TRAIP functions as an oncogene in hepatocellular carcinoma via Rb/EZH2/p21 signaling pathway

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Research

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Abstract

Background The TRAF-interacting protein (TRAIP) has been identified as a master regulator of DNA damage and implicated in the progression of human cancers. Yet the underlying mechanism of TRAIP-mediated malignant phenotype remains unclear.

Methods The expression of TRAIP in hepatocellular carcinoma (HCC) was examined by qRT-PCR, western blot and immunohistochemistry. The clinical significance of TRAIP was determined by Kaplan-Meier survival analyses. The biological function and the underlying mechanism of TRAIP in HCC progression were investigated, using cellular and molecular biological experiments.

Results Here, we show that TRAIP is upregulated in HCC and functions as an oncogene via Rb/EZH2/p21 signaling. Overexpression of TRAIP, at both mRNA and protein levels, is correlated with more aggressive clinicopathological features, and unfavorable overall and disease-free survivals. *In vitro* experiments demonstrate that ectopic expression of TRAIP enhances, whereas knockdown of TRAIP attenuates HCC cell proliferation. Further data show that TRAIP interacts with SPAG5 in HCC cells, which results in the stabilization of TRAIP protein. TRAIP overexpression suppresses the expression of Rb, subsequently leading to the increase of EZH2 and decrease of p21. Re-expression of Rb and p21 significantly inhibits TRAIP-mediated cell growth.

Conclusion Collectively, our findings suggest TRAIP exert oncogenic activity and have prognostic and therapeutic potential in HCC.

1. Background

Hepatocellular carcinoma, representing approximate 80% of liver caners, is one of the leading causes for cancer-related death in many parts of the world [1, 2]. Rational approaches have been developed to prevent HCC and curb the overall mortality [3]. However, due to the majority of patients were diagnosed at an advanced clinical stage, especially in Eastern Asia and sub-Saharan Africa, cancer-specific mortality still continues to climb [3]. Substantial advances have been made to understand the detail mechanism of tumor transformation and HCC progression [4, 5]. Accumulating findings of factors contributing to the development of targeted drugs, systemic therapy for HCC patients has made great progress [4, 5]. Undoubtably, identification of potential prognostic and therapeutic markers is critical for decreasing the global burden of HCC.

Genomic instability, one of the key hallmarks of human cancer, is attributed to the defects in DNA damage response and replication stress. Those defects have been potentially exploited to develop effective cancer therapy and thereby improve patient outcomes [6]. Tumor necrosis factor receptor-associated factor (TRAF) interacting protein (TRAIP) has been recently identified as a master regulator of DNA damage [7, 8]. TRAIP interacts with PCNA and is involved in the regulation of cell proliferation and apoptosis [9–11], partly through its activity of E3 ubiquitin ligase [12]. Contradictory data have been reported for the role of TRAIP in human cancers. On one hand, TRAIP enhances DNA damage–induced

NEMO ubiquitination to suppress the myeloma cell proliferation [13]. TRAIP is upregulated by acetylation of mutp53^{R158G} to inhibit the activation of oncogenic NFκB signaling to induce cell apoptosis in lung cancer [14]. On the other hand, TRAIP works with Aurora A to modulate NFκB signaling to enhance the chemoresistance in Acute Myeloid Leukemia [15]. Knockdown of TRAIP in HCC led to cell apoptosis and G1/S phase arrest [16]. TRAIP is targeted by miR-4775 and promotes cell growth and migration in lung cancer [17]. These data may suggest the biological function of TRAIP affecting cancer progression depends on cellular content.

Using tissue microarray-based histochemistry assay and *in vitro* cell experiments, we intended to determine the expression and clinical significance of TRAIP in HCC, and to investigate the role of TRAIP in HCC cell proliferation and the underlying mechanism. Our data suggest TRAIP as an oncogene to promote HCC progression and a promising prognostic factor for the patients with HCC.

2. Materials And Methods

2.1 Patients

Clinical samples including 53 pairs of fresh tissues and 384 paraffin-embedded tissues were collected in Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China. Prospective collection of clinicopathological data of the 384 patients with HC were obtained. All patients in this study provided written informed consents and received no chemotherapy or radiotherapy before surgical resection. This study was approved by the SYSUCC Institute Research Ethics Committee. Gene Expression Omnibus (GEO), Oncomine, The Cancer Genome Atlas (TCGA) databases were used to confirmed the upregulation of TRAIP in HCC.

2.2 Cell culture and transfection

The HCC cell lines (LM3, MHCC97L, MHCC97H, PLC8024, HepG2, Huh7, Hep3B) were purchased from the Cell Resource Center, Chinese Academy of Science Committee (Shanghai, China), and cultured by Dulbecco's modified Eagle's medium (DMEM) (Gibco, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone, Logan, UT). HepG2 and Huh7 cells were transfected with overexpression vector encoding full-length human TRAIP cDNA or TRAIP shRNAs, using Lipofectamine 2000 (Invitrogen). pcDNA 3.2(+) vector and scramble shRNA were used as negative controls. Cells were then selected by 500 mg/L G418 for 14 days to construct stable cell lines. The targeted sequences for TRAIP shRNAs and EZH2 siRNA were as followings: TRAIP shRNA #1, 5'-UUACACCUCAGGCUGGUCCCG-3'; TRAIP shRNA #2, 5'-CCCAGCATGGTTACTACGAAA-3'; EZH2 siRNA, 5'-AGUCUCAUGUACGCTGACUCUG-3'.

