p53 Induction Prevents Accumulation of Aberrant Transcripts in Cancer Cells

Caroline Moyret-Lalle, Cyril Duriez, Joris Van Kerckhove, Christel Gilbert, Qing Wang, and Alain Puisieux

Département d’Oncologie Fondamentale et Appliquée, INSERM Unité 453, Centre Léon Bérard, 69008 Lyon, France

Abstract

Loss of fidelity of the splicing process occurs during tumor progression and can have a deleterious effect on genes like tumor suppressor genes. It was reported recently that the presence of aberrant transcripts of the TSG101 gene in breast cancer cells was associated with the mutation of the p53 tumor suppressor gene. On the basis of this observation, we have analyzed TSG101 transcript patterns in p53-active and p53-inactive cells. Using several isogenic cellular models, we demonstrate that the induction of p53 in cancer cells leads to a significant decrease of aberrant transcripts levels. This indicates a novel implication of p53 in the regulation of the splicing process.

Introduction

Tumor development is driven by genetic and epigenetic events leading to the alterations of oncogenes and tumor suppressor genes. During the last few years, several reports have demonstrated that carcinogenesis induced a deregulation of the process of alternative splicing (1). Tumor suppressor genes can be affected by such abnormalities, suggesting that splicing defects may participate in the malignant transformation. The TSG101 gene was first identified as a candidate tumor suppressor gene, based on the observation of intragenic deletions in breast cancers (2). These results were denied by later studies that failed to demonstrate any genomic rearrangements of the gene (1, 3). However, it is now clear that TSG101 is a frequent target of splicing defects. Although aberrant transcripts may be found in normal tissues, the frequency of TSG101 splicing abnormalities increases during transformation and are consistently observed in breast, cervix, prostate, liver, and gastrointestinal cancers compared with adjacent normal tissues (2, 4–6). Variant transcripts found in tumors are generally shorter than the wild-type mRNA. They are generated by exon skipping and alternate RNA processing events. Increasing levels of variant transcripts in tumors could reflect a progressive loss of stringent splice control function or an extended alternative splicing during tumor progression. Several studies have reported a correlation between the presence of aberrant transcripts and tumor grades (1, 4, 5, 7). Recently, Turpin et al. (8) have observed, in breast cancers, an association of TSG101 aberrant spliced products to p53 mutation. Overall, these results suggest that an increasing relaxation of TSG101 splicing fidelity occurs during malignant progression. To address the specific question of whether p53 function can interfere with an alternative splicing process, we have analyzed the presence of aberrant TSG101 transcripts in different isogenic cell models differing only in p53 status.

Materials and Methods

Cell Lines and Treatments. Parental cell lines used in these studies were obtained from the American Culture Collection (Rockville, MD). Breast HBL100 and colon HCT116 cancer cells were grown in McCoy’s medium (Life Technologies, Inc.) supplemented with 10% FCS. Breast adenocarcinoma MCF7 and MDA-MB-231 cells and colon carcinoma EB cells were maintained in DMEM (Life Technologies, Inc.) containing 10% FCS. Hepatocarcinoma HepG2 and Hep3B cells were maintained in Eagle’s MEM (Life Technologies, Inc.) containing 10% FCS. Esophageal cancer TE-1 cells were cultured in RPMI 1640 supplemented with 10% FCS. Parental MCF7, HBL100, HepG2, and HCT116 cell lines express an endogenous wild-type p53 gene. MDA-MB-231 and TE-1 cells express a mutant p53, whereas Hep3B exhibits an homozygous deletion of the gene. Cells were plated at 10^6 cells/100-mm dish 24 h before radiation treatment. Cells were exposed to γ-irradiation at 6 Gy (a high energy X-irradiation, 5 or 10 MV, at ~3 Gy/min).

RNA Isolation and RT-PCR. Total RNA was extracted using TRI-Reagent (Sigma Chemical Co.). cDNA was synthesized from 3 μg of total RNA with first-strand cDNA synthesis kit (Amersham Pharmacia Biotech). The reaction mixture was incubated at 37°C for 1 h, and cDNA was stored at −70°C. Nested RT-PCR reactions were carried out using the conditions described by Li et al. (2). P1 (5′-CGGGTGTCCGAGGCGAGCTCAAAGAAA-3′) and P2 (5′-CTCTCAGCTGGATCAGAAGCTGCTTGGTACAGCTGGCTTGG-3′) primers were used for the first PCR round for 30 cycles. Second-round PCR for 25 cycles was performed using two nested primers, P3 (5′-GCCGACCTCAAGAAAATGGTGGCTCAAG-3′) and P4 (5′-TCACTGAGAAGCCGCAGCTTGGTTGGCTTGG-3′). The RT-PCR products were analyzed in 1.5% agarose gel.

cDNA Subcloning and Sequencing. Aberrant PCR products were cut out and purified with the Concert Rapid gel extraction system (Life Technologies, Inc.). Purified cDNAs were cloned in the pGEM-T vector (Promega Corp.) and sequenced using an ABI PRISM 377 DNA sequencer (Perkin-Elmer). Sequencing was carried out in both directions with the P3 and P4 primers.

