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Identification of the core promoter of STK11 gene and its transcriptional regulation by p53

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Abstract

Peutz-Jeghers syndrome (PJS) is an autosomal dominant disease characterized by mucocutaneous pigmentation and hamartomatous polyps. Most cases of PJS involve the inactivation of germline mutations in the serine/threonine kinase gene *STK11* which is also known as *LKB1*. The function of STK11 was previously linked to the tumor suppressor p53 and was shown to activate the p53 target p21/WAF1. Recently, STK11 was reported to be interacting with p53 physically in the nucleus and it can directly or indirectly phosphorylate p53. Here we characterized the 5′-flanking region of human *STK11* gene and identified a 161-bp fragment with promoter activity. Sequence analysis, mutagenesis and gel shift studies revealed a binding site of Sp1 and p53, which affects the promoter activity. Mutation analyses showed that this fragment was required for p53-mediated transcriptional activation. This transcriptional activation was further confirmed by real-time quantitative RT-PCR and Western blot analysis. Transient transfection of p53 expression plasmid into fetal liver cell lines increased *STK11* mRNA and protein levels. In conclusion, our results reveal a new role for p53 in elevating *STK11* gene expression via a positive feedback pattern.

Keywords: Peutz-Jeghers syndrome; STK11; p53; Promoter transcriptional regulation; Tumor suppressor

1. Introduction

Peutz-Jeghers syndrome (PJS) was firstly described by Johannes Peutz [1] in 1922, and further characterized by Harold Jeghers and his coworkers [2] in the 1940s. This disease is inherited in an autosomal dominant fashion. Patients with PJS develop benign hamartomatous polyps (overgrowth of differentiated tissues), especially in the gastrointestinal tract, as well as marked cutaneous pigmentation of the mucous membranes. Another key feature of PJS is a greatly increased risk of developing malignant tumors in multiple tissues [3–5]. Most cases of PJS involve inactivating germline mutations in the serine/threonine kinase gene *STK11* which is also known as *LKB1* [6,7]. STK11 protein seems to be a master kinase involved in the control of cell cycle arrest, p53-mediated apoptosis, Wnt and TGF-β signaling, Ras-induced transformation, energy metabolism and cell polarity [8]. Studies revealed the expression level of *STK11* changes in different tissues and stages during the individual development, and the expression of *STK11* is precisely controlled. Despite extensive characterization of *STK11* mutations in human cancers and a relatively good understanding of the molecular roles of STK11 in the control of cellular processes, little

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is known about regulation modes of *STK11*. In the present study, we identified and characterized the promoter region of *STK11* gene, and we demonstrated that transcription factors Sp1 and p53 could bind to −1256 to −1252 and −1220 to −1196 regions of *STK11* gene, respectively. Over-expression of p53 resulted in elevated levels of STK11 in L02 cell line (fetal liver cell line, which has endogenous STK11 expression). Our results provided the evidence that p53 plays a significant role in the transcriptional activation of the *STK11* gene.

2. Materials and methods

2.1. Bioinformatics analysis

The sequences of *STK11* transcripts for bioinformatics analysis were searched from GenBank, Ensemble and UCSC.

Human *STK11* CpG island analysis was performed by MethPrimer (http://www.urogene.org/methprimer/index1.html), and transcription start site was determined by Dragon Gene Start Finder (DGSF) at website http://sdmc.lit.org.sg/promoter/dragonGSF1_0/genestart.htm.

STK11 promoter region was predicted by using softwares CONSITE (http://www.phylofoot.org/) and PromoterInspector (http://www.genomatix.de/cgi-bin/eldorado/main) with *STK11* gene sequence of from −4000 to −1 as the target sequence.