2.3 Quantitative real-time polymerase chain reaction (qRT-PCR)

The mRNA expression level of TRAIP was determined by qRT-PCR, using a SYBR Green real-time assay. Total RNAs were extracted from cells using the Trizol reagent (Invitrogen, CA, USA) according to the manufacturer's instruction. One microgram of RNA sample was reverse transcribed using the Superscript III enzyme (Invitrogen, CA, USA) to obtain single-stranded cDNA. Real-time PCR was then performed on cDNA in an iQ Sybr Green Supermix (Bio-Rad) with gene-specific primers. The following primers were used: TRAIP, forward: 5'-TGCTGTCTCAAACCACTGAA-3' and reverse: 5'-CACTATCTGCTCCGACTTCTTC-3'; β-actin, forward: 5'-TGGCACCCAGCACAATGAA-3' and reverse: 5'-CTAAGTCATAGTCCGCCTAGAAGCA-3'.

2.4 Western blot

Expression of TRAIP, Rb, p21, EZH2, p53 and SPAG5 was determined by western blot, using the protocol as in our previous study [18]. The primary antibodies were listed as followings. TRAIP (1:500, #ab80170, Abcam), SPAG5 (1:500, Sigma-Aldrich), EZH2 (1:1000, #5246, Cell Signaling Technology), p21 (1:1000, #2947, Cell Signaling Technology), p53 (1:1000, #2524, Cell Signaling Technology), Rb (1:1000, #9309, Cell Signaling Technology), β-actin (1:1000, #4970, Cell Signaling Technology).

2.5 Immunohistochemistry (IHC)

IHC was used to examine the expression of TRAIP in HCC tissues. Paraffin embedded sections were dewaxed in xylene (3 × 5 min) and dehydrated in ethanol series (3 min in 100% ethanol, 1 min in each of 95% and 70% ethanol). Sections were washed in PBS and endogenous peroxidases were blocked with 3% H_2O_2 for 10 min. The tissue sections were subjected to antigen retrieval by pressured cooking in 10 mM citrate buffer for 3 min, and then incubated with serum blocking solution for 20 min to block nonspecific binding, followed by incubation with primary antibodies for 2 h at room temperature. After rinsing in PBS for 10 min, the sections were incubated with the biotinylated secondary antibody for 1 h and further incubation with the Streptavidin Biotin complex. Reactivity was developed in chromogen DAB (3,3-diaminobenzidine) solution. The signal was enhanced by applying the solution of $CuSO_4$ and NaCl for 5 min. Finally, the sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted. All sections were observed under light microscopy and the staining intensities were assessed by two independent pathologists (Yang YF and Cao Y). Nucleus staining was graded for intensity (0-negative, 1-weak, 2-moderate, and 3-strong) and percentage of positive cells [0, 1 (1–24%), 2 (25–49%), 3 (50–74%), and 4 (75–100%)] with discrepancies resolved by consensus. The H-scores for tumors with multiple cores were averaged. The median IHC score was used to define high FMNL1 expression and low FMNL1 expression groups.

2.6 Colony formation

Stable cells with TRAIP overexpression or knockdown were culture in 6-well plates at a density of 1.0×10^3 per well by medium plus G418 for 10 days. Colonies were fixed with methanol, stained with 0.1% crystal violet, pictured, and counted under a microscope.

2.7 5-Ethynyl-2'-deoxyuridine (EdU) staining

The effect of TRAIP on HepG2 and Huh7 cell proliferation was assessed by EdU staining, using the KeyFluor488 Click-iT EdU Imaging Kit (KeyGEN BioTECH, Nanjing, China).

2.8 Coimmunoprecipitation (co-IP)

Proteins were extracted by radioimmunoprecipitation assay buffer supplemented with proteinase inhibitor cocktail. Primary antibodies were added for 2.5 h. Protein A/G beads were added for an additional 2 h. Precipitated proteins were dissolved in SDS loading buffer and fractionated by SDS-PAGE.

2.9 Statistics

Difference of TRAIP expression in HCC and nontumorous tissues was revealed by Student's t-test. Data are mean and SEM from three independent *in vitro* experiments. Significance between groups was calculated by the Student's t-test. Survival analyses were conducted by KaplanMeier analyses (log-rank test). Differences were considered significant for *P*-values less than 0.05.

3. Results

3.1 TRAIP expression is increased in HCC tissues

The expression of TRAIP was dysregulated in human cancers. According to TCGA data, TRAIP mRNA was upregulated in most of the gastrointestinal cancers, including colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), liver hepatocellular carcinoma (LIHC), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), and stomach adenocarcinoma (STAD). The biggest difference was recorded in HCC (Supplementary Fig. 1). Therefore, we then examined the mRNA expression of TRAIP in 53 pairs of fresh HCC specimens. qRT-PCR results showed that TRAIP mRNA level in HCC was much higher than that in nontumorous tissues. In most of the individuals in SYSUCC cohort, TRAIP mRNA expression was upregulated (Fig. 1A). Similarly, the mRNA expression of TRAIP in HCC tissues of TCGA cohort was significantly increased, compared with the nontumorous tissues (Fig. 1B). Strikingly, the advanced tumor (higher tumor stage, higher pathological grade, tumor metastasis, higher serum AFP level), the higher level of TRAIP mRNA expression (Fig. 1C-F). The increase of TRAIP mRNA in HCC was also confirmed in other studies in Oncomine database (supplementary Fig. 2).

