Functional repair of p53 mutation in colorectal cancer cells using trans-splicing

Supplementary Material

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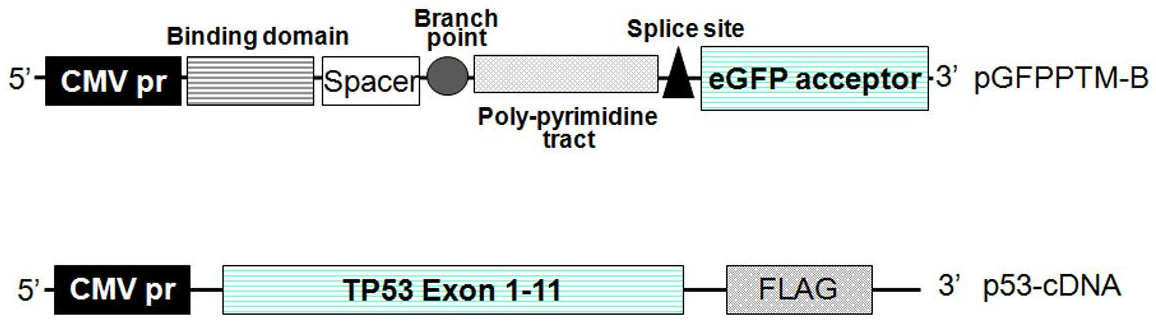

Figure S1: Schematic representation of the plasmids encoding eGFP pre-trans-splicing molecules (PTM) and p53 cDNA. The plasmid (pGFPPTM-B) contained CMV promoter, a binding domain, spacer, branch point, poly-pyrimidine tract, splice acceptor site, eGFP acceptor (GenBank accession # U55763.1 nt 995-1410) and Flag-tag. The binding domain is antisense to intron 7 of p53 pre-mRNA. The plasmid of p53 cDNA contained CMV promoter, p53 cDNA (GenBank accession # NM_000546 nt 198-1379) and Flag-tag, which was used as positive control.

