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The p53 status of Chinese hamster V79 cells frequently used for studies on DNA damage and DNA repair

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ABSTRACT

Chinese hamster lung fibroblast V79 cells have been widely used in studies of DNA damage and DNA repair. Since the p53 gene is involved in normal responses to DNA damage, we have analyzed the molecular genetics and functional status of p53 in V79 cells and primary Chinese hamster embryonic fibroblast (CHEF) cells. The coding product of the p53 gene in CHEF cells was 76 and 75% homologous to human and mouse p53 respectively, and was 95% homologous to the Syrian hamster cells. The V79 p53 sequence contained two point mutations located within a presumed DNA binding domain, as compared with the CHEF cells. Additional immunocytochemical and molecular studies confirmed that the p53 protein in V79 cells was mutated and nonfunctional. Our results indicate that caution should be used in interpreting studies of DNA damage, DNA repair and apoptosis in V79 cells.

INTRODUCTION

The V79 cell has been widely used in studies on X-ray, UV radiation and oxidizing agent induced DNA damage and DNA repair. Over 300 primary studies using V79 cells have been published in the past 20 years (1–7).

The protein product of p53 gene has been suggested to act as 'the guardian of the genome' (8). Evidence suggests that p53 temporarily halts the cell cycle in response to DNA damage to allow time for DNA to be repaired (9). For example, p53 is activated in response to DNA damage, and overexpression of wild type p53 induces a pronounced accumulation of the *mdm2* gene product at mRNA and protein levels (10,11). It has also been suggested that p53 works through Gadd45 and perhaps can directly stimulate the repair machinery as well (12). Another function of the p53 product is to mediate the apoptosis (13,14).

To clarify the role of the p53 tumor suppresser gene product in the response of V79 cells to DNA damage, p53 cDNA of V79 cells was cloned and sequenced. p53 cDNA was also collected from Chinese hamster embryonic fibroblast (CHEF) cells (at early passage P7, known to contain wild type p53) and used as the control. In this report we compare the p53 cDNA sequence of CHEF and V79 cells, check the homology between different species, and define the mutations in V79 cells. Immunohistochemical and RNA dot blot analyses were also used to determine the biological function of p53 in V79 cells. These studies have implications for the interpretation and generalizability of studies of the mutagenicity of DNA damaging agents to V79 cells.

MATERIALS AND METHODS

Cells and cell lines

CHEF/P7 cell was a gift from Dr John Lehman (Albany Medical College). V79 cell at low passage was maintained as previously described (6). The $p53^{-/-}$ human promyelocytic HL-60 cells (15) and mouse embryo fibroblasts (MEF) known to have wild type p53 (16) were generously provided by Dr Robert Carroll (NYUMC).

Immunohistochemical analysis and RNA dot blotting assay of p53 protein

The status of p53 protein in V79 and CHEF cell was analyzed by immunocytochemistry (17) using the p53 monoclonal antibody DO-1 (Santa Cruz Biotechnology) and pAb240 (Oncogene Science). The detection of the *mdm2* mRNA level was performed by the modified RNA dot blotting method described by Kline *et al.* (18). Two oligonucleotides homologous to the hamster *mdm2* cDNA were synthesized (5′-CCAGCTTCGGAACAAGAGAC-3′ and 5′-GGTGGAAGGGGAGGATTCATT-3′) and used as the primers. The PCR product from the two *mdm2* primer sets then was used as the probe. The β-actin primer sets were purchased from CLONTECH and their PCR product was used as an internal control probe. The densitometric analysis was done using the 'NIH Image 1.6 on the Macintosh' program.

cDNA library screening, sequencing and analysis

The constructed cDNA library from each cell line was selected with a PCR amplified probe using the primer set suggested by Legros (19). Sequencing was carried out directly on the positive cDNA clones using method described elsewhere (20). To preclude the possibility that *Taq* polymerase errors might be interpreted as mutations, the p53 coding region from the mutiple cDNA clones

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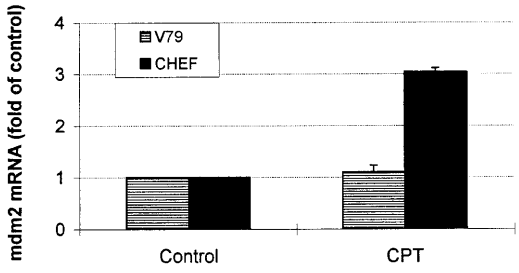

Figure 1. Densitometric analysis of *mdm2* mRNA induction by camptothecin in CHEF cells and V79 cells from RNA dot blotting experiment. The relative amount of *mdm2* mRNA is normalized relative to β-actin mRNA for each condition. The data plotted is the average of three determinations.

was sequenced twice to confirm the mutations in the V79 and CHEF cells. The analysis programs used were 'ALIGN' from EERIE-Nimes, France and 'BCM Multiple sequence alignments program-CLUSTAL-W' at Baylor College of Medicine (21,22).