2.2. Construction of plasmids and site-directed mutagenesis

The 5′-flanking regions of the *STK11* gene were amplified by PCR from human peripheral blood lymphocyte genomic DNA and cloned into pGL3-Basic vector. There was a *Kpn* I recognition site in the forward primers (F-1943, F-1543, F-1425 and F-1322) and a *Xho* I recognition site in the reverse primers (R-1160, R-1039 and R-918) (Table 1). The fragments with site-directed mutations were produced by PCR with primers (sp1-mut1 and p53-mut) (Table 1) using "megaprimer" method [9]. The plasmid pcDNA3.1-myc-his-B(-)-p53 was generated by subcloning p53 from pGEM-p53 (provided by Zhang Yakun in the National Lab of Medical Gennetics of China) into pcDNA3.1-myc-his-B(-) (Invitrogen). All constructs were sequenced on the ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems).

2.3. Cell culture, transient transfection and luciferase reporter assays

HeLa cells without endogenous STK11 [10] and L02 cells, a fetal liver cell line with endogenous STK11 from CCTCC, were grown in DMEM supplemented with 10% newborn calf serum, 2 mM L-glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37 °C in a 5% CO_2 incubator. Cells were seeded in 24-well cell culture plates and transfected at 80% confluence using Lipofectamine 2000 Transfection Reagent (Invitrogen) according to the manufacturer's instruction. Thirty-six hours after transfection, the cells were lysed and the luciferase activity of the cells was measured using the Dual-Luciferase Assay System (Promega). The assay was repeated three times.

2.4. Electrophoretic gel mobility shift assays

The oligonucleotides containing wild type or mutated putative transcription factor binding sequences (Sp1-mut1, Sp1-mut1-mut2 and p53-mut) (Table 1) were synthesized. The forward and reverse oligonucleotides were combined and heated to 95 °C for 10 min and then cooled

Table 1
Sequences of primers for PCR and the oligonucleotides for EMSA

Primer or oligonucleotides	Sequences(5′–3′)	Purpose
F-1943	GGggtaccACTGGGGAGGCTGAGGCAGGAG	Plasmid construction
F-1543	GGggtaccGCTCCTTCCAGGTCCGCAAG	Plasmid construction
F-1425	GGggtaccTCACAGCCGTGGCCTCGTCTC	Plasmid construction
F-1322	GGggtaccCCCAAGCGCCGACCAATCGC	Plasmid construction
R-1160	CCGctcgag GGACACCGCACGCCC	Plasmid construction
R-1039	CCGctcgagCACCCGCGCTCCCTTC	Plasmid construction
R-918	CCGctcgagGGGTTCCCGAAGGTGCCG	Plasmid construction
Sp1-mut1	TCAGCGGCGTCAGGGCGGGCAGAG	Plasmid construction and EMSA
Sp1-mut1-mut2	TCAGCGGCGTCAGAGCGTGCAGAG	EMSA
p53-mut	AACGGGTGGGCGCATCGTCCTCGC	Plasmid construction and EMSA
Sp1-consensus	ATTCGATCGGGGCGGGGCGAGC	EMSA
Sp1-wt	TCAGCGGCGGCGGGGCGGGCAGAG	EMSA
P53-consensus	GAACATGTCCGGACATGTTC	EMSA
P53-wt	AACGGGTGGGCACGTCGTCCTCGC	Plasmid construction and EMSA
STK11-F	CTGAGTACGAACCGGCCAA	Real-time quantitative PCR
STK11-R	CTACGGCACCACAGTCATG	Real-time quantitative PCR
Beta-actin-F	AGCACAGCCTGGATAGCAAC	Real-time quantitative PCR
Beta-actin-R	AATCTGGCACCACACCTTCT	Real-time quantitative PCR

Lowercase letters are the restriction endonuclease recognition sites, underlined are the bases which were site-directed mutated.

