

Differential Expression of TAp63 Gene in Normal and Tumor Cell Lines

Dan Han

Department of Biology, Fordham University, Bronx, New York 10458
adahan@fordham.edu

Abstract:

The p53 gene that mediates cellular response to DNA damage, e.g., cell cycle arrest or apoptosis is a well-known anti-oncogene. Based on gene sequence homologies, p63 gene and p73 gene are also identified recently. Transactivating isoforms, such as TAp63 shows TSG properties similar to p53. In this experiment, the expression of TAp63 in two cell lines: HeLa and Wi38 are tested by isoform specific RT-PCR. Higher mRNA level of the TAp63 is detected in HeLa than in Wi38. Sequencing is done to confirm the RT-PCR product. We propose that in HeLa the anti-tumor activity of TAp63 is weakened by some unknown mechanism.

Introduction:

P63 gene, a member of p53 superfamily, located at 3q27. As a homologue of P53, P63 has 6 isoforms (Kaghad *et al.*, 1997; Yang *et al.*, 1998; Benard *et al.*, 2003). The transactivation (TA) isoforms consist of 3 domains: an acidic transactivation N-terminal domain, a central DNA binding domain and a C-terminal oligomerization domain (Figure 1). The DNA binding domains of P63 and P53 share over 60% identical primary amino acid sequences. And they share 25% identity in TA domain. Some P53 target genes including Bax, MDM2 and p21 can be transactivated by P63 TA isoforms (Jost *et al.*, 1997; Yang and Kaghad, 2002).

The ΔN isoforms are produced from an intronic promoter, contain the same DNA binding and C-terminal oligomerization domains as the TA isoforms but lack a transactivation domain. Without the transactivation activity, ΔN isoforms function as a dominant-negative manner, inhibit TAp63 and other p53 family members. Different splicing in C-terminal of both TA and ΔN isoforms yields TAp63 α , β , γ and ΔN p63 α , β , γ 6 isoforms, whose detailed functions are not well understood yet. (van Bokhoven and Brunner, 2002)

P53 tumor suppressor gene properties is due to its DNA binding and transactivation of target genes which specify cell cycle and apoptosis. TAp63 protein, when over-expressed in human cells, also binds to p53 target gene, and induces cell cycle arrest, differentiation and apoptosis in a p53-like manner. (Jacobs *et al.*, 2005; Mills, 2006) And ΔN p63 isoforms, unlike TAp63 isoforms which acts as tumor suppressors, act as oncogenes. (Lee, *et al.*, 2006)

In this study TAp63 expression is tested in normal and tumor cell lines by RT-PCR using isoform-specific primers.

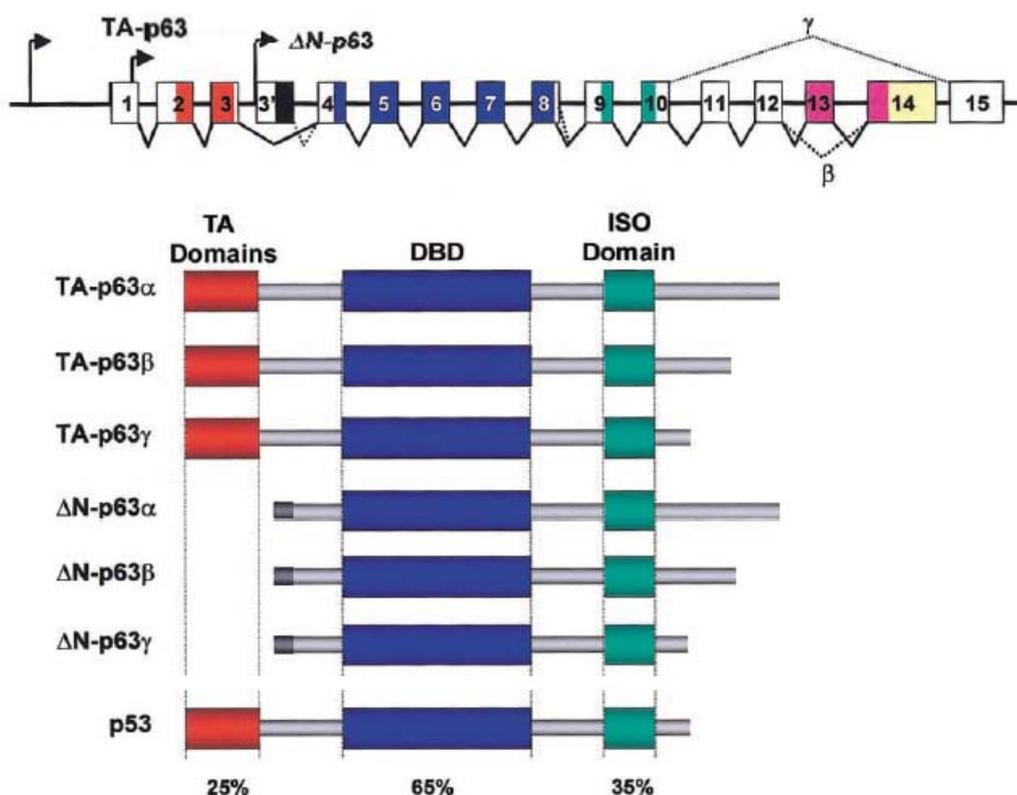


Figure1 Structure of P63 isoforms. TAp63 isoforms and ΔNp63 isoforms uses different transcription initiation sites (arrows) and alternative splicing, to generate different mRNAs. Several protein domains can be distinguished; of these, the TA domains(Transactivation Domain), the DBD(DNA Binding Domain), and the ISO domain (Tetramerization Domain) are highly homologous to the corresponding domains in p53.(van Bokhoven and Brunner, 2002)

