Monoclonal Antibody Register

New Monoclonal Antibodies Recognizing the p53 Tumour Suppressor Protein Homologue p73

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Background

The *p53* tumour suppressor gene is the most frequent target for genetic alterations in human tumours (Nigro et al., 1989; Hollstein et al., 1991) and encodes a nuclear phosphoprotein which plays a key role in cell cycle regulation. This protein protects cells from undergoing tumorigenic alterations by inