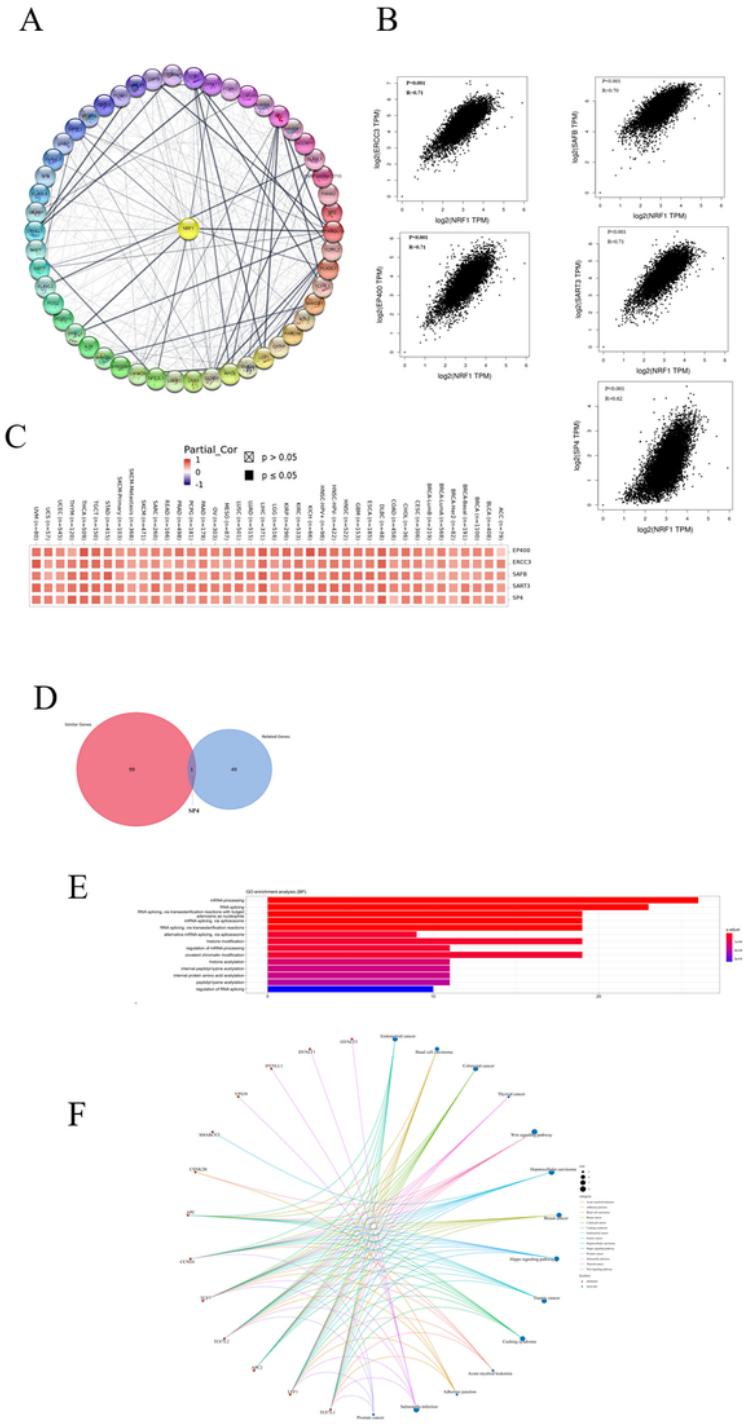

Figure 6

NRF1-related gene enrichment analysis. (A) The protein binding to NRF1 determined by the ascertained was collected through the String website. (B) The first 100 genes related to NRF1 in TCGA project were gleaned through GEPIA2 website, and the expression relationship between NRF1 and picked target genes EP400, ERCC3, SAFB, SART3 and SP4 was analyzed. (C) The selected genes and the heat map data associated with different cancers. (D) Cross analysis of NRF1 binding and related genes. NRF1 binding

and interacting genes were used for molecular functional enrichmentin in GO analysis(E) and KEGG pathway analysis(F).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.docx
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