Materials and Methods:

RNA samples

RNA from Wi38 (Human lung fibroblast cell line) and Hela (Human epithelial carcinoma cell line) were provided by Jinsong Qiu (Dr. Rubin's lab). Then RNA samples were diluted to 20ng/ul and stored at -80°C

Primers:

Primers specific to TAp63 were designed. The forward primer: 5' tgttcagttcagccattga 3' is located at exon 3 in the transactivation domain; the reverse primer: 5' gctggaaggacacgtcgaa 3' is located at exon 4 in the DNA binding domain. The length of intron 3 is 69k. Predicted RT-PCR product is 349bp .Primers specific to GAPDH were provided by Jinsong Qiu (Dr. Rubin's lab). All primers were diluted to 10pmol/ul.

RT-PCR:

RT-PCR was performed using Qiagen One-step RT-PCR kit. 10ng RNA was used as template in a total volume 15ul reaction. One-step RT-PCR used the following protocol: RT— 50°C for 30min, 95°C for 15mins, PCR reaction—94°C for 30sec, 59°C for 30sec, 72°C for 30sec.

RT-PCR product purification:

The RT-PCR product was purified using Qiagen's PCR purification kit. The concentration of the purified DNA is 25ng/ul

Sequencing:

RT-PCR product was sequenced using TAp63 primers by the Sanger's dideoxy method. The sequencing protocol was: 94°C for 3 min followed by 35 cycles of 94°C for 30sec, 58°C for 30sec, 72°C for 1min followed by a hold period at 4°C. Then 4ul of stop solution was added to each reaction tube. The products were run on a denaturing polyacrylamide gel electrophoresis and the nucleotide sequence was visualized by autoradiography.

Results:

TAp63 mRNA level difference in two cell line:

The mRNA level of TAp63 in two cell lines, HeLa and Wi38, were tested by RT-PCR in this experiment using transactivation isoform specific p63 primers. GAPDH was used as the control (Figure 2). The mRNA level of TAp63 in HeLa was higher than in Wi38, suggesting more expression of this gene in HeLa.

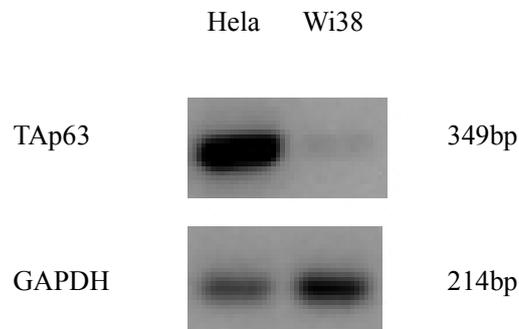


Figure2 RT-PCR analysis of TAp63 expression. The mRNA level of TAp63 in two cell lines, HeLa and Wi38, were tested by RT-PCR in this experiment using transactivation isoform specific p63 primers. GAPDH was used as the control. Compared to Wi38, HeLa have a higher TAp63 expression.

Sequencing:

RT-PCR product was confirmed to be part of TAp63 by sequencing (Figure 3). The DNA sequence of the RT-PCR product was determined by dideoxy method of sequencing. Analyses in Genbank revealed that the 349bp product has greater than 99% homology with part of TAp63 gene (AF075432).

RT-PCR product	1	TGTTTCAGTTCAGCCCATTTGACTTGAACCTTTGTGGATGAACCATCAGAAGATGGTGCGACA	60
AF075432	82	 TGTTTCAGTTCAGCCCATTTGACTTGAACCTTTGTGGATGAACCATCAGAAGATGGTGCGACA	141
RT-PCR product	61	AACAAGATTGAGATTAGCATGGACTGTATCCGCATGCAGGACTCGGACCTGAGTGACCCC	120
AF075432	142	 AACAAGATTGAGATTAGCATGGACTGTATCCGCATGCAGGACTCGGACCTGAGTGACCCC	201
RT-PCR product	121	ATGTGGCCACAGTACACGAACCTGGGGCTCCTGAACAGCATGGACCAGCAGATTCAGAAC	180
AF075432	202	 ATGTGGCCACAGTACACGAACCTGGGGCTCCTGAACAGCATGGACCAGCAGATTCAGAAC	261
RT-PCR product	181	GGCTCCTCGTCCACCAGTCCCTATAACACAGACCACGCGCAGAACAGCGTCACGGCGCCC	240
AF075432	262	 GGCTCCTCGTCCACCAGTCCCTATAACACAGACCACGCGCAGAACAGCGTCACGGCGCCC	321
RT-PCR product	241	TCGCCCTACGCACAGCCAGCTCCACCTTCGATGCTCTCTCTCCATCACCCGCCATCCCC	300
AF075432	322	 TCGCCCTACGCACAGCCAGCTCCACCTTCGATGCTCTCTCTCCATCACCCGCCATCCCC	381
RT-PCR product	301	TCCAACACCGACTACCCAGGCCCGCACAGTTTCGACGTGTCTTCCAGC	349
AF075432	382	 TCCAACACCGACTACCCAGGCCCGCACAGTTTCGACGTGTCTTCCAGC	430