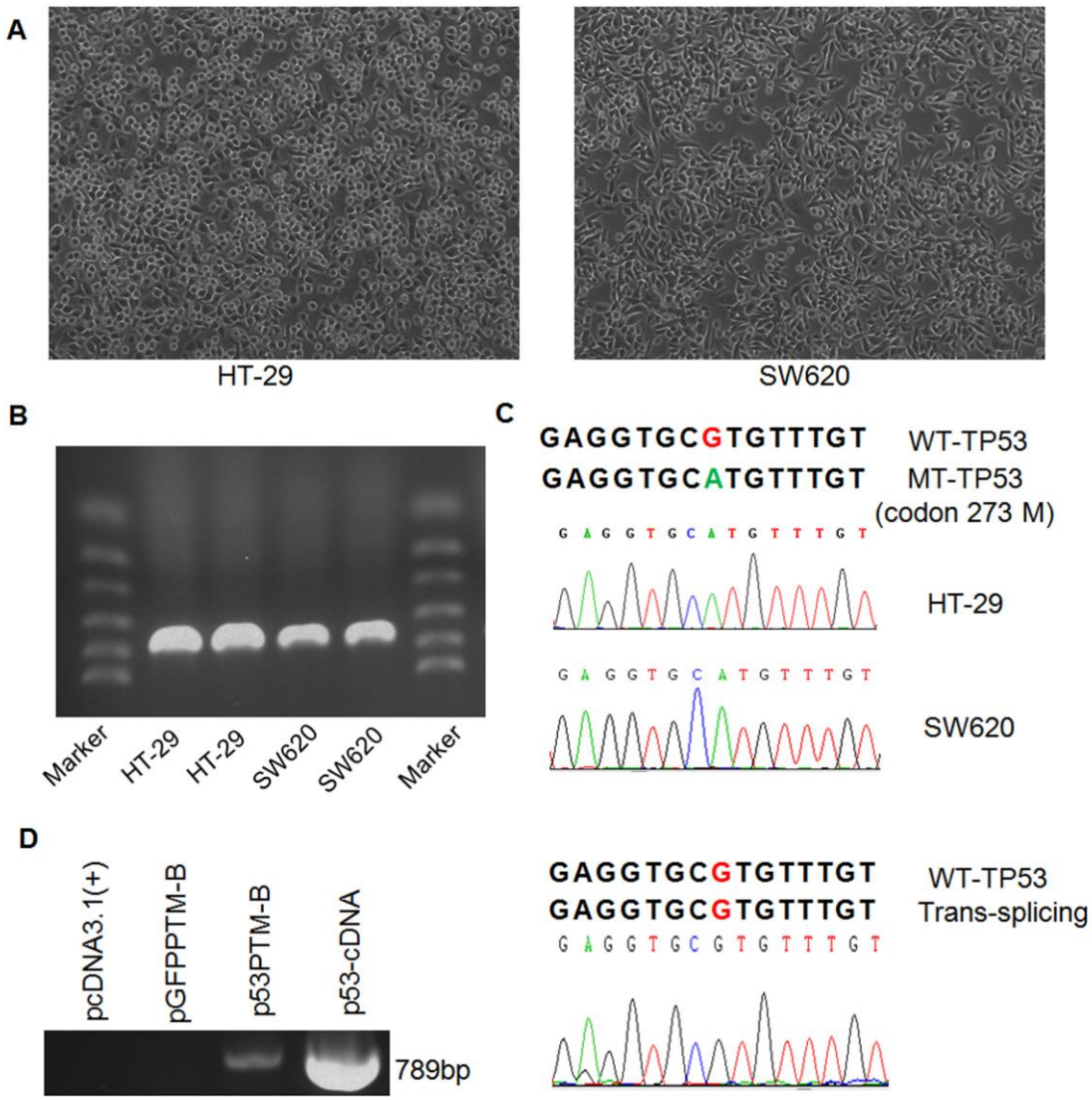

Figure S2: The detection of trans-splicing-mediated repair of mutant p53 transcripts in transfected SW620 cells.

A. The micrographs of colorectal cancer cell lines HT-29 and SW620.

B. RT-PCR (Reverse transcription-polymerase chain reaction) was performed to amplified part of TP53 cDNA on total RNA isolated from colorectal cancer cell lines HT-29 and SW620. The primers used for RT-PCR (TP53-FP and TP53-RP) were listed in Supplementary Table1.

C. DNA sequence results of the RT-PCR products amplified from HT-29 and SW620 cells. The results confirm there is a G to A mutation in codon 273 of the p53 gene resulting in an Arg to His substitution in HT-29 and SW620 cells.

D. Detection of trans-spliced p53 RNA in SW620 cells transfected with p53-PTM-B. The result of RT-PCR showed the generation of trans-spliced p53 RNA in SW620 cells transfected with p53-PTM-B. DNA sequence analysis of trans-spliced p53 RNA confirmed that the mutation in codon 273 of the p53 gene was repaired by trans-splicing.

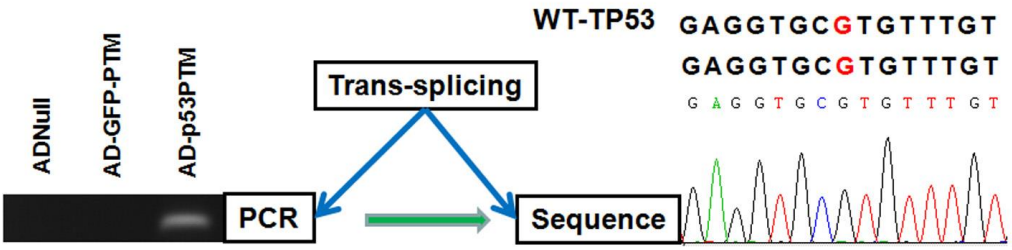

Figure S3: The detection of trans-splicing-mediated repair of mutant p53 transcripts in HT-29 cells infected with adenovirus vector expressing p53-PTM. RT-PCR analysis evaluated trans-spliced p53 transcripts in HT-29 cells infected with Ad-p53-PTM. HT-29 cells were infected with recombinant adenovirus expressing p53-PTM or the controls; 48 hours after infection, RNA isolated from infected cell was subjected to RT-PCR analysis for trans-spliced p53 transcripts. The result demonstrated that trans-spliced p53 mRNA was only detected in HT-29 cells infected with Ad-p53-PTM (left panel). DNA sequence analysis of the RT-PCR product demonstrated mutant site of p53 in codon 273 was repaired (right panel).