slowly to room temperature. Double-stranded oligonucleotides were end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase and purified with the Microspin G-25 columns (Amersham Pharmacia). Binding reactions (15 μl) contained 5 pmol labeled probe, 10 mM Hepes (pH 8.0), 60 mM KCl, 4 mM $MgCl_2$, 0.1 mM EDTA, 100 μg/ml BSA, 0.25 mM DTT, 2.5% glycerol and 5 μg nuclear protein extract. The reaction mixture was incubated at 25 °C for 45 min, then resolved on 4% non-denaturated polyacrylamide gels. Gels were dried and exposed to Kodak X-ray films at −80 °C, and signals were detected by autoradiography. Competition reactions were performed by preincubating the nuclear protein extract with 50-fold molar excess of unlabeled competitor DNA prior to addition of the radioactive probe. The super-shift assay was performed by addition of a monoclonal antibody against p53 (2.0 μg) (BD Bioscience).

2.5. Real-time quantitative RT-PCR

Real-time quantitative RT-PCR was conducted to quantify the expression level of *STK11* gene. Total RNA was isolated from L02 cells 24 h or 36 h after transfection with 5 μg of either empty vector or p53 expression plasmid. All RNA samples were treated with DNase I for 30 min at 37 °C to remove any contamination of DNA prior to RT-PCR. Total RNA was reverse transcibed to cDNA by using Reverse Transcriptase Kit (Promega, USA). Beta-actin was used as the internal control to normalize template loading quantity. Real-time quantitative RT–PCR was performed in triplicate using the ABI PRISM 7900HT Detection System (Applied Biosystems, USA). The primers used (STK11-F, STK11-R, Beta-actin-F and Beta-actin-R) are listed in Table 1. Each reaction contained 1 μl cDNA, 15 μl SYBR Green I PCR Master Mix (Applied Biosystems, 430447), 900 nmol forward primer, 900 nmol reverse primer, in a final volume of 30 μl. This mixture was distributed to 10 μl/well. The thermal cycle used was 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 45 s at 60 °C. Relative quantification of gene expression was calculated using the method described in Ref. [11]. All results were confirmed by three independent experiments.

2.6. Immunoblotting

Total protein was isolated from L02 cells at 24 h or 36 h after transfection of pcDNA3.1-myc-his-B(-)-p53. Cell lysates (10 μg) were heated in SDS sample buffer and subjected to SDS–PAGE and electrotransferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were blocked for 1 h in PBS buffer, which contained 5% skimmed milk and 3% BSA, and then incubated with antibodies against STK11 and Beta-actin in PBS for 1 h at room temperature. Detection of proteins was performed using horseradish peroxidase-conjugated secondary antibodies and the enhanced chemiluminescence reagent.

3. Results

3.1. Bioinformatics analysis

By searching and comparision of nucleotide sequences in GenBank, Ensemble and UCSC, we identified one mRNA sequence (GenBank Accession No. AF217978), with its 5′ end located at −916 bp upstream the translation initiation codon. Moreover, the promoter region of human *STK11* was predicted to be from −1403 bp to −540 bp by Promoter Inspector software or from −1680 bp to −540 bp by CONSITE software. The analyses suggested that the promoter region of this gene is most likely located in the region from −1680 to −540. According to the bioinformatics analysis, we constructed six candidate promoters with different deletions (Fig. 1).

3.2. Identification of transcriptional regulatory region of *STK11* gene

To study the transcriptional regulation of *STK11*, we amplified the six fragments with different lengths of the 5′ flanking region of *STK11* gene and subcloned them individually into a promoterless luciferase plasmid, pGL3-Basic (Fig. 1). These constructed plasmids were transfected