RESULTS AND DISCUSSION

The V79 cell line has been widely used to study the toxicity, mutagenicity and repair of a wide variety of DNA damaging agents. We have utilized these cells to study the toxicity of the thymidine analogue 5-hydroxymethyl-2′-deoxyuridine (hmdUrd), and to study the repair of hydroxymethyluracil (hmUra) from DNA (5,6). We have found that hmdUrd is able to induce apoptosis in the V79 cells but not in V79*mut1* (hmUra-DNA glycosylase deficient line) cells (23). Since the p53 protein is elevated in response to other types of DNA damage (24), is involved in binding to and reannealing strand breaks (25), and is integrally involved in apoptosis (13), it became necessary to ask whether the p53 gene and its expression were altered in these cells.

First, we evaluated the status of p53 in the V79 and CHEF/P7 cells by immunocytochemistry. V79 cells stained strongly positive with the anti p53 monoclonal antibody PAb-240. PAb-240 recognizes the epitope between AA213 and 217 which is exposed only when p53 is denatured or mutated. PAb-240 did not stain either CHEF cells, HL-60 cells ($p53^{-/-}$) or MEF cells (which have only wild type p53). Although antibody p53 DO-1 recognizes both wild type and mutant type of p53, the strongly positive staining inside the nucleus of V79 cells suggested the p53 protein was mutated in V79 cells. In contrast, CHEF cells showed very weak staining mainly in the cytoplasm, indicating wild type p53. This result was confirmed by Dr Lehman (personal communication) and is consistent with other studies (26).

Next, we cloned and sequenced the complete cDNA from the CHEF and V79 cells. The CHEF sequence contained 2041 bp (GenBank No. Y08900), while the V79 cDNA contained 2073 bp (GenBank No. Y08901). These two cell lines showed 98% homology to each other. Two mutations were found in the coding region of the p53 cDNA (#136 leucine [C<u>T</u>A]→glutamine [C<u>A</u>A]; #138 cysteine [TG<u>C</u>]→tryptophan [TG<u>G</u>]). These mutations, within the p53 DNA binding domain, presumably affect the ability of p53 to bind to DNA and thus regulate gene expression (27). The mutated sites in V79 cells are located within the evolutionary conserved box #2 (27,28) and is within one of the 'hotspots' found in human p53 described by Vogelstein and Kinzler (29). When compared with the Syrian hamster cell (GenBank No. M75144), the Chinese hamster cells showed 91% identity of the cDNA and 95% homology (only 19 amino acid sequences are different) in the coding region. None of these amino acid differences were found in five major conserved domains. When compared with other species, CHEF cells showed 76 and 75% homologous to human (30) and mouse (31,32) respectively.

Since p53 protein is able to activate transcription of the *mdm2* gene and elevates its mRNA level (11), we demonstrated that V79 cells do not have a functional p53 product by showing that these cells failed to induce *mdm2* gene product with camptothecin (CPT) treatment (Fig. 1). The control, CPT treated CHEF cells, generated an obvious induction of *mdm2* mRNA and is consistent with previous studies with normal human fibroblasts lines (11). This finding suggested that two point mutations found in the DNA binding domain of the p53 protein in V79 cells could possibly cause the loss of the ability to bind specific p53-binding sequences and thereby limit the ability of p53 to regulate the expression of other genes (27,29).

Our results therefore indicate that V79 cells, a widely used cell line for studies of DNA repair and DNA damage, do not express normal p53 protein and fail to show induction of *mdm2* gene product response to DNA damage. V79 cells are known to be immortal, have a shortened cell cycle, and are readily mutagenized to make stable mutant lines deficient in DNA repair enzymes and related DNA damage response functions. While these properties have made these cells extremely useful, they also raise questions about the generalizability of results obtained. As a consequence, studies of mutagenesis and related studies of DNA damage and DNA repair in these cells thus must, therefore, be interpreted with caution as a result of the disruption of normal DNA damage response pathways.

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