Table S1: Primers used for plasmids construct, the detection of trans-splicing and the amplification of p53 cDNA.

Primer names	Purpose	Sequences (5' to 3')
TP53-FP	Amplification of p53 part cDNA used for the detection of p53 mutation	CCCCACCATGAGCGCTGCTC
TP53-RP	Amplification of p53 part cDNA used for the detection of p53 mutation	CGCTCACGCCCACGGATCTG
TP53-S-P	Sequencing primer used for the detection of p53 mutation	ATGAGCCGCCTGAGGTTGG
BD-A-FP	Amplification of binding domain A	CGC GGATCC AACCACCCTTGTCCTTTCTG
BD-A-RP	Amplification of binding domain A	CCG GAATTC ACCCATCCACCTCTCATCACATCC
BD-B-FP	Amplification of binding domain B	CGC GGATCC AGGAAAAGAGAAGCAAGAGGCAGT
BD-B-RP	Amplification of binding domain B	CCG GAATTC TGTGGCTTCTCCTCCACCTACCT
p53-E8-11-FP	Amplification of exon 8-11 of p53	ATAAGAAT GCGGCCGC TCTTCTTTTTTTTGCAGTGGTAATCTACTGGG
p53-E8-11-RP	Amplification of exon 8-11 of p53	CCGCTCGAGCTA**CTTGTCATCGTCGTCCTTGTAGTC**GTCTGAGTCAGGCCCTT
p53-cDNA-FP	Amplification of p53 cDNA	CGCGGATCCGCCACCATGGAGGAGCCGCAG
p53-cDNA-RP	Amplification of p53 cDNA	CCGCTCGAGCTA**CTTGTCATCGTCGTCCTTGTAGTCGTC**TGAGTCAGGCCCTT
PF	Detection of trans-spliced p53 RNA	CAAGACCTGCCCTGTGCAGC
PR	Detection of trans-spliced p53 RNA	ACTTGTCATCGTCGTCCTTG

FP, forward primer; RP, reverse primer. BamHI:GGATCC; EcoRI:GAATTC; NotI:GCGGCCGC; XhoI:CTCGAG;

FLAG: **CTT GTC ATC GTC GTC CTT GTA GTC.**

Table S2: Primers used in SYBR Green qRT-PCR.

Primer names	Sequences (5’ to 3’)
Cyclin A2-FP	CAGACGGCGCTCCAAGAGGA
Cyclin A2-RP	AGGGGTGCAACCCGTCTCG
Cyclin B1-FP	TCCGAGTCACCAGGAACTCGAAAA
Cyclin B1-RP	AGTCCGGGCTTGGAGGTTGC
Cyclin D1-FP	GCGTACCCCGATGCCAACCT
Cyclin D1-RP	CCTCGCAGACCTCCAGCATCC
Cyclin E1-FP	CAGGGGCGTCGCTGATGAAGA
Cyclin E1-RP	TGCTCGGGCTTTGTCCAGCAA
Bcl-2-FP	CGGAGGCTGGGATGCCTTTGT
Bcl-2-RP	AGCTCCCACCAGGGCCAAAC
p21-FP	CACTCAGAGGAGGCGCCATGT
p21-RP	GCCCGCCATTAGCGCATCAC
Bax-FP	GCAAACTGGTGCTCAAGGCCC
Bax-RP	TCTCACCCAACCACCCTGGTCT
PUMA-FP	TGGGACTCCTGCCCTTAC
PUMA-RP	GGCTGGGAGTCCAGTATG
MDM2-FP	GCGCCCCGTGAAGGAAACTG
MDM2-RP	TGCACATTTGCCTGCTCCTCAC
Caspase-3-FP	GGCGGTTGTAGAAGAGTTTCGTGA
Caspase-3-RP	ACCGAAAACCAGAGCGCCGA
PARP1-FP	GATTGCTGTGGCACGGGTCC
PARP1-RP	TAGCCTGTCACGGGCGCTTC
GAPDH-FP	CCCCAGCAAGAGCACAAGAG
GAPDH-RP	GCACAGGGTACTTTATTGATGGTAC

FP, forward primer; RP, reverse primer