Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pathology – Research and Practice 201 (2005) 109–115
PATHOLOGY RESEARCH AND PRACTICE
www.elsevier.de/prp

ORIGINAL ARTICLE

Correlation between DNA alterations and p53 and p16 protein expression in cancer cell lines

Yoshihiro Murai*, Shinichi Hayashi, Hiroyuki Takahashi, Koichi Tsuneyama, Yasuo Takano

Department of Pathology, Faculty of Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan

Abstract

To investigate the interaction between DNA abnormalities, p53 and p16 gene mutations, and methylation and protein expression, 20 cancer cell lines were examined by Western blotting. A clear relation was found to exist between p53 accumulation and mutation status. Of 20 cell lines examined, 14 demonstrated p53 homozygous mutations in exons 3–10, including 12 missense mutations, one nonsense mutation, and one frameshift mutation. Overexpression of p53 was always linked to missense mutations in exons 6–8. Intermediate expression of p53 was noted in cells with missense mutations or polymorphism to proline at codon 72 in exons 4–5, whereas there was slight or no visible expression in wild type cells and in cells with nonsense and frameshift mutations. DNA aberration in the p16 promoter gene correlated significantly with protein expression of the p16 suppressor gene. Overexpression was noted in six cell lines, intermediate expression in two, and slight or no visible expression in 12. Methylation-caused disappearance of p16 protein was noted in 40% (8/20) of the cell lines. Of six cell lines overexpressing p16 protein, two could be amplified with primers for both unmethylated and methylated forms in a methylation-specific RCP analysis. One cell line with no visible expression could also be amplified with both primers. Overexpression or disappearance of p16 protein may readily occur when one of two alleles has been methylated.

Keywords: P53; Mutation; P16; Methylation

Introduction

The wild type p53 (wt p53) tumor suppressor is a cell cycle checkpoint regulatory protein [6,11]. It is normally detectable at only low levels, which is partly due to its short half-life [8,9]. However, in response to DNA damage, wt p53 is stabilized and accumulates, leading to cell cycle arrest, allowing for repair of DNA damage or apoptotic cell death [6]. Another regulatory protein of essential significance is p16, encoded by the INK4a tumor suppressor gene on chromosome 9p21, which inactivates the function of cdk4 or cdk6, which normally phosphorylates the retinoblastoma protein [7,16]. This step is necessary for cell cycle progression from G1 into S phase. Therefore, p53 and p16INK4a act in the two main cell-cycle control pathways. Aberrant expression of p53 and/or p16 protein has been reported to occur in various malignancies [2,12], and mutant type p53 (mt p53) frequently accumulates in cancer cells because of its increased stability [10]. The expression of p16 protein, on the other hand, is suppressed by methylation [17,19,20]. The aim of the present study was to assess the association between p53 and p16 expression in a series of cancer cell lines and to evaluate its correlation with DNA aberrations.

*Corresponding author. Tel.: 076 434 2281; fax: 076 434 5016.
E-mail address: ymp@ms.toyama-mpu.ac.jp (Y. Murai).

0344-0338/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.prp.2005.01.002

Materials and methods

Cell lines and DNA

Twenty cancer cell lines (obtained from five lymphomas/leukemias, four stomach cancers, five lung cancers, and six uterine cancers) were examined. Tanoue (B-lineage acute lymphoblastic leukemia), MOLT-4 (T cell leukemia), CCRF-HSB-2 (HSB2; T cell leukemia), BALL-1 (B cell leukemia), four stomach cancer lines (HuG1-N, MKN28, MKN45, and HGC-27), and five lung cancer lines (AOI, RERF-LC-KJ(KJ), PC-14, Lu-139, and MS-1) were purchased from RIKEN Cell Bank (Japan), Raji, HeLa, and the other five uterine cancer (HEC-1A, HEC-1BE, HHUA-1, HOUA-1, and Ishikawa) cell lines were kindly provided by the Departments of Pediatrics, Radiological Sciences, and Gynecology of our university. Genomic DNA was obtained from these cell lines according to an established method.

Western blotting

Cells were homogenized in 10 mM PBS and centrifuged at 14,000 rpm for 30 min. The supernatants were mixed with 2 × SDS sample buffer (125 mM Tris-HCl buffer pH 6.8, containing 4% sodium dodecyl sulfate (SDS), 10% 2-mercaptoethanol, 10% glycerol, and 0.01% bromophenol blue) and then boiled for 5 min. Proteins (15 μg) were electrophoresed on 15% SDS-poly acrylamide gels at 200 V for 50 min and transferred onto 0.45 mm polyvinylidene fluoride membranes (Immobilon-P, Millipore, USA), using a semidry system (Bio-Rad, Japan) at 15 V for 30 min. The membranes were incubated with primary antibodies [p53 (DO7, DAKO, Denmark), p16 (50.1, Santa Cruz Biotechnology, USA), and α-tubulin (DM1A, NeoMarkers, USA)], followed by exposure to horseradish peroxidase-conjugated goat anti-mouse immunoglobulins (DAKO) in 5% skimmed milk. Specific binding was determined with enhanced chemiluminescence (PerkinElmer Life Sciences, USA) on X-ray films (Fuji RX-U, Japan).

Reverse transcriptase polymerase chain reactions and DNA direct sequencing

Total RNA was extracted by the phenol and guanidinium thiocyanate method, and 1 μg of aliquots was dissolved in 20 μl of reaction buffer containing reverse transcriptase (RAV-2; Takara, Japan), random primers, and ribonuclease inhibitor (Takara). After incubation at 42 °C for 1 h, cDNAs were obtained and amplified in a final volume of 100 μl of reaction mixture containing 2U of Taq polymerase, a dNTP mixture (Takara), and primers. Exons 3–10 of the p53 gene were analyzed using the oligonucleotide primers listed in Table 1. The conditions for the polymerase chain reaction (PCR) were as follows: 94 °C for 3-min denaturation, followed by 94 °C for 20 s, and annealing at 64 °C for 20 s for 35 cycles. Amplification was confirmed by 2% agarose gel electrophoresis, and the PCR products were purified with MicroSpin™ Columns (Amersham Pharmacia Biotech, UK) and directly

Table 1. Primer sequences and PCR conditions for p53 and MSP analysis

Gene and primer set	Primer sequence	Product basepair	Anneal temp (°C)
p53			
Exons 2–4	5′-ATGGAGGAGCCGCAGTCAGAT-3′ 5′-CTGTCCCAGAATGCAAGAAG-3′	358	64
Exons 4–6	5′-CTACCAGGGCAGCTACGGTT-3′ 5′-CATCGCTATCTGAGCAGCGCT-3′	254	64
Exons 6–7	5′-CACATGACGGAGGTTGTGAG-3′ 5′-CTGGAGTCTTCCAGTGTGAT-3′	281	64
Exons 7–9	5′-TGCCCTATGAGCCGCCTGAG-3′ 5′-CTGAAGGGTGAAATATTCTCC-3′	341	64
Exons 9–11	5′-GGGAGCCTCACCACGAGCTG-3′ 5′-TCAGTCTGAGTCAGGCCCTT-3′	305	64
p16-M	5′-TTATTAGAGGGTGGGGCGGATCGC-3′ 5′-GACCCCGAACCGCGACCGTAA-3′	150	65
p16-U	5′-TTATTAGAGGGTGGGGTGGATTGT-3′ 5′-CAACCCCAAACCACAACCATAA-3′	151	60

M, methylated-specific primers; U, unmethylated-specific primers.