Results and Discussion

Presence of the Δ154–1054 TSG101 Splice Variant in Cancer Cells. To study the presence of TSG101 splice products in relation with p53 status, we first examined the expression TSG101 transcripts by nested RT-PCR as described by Li et al. (2) in wild-type and mutant p53 cancer cell lines derived from breast (MCF7, HBL100, and MDA-MB-231), colon (HCT116), and liver (HepG2 and Hep3B) tumors. Using primers flanking the TSG101 coding region, the predicted 1192-bp full-length transcript (Fig. 1) was observed in all cell lines after the first round of PCR amplification. After the second round of PCR, in addition to the 1145-bp, normal-sized TSG101 transcript, bands of smaller size were detected in all cell lines (data not shown). An ~250-bp transcript was observed in most cases as a major aberrant RT-PCR product (Figs. 1 and 2). Southern blot analysis of the RT-PCR products, using full-length TSG101 cDNA as a probe, confirmed the authenticity of this variant TSG101 splice product (data not shown). Sequence analysis revealed that this fragment (244 bp) was the most frequent variant TSG101 form identified previously in tumors (1, 9, 10). It corresponds to a deletion of 900 nucleotides (nucleotides 154-1054). The deletion junction contains a donor site-like sequence within the coding exon 1 and an acceptor site-like
sequence within the coding exon 5 (Fig. 1). This deletion encompasses the segment encoding the coiled-coil domain of the TSG101 protein. The resulting aberrant transcript alters the open reading frame and introduces several in-frame termination codons.

Levels of TSG101 Spliced Variants Decrease in Stress-induced p53 Cells. Wild-type p53 (wtp53) is present at extremely low levels in most cells. In response to DNA damage, the half-life of p53 is increased, and the protein accumulates in the nucleus where it can act as a transcriptional factor (11). To determine whether activation of p53 function could be involved in regulation of TSG101 splicing, TSG101 product patterns were studied after X-irradiation, using nested RT-PCR in three breast cancer cell lines (MCF7, HBL100, and MDA-MB-231). As expected, the aberrant TSG101 product Δ154–1054 was amplified alongside the normal transcript in nonirradiated MDA-MB-231. As expected, the aberrant transcript significantly decreased in wtp53 cell lines, whereas it remained high in the mutant p53 cell line (Fig. 2). This observation suggested a p53-dependent mechanism. To assess the specific role of p53, we performed a detailed kinetic analysis of TSG101 transcripts in response to γ-irradiation in MCF7 and HCT116-derived cell lines, exhibiting an inactivation of endogenous p53. MCF7/MDD2 cells were generated by stably transfecting breast adenocarcinoma MCF7 cells with a dominant-negative p53 miniprotein (12, 13). This COOH-terminal segment of p53 forms stable oligomers with endogenous p53, leading to the abrogation of sequence–specific DNA binding. MCF7/MN1 cells were generated from MCF7 cells by transfection of the antibiotic resistance gene alone. Endogenous wtp53 was inactivated in HCT116 colon cancer cells by homologous recombination (14). As it was shown in parental cell lines, exposure to X-rays led to a decrease of the Δ154–1054 truncated transcript levels in p53-active cell lines [MN1 and HCT116 p53(+/+)], whereas levels of the full-length transcript remained unchanged. In contrast, levels of the abnormal transcript were unaffected in the two p53-inactive cell lines [MDD2 and HCT116 p53(−/−); Fig. 3].

Induction of p53 Alone Is Not Sufficient to Down-Regulate the Δ154–1054 TSG101 Truncated Transcript. To further study the regulatory role of p53, we tested the pattern of TSG101 transcripts in three cellular models displaying an inducible p53 activity:

(a) An expression plasmid encoding a temperature-sensitive (ts) version of mutant p53 (murine p53 Val135) under the control of a constitutive promoter was stably transfected into Hep3B, a human hepatocarcinoma cell line that contains no endogenous p53 protein. As described previously, ts-p53 protein is inactive at 39°C but is transcriptionally active upon shift to the permissive temperature of 32°C (15).

(b) The esophageal cancer cell line TE-1 expresses an endogenous...
mutant p53 (amino acid 272, methionine to valine) that functions as a ts-p53 (16, 17).

(c) The third cellular model, EB1, was derived from the human colon carcinoma cell line EB after stable transfection with a construct containing the wild-type p53 cDNA under the control of the metallothionein MT-1 promoter (18).

In all models, p53 induction leads to a cell cycle arrest (data not shown). The Δ154–1054 truncated transcript was detected in addition to the normal transcript in the three noninduced cell lines. Levels of truncated transcripts were not modified after p53 induction (Fig. 4), suggesting that wtp53 expression is necessary but not sufficient to modulate expression of TSG101 aberrant transcripts.

Our results indicate that stress-activated p53 is able to modulate the TSG101 splicing process. The underlying mechanism remains to be elucidated. The lack of change in TSG101 transcripts pattern after experimental induction of p53 (ts-p53; inducible expression) leads to two main remarks: (a) this suggests that p53 effects depend either on specific posttranslational modifications induced by genotoxic treatments or on stress-induced coactivators; and (b) this indicates that the variation of the TSG101 spliced transcript levels is not a secondary consequence of the p53-dependent cell cycle arrest. Consistently, γ-irradiation triggers a similar decrease of aberrant transcript levels in p21Waf1-proficient and -deficient HCT116 cells (data not shown).

Thus, the activation of this cyclin kinase inhibitor, which is a major p53 target gene, is not required for the p53-dependent modulation of splicing process.

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References

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