The protein expression of TRAIP was next examined to confirm the upregulation of TRAIP in HCC tissues. Nine pairs of clinical fresh specimens were used in western blot analyses to compare the expression of TRAIP protein in HCC and nontumorous tissues. In line with the expression pattern of mRNA, TRAIP protein expression was noticeably increased in HCC tissues, compared with the corresponding adjacent liver tissues (Fig. 1G). A large cohort containing 384 patients with HCC was recruited for IHC staining. Results showed that TRAIP was expression in both cytoplasm and nucleoplasm. In 78.6% (302/384) of the cases, TRAIP expression in HCC was higher than that in nontumorous tissues (Fig. 1H). These data suggest that TRAIP was upregulated in HCC.

3.2 High expression of TRAIP is correlated with poor prognosis in HCC

Analyses from TCGA cohort indicate that TRAIP expression was associated with tumor progression. We next determined the clinical value of TRAIP in HCC. Patients were separated into two groups (Low and High TRAIP), according to median IHC score in SYSUCC cohort and median mRNA expression value in TCGA cohort. Kaplan-Meier analyses were conducted. High expression of TRAIP was significantly correlated with shorter overall and disease-free survival in both SYSUCC (Fig. 2A) and TCGA (Fig. 2B) cohort, indicating that both mRNA and protein expression of TRAIP were helpful to differentiate patients with good or poor prognosis. Further analyses using TCGA data revealed the prognostic implication of TRAIP mRNA in multiple cancers. Significant correlation of high TRAIP mRNA expression and unfavorable overall and disease-free survivals was found in adrenocortical carcinoma (ACC), renal clear cell carcinoma (KIRC), HCC (LIHC) and sarcoma (SARC). Collectively, TRAIP may serve as potential prognostic factor in many types of human cancers.

3.3 TRAIP promotes cell proliferation in HCC

Since TRAIP expression was associated with tumor progression, we next investigated the biological function of TRAIP in HCC cell proliferation. The expression of TRAIP was determined in seven HCC cell lines and the immortalized liver cell line (L-02). According to the result of western blot, HepG2 and Huh7 cells were chosen for the further functional experiments (Fig. 3A). TRAIP overexpression vector and shRNAs were introduced into the two cell lines, using Lipofectamine 2000, and then selected by G418 to construct stable cell lines. The overexpression or knockdown of TRAIP mRNA and protein were confirmed by qRT-PCR and western blot (Fig. 3B&C). Cell counting experiment showed that cell proliferation was enhanced by TRAIP overexpression, but suppressed by TRAIP silence (Fig. 3D). Colony formation was performed to demonstrate that cells with TRAIP overexpression were able to form more and larger colonies in 35 mm plates, whereas HCC cells lacking TRAIP expression hardly grew (Fig. 3E). The effect of TRAIP in HCC cell proliferation was validated by EdU staining showing that more proliferating cells were found in TRAIP-expressing HCC cells (Fig. 3F). These data suggest the oncogenic role of TRAIP in HCC cells.

3.4 TRAIP interacts with SPAG5 in HCC cells

Using gene set enrichment analysis (GSEA) based on TCGA data, we found that many signaling pathways, such as Spermatogenesis and Spliceosome (Fig. 4A&B), were commonly activated in HCC cases with either TRAIP or SPAG5 overexpression. Our previous study demonstrated that SPAG5 functions as an important oncogene in HCC. Thus, we intended to investigate whether TRAIP corporates with SPAG5 to promote HCC progression. Correlation analyses in TCGA cases showed a positive correlation between TRAIP and SPAG5 expression (Fig. 4C). In paired HCC tissues collected from SYSUCC, high TRAIP expression was significantly associated with increased expression of SPAG5, at

both mRNA and protein levels (Fig. 4D&E). We next performed confocal analysis to observe the colocalization of the two proteins in cytoplasm in HepG2 and Huh7 cells (Fig. 4F), suggesting a direct binding of TRAIP and SPAG5. Data of coIP experiments presented that TRAIP and SPAG5 were detectable in the precipitant mediated by SPAG5 or TRAIP antibody in both HCC cell lines (Fig. 4G). Since TRAIP was reported as a E3 Ubiquitin ligase, we suspected that TRAIP contributed to the protein stability of SPAG5. Surprisingly, the interaction of TRAIP and SPAG5 led to the enhanced half-life of TRAIP but not SPAG5. TRAIP protein was fast degraded in cells with SPAG5 knockdown (Fig. 4H). These data suggest that TRAIP was stabilized via interaction with SPAG5 in HCC cells.

3.5 TRAIP triggers Rb/EZH2/p21 signaling in HCC cells

Current literatures reported contradictory roles in human epithelial and cancer cells through distinct mechanisms. We next determined to uncover the signaling pathway through which TRAIP promoted HCC cell proliferation. GSEA based on TCGA data indicated that Rb/EZH2, but not p53 pathway, was activated in HCC cases with TRAIP overexpression (Fig. 5A-C). To test whether TRAIP triggers Rb/EZH2 signaling, western blot was performed to examine the alteration of expression of relevant proteins. In HCC cells with TRAIP overexpression, an increase of EZH2 and decreases of Rb and p21 were detected (Fig. 5D). Previous studies demonstrated that Rb was able to modulate the expression of EZH2, and EZH2 was capable of suppressing the expression of p21 in a p53-independent manner. Rb was upregulated in cells with TRAIP silence, resulted in the downregulation of EZH2 and upregulation of p21 (Fig. 5E). We next examined whether Rb/EZH2/p21 signaling mediated the oncogenic function of TRAIP. Colony formation and EdU staining were performed. Overexpression of Rb and p21, and knockdown of EZH2 in TRAIP-overexpressing cells markedly decreased the colony formation and EdU-positive cells (Fig. 5F&G). These findings indicate TRAIP functions as an oncogene in HCC, at least partly, via regulation of Rb/EZH2/p21 signaling pathway.