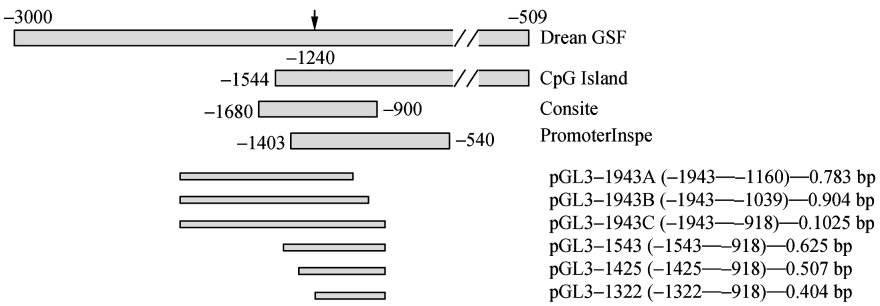

Fig. 1. CpG island in *STK11* gene and gene transcription start site. The CpG island region was predicted to be from −1544 bp to +509 bp and transcription start site was determined at −1240 bp (arrow). The promoter region of human *STK11* was predicted from −1403 bp to −540 bp by Promoter Inspector software or from −1680 bp to −540 bp by CONSITE software. The translation initiation site was assigned as +1 for numbering. The six fragments with different lengths of the 5′-flanking region of the STK11 gene are indicated.

into HeLa cells and L02 cells, and the luciferase activity in the cells driven by different *STK11* fragments was assayed. HeLa cells and L02 cells have similar luciferase activity level (Fig. 2). The cells transfected with the longest *STK11* gene 5′-flanking region (1025 bp fragment) had approximately 140-fold luciferase activity compared with the negative control of HeLa cells and 150-fold for L02 cells. In order to find the *STK11* promoter region, six fragments with different deletions were transferred into the above two cell lines and it was found that different deletions at both ends of the 1025 bp fragment either increased or decreased luciferase activity, suggesting the existence of both *cis*-acting and *trans*-acting elements in the region from −1943 bp to −918 bp. When the 3′ end of the fragment was kept unchanged at −918 bp, deletion from −1943 bp to −1425 bp at the 5′ end had no obvious effect on promoter activity, while, when the 5′ ends were kept at −1943 bp, deletion from −918 bp to −1160 bp remarkably increased the promoter activity (promoter activity is in an order of pGL3-1943A>pGL3-1943B>pGL3-1943C), suggesting that the 3′ end of the promoter was located at 1160 bp. Luciferase activity driven by the pGL3-1322 fragment was about 15-fold and 25-fold of the basic control in HeLa cells and in L02 cells, respectively, suggesting that the 3′end of the promoter was located at −1322 bp. Consequently, the core sequence of the promoter was localized to the region from −1322 bp to −1160 bp.

3.3. The STK11 gene promoter contains Sp1 and p53 binding sites

The sequence shown in Fig. 1 covers the partial 5′-flanking region of the human *STK11* gene. To identify potential transcription factor binding sites in the *STK11* promoter region, we first analyzed this sequence using MatInspector2.2 software (Genomatrix). We focused our analysis on the region with basic promoter activities. Prediction analysis revealed that this region possesses two Sp1 and one p53 binding sites (Fig. 3).

To investigate the binding of Sp1 and p53 to the promoter region, we performed gel shift assays. For Sp1, a double-stranded oligonucleotide sequence corresponding to the *STK11* promoter region from −1264 bp to −124 bp was synthesized and labeled as the probe. As shown in Fig. 4a (lane 3), a protein–DNA complex was detected after incubation of the probe with nuclear protein extract of HeLa cells. A classic Sp1 binding oligonucleotide probe (Sp1-consensus) was also shown to form a protein–DNA complex that migrated at the same position as the putative Sp1 wild type probe (Sp1-wt) which was based on *STK11* promoter sequence (Fig. 4a, lane 2). This probe–protein complex formation was dramatically competitively inhibited by Sp1-consensus (Fig. 4a, lane 4) and Sp1-wt (Fig. 4a, lane 6). In contrast, this probe–protein complex formation was not affected by competition from