Figure 3 Sequence alignment of the RT-PCR product in reference with the p63 NCBI reference sequence. The DNA sequence of the RT-PCR product, which has greater than 99% homology with part of TAp63 gene (AF075432), was determined by dideoxy method of sequencing.

Discussion:

TAp63, reported as an anti-oncogene in many publications, was expected to have lower expression in tumor cell line than in normal cell line. But in this experiment, the expression of this gene was higher in HeLa (Human epithelial carcinoma cell line) than in Wi38 (Human lung fibroblast cell line). A possible reason for this result is that TAp63 is an anti-oncogene but not necessarily have high level of expression in all normal cell types. In HeLa, the level of TAp63 is higher than in Wi38 but maybe still at a low level which can not give enough anti-tumor activity. Further work may concern about transfection of TAp63 whether can induce cell cycle delay or apoptosis in HeLa.

TAp63 is critical for the development of epithelial tissues, and its high level expression is observed in many kinds of normal epithelial cells (Di Como et al., 2002). That may help to explain the higher level in HeLa (Human epithelial carcinoma cell line), but due to its anti-tumor activity, further work maybe can tell if there is different expression level of TAp63 between normal epithelial cells and HeLa. Also study about TAp63 expression in more types of cells can give a more detailed pattern of TAp63 expression in different cell types.

This work was looking at the mRNA level, but not the protein level. There may be posttranscriptional regulations which can decrease the P63 protein level, or non-functional P63 proteins come from the mutation of the gene in HeLa. Immunology methods, like western blot and sequencing can be used in further study.

Another explanation of the higher expression of TAp63 in tumor cell line is that the Δ Np63 isoforms were also expressed at a high level together with the TA isoforms. Δ Np63 isoforms contain the same DNA binding as the TA isoforms but lack a transactivation domain, so function

as a dominant-negative manner, inhibit TAp63 and other p53 family members. Δ N Isoforms specific RT-PCR may work in further study on this topic.

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References:

1. Benard J, Douc-Rasy S, Ahomadegbe JC. 2003. TP53 family members and human cancers. *Hum Mutat.* Mar; 21(3):182-91.
2. Di Como CJ, Urist MJ, Babayan I, Drobniak M, Hedvat CV, Teruya-Feldstein J, Pohar K, Hoos A, Cordon-Cardo C. 2002. p63 expression profiles in human normal and tumor tissues. *Clin Cancer Res.* 8(2):494-501.
3. Jacobs WB, Govoni G, Ho D, Atwal JK, Barnabe-Heider F, Keyes WM, Mills AA, Miller FD, Kaplan DR. 2005. p63 is an essential proapoptotic protein during neural development. *Neuron.* 8;48(5):743-56.
4. Jost CA, Marin MC, Kaelin WG. 1997. P73 is a simian (correction of human) p53-related protein that can induce apoptosis. *Nature* 389:191–194.
5. Kaghad M, Bonnet H, Yang A, Creancier L, Biscan JC, Valent A, Minty A, Chalon P, Lelias JM, Dumont X, Ferrara P, McKeon F, Caput D. 1997. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell* 90:809–819.
6. Lee HO, Lee JH, Choi E, Seol JY, Yun Y, Lee H. 2006. A dominant negative form of p63 inhibits apoptosis in a p53-independent manner. *Biochem Biophys Res Commun.* 26;344(1):166-72. Epub 2006 Apr 17.
7. Mills AA. p63: oncogene or tumor suppressor? 2005. *Curr Opin Genet Dev.* 16(1):38-44. Review.
8. Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dotsch V, Andrews NC, Caput D, McKeon F. 1998. p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating death-inducing and dominant negative activities. *Mol Cell* 2:305–316.
9. Yang A, Kaghad M, Caput D, McKeon F. 2002. On the shoulders of giants: p63, p73 and the rise of p53. *Trends Genet* 18:90–95.
10. Ying HQ, Chang D, Zheng HW, McKeon F, and Xiao ZX. 2005. DNA-binding and

transactivation activities are essential for TAp63 protein degradation. *Mol Cell Biol.* 25(14):6154-64

11. van Bokhoven H, Brunner HG. 2002. Splitting p63. *Am J Hum Genet.* 71(1):1-13.