4. Discussion

Defects in the DNA damage response (DDR) contributes to the genomic instability, and consequently aids in cancer initiation and progression [19]. Understanding the factors involved in DDR offers targetable vulnerabilities relatively specific to cancer cells, which may bring potential benefit to clinical management [20]. TRAIP, as a key regulator in DDR, exerts paradoxical activities in cancer progression. In this study, TRAIP expression is significantly increased in HCC tissues, and positively correlated with poor outcomes. TRAIP exhibits oncogenic activity to promote HCC cell proliferation via Rb/EZH2/p21 signaling pathway.

Similar results were obtained in HepG2 cells with wildtype p53 and Huh7 cells with mutant p53, indicating that the malignant behavior of TRAIP is p53-independent. Data of western blot demonstrated that either overexpression or knockdown of TRAIP led to the alteration of wildtype or mutant p53 expression in HCC cells. Interestingly, a recent paper reported that treatment of belinostat/cisplatin markedly increased the binding of mutant $p53^{R158G}$, but not the wildtype p53, to the promoter of TRAIP to increase its expression [14]. The chemotherapy-induced TRAIP upregulated the expression of TRAF2 to

block the activation of NFκB, and finally sensitize cancer cells to chemotherapy. Strikingly, upregulation of TRAIP mediated by the acetylation of $p53^{R158G}$ induced the expression of p21 in $p53^{-/-}$ cells. Our data also showed that ectopic expression of TRAIP in HCC cells dramatically upregulated p21 via impeding the Rb-mediated suppression of EZH2. Several studies reported the relationship of Rb, EZH2 and p21 in human cancers [21, 22]. Yet the detailed mechanism of TRAIP-mediated regulation of Rb/EZH2/p21 axis required further investigation.

Previous study showed that TRAIP participated in cell cycle regulation in HCC, but the mechanism remains unknown [16]. Data in our study that TRAIP-induced downregulation of Rb and p21, two important genes controlling the process of cell cycle, may provide hints. In addition, in this study, we also found that TRAIP was able to interact with SPAG5 that has been reported essential for cell division [23, 24]. The direct binding of TRAIP and SPAG5 resulted in the stabilization of TRAIP protein. Current literatures reported the upstream regulation of TRAIP in cancer cells. LncRNA SLC7A11-AS1 induced TRAIP expression via sponging miR-4775 that inhibited TRAIP transcript [17]. Mutant $p53^{R158G}$ increased the expression of TRAIP during the cytotoxic stress [14]. E2F1 was identified as a transcriptional factor for TRAIP in Hela cells [25]. Interestingly, Rb gene was proved to be able to downregulate the TRAIP mRNA transcription [25], which may suggest a negative feedback loop for TRAIP and Rb. Furthermore, SPAG5 was capable of trigger c-Myc/E2F1 and PI3K/ATK pathways that act as the upstream of TRAIP [24, 26]. Collectively, TRAIP may form several feedback loops with famous oncogenes or tumor suppressors, making TRAIP as an ideal therapeutic biomarker in cancer.

5. Conclusion

In summary, we found that TRAIP expression was markedly increased in two independent cohorts containing over 700 patients with HCC. The high expression of TRAIP mRNA and protein were correlated with unfavorable overall and disease-free survivals. TRAIP overexpression promotes HCC cell proliferation via Rb/EZH2/p21 signaling. Taken together, our findings suggest TRAIP as a promising prognostic factor and an oncogene to promote HCC growth.

Declarations

Ethics approval and consent to participate: All patients in this study provided written informed consents. This study was approved by the SYSUCC Institute Research Ethics Committee.

Consent for publication: All authors have read the manuscript and approved the submission.

Availability of data and material: The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests: All authors declare no conflict of interest.

Author contributions: Conception and design of the study: Zhang CZ, Shen HM; Generation, collection, assembly, analysis of data: Luo RZ, Li QL, Zhang MF, Zhang PW, Shen HM, Zhang CZ; Drafting and revision of the manuscript: Luo RZ, Zhang CZ, Shen HM; Approval of the final version of the manuscript: all authors.

Abbreviations: HCC, hepatocellular carcinoma; TRAIP, TRAF-interacting protein; GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas; DMEM, Dulbecco's modified Eagle's medium; IHC, Immunohistochemistry; EdU, 5-Ethynyl-2'-deoxyuridine; co-IP, Coimmunoprecipitation; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; LIHC, liver hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; GSEA, gene set enrichment analysis; DDR, DNA damage response

