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Original Article

Discovery and characterization of a novel splice variant of the p53 tumor suppressor gene in a human T cell leukemia cellline

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Abstract: Alternative splicing produces multiple mRNA variants of *TP53* which have diverse biologic functions. In this study, we identified a novel splice variant of *TP53* lacking a 200 nt portion of exon 4 (p53ΔE4p) from a human leukemia T cell line. No protein product of p53ΔE4p was identifiable by western blot; however, forced expression of the variant in HEK-293T cells expressing wild-type p53 could inhibit cell proliferation and promote cell death. Interestingly, this novel variant also significantly enhances the expression of reporter genes. Moreover, transcriptome analysis showed that genes related to *DNA binding* and *regulation of transcription by RNA polymerase II* function were significantly upregulated following p53ΔE4p transfection, suggesting a role for this variant in the regulation of gene expression.

Keywords: TP53, splice variant, Jurkat, expression regulation, transcriptome

Introduction

The p53 protein is an important regulator of various cellular metabolic processes [1]. It can be induced by diverse signals such as DNA damage, nutrient starvation, heat shock, virus infection, and oncogene activation, and plays an important role in transcriptional regulation by specifically binding p53 response elements (p53REs) in the promoters of target genes, so as to induce apoptosis, DNA repair, or cell cycle arrest to maintain genetic stability and prevent cancer [2-5].

Alternative splicing produces multiple transcript variants and isoforms, and is a common way for eukaryotic genes to expand their biologic functions. The human *TP53* gene produces at least nine RNA transcripts encoding 12 isoforms (p53α, p53β, p53γ, Δ40p53α, Δ40p53β, Δ40p53γ, Δ133p53α, Δ133p53β, Δ133p53γ, Δ160p53α, Δ160p53β and Δ160p53γ) generated by alternative splicing, use of alternate translation initiation codons, or alternate promoters. Not only are these p53 isoforms differentially expressed in different cancer types, but they also have different transcriptional activities and anti-cancer effects, which can affect a variety of biologic functions [6-11]. p53β and p53γ regulate the cellular response to modulation of alternative splicing pre-mRNA pathway by a small drug inhibitor [12]. Co-transfection of p53 and p53β slightly increases p53-mediated apoptosis, while co-transfection of p53 and Δ133p53 strongly inhibits p53-mediated apoptosis in a dose-dependent manner [13]. Moreover, Δ40p53α oligomerizes with full-length p53 results in negative regulation of p53's transcriptional and growth-suppressive activities [14]. In addition, Δ133p53α and Δ40p53α have been evaluated as potential biomarkers in serous ovarian cancer patients [15]. Thus, each of the various p53 isoforms may possess unique biologic activities.

In this study, we identify a novel mRNA variant of the *TP53* gene generated by alternative splicing in the Jurkat leukemia cell line. Because this variant has a 200 bp sequence excision in exon 4, it was named p53ΔE4p. To further understand the biological function of p53ΔE4p, expression analysis and transfection experiments were performed. No protein product of p53ΔE4p was identified; however, after transfection into HEK-293T cells expressing wild-type p53 protein, p53ΔE4p exhibited an inhibitory effect on cell proliferation and promoted cell death. Interestingly, expression of p53ΔE4p was found to significantly enhance expression of EGFP downstream of the h-CMV promoter. Transcriptome analysis showed that the genes related to *DNA binding* and *regulation of transcription by RNA polymerase II* increased significantly in cells transfected with p53ΔE4p. In addition, there were significant changes in the expression of a large number of unannotated genes, indicating that p53ΔE4p may significantly affect cell metabolism and regulate gene expression.

Materials and methods

Cell lines and cell culture

Jurkat cells (Clone E6-1, ATCC®TIB-152™) were purchased from ATCC. HEK-293T cells (human embryonic kidney cells) were purchased from the Stem Cell Bank of the Chinese Academy of Sciences (serial number: SCSP-502). Cells were cultured in RPMI-1640 (Jurkat) or DMEM (HEK-293T) medium (Thermo Fisher Scientific, Gibco, Grand Island, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific, Gibco, Grand Island, USA) and 1% Penicillin-Streptomycin (Thermo Fisher Scientific, Gibco, Grand Island, USA) at 37°C and 5% CO_2.

Cloning of novel splice variant and construction of recombinant plasmid

Total RNA from Jurkat cells was extracted using TRIzol (Takara, Dalian, China) and cDNA was synthesized using Hifair®II 1st Strand cDNA Synthesis SuperMix (YEASEN, Shanghai, China). PCR amplification was performed with 5'-GCTCCGGGGACACTTTGCGTTCG-3' (forward) and 5'-AGAGATGGGGGTGGGAGGCTGTCA-3' (reverse) primers. The fragment was inserted into the pGEM®-T Easy Vector (Promega, Wisconsin, USA) and the recombinant DNA was transformed into DH5α (KT Health, Shenzhen, China). After sequencing analysis, positive clones were chosen for scale-up culture, and the plasmids were extracted and digested with Sac II and Sal I (Takara, Dalian, China). The cut fragment (~1160) was ligated into pIRES2-EGFP (also digested with Sac II and Sal I) to obtain the recombinant pIRES2-p53ΔE4p plasmid.

Cell transfection

According to the instructions provided with the calcium phosphate cell transfection kit (Beyotime Biotechnology, Shanghai, China), 7.0×10^5 HEK-293T cells/well were inoculated into a 6-well plate, and reached ~80% confluency 24 h after plating. The medium was replaced with fresh antibiotic-free medium about 30 minutes before transfection. The DNA-calcium chloride solution was made by adding 5 μg plasmid DNA [1 μg/μl] to 100 μl of calcium chloride solution followed by incubation for 2 minutes before addition to 100 μl of BBS solution and incubation at room temperature for 20 minutes. 205 μl DNA-calcium chloride-BBS mixture was added dropwise to each well. After 10 hours, the medium was replaced with fresh complete culture medium. Gene expression was detected approximately 48 hours after transfection.

Western blot

RIPA lysate buffer (Beyotime Biotechnology, Shanghai, China) was used for protein extraction and protein content was quantified by BCA (Beyotime Biotechnology, Shanghai, China). After SDS-PAGE, proteins were transferred to PVDF membranes, which were then incubated with primary antibodies followed by secondary antibodies. The anti-β-actin primary antibody used was β-actin (4D3) monoclonal antibody (Bioworld Technology, Nanjing, China), and the secondary antibody was HRP-conjugated Goat Anti-Mouse IgG (BBI Life Science, Shanghai, China). The anti-p53 primary antibody was S46 (Abgent, Suzhou, China), and the secondary antibody was Peroxidase-Conjugated Goat Anti-Rabbit IgG (YEASEN, Shanghai, China). Finally, a high-sensitivity ECL developing solution (YEASEN, Shanghai, China) was used for detection.

Cell viability test

HEK-293T cells were inoculated into 96-well plates (8000 cells/well) followed by transfec-

Table 1. Primers used for quantitative Real-time reverse transcription-PCR (qRT-PCR)

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')
GAPDH	TCCTGCACCACCAACTGCT	TCACGCCACAGTTTCCCGGAG
TP53	TGCCCTATGAGCCGCCTGA	CGCACCTCAAAGCTGTTCCG
EGR2	AAGCCCTTCCAGTGTCGGAT	CCTCTCATCACTCCGGGCAA
CEBPB	GCCTCTCCACGTCCTCCTCGTCCA	CGCTGTGCTTGTCCACGGTCT
NLRC5	AGTCAGTTTTCGGGACAACCA	GTGACTGCCACCCTTGCC
IRF4	AGAGCATCTTCCGCATCCC	TGGCTCCGCTCAACCAGT

tion the next day. At 48 h after transfection, 10 μl of MTT (Biofroxx, Guangzhou, China) solution was added to each well. After 4 h, the supernatant was removed, 100 μl of DMSO (Biofroxx, Guangzhou, China) was added, the plate was incubated with shaking for 10 min. MTT absorbance was measured at OD570 nm using a 96-well plate reader.

