Indian J.Sci.Res.2(4) : 135-136, 2011

Short Communication

A NOVEL P53 TARGET GENE IDENTIFIED

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ABSTRACT

Relation between cancer and p53 has been reported in various studies since 1980s. Owing to the critical role of p53 in controlling cancer, researchers have been working hard to identify and target p53 genes with the view of designing drugs to suppress tumour growth and yet restore the protein function. In this study we report as a new p53 target gene. A primer has been designed for LOC340109 and its expression was studied on p53 and U2OS-ADR cell lines. U2OS-ADR cells are human osteosarcoma cells bearing wild p53 treated with chemotherapy drug, Adriamycin. RT PCR was conducted and the products were then analysed on 1% agarose gel. LOC340109 over expressed in p53 cells. LOC340109 expressed equally in all the U2OS-ADR cells suggesting that LOC340109 is a p53 target gene but has no effect by Adriamycin treatment. Hereby, form our preliminary identification study, we report LOC340109 as a new p53 target gene.

KEY WORDS: p53, cancer, Adriamycin

Cancer remains to be one of the biggest threats to longevity of mankind till day. Several metabolic factors have been related to cancer. P53 protein which is a tumor suppressor is associated with approximately 50 to 55 percent of human cancers. The p53 protein either prevents or initiates cell death thus acting as a checkpoint in the cell cycle. Role of p53 is critical in cancer as the latter is associated with uncontrolled proliferation of cells. Normally, p53 controls cell division. Any mutation in the p53 protein leads to uncontrolled cell division which may result in tumour formation (Rousseau et al., 2011) Mutations in p53 are observed in about half of cancer cases, making the protein an important target in the development of new cancer therapies. Successful development of effective therapy always revolves around identification of appropriate p53 target genes. In this study we carried out preliminary identification of LOC340109 as a new p53 target gene.

MATERIALS AND METHODS

A primer was designed for LOC340109 gene using software primer5.The sequence is: LOC340109-F295'tgtagcaggaacccctcttg3'; LOC340109-R2635'cgcctggtaaaacaaccagt3'. The designed primers were then synthesized and purchased from Takara (Dalian, China). U2OS are human osteosarcoma cells bearing wild p53.U2OS cells were treated with Adriamycin and collected by trypsination at ohr, 3hr, 6hr, 12hr, 24hr and 48 hr. Later, total RNA was isolated from the U2OS-ADR cells using the Trizol reagent (MRC, USA) according to the manufacturer's instructions (Wu et al., 2003). 5 µL of total RNA was converted to complementary DNA (cDNA) with Revert Aid™ First Strand cDNA Synthesis Kit. An empty Pcdna was used as negative control to compare with p53,p63 and p73 cells. GAPDH was used as positive control, fig 1.

RT-PCR was conducted to check the expression of LOC340109 on p53 cell lines as well as U2OS-ADR cells. PCR conditions maintained are- 2 min at 94° C; 15 sec at 94° C; 15 sec at 60 ° C; 20 sec at 60 ° C; 3 min at 72° C. The products were then analysed on 1% agarose gel.

RESULTS AND DISCUSSION

The results obtained shows that LOC340109 over p53 protein as compared to pcdna, p63 and p73. No change in the expression of LOC340109 has been noticed in U20S-ADR cells between 0hr to 48hr. Thus we conclude reporting two things which are, firstly the over expression of p53 protein by LOC340109 reports LOC340109 to be a novel p53 target gene (fig 2). Secondly, no change in the expression of LOC340109 in U2OS-ADR cell, (fig. 3)

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further confirms that LOC340109 is a p53 target gene, and is unaffected by Adriamycin. U2OS, (wild-type for p53) is capable of undergoing either p53 dependent apoptosis or cell cycle arrest by radiation (Allan and Fried,1999). Since U2OS is found to be insensitive to Adriamycin, other chemotherapy drug may be tried to suppress the expression

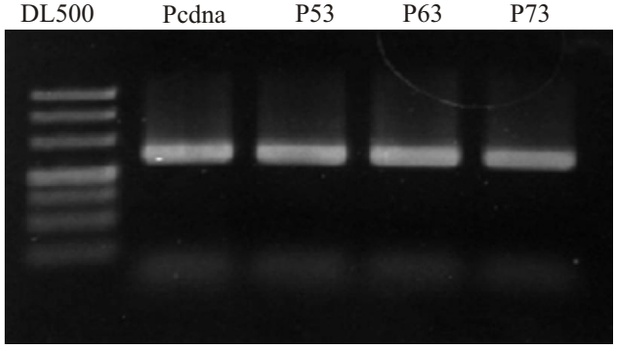

Fig 1: RT PCR analysis of GAPDH on p53 cell line. GAPDH is used as positive control.

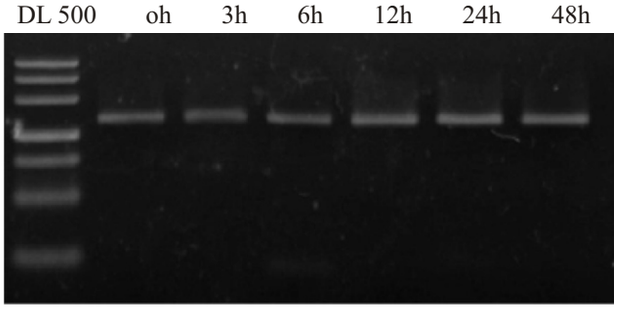

Fig 3: RT analysis of expression of LOC340109 on U2OS cells from 0hr to 48 hr after treatment with adriamycin .

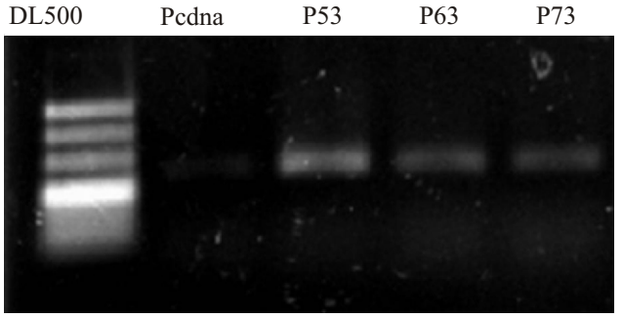

Fig 2: RT PCR analysis of LOC340109 on p53 cell line.

of LOC340109 inU20S cells. For now we suggest that our pilot experiment reports LOC340109 as a p53 target gene. This gene can be targeted to design effective chemotherapy to knockdown its expression in human osteoscarcoma.

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