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Figures

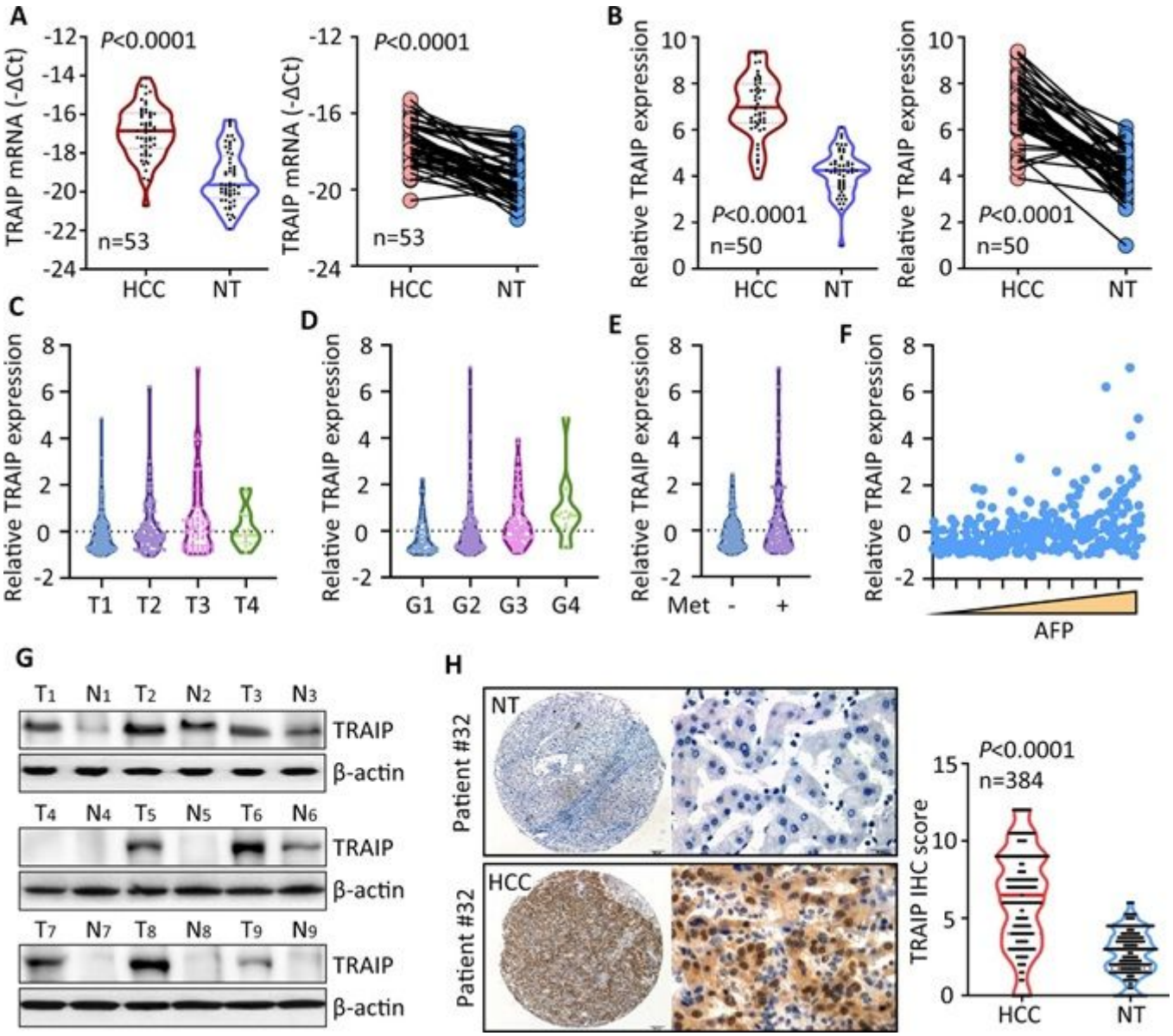

Figure 1

TRAIP expression is increased in HCC. A. The mRNA expression of TRAIP was examined by qRT-PCR in 53 pairs of HCC and nontumorous (NT) fresh tissues in SYSUCC cohort. The -ΔCt values were indicated in the left panel, and the upregulation of TRAIP mRNA was indicated in the right panel. B. The increased expression of TRAIP mRNA was validated in 50 paired HCC samples in TCGA cohort. C-F. The relationship

between TRAIP mRNA expression and tumor T stage (C), tumor grade (D), tumor metastasis (E) and serum AFP level (F) was determined in TCGA cohort. G. The protein expression of TRAIP was examined by western blot in HCC (T) and nontumorous (N) tissues. β-actin was used as a loading control. H. The increased expression of TRAIP protein was confirmed in a large cohort containing 384 pairs of HCC and nontumorous paraffin-embedded tissues. Representative images were shown in the left panel, and the IHC scores were shown in the right panel.