Cell death assay

Adherent cells were detached using trypsin (Thermo Fisher Scientific, Gibco, Grand Island, USA), and washed with pre-chilled PBS. After centrifugation the supernatant was removed, and the cells were resuspended with 100 μl 1× Annexin V Binding Buffer (BD Pharmingen, USA) mixed with 5 μl propidium iodide (PI) (BD Pharmingen, USA), and incubated in the dark for 15 min, following by the addition of 400 μl 1× Annexin V Binding Buffer and the rate of cell death was assessed using flow cytometry.

Cell cycle experiment

Cells were centrifuged and the supernatant was removed. After washing with pre-chilled PBS, the cells were fixed by adding pre-chilled 70% ethanol and placed at 4°C for 3 h. Cells were washed with pre-chilled PBS again prior to staining with 0.5 ml of PI solution (Beyotime Biotechnology, Shanghai, China) and incubation at 37°C in the dark for 30 min. Cell cycle was then analyzed by flow cytometry.

Observation and analysis of reporter gene EGFP

At 48 h after transfection, cells were observed using an inverted fluorescence microscope and confocal laser microscope. The transfection efficiency and mean fluorescence intensity (MFI) were assessed by flow cytometry.

Transcriptome analysis

At 48 h after transfection, the cells were collected and sent to ShangHai Majorbio for transcriptome analysis. High-throughput sequencing was used to analyze the data, which were compared to the eukaryotic reference genome. To quantify transcript expression levels, the RNA-seq (RNA-sequencing) data was normalized to obtain a TPM (Transcripts Per Million reads) value. We applied DEGseq to filter the DEGs (differentially expressed genes). Following statistical analysis, we screened the DEGs by the following criteria: fold change > 1.50 or fold change < 0.67, FDR < 0.05, *P* value = 0.05. The DEGs were subjected to GO (gene ontology) function enrichment analysis, and the interaction gene/protein retrieval tool (STRING) algorithm was used to analyze the functional interaction network of DEGs to identify key genes for further research.

Real-time PCR

SYBR green (KT Health, Shenzhen, China) was used for real-time PCR analysis using primers listed in **Table 1**. The expression level of genes was calculated based on the C*p* value according to the $2^{-\Delta\Delta Ct}$ formula.

Immunofluorescence assay

HEK-293T cells were cultured at a density of 7.0×10^5 cells/well on coverslips placed in six-well plates. At 48 h after transfection, the cells were fixed with 4% paraformaldehyde and stained with anti-p53 rabbit pAb (GB-11626) (Servicebio, Wuhan, China) and DAPI (Servicebio, Wuhan, China). The slices were observed using a fluorescence microscope and images were collected.

Statistical analysis

Charts were drawn with GraphPad Prism 7.0. Data were analyzed with SPSS 19.0. All data were first tested for normality and homogeneity of variances. Data are presented as the mean ± standard error of the mean (SEM). The quantitative data were analyzed using an independent-sample *t*-test for comparison between two groups, and $P < 0.05$ was considered significant.

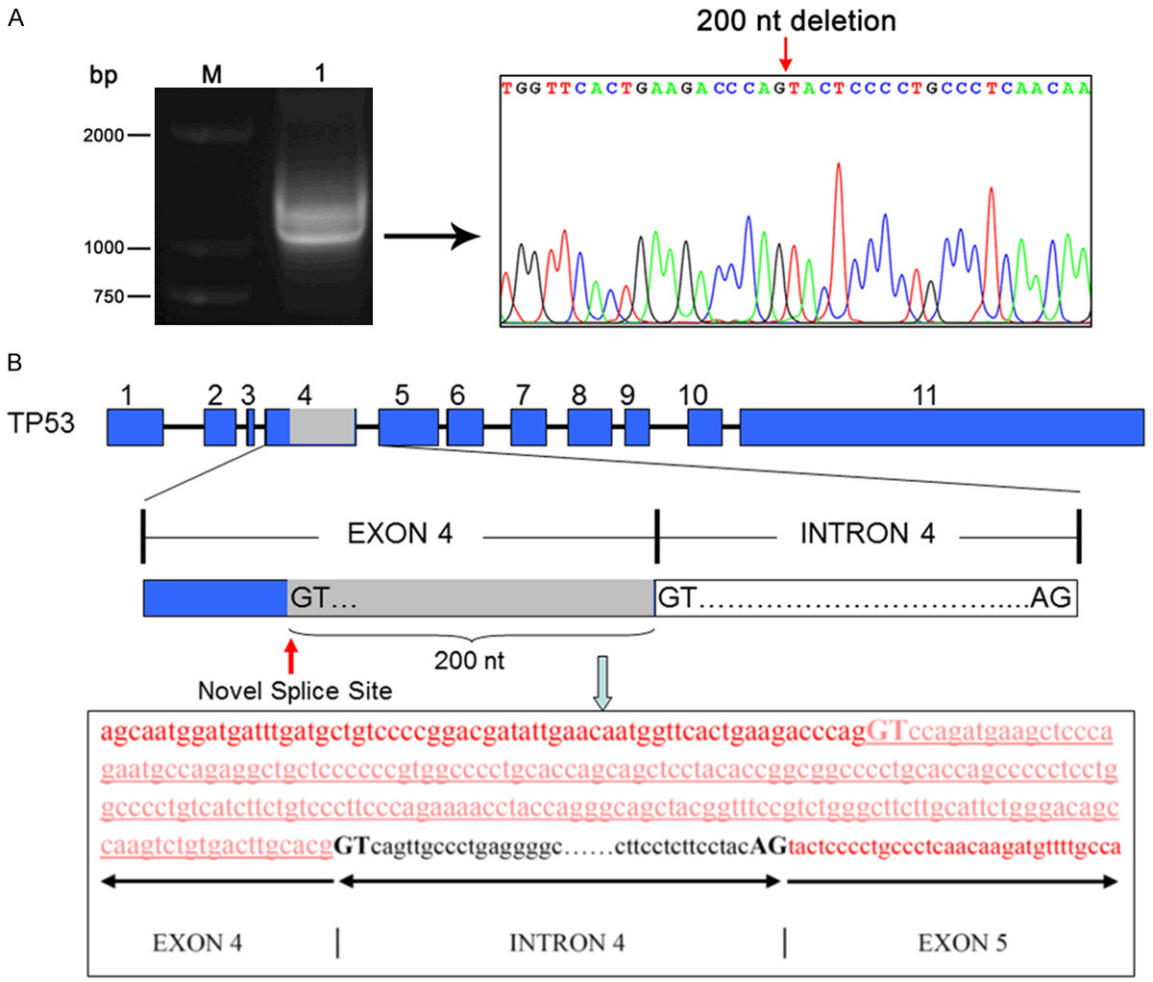

Figure 1. Amplification and sequence analysis of the TP53 gene in Jurkat cells. A. RT-PCR was performed to amplify the p53 gene of Jurkat cells. M: Marker, 1: p53 amplification products. B. The smaller band was excised and cloned into T-vector for sequence analysis. Sequence alignment indicated that this amplification product is a novel p53 transcript lacking a 200 bp sequence in exon 4, which results in frameshift mutations and premature termination.