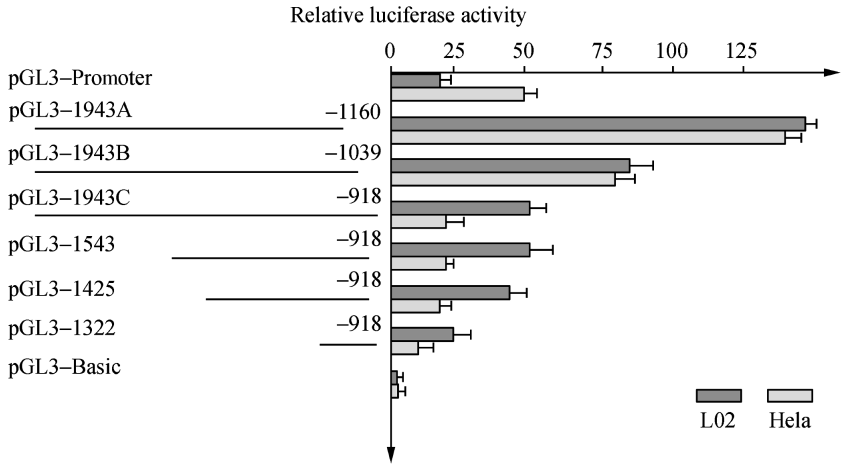

Fig. 2. Luciferase activity in cells measured 36 h after transient transfection with a luminometer. *Renilla* luciferase activity expressed by phRL-SV40 was used to normalize the transfection efficiency. The values represent means ± standard errors ($n \geqq 3$). These data showed that every fragment had the promoter activity both in HeLa cells and in L02 cells.

Fig. 3. Potential transcription factor binding sites in the *STK11* promoter region. The potential transcription factor binding sites were framed and the second Sp1 binding site (Sp1-2) was underlined.