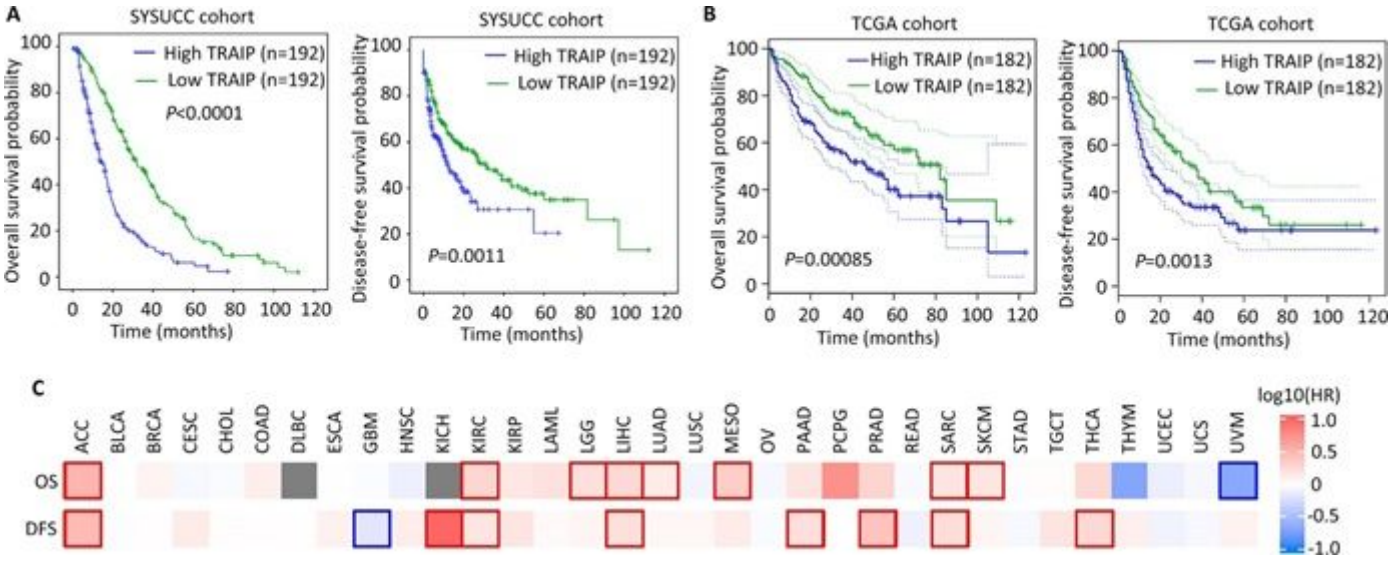

Figure 2

High expression of TRAIP was correlated with poor prognosis in HCC. A. Patients were defined as high and low TRAIP, according to the median of TRAIP IHC score. Kaplan-Meier survival analyses were conducted to indicated the value of TRAIP protein expression in the post-surgical overall (left panel) and disease-free (right panel) survivals in SYSUCC cohort. B. The impact of TRAIP mRNA expression in prediction of HCC patients' prognosis was investigated in TCGA cohort. High and low TRAIP was defined by the median expression of TRAIP mRNA. C. High expression was frequently associated with unfavorable prognosis in TCGA cancers. The p values of Kaplan-Meier survival analyses were indicated. The significant ones were framed. LIHC, liver hepatocellular carcinoma.