Results

Cloning and sequencing analysis of the TP53 gene from Jurkat cells

Total RNA was extracted from Jurkat cells and reverse transcription was performed to generate cDNA. Next, p53 was amplified from the cDNA using specific primers (described above) and the products were subjected to agarose-gel electrophoresis. Two bands longer than 1000 bp were identified by gel electrophoresis (**Figure 1A**). The amplification product of the full-length p53 transcript with the primers described in this study is expected to be 1325 bp, corresponding to the larger band on our gel. The smaller band was extracted and cloned into the T-vector for sequence analysis. Sequence alignment revealed that the sequence is a p53 transcript lacking 200 nt in exon 4. This was caused by a new splice site inside exon 4 and resulted in frame-shift mutations and premature termination (**Figure 1B**). Thus, this novel transcript was named as p53ΔE4p and its sequence in comparison to TP53 is shown in Supplementary Figure 1.

Detection of p53ΔE4p expression products

Sequence analysis revealed the potential for p53ΔE4p to encode an 80 amino acid product (about 10 kDa) (**Figure 2A**). The putative 58 amino acid sequence before the frameshift mutation corresponds to the TAD region (most

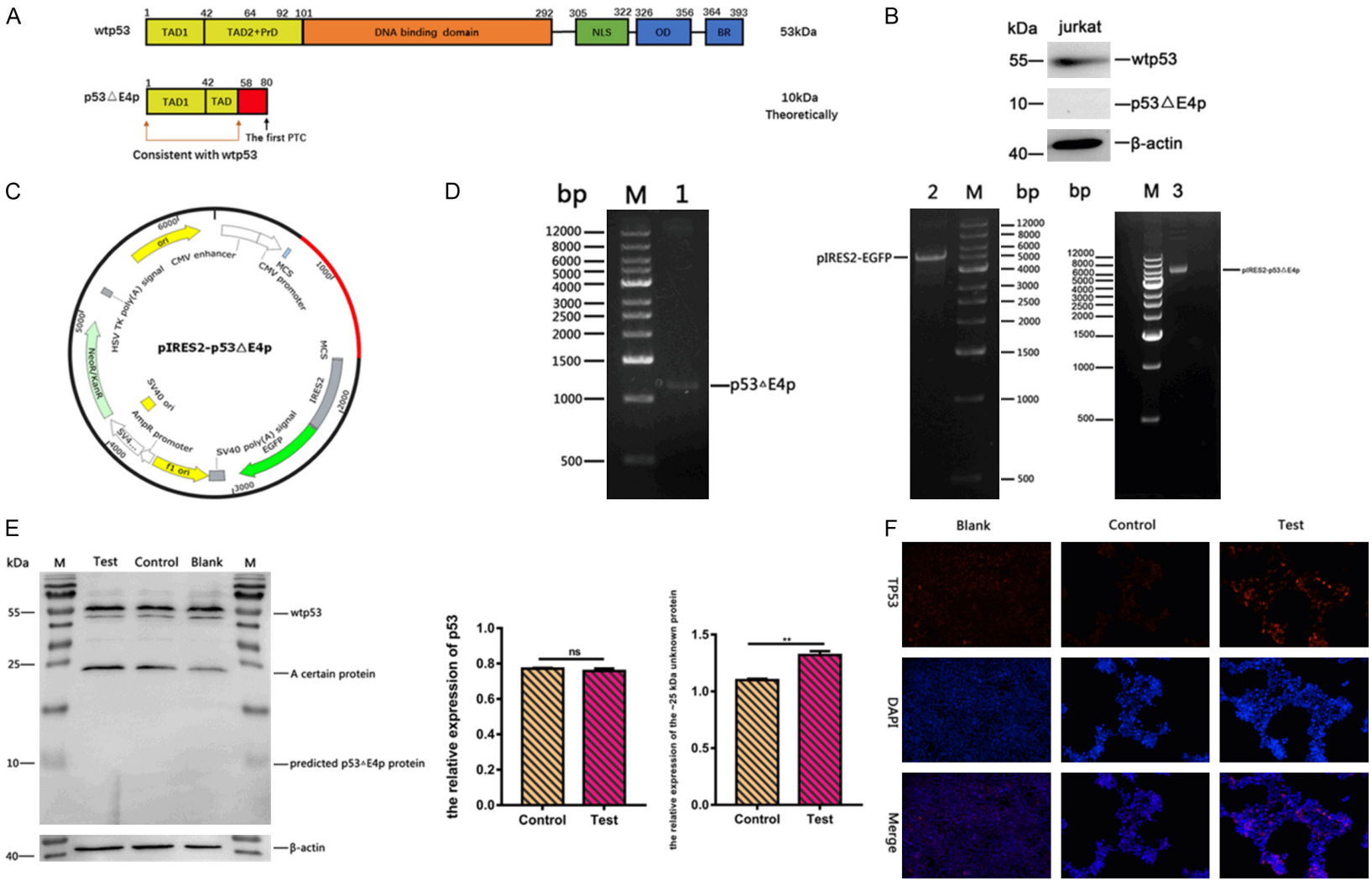

Figure 2. Detection and analysis of p53ΔE4p expression products. (A) Putative protein structure of p53ΔE4p based on the base sequence. (B) Western blotting analysis of p53 proteins in Jurkat cells. (C) Schematic diagram of recombinant pIRES2-p53ΔE4p plasmid (the inserted p53ΔE4p gene is shown in red). (D) Construction of the recombinant pIRES2-p53ΔE4p plasmid (M, marker; 1, p53ΔE4p; 2, pIRES2-EGFP; 3, pIRES2-p53ΔE4p) (E) Western blotting analysis and (F) Immunofluorescent staining of p53 proteins in HEK-293T cells. M: marker, Test: HEK-293T transfected with pIRES2-p53ΔE4p, Control: HEK-293T cells transfected with pIRES2-EGFP, Blank: HEK-293T.