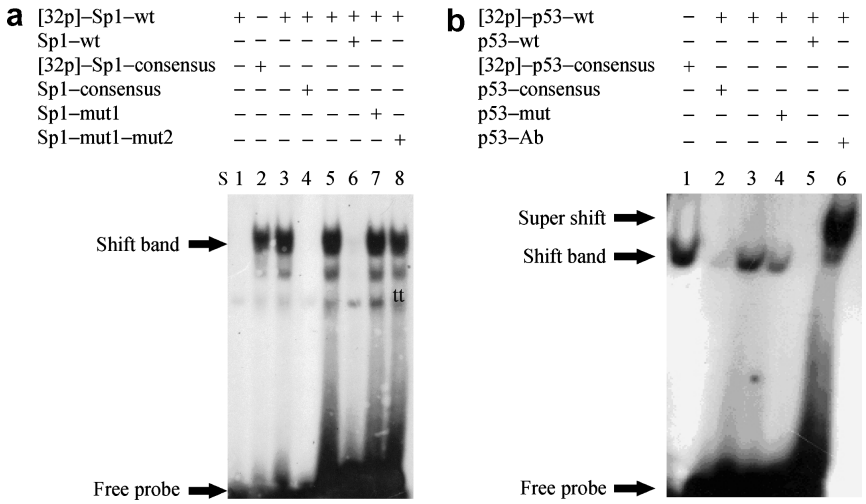

Fig. 4. Electrophoretic mobility shift assay using the putative Sp1 or p53 binding site sequences as the probes. **(a)** [^{32}p]-Sp1-wt and Sp1-wt stand for ^{32}p -labeled and non-labeled putative Sp1 wild type probes, respectively, which were based on *STK11* promoter sequence. [^{32}p]-Sp1-consensus and Sp1-consensus stand for ^{32}p-labeled and non-labeled Sp1-consensus, respectively. Sp1-1-mut1 is the mutant probe containing mutation in the first putative Sp1 binding site: Sp1-1-mut1-mut2 is the mutant probe containing mutations in both putative first Sp1 binding site and the second putative Sp1 binding site. Lanes 2 and 3 show both [^{32}p]-Sp1-consensus and [^{32}p]-Sp1-wt could form the protein–DNA complex with nuclear protein extract. Lanes 4 and 6 show that the protein–DNA complex was dramatically competitively inhibited by the Sp1 consensus and the putative Sp1 binding sequence existing in *STK11*. Lanes 5, 7 and 8 show the probe–protein complex formation was not affected by competition from an AP2 consensus probe and mutant probes Sp1-mut1 and Sp1-mut1-mut2. **(b)** [^{32}p]-p53-wt and p53-wt stand for ^{32}p -labeled and non-labeled putative p53 wild type probe, respectively, which were based on *STK11* promoter sequence. [^{32}p]-p53-consensus and p53-consensus stand for ^{32}p-labeled and non-labeled p53-consensus, respectively. p53-mut is the mutant probe containing mutation in the putative p53 binding site, and p53-Ab is the antibody of p53. Lanes 1 and 3 show both [^{32}p]-p53-consensus and [^{32}p]-p53-wt could form the protein–DNA complex with nuclear protein extract. Lanes 2 and 5 show the protein–DNA complex was dramatically competitively inhibited by p53-consensus and p53-wt, respectively. Lane 4 shows the probe–protein complex formation was not affected by p53-mut. Lane 6 shows a super-shift band of DNA–protein–antibody complexes was formated by [^{32}p]-p531-wt, p53-Ab and nuclear protein extract.

an AP2 consensus probe (Fig. 4a, lane 5) and mutant probes (Sp1-mut1 and Sp1-mut1-mut2) (Fig. 4a, lane 7; lane 8), respectively. For p53, a double-stranded oligonucleotide sequence corresponding to the *STK11* promoter region from −1217 bp to −1194 bp was synthesized and labeled as the probe (p53-wt). A protein–DNA complex was detected after incubation of the probe with HeLa nuclear protein extract that from the cells transfected with p53 expression plasmids. A classic p53 binding oligoncleotide probe (p53-consensus) was also shown to form a protein–DNA complex that migrated at the same position of putative p53 wild type probe (p53-wt). This complex formation was dramatically competitively inhibited by p53-consensus (Fig. 4b, lane 2) and p53-wt (Fig. 4b, lane 5). In contrast, this probe–protein complex formation was not affected by competition from a mutant probe (Fig. 4b, lane 4). A super-shift bind of the DNA–protein–antibody complexes was observed by the addition of p53 monoclonal antibody (2.0 μg, BD) (Fig. 4b, lane 6). Together, these results indicate that the identified Sp1-1 and p53 binding elements are able to form a complex with nuclear Sp1 and p53 protein *in vitro*.

To further define how the potential p53 transcription factor affects the *STK11* promoter activity, we mutated these potential transcriptional factor binding sites individually. The mutated fragments were cloned into pGL3-Basic luciferase reporter vector, followed by promoter activity analysis. Mutations of the Sp1 sites had little effect on the promoter activity (Fig. 5), while mutation of the p53 site resulted in a dramatic decrease of the luciferase activity (6.8% of the wild type control).

These results suggest that Sp1 and p53 are potential transcriptional factors regulating *STK11* transcription. Sp1 plays a role in the basal transcription of *STK11*, and p53 plays a significant role in the transcriptional activation of the *STK11* gene.

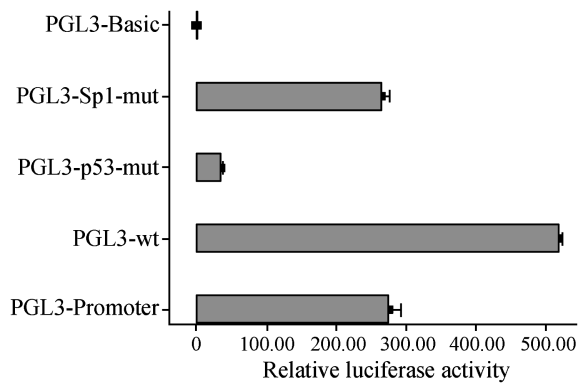

Fig. 5. Relative luciferase activity in HeLa cells transfected with Sp1 and p53 mutated fragments. Mutations of the Sp1 site had little effect on the promoter activity (52.1% of the wild type control). Mutation of the p53 site resulted in a dramatic decrease of the luciferase activity (6.8% of the wild type control).

