Report

Comparison of p53 mutational status with mRNA and protein expression in a panel of 24 human breast carcinoma cell lines

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Summary

We analyzed the p53 mutational status, mRNA and protein expression in 24 human breast carcinoma cell lines. Following measurement of their DNA content with flow cytometry, we ascertained the copy numbers of the centromere of chromosome 17 (cen17) and p53 with fluorescence in situ hybridization (FISH). A functional yeast assay (FASAY) was used to screen for inactivating mutations. Positive results were subsequently verified by DNA sequencing. Finally, we assessed the mRNA expression with a competitive reverse transcription-polymerase chain reaction (RT-PCR) assay and the protein expression with immunocytochemical staining, western blot, and quantitative flow cytometry. The DNA content of the cell lines ranged from 0.85 to 2.58. Nine cell lines had concordant copy numbers (between two and four) of p53 and cen17, whereas 12 had more, and three less cen17 than p53 copies. The FASAY was successful in all but one cell line and revealed the presence of mutated alleles in 16 of them, 13 cell lines expressed only the mutated, and three both the mutated and the wild-type alleles. The mutations were comprised of 11 missense, two nonsense, and three frameshift mutations. Immunocytochemical staining, western blot and quantitative flow cytometry yielded comparable p53 protein expression results. However, both the mRNA and the protein expression levels varied considerably in the different cell lines and no consistent pattern with regard to the respective p53 mutational status became evident. The results obtained in these breast carcinoma cell lines indicate that no clear-cut linear relationship exists between the p53 mutational status and the extent of its respective mRNA and protein expression. Therefore, direct DNA analyses and functional assays remain the only methods for the reliable detection of p53 mutations.

Introduction

The tumor suppressor gene p53, that is located on the short arm of chromosome 17, plays an important role in the control of the cell cycle, DNA repair, and activation of apoptosis [1]. It encodes a nuclear phosphoprotein that functions as a transcriptional regulator through sequence specific DNA binding [2]. In response to DNA damage, cells with wild-type p53 genes exhibit a rapid increase in p53 protein levels and a correlated temporarily G1 arrest [3]. This G1 arrest provides the time necessary for repair processes to take place before the DNA is replicated in the S phase [4]. In case proper DNA repair is not possible, p53 drives the cell into apoptosis [1]. p53 gene mutations are amongst the most common genetic abnormalities described in human cancer in general and in human breast cancer in particular [5]. Their central role in carcinogenesis derives from the fact that they alter the control of the cell
cycle that results in an increased genetic instability [4]. In addition, the impairment of the p53-dependent apoptotic pathway increases the resistance to DNA-damaging agents [6, 7]. Nevertheless, the prognostic significance of such mutations, particularly in breast cancer, is still a matter of dispute. Some authors consider them as an independent, adverse prognostic indicator for overall and disease-free survival [8, 9], whereas others judge their prognostic value as overrated [10]. One explanation for these controversial opinions may lie in the fact that the functional impairments exerted by these mutations seem to differ depending on the particular affected region of the gene [11].

Various studies on p53 status and function in a limited number of breast carcinoma cell lines are available [12]. However, a comprehensive overview describing the p53 status in a large panel of commonly used cell lines is missing. Therefore, we opted to define the p53 mutation patterns and study their consequences on the associated mRNA and protein expression in a panel of 24 breast carcinoma cell lines.

Materials and methods

Cell lines

The breast carcinoma cell lines, MCF-7, DU4475, ZR-75-1, UACC-812, MDA-MB-175-VII, ZR-75-30, UACC-893, MDA-MB-468, MDA-MB-157, MDA-MB-435S, MDA-MB-231, BT-20, BT-474, MDA-MB-436, HS 578.T, BT-549, T-47D, MDA-MB-453, CAMA-1, SK-BR-3, MDA-MB-361, BT-483, MDA-MB-134-IV, and HBL-100, a breast epithelial cell line derived from the milk of a nursing mother were obtained from the American Type Culture Collection (Rockville, MD, USA). HBL-100 has been reported to become increasingly tumorigenic at higher passages and should not be considered normal [13, 14]. All cell lines were cultured according to the supplier’s instructions.

DNA index analysis

Cryopreserved cells were thawed and stained with the Cycle TEST™ PLUS DNA Reagent Kit (Becton Dickinson, San Jose, CA, USA) according to the manufacturer’s recommendations. Flow cytometric analysis was performed with a fluorescence-activated cell sorter (FACS) Calibur and Cell Quest Software (Becton Dickinson, San Jose, CA, USA).

Fluorescence in situ hybridization (FISH)

Cell cultures were treated with 0.08 µg/ml colcemid (Life Technologies, Paisley, UK) over night and then trypsinized. Cells were harvested, fixed and slides prepared according to standard procedures. Dual-color FISH was performed with probes specific for D17Z1 (cen17) and p53 (17p13.1) (both from ONCOR, Inc., Gaithersburg, MD, USA). Slides were pretreated and hybridized according to the manufacturer’s recommendations. The probes were detected with a sheep-anti-dig-FITC (Roche, Vienna, Austria), rabbit-anti-sheep-FITC (DAKO, Glostrup, Denmark), and mouse-anti-biotin-CY3 (Jackson, Immunoresearch laboratories Inc., West Grove, PA, USA), respectively. After detection, slides were mounted with Vectashield (Vector Laboratories Inc., Burlingame, CA, USA) containing 500 ng/ml DAPI. Slides were analyzed with an Axioplan fluorescence microscope (Zeiss) that was equipped with a cooled CCD-camera (Photometrix) and appropriate filter sets. Up to 100 metaphases and at least 200 interphase nuclei were scored in each sample.

Yeast-based functional assay (FASAY) and sequencing

A FASAY was used to determine the transcriptional activity of the separated p53 alleles and by that the presence of inactivating mutations [15]. p53 mRNA was reverse transcribed, PCR-amplified and co-transformed into Saccharomyces cerevisiae with a linearized yeast homologous recombination expression vector that contains the 5’ and 3’ ends of the p53 open reading frame. Wild-type p53 activates transcription of the yeast ADE2 gene that encodes the phosphoribosyl-aminomimidazole carboxylase. If present, wild-type p53 results in white colonies, while mutated alleles that abrogate transcriptional activity result in smaller, red colonies. At least three selected red colonies were resuspended in 20 µl water and 2 µl of this suspension were used for sequencing as previously described [16]. Briefly, p53 exons were amplified by PCR using four overlapping primer pairs. The products were sequenced directly using an ALFexpress DNA sequencer and Thermo Sequenase fluorescent labeled primer cycle sequencing kit (Amersham Pharmacia Biotech, Uppsala, Sweden). The presence of the