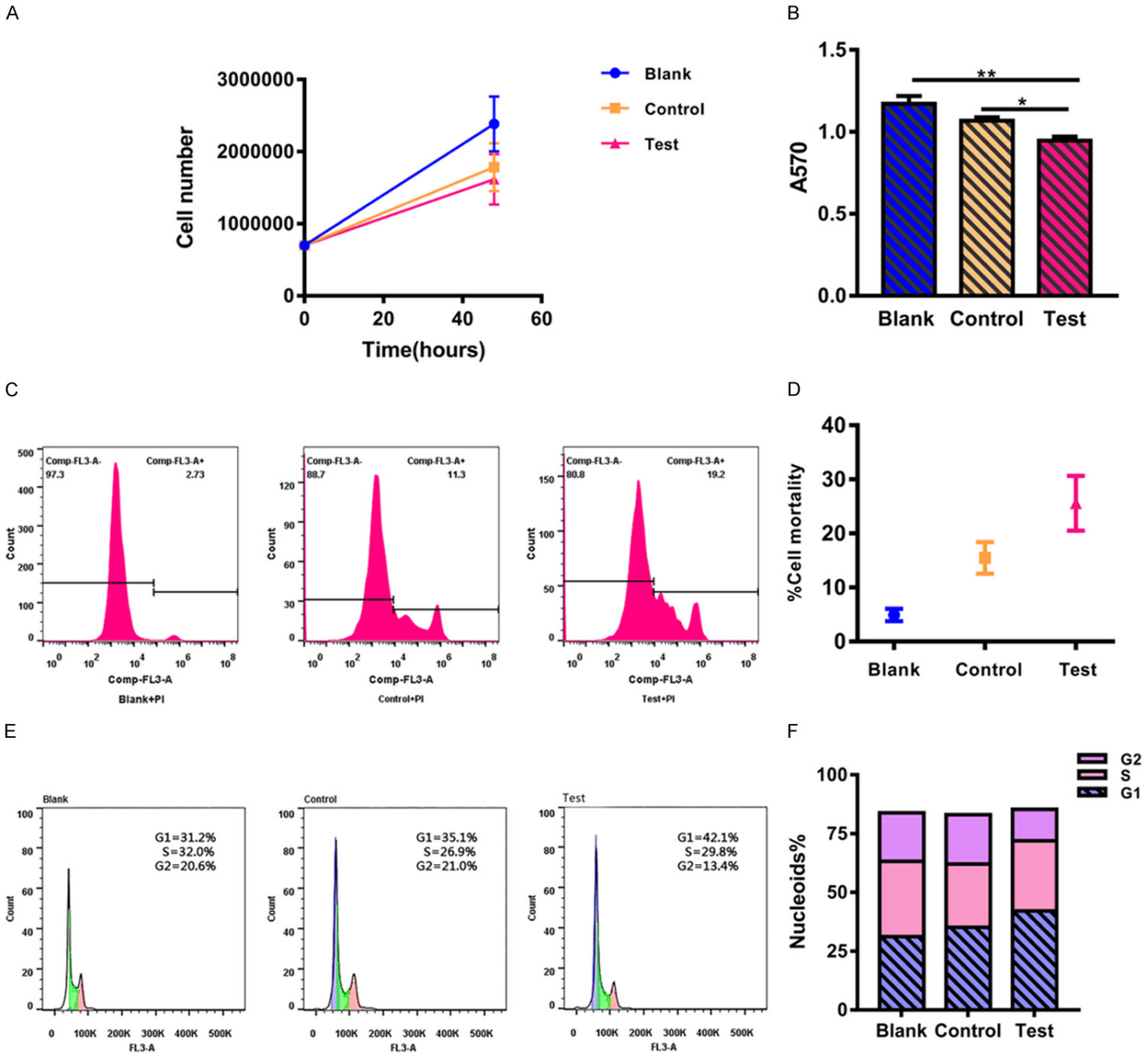
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Figure 3. Analysis of the proliferation, cell cycle, and mortality of p53ΔE4p-transfected HEK-293T cells, which also express wild-type p53, 48 hours after transfection. (A) Cell growth curve ($P > 0.05$, Mean ± SEM, n = 3) and (B) MTT assay for each group, *: $P < 0.05$, **: $P < 0.01$, Mean ± SEM, n = 3. (C) Analysis of mortality by PI staining and flow cytometry. (D) The mortality ratio of each group was calculated, $P > 0.05$, Mean ± SEM, n = 3. (E) Detection and (F) analysis of cell cycle in each group. Test: HEK-293T transfected with pIRES2-p53ΔE4p, Control: HEK-293T cells transfected with pIRES2-EGFP, Blank: HEK-293T.

are TAD1). In order to detect the p53ΔE4p protein products, Jurkat cell extracts were subjected to western blotting with polyclonal anti-p53 antibody (S46). However, there was no band corresponding to the target protein at the expected size (~10 kDa) (**Figure 2B**).

To further confirm expression of p53ΔE4p, this novel splice variant was inserted into the Sac II and Sal I restriction sites of pIRES2-EGFP to construct the recombinant eukaryotic expression vector, pIRES2-p53ΔE4p (**Figure 2C**, **2D**). This vector was transfected into HEK-293T cells and 48 hours after transfection, cells were lysed and lysates were used for western blotting with S46 anti-p53 antibody, which recognizes the sequence between amino acids 24 and 53 of human p53 protein. However, there was also no band at ~10 kDa (**Figure 2E**). These results indicate that the novel splice variant p53ΔE4p does not express a detectable protein product.

Interestingly, although the full length p53 protein band at ~53 kDa was unchanged, the density of the protein band at ~25 kDa significantly increased after p53ΔE4p transfection (**Figure 2E**). Since there is no similar molecular weight p53 isoforms with the same 24-53 amino acid sequence of wild-type p53 protein, we speculate that this protein is either a new p53 isoform or another protein that can be cross-recognized by S46 polyclonal antibody. Furthermore, an immunofluorescence assay was performed to stain the p53 protein using a rabbit polyclonal antibody (GB11626) in HEK-293T cells transfected with p53ΔE4p. Significantly enhanced p53 staining was found after transfection of this novel variant, with the signal mainly located in the nucleus, which could be due to non-specific staining similar to that in the western blot (**Figure 2F**).

The proliferation, cell cycle, and mortality of HEK-293T cells expressing wild-type p53 were significantly affected by p53ΔE4p

Analysis of cell growth and viability by MTT assay suggested that the proliferation of HEK-293T cells is inhibited by p53ΔE4p (**Figure 3A**, **3B**). Flow cytometric analysis after PI staining revealed a statistically significant increase in PI^+ cells in p53ΔE4p-transfected HEK-293T cells (**Figure 3C**, **3D**). In addition, cell cycle experiments indicated that p53ΔE4p induces G1 arrest in HEK-293T cells (**Figure 3E**, **3F**). Together, these results suggest that p53ΔE4p may affect the proliferation of HEK-293T cells by inducing G1 arrest and cell death.

Transfection of p53ΔE4p enhances expression of the exogenous EGFP reporter gene

Interestingly, we found that transfection of p53ΔE4p into HEK-293T markedly increased the expression of the EGFP gene located on the same plasmid. **Figure 4A** shows that the fluorescence intensity of EGFP observed by inverted fluorescence microscope was significantly enhanced in HEK-293T cells transfected with p53ΔE4p compared with the control group. The results of flow cytometry showed that while the transfection efficiency between two groups was similar, the MFI was significantly different (**Figure 4B**, **4C**). These findings were confirmed through confocal laser scanning microscopy (**Figure 4D**, **4E**).

Gene expression in HEK-293T cells is affected by p53ΔE4p

HEK-293T cells transfected with p53ΔE4p were subjected to eukaryotic reference transcriptome analysis (fold change > 1.50 or fold change < 0.67, *P* value = 0.05, FDR < 0.05). Through this analysis it was found that transfection of p53ΔE4p results in the significant changes in the expression of 291 genes in HEK-293T cells. A cluster heat map and volcano map were used to show the distribution of differentially expressed genes (DEGs) between groups (**Figure 5A**, **5B**). It is worth noting that most of the DEGs are unannotated genes (Supplementary Material 2).