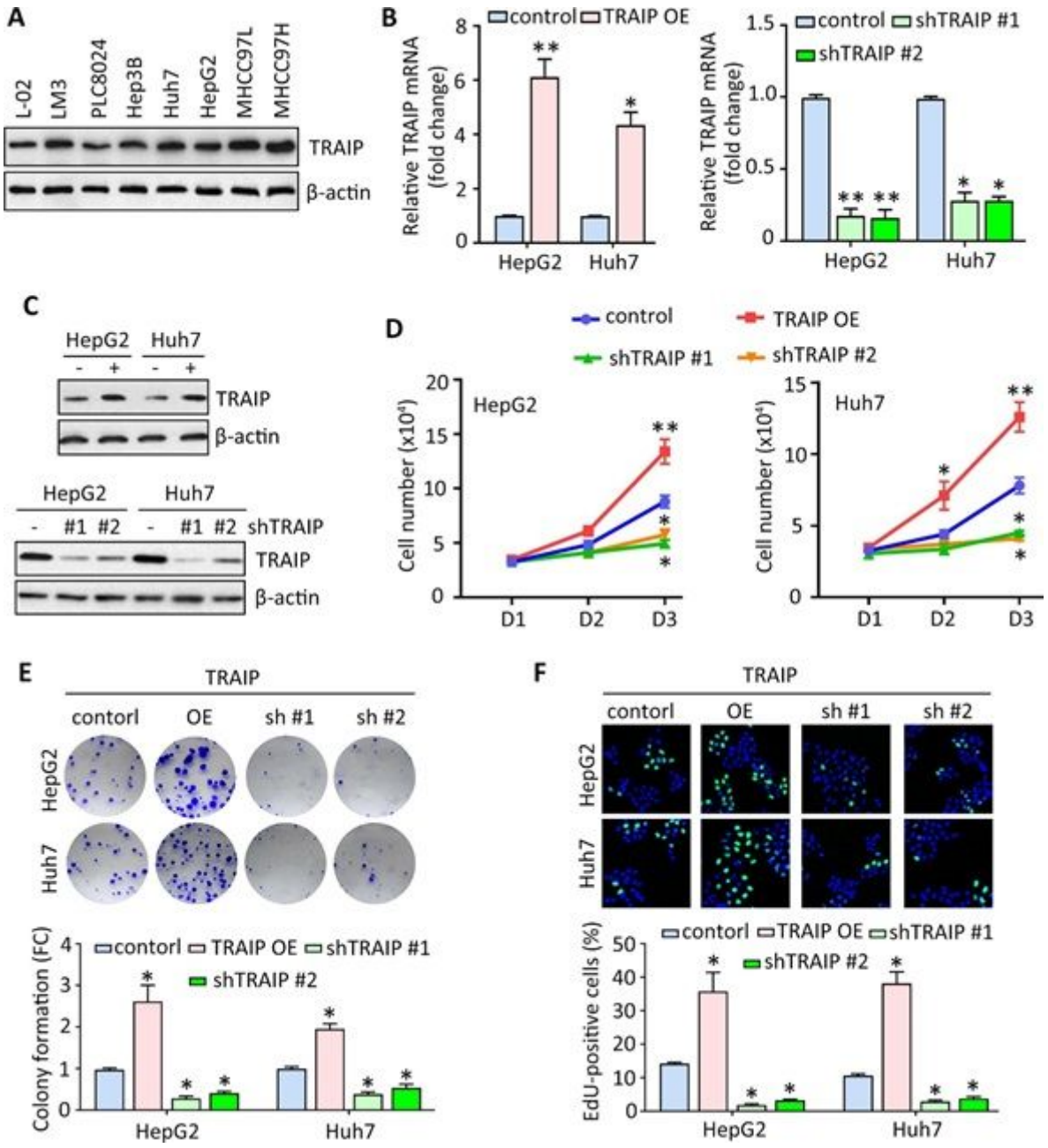

Figure 3

TRAIP promotes HCC cell proliferation. A. The protein expression of TRAIP in HCC and immortalized human hepatocyte cell line (L-02) was examined by western blot. B. HepG2 and Huh7 cells were transfected with TRAIP overexpression vector or shRNAs for 36 h. The mRNA level of TRAIP was determined by qRT-PCR. *P<0.05. **P<0.01. C. The overexpression and knockdown of TRAIP protein in HCC cells were confirmed. D. Stable cells with TRAIP overexpression or silencing were cultured in 6-well plates for 3 days. The cell number was counted and indicated by growth curve. *P<0.05. **P<0.01. E. Stable cells were incubated into 6-well plates for 10 days. Colonies were stained by 0.1% crystal violet. Representative images of colony formation were shown, and the relative fold change (FC) was indicated. *P<0.05. F. Stable cells were stained by EdU and DAPI, and pictured under a fluorescence microscope. The

proliferative cells were indicated by green spots. The percentages of EdU-positive cells were shown. *P<0.05.

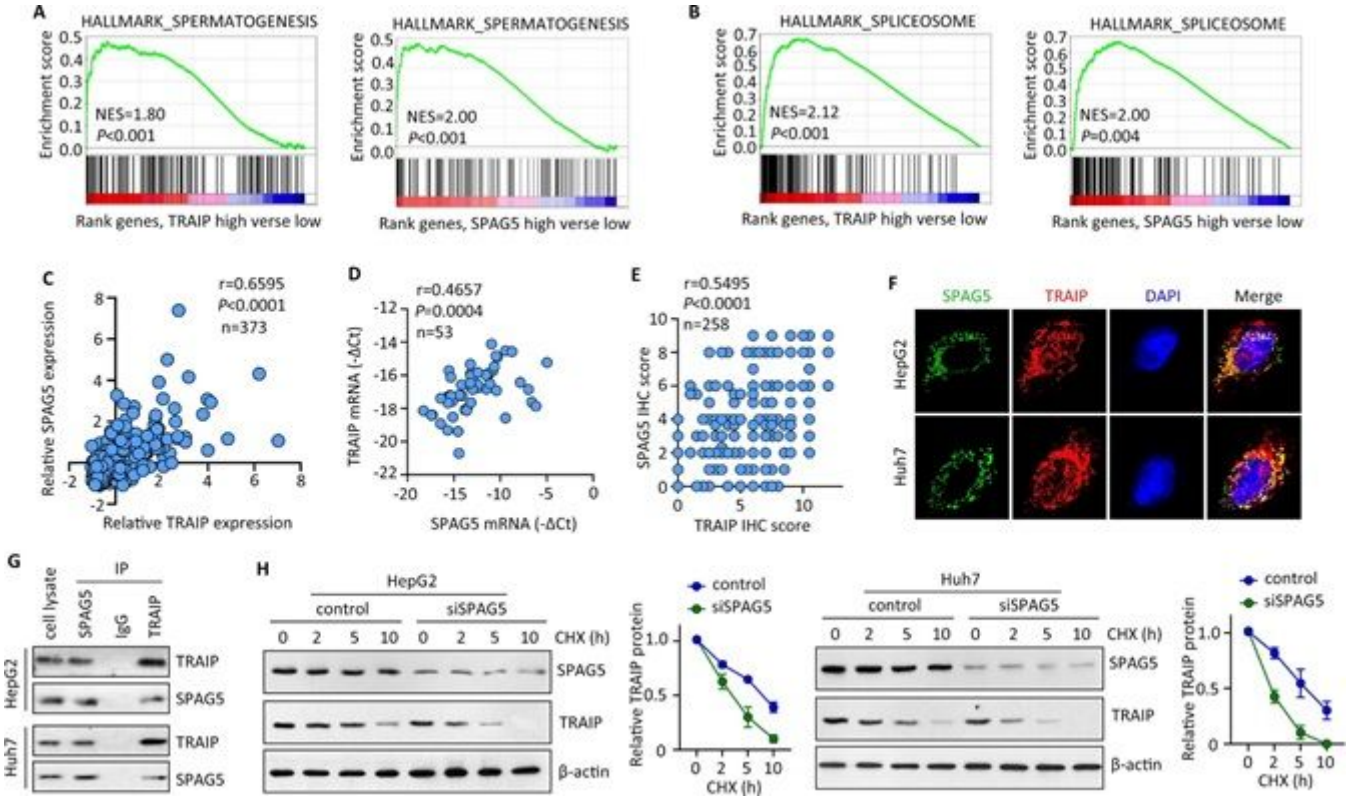

Figure 4

TRAIP interacts with SPAG5 in HCC cells. A,B. Gene set enrichment analysis (GSEA) was conducted using TRAIP and SPAG5 expression in TCGA cohort. Pathways of Spermatogenesis (A) and Spliceosome (B) were enriched patients with high expression of TRAIP or SPAG5. C,D. The correlation of TRAIP and SPAG5 mRNA expression was determined in patients of TCGA (C) and SYSUCC (D) cohorts. E. Protein expression was examined by IHC in 258 patients in SYSUCC cohort. Pearson correlation analysis was used to valid the positive correlation of TRAIP and SPAG5. F. The cellular colocalization of TRAIP and SPAG5 was checked by confocal experiment. G. Total proteins were extracted and incubated with primary antibody of TRAIP or SPAG5. Precipitate was subjected to western bot to determine the existent of TRAIP and SPAG5. Antibody for IgG was used as negative control for immunoprecipitation. H. Cells transfected with SPAG5 siRNA for 36 h was treated with 20 μg/mL cycloheximide (CHX) for variety period. The expression of SPAG5 and TRAIP was examined and the relative TRAIP protein was indicated.