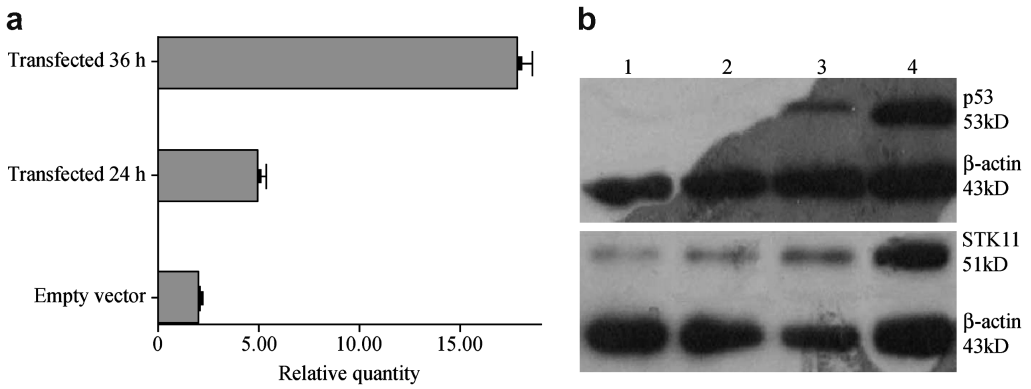

Fig. 6. Effects of p53 over-expression on the expression of *STK11* in L02 cells. (a) Real-time quantitative PCR revealed that STK11 mRNA was increased 2.27-fold of that of empty expression vector at 24 h after the plasmid pcDNA3.1-myc-his-B(-)-p53 transfection. After 36 h of transfection, STK11 mRNA was remarkably increased (about 7.23-fold than that of empty expression vector). (b) Western blot analysis of STK11 protein expressed in L02 cells. Lane 1, without pcDNA3.1-myc-his-B(-)-p53 transfection; lane 2, empty expression vector (pcDNA3.1-myc-his-B(-)) transfection; lane 3, 24 h after the pcDNA 3.1-myc-his-B(-)-p53 transfection; lane 4, 36 h after the pcDNA 3.1-myc-his-B(-)-p53 transfection.

3.4. STK11 gene mRNA and protein are elevated in response to p53 over-expression

To further examine the effects of p53 on endogenous *STK11* expression, we constructed the p53 expression plasmid. Transient transfection was carried out using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. Effects of p53 over-expression on the relative expression of *STK11* in L02 cells were analyzed by real-time quantitative RT-PCR. *STK11* expression was increased about 2.27-fold of that in the cells transfected with empty vector at 24 h. At 36 h, *STK11* mRNA was remarkably increased, which was about 7.23-fold of that in cells transfected with empty vector (Fig. 6a). These results were further confirmed by Western blotting (see Fig. 6b). STK11 protein level in the L02 cells increased after transient transfection of p53 expression plasmid, implying that p53 could regulate *STK11* gene expression at the transcriptional level.

4. Discussion

Over the past years, STK11 has shown an aptitude for multitasking. The first STK11 binding partner Brg1 was identified six years ago [12]. More recent evidence showed that STK11 is involved in Wnt signaling [13,14], cell polarity [15,16], cell cycle arrest [17], chromatin remodeling and energy metabolism [18–20], all of which may require the tumor suppressor function of this kinase and/or its catalytic activity. The biological networking capability of STK11 is palpable. There are probably additional pathways, yet to be described, in which STK11 is involved through protein–protein interactions and/or through *trans*-phosphorylation events. Although we have a relatively good understanding of the molecular roles of STK11 in the control of cellular processes, little is known about how *STK11*'s transcription is regulated. Discovery of its regulatory network is very important. Therefore, in this study we identified a 161-bp fragment upstream of the putative translational start codon as a critical *STK11* promoter region, in which several potential transcriptional factor binding sites were characterized. However, only a p53 binding site was shown to be essential for *STK11* transcription regulation, namely mutation of this p53 site resulted in abolishment of the 265-bp fragment's transcriptional activity (6.8% of the wild type). Furthermore, electrophoretic mobility shift assays demonstrated the binding of p53 to the *STK11* promoter *in vitro*. In addition, over-expression of p53 by transient transfection significantly increased both mRNA and protein expression levels of *STK11*. Together, these results demonstrated that expression of *STK11* is regulated by p53 transcriptional factor.