GO (Gene Ontology) functional enrichment analysis of DEGs (enriched genes ≥ 10) revealed that the main biological processes (BP) enriched were *regulation of transcription by*

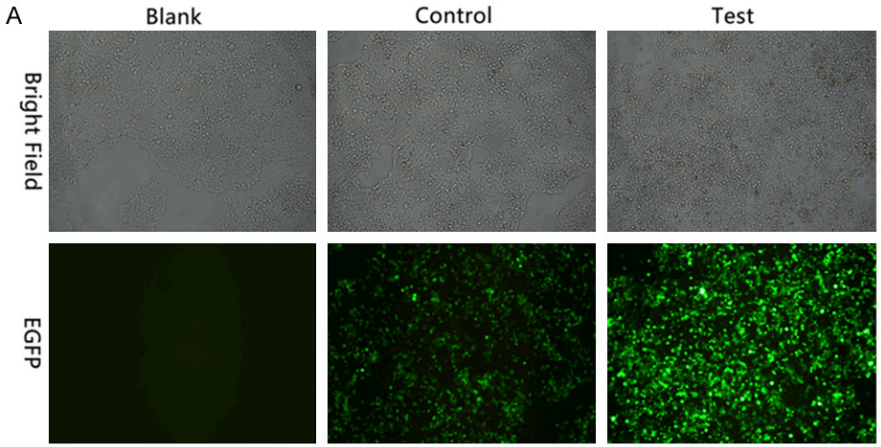
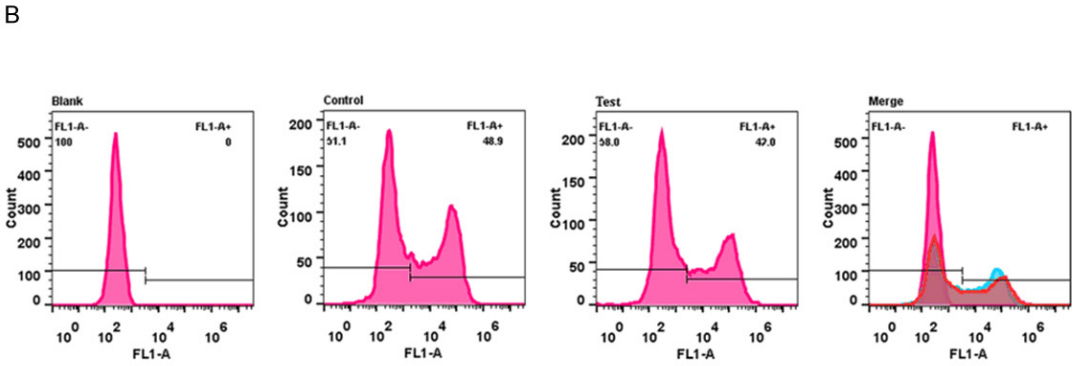
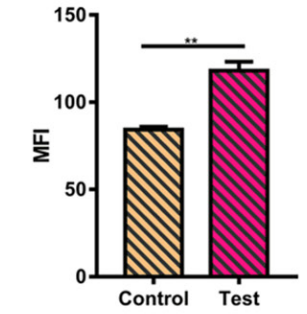
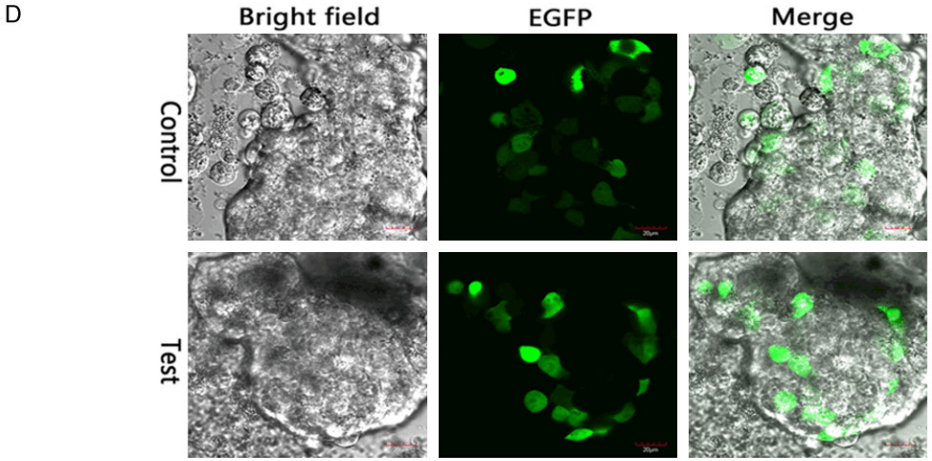
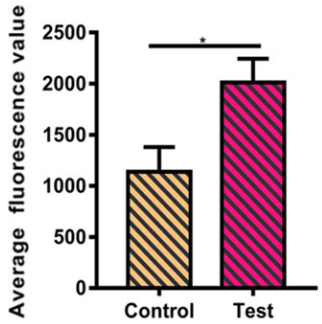


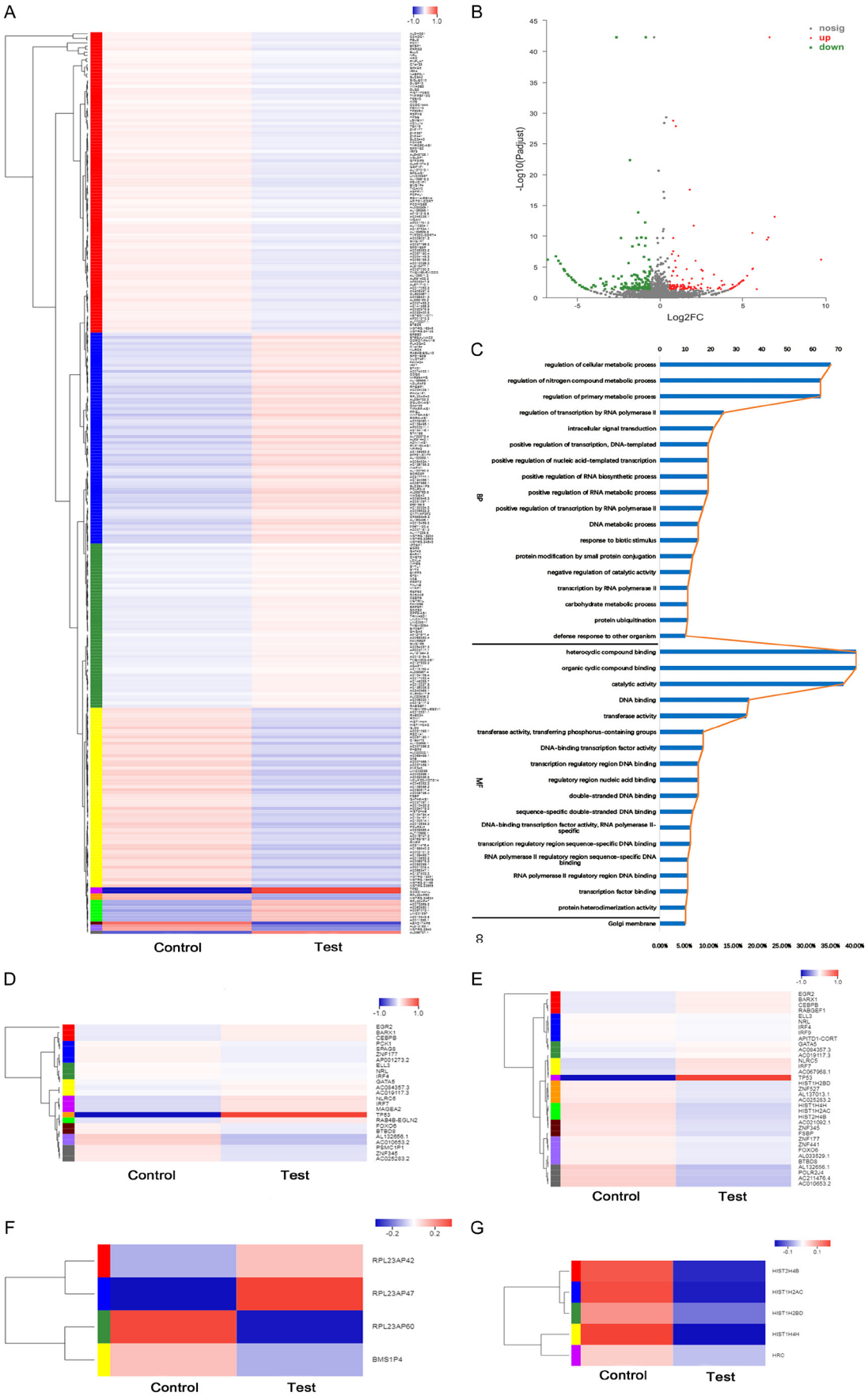
Figure 4. Promotion of the expression of the exogenous reporter gene, EGFP, following transfection of p53ΔE4p into HEK-293T cells. (A) A fluorescent inverted microscope was used to observe the expression of EGFP in HEK-293T cells transfected with p53ΔE4p 48 hours after transfection. (B) Transfection efficiency and (C) MFI of EGFP in cells was measured by flow cytometry. **: P < 0.01, Mean ± SEM, n = 3. (D) Expression of the EGFP reporter gene was observed by confocal laser scanning microscopy, and (E) the average fluorescence intensity of EGFP was calculated. *P < 0.05, Mean ± SEM, n > 3. Test: HEK-293T transfected with pIRES2-p53ΔE4p, Control: HEK-293T cells transfected with pIRES2-EGFP, Blank: HEK-293T.