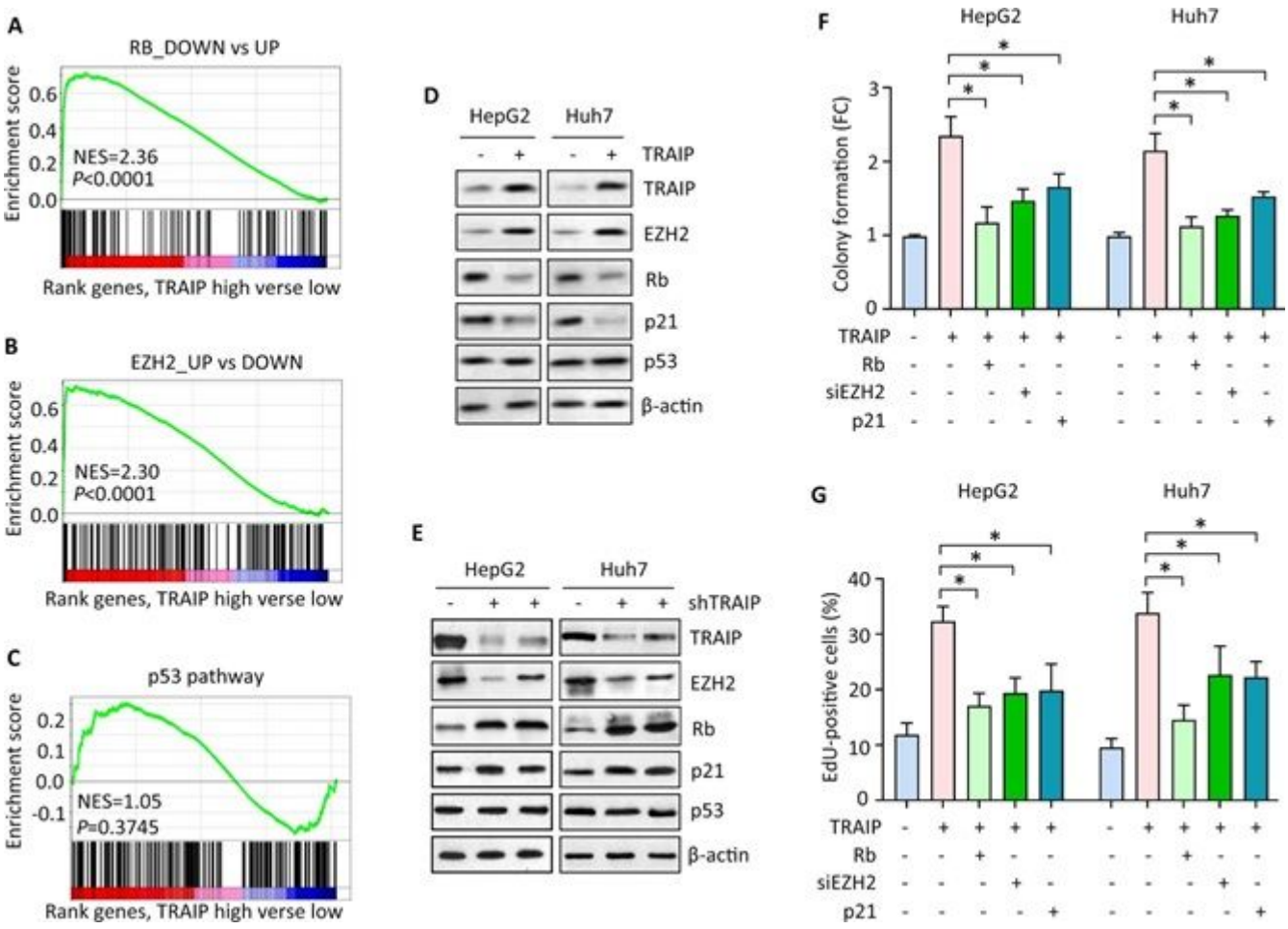

Figure 5

TRAIP exerts oncogenic activity via Rb/EZH2/p21 signaling pathway. A-C. GESA indicates that Rb signaling (A) was inhibited and EZH2 signaling (B) was activated in HCC patients with high expression of TRAIP in TCGA cohort. No significant alterations occur in p53 pathway (C). D. Cells were transfected with TRAIP overexpression vector to construct stable cell lines. The expression of TRAIP, EZH2, Rb, p21 and p53 was determined by western blot in HepG2 and Huh7 cells. E. TRAIP knockdown downregulated the expression of EZH2, but upregulated the expression of Rb and p21. F. Cells with TRAIP overexpression were transfected with EZH2 siRNA, overexpression vector for Rb or p21 every three days. Colony formation were performed to examine the effect of Rb/EZH2/p21 signaling on cell growth. G. EdU staining was performed in cells treated as described in F to validate the role of Rb/EZH2/p21 in HCC cell proliferation. *P<0.05.

Supplementary Files

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

- SIFigure1.jpg
- SIFigure2.jpg