Assignment of tumor protein p53 induced nuclear protein 1 (TP53INP1) gene to human chromosome band 8q22 by in situ hybridization

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Rationale and significance

TP53INP1, formerly called either TEAP (thymus-expressed acidic protein) or SIP (stress-induced protein), was identified by microarray technology in mouse. It is a novel gene plausibly involved in the function of thymus (Carrier et al., 1999) and is induced by stress during the acute phase of pancreatitis (Tomasini et al., 2001). The human TP53INP1 counterpart (also called p53DINP1: p53-dependent damage-inducible nuclear protein 1, Okamura et al., 2001) and SIP (Tomasini et al., in press) is a proapoptotic gene induced through TP53 activation. This gene is a stress-induced gene with potential antitumoral properties. We report here the localization of TP53INP1 gene to human chromosome 8q22, in a region that shows conserved synteny with region A1–A2 of the murine chromosome 4 where the mouse gene was mapped (Carrier et al., 2000).

Materials and methods

Identification of a human cosmid specific to TP53INP1

The human chromosome 8 specific cosmid library (Los Alamos National Laboratory), obtained from the Resource Center of the German Human Genome Project, was screened with the 0.833-kb and 0.795-kb *Bam*HI-*Sal*I insert cDNA fragment (AF409115) corresponding to the 5' coding region of TP53INP. The hybridization by using the probe labeled with ³²P-dCTP in random priming reactions led to the identification of the cosmid LANLc151H2152Q3.

Fluorescence in situ hybridization on the human genome

Metaphase spreads were prepared from phytohemagglutinin-stimulated human lymphocytes, cultured at 37 °C for 72 h. 5-Bromodeoxyuridine (60 µg/ml) was added for the final 7 h of culture to ensure chromosome R-banding of good quality.

The TP53INP1 cosmid clone was labeled by random priming with biotin 14-dCTP (Bioprime DNA labeling system, Invitrogen). Hybridization to chromosome spreads was done with standard protocol (Pinkel et al., 1986). For each slide, 70 ng of labeled DNA was used. Before hybridization, the probe was annealed with a 150-fold excess amount of Cot-1 human DNA (Gibco-BRL) for 45 min at 37 °C to compete the aspecific repetitive sequences.

The hybridized probe was detected by means of fluorescence isothiocyanate-conjugated avidin (Vector laboratories). Chromosomes were counterstained and R-banded with propidium iodide diluted in antifade solution pH 11.0 as described in Lemieux et al. (1992).

Probe name: LANLc151H2152Q3

Probe type: cosmid clone

Insert size: 40 kb

Vector: SuperCos1Lawrist 7

Proof of authenticity: Southern blot hybridization; PCR amplification

Gene reference: GenBank AF409114, AF409115

Results

Mapping data

Number of cells examined: 30

Number of cells with specific signal: 1 (0), 2 (0), 3 (2), 4 (28) chromatids per cell

Mapping by FL

Number of chromosomes examined: 60

Mean location: chr 8 bands encompassed: q22.1, q22.2, q22.3

Range: 2:3 and 1:3 of specific signals were located on bands q22.1–2 and q22.3, respectively.

The TP53INP1 gene sequence is part of *Homo sapiens* chromosome 8 working draft sequence segment (NT_008046.8),

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Fig. 1. FISH mapping of the TP53INP1 probe to a human metaphase. The green fluorescent signals correspond to the FITC-TP53INP1 probe, located at the 8q22 band. Chromosomes are counterstained in red with propidium iodide.

region: 98,088–98,117 K (NCBI map viewer: http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search). Serine/Threonine kinase (STK3/MST2; Creasy and Chernoff, 1995), and Cyclin E2 (CCNE2; Gudas et al., 1999), are the known flanking genes (96,355–97,098K and 98,137–98,168K regions, respectively).

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