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positive regulation of nucleic acid-templated transcription
positive regulation of RNA biosynthetic process
positive regulation of RNA metabolic process
positive regulation of transcription by RNA polymerase II
DNA metabolic process
response to biotic stimulus
protein modification by small protein conjugation
negative regulation of catalytic activity
transcription by RNA polymerase II
carbohydrate metabolic process
protein ubiquitination
defense response to other organism
heterocyclic compound binding
organic cyclic compound binding
catalytic activity
DNA binding
transferase activity
transferase activity, transferring phosphorus-containing groups
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RPL23AP47
RPL23AP60
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Figure 5. Transcriptome analysis of HEK-293T cells transfected with p53ΔE4p. Transcriptome analysis using a eukaryotic reference was performed (analysis parameters were fold change > 1.50 or fold change < 0.67, *P* value = 0.05, FDR < 0.05). (A) Cluster heat map and (B) volcano map displaying the distribution of 291 DEGs. (C) GO function enrichment analysis of 291 DEGs was performed (enriched genes ≥ 10). Cluster heat map analysis for DEGs related to (D) *regulation of transcription by RNA polymerase II*, (E) *DNA binding*, (F) *ribosomes* and (G) *histones* are displayed separately.

RNA polymerase II, *positive regulation of transcription*, *positive regulation of nucleic acid-templated transcription*, *positive regulation of RNA metabolic processes*, and *regulation of cellular metabolic processes*. The molecular functions (MF) found to be enriched included *DNA binding*, *DNA-binding transcription factor activity*, *transcription regulatory region DNA binding*, *regulatory region nucleic acid binding*, and *RNA polymerase II-specific* (**Figure 5C**). We performed cluster heat map analysis for the DEGs related to *regulation of transcription by RNA polymerase II*, *DNA binding*, *ribosome,* and *histone* (**Figure 5D-G**).

Furthermore, we analyzed the GO term (STRING) to identify relationships between proteins (**Figure 6A**, **6B**). According to the protein-protein interaction network, it was found that each enriched GO term focused on TP53, EGR2, CEBPB, NLRC5, IRF4, and IRF7. Interaction maps for these five genes revealed that they are related to functions such as *positive regulation of transcription by RNA polymerase II* and *DNA binding* (**Figure 6C**). Based on the qPCR results, it was found that the relative expression levels of TP53, CEBPB, NLRC5 and EGR2 genes were higher in the test group than in the control group, while IRF4 expression was decreased (**Figure 6E**), which was consistent with the results of transcriptome analysis (**Figure 6D**). The h-CMV promoter, which regulates EGFP expression in the pIRES2-p53ΔE4p plasmid, was subjected to transcription factor binding site prediction (PROMO database, factors predicted within a dissimilarity margin less or equal than 5%). The results showed that there were 34 possible transcription factor binding sites in the h-CMV promoter, including CEBPB, CEBPA, and p53. Thus, this implies that the h-CMV promoter may be affected by these transcription factors resulting in enhanced transcriptional activity (**Figure 6F**).