Previous data have linked STK11 and p53: STK11 and p53 physically associate in the cells; disruptions of STK11 in mouse embryonic fibroblasts reduce p53 levels [21]. It has also been reported that a p53–STK11 complex enhances the p53-mediated program for apoptosis [22]. Recently, a knock-out mouse study showed that *STK11* $\pm$ /p53 $\pm$ mice display earlier tumor formation, increased tumor incidence, and a dramatically reduced life span compared with mice with either *STK11* or p53 single gene knock-out, suggesting cooperation between *STK11* and p53 in tumor suppression *in vivo* [23]. STK11 arrests the cell cycle in p53-dependent manner; activation of p21/WAF1 promoter by STK11 requires wild type p53 [17]. Lately it was shown that STK11 physically associates with p53 in the nucleus and directly or indirectly phosphorylates p53 Ser15 and p53 Ser392. Further, these two p53 residues are required for STK11-dependent cell cycle G1 arrest. Chromatin immunoprecipitation analyses showed that STK11 is recruited directly to the p21/WAF1 promoter in a p53-dependent fashion [24].

The rapid signal transduction pathway responds to DNA damage by the activation of p53 and AMP-activated protein kinase (AMPK), which in turn activates TSC2 via phosphorylation. This inactivates RhEB and then mTOR and shuts down translation while turning on autophagy. These events are p53-dependent in a cell

after DNA damage. The dependence on an active AMPK was demonstrated by using an inhibitor of this kinase [25,26]. The STK11 has been shown to be the major upstream kinase of AMPK and directly phosphorylates Thr172 in the activation of the AMPK network, a modification that is absolutely required for AMPK catalytic activity. However, a clear molecular mechanism linking these proteins has been lacking. In the present study, we demonstrated that the p53 transcriptional factor could transactivate the *STK11* gene expression. While no explicit explanations for these perplexing results can be provided at present, we speculate that: First, STK11 is the major upstream kinase of AMPK which is involved in phosphorylating p53 and inducing cell apoptosis via p53 pathway. Second, STK11 directly or indirectly phosphorylates p53 Ser15 (previously shown to be phosphorylated by AMP-dependent kinase). Our results also showed that p53 could transactivate *STK11*, resulting in the elevation of *STK11* transcripts and protein levels in HeLa and L02 cells. Thus STK11 could form a positive feedback loop for p53 signaling network. It was reported that STK11 negatively regulates mTOR signaling pathway [26–28], so, we assume that there is a rapid pathway of DNA damage – p53 activation – STK11–AMPK activation and TOR and its downstream target gene activation. PJS patients have detectable mutations in their *STK11* gene, which leads to loss of the function carried by *STK11* gene and the interruption of the STK11–AMPK–TOR pathway. Thus, differentiated tissues overgrow to develop benign harmartomatous polyps. With more STK11 signaling pathways identified, a more profound understanding of mechanisms that lead to PJS and associated malignancies will give rise to the development of targeted cancer treatments.

In conclusion, we cloned human Peutz-Jeghers syndrome causative gene *STK11* promoter and identified a p53 binding site in *STK11* promoter region. Over-expression of p53 by transient transfection stimulated the transcription and translation of STK11. Hence, we conclude that p53 plays an important role in transcriptional activation of *STK11* gene via the *cis*-element in the core promoter region.

Acknowledgements

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