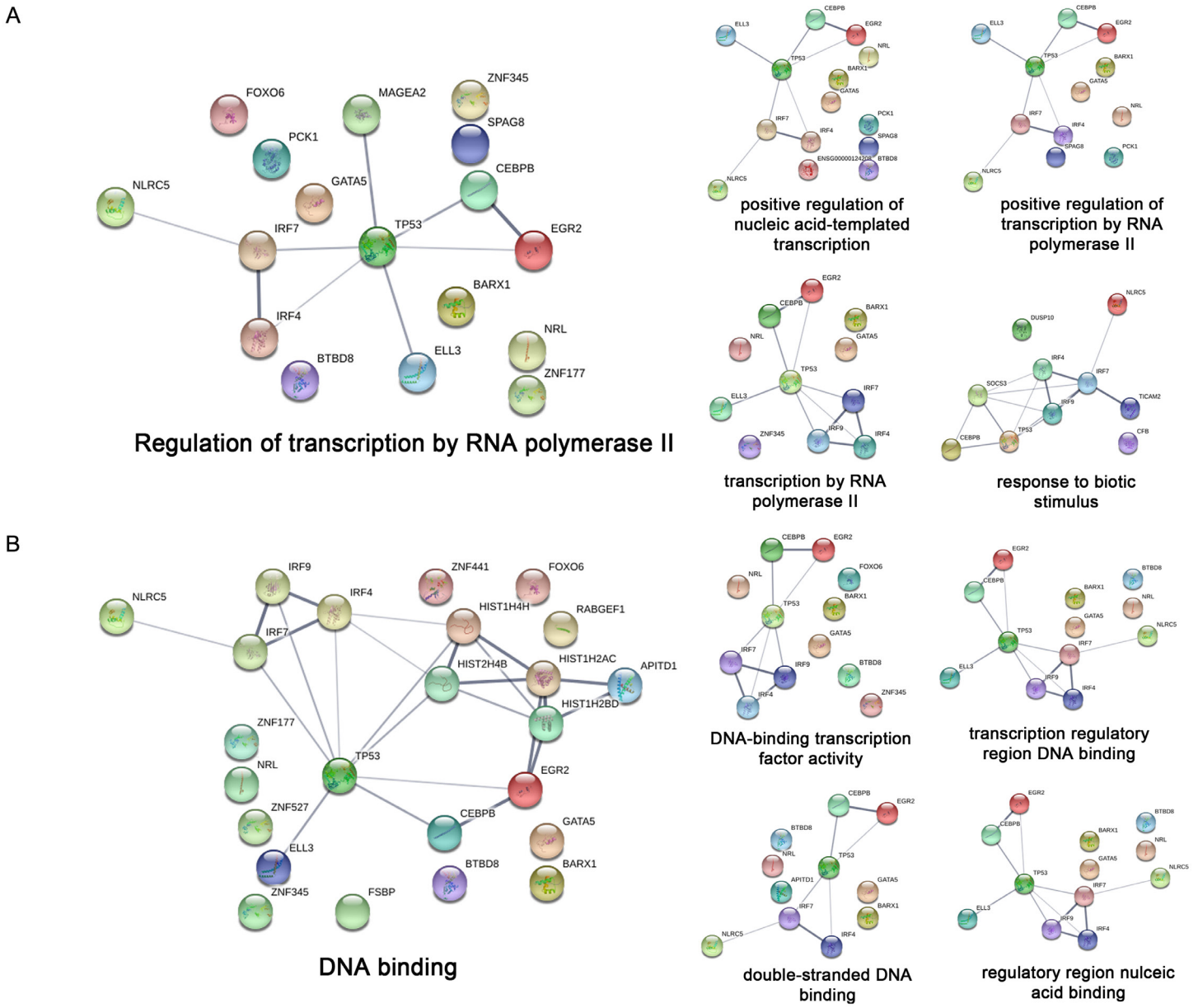
Discussion

The *TP53* gene was first discovered in 1979. Because it is proline rich, the electrophoretic mobility of this protein is retarded in SDS-PAGE experiments and it runs at about 53 kDa. Therefore, it was referred to as p53 [16, 17]. While *TP53* was initially classified as an oncogene, further research revealed that wild-type p53 could inhibit cell growth and prevent the development of cancer by inducing cell cycle arrest or apoptotic programs, and thus, it is now considered an important tumor inhibitor. As a tumor suppressor gene, *TP53* can reduce the cell's ability to proliferate under adverse conditions by responding to various cellular stress signals such as DNA damage, hypoxia, nutrition deprivation, and oncogene expression by promoting apoptosis, cell cycle arrest, aging, and DNA repair. Therefore, *TP53* plays an important role in tumor growth, development, and human health [11, 18, 19].

TP53 encodes several mRNA variants through alternative transcription start sites, promoters, and splicing [20, 21]. Some p53 isoforms may interact with full-length p53 and weaken or enhance its function while others may have independent biologic functions [22-25]. In-depth research has led to the continuous identification of p53 isoforms and their respective functions.

The p53ΔE4p variant identified in this study lacks a 200 bp stretch of exon 4, which leads to a frameshift mutation and premature termination. The main functional domain of p53, the TAD region, regulates gene transcription and is contained within the putative 58 aa of p53ΔE4p [26-28]. However, we found no evidence of the production of a protein product from p53ΔE4p by western blotting with the S46 antibody, which reacts with amino acids 24-53 of human full-length p53. Thus, this suggests that this variant may function as a non-coding RNA.

Although p53ΔE4p does not appear to express a protein product, its transfection does increase the expression of some p53-related proteins (**Figure 2E**). Moreover, p53ΔE4p can also inhibit cell proliferation, cause increased cell death, and induce G1 phase arrest when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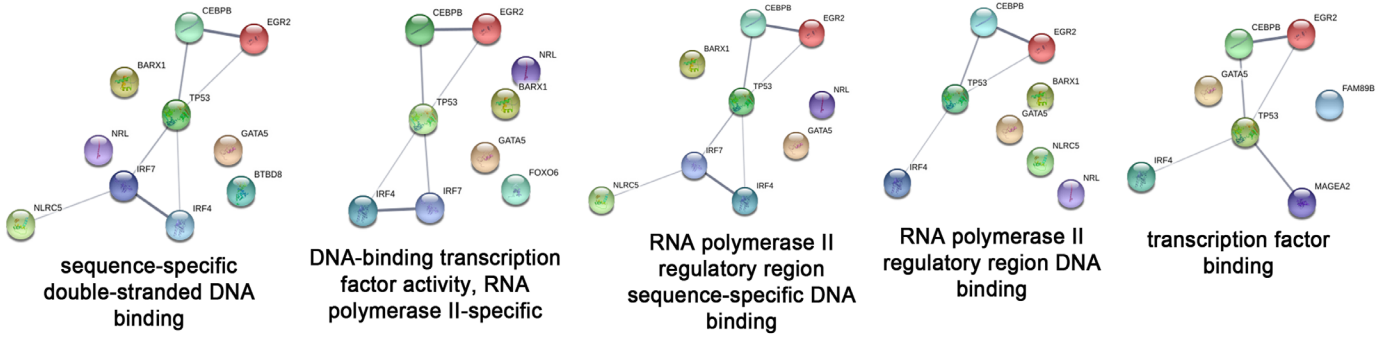
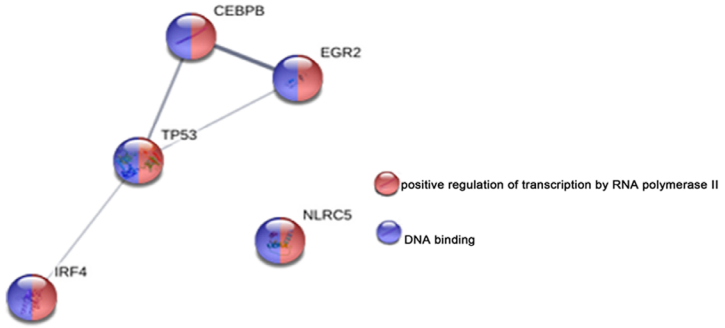
HIST1H2BD
ZNF177
TP53
NRL
EGR2
ZNF527
CEBPB
GATA5
ELL3
BTBD8
BARX1
ZNF345
FSBP
DNA binding
DNA-binding transcription factor activity
transcription regulatory region DNA binding
double-stranded DNA binding
regulatory region nulceic acid binding



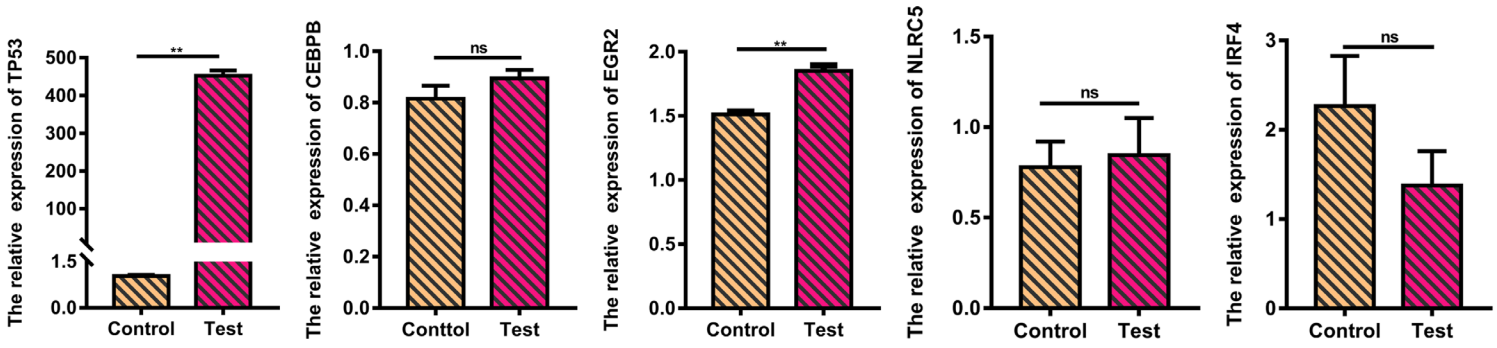
C



D

Gene	TPM	
	Control	Test
TP53	101.52	11534.87
CEBPB	6.96	11.52
EGR2	1.65	2.95
NLRC5	1.24	3.9
IRF4	0.25	0.07

E



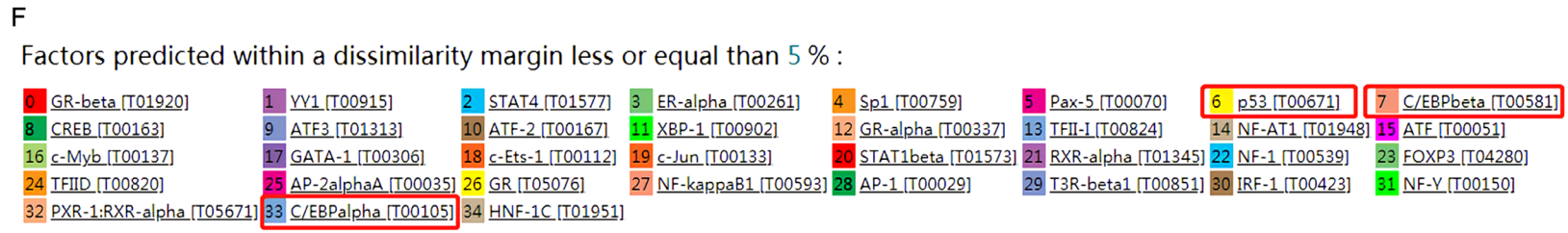


Figure 6. Analysis of several important p53-interacting DEGs. A. String analysis of main GO term and branch GO term of BP (Biological Process). B. String analysis of main GO term and branch GO term of MF (Molecular function). C. String analysis of five key genes (TP53, EGR2, CEBPB, NLRC5, and IRF4). D. Expression analysis of five key genes based on transcriptome data. E. Expression confirmation of five genes by real-time PCR. **: $P < 0.01$, ns: $P > 0.05$, Mean ± SEM, n = 3. F. Prediction of possible transcription factor binding sites within the h-CMV promoter. Test: HEK-293T cells transfected with pIRES2-p53ΔE4p, Control: HEK-293T cells transfected with pIRES2-EGFP.

transfected into HEK-293T cells (**Figure 3**). Interestingly, observation of EGFP by fluorescent microscopy and flow cytometry showed that p53ΔE4p can significantly promote the expression of this exogenous reporter gene regulated by the h-CMV promoter. In addition, according to transcriptome analysis, the expressions of EGR2, CEBPB, NLRC5, IRF4, all related to *DNA binding* and *regulation of transcription by RNA polymerase II*, were found to be influenced by p53ΔE4p (**Figure 6C-E**). Additional studies have shown that there is a close relationship between these five genes. For example, C/EBPB-2, a CEBPB isoform, effectively binds to the p53 promoter and induces its expression [29]. C/EBPB-2 was found to have the largest binding activity on the p53 promoter, and had the largest effect on promoter activity in co-transfection experiments, causing a 15-fold increase in activity of the p53 promoter [30]. In addition, the transcription factors CEBPB and EGR2 promote adipogenesis, while IRF4 has a negative effect on adipogenesis [31-33]. The prediction of the transcription factor binding site for the h-CMV promoter showed a potential for recognition by CEBPB, CEBPA, and p53 (**Figure 6F**). These results suggest that the h-CMV promoter may be affected by the increased transcription factor expression, thus enhancing EGFP expression. **Figure 5G** shows decreased expression of several histones following transfection of p53ΔE4p, which also implies the existence of an active transcription state in the cell. However, excessive protein expression could cause the cell to be overloaded, resulting in the inhibition of cell growth and increase of mortality (**Figure 3**).

In summary, we identified a novel *TP53* splice variant, p53ΔE4p, in a human leukemia T cell line, and found that it can affect the proliferation of p53 wild-type HEK-293T cells, enhance the expression levels of certain proteins, and increase the expression level of EGFP regulated by h-CMV promoter. Furthermore, transcriptome analysis showed that p53ΔE4p may affect some cellular functions, such as *DNA binding*, *regulation of transcription by RNA polymerase II*, and the expression of many unannotated genes. These results lay a foundation for further research on p53ΔE4p, increase the pool of known p53 variants, and provide additional insight into the biologic function of the *TP53* gene.

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Disclosure of conflict of interest

None.

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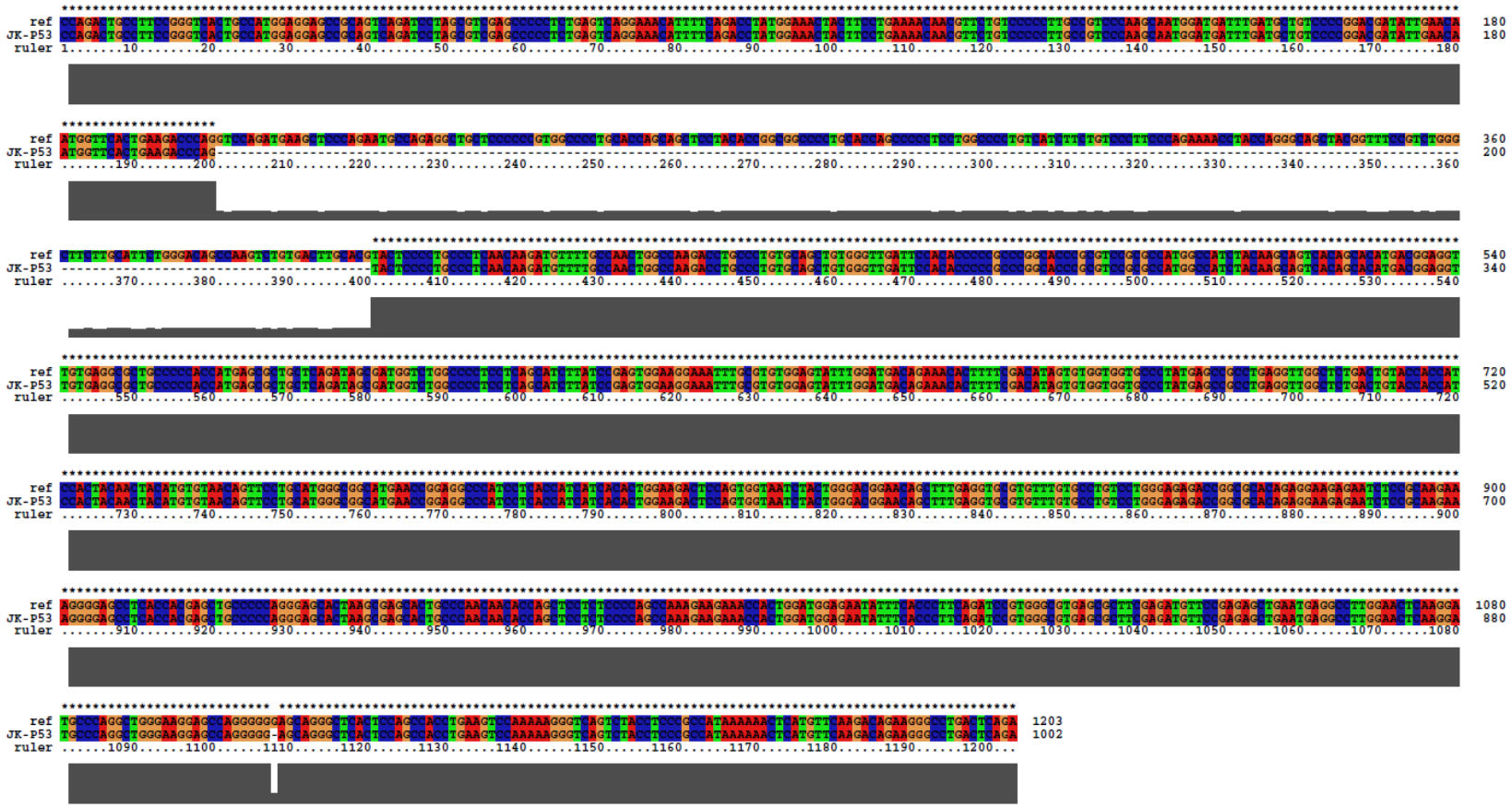

Supplementary Figure 1. p53ΔE4p's sequence in comparison to TP53. Ref: TP53 gene's sequence, JK-p53: p53ΔE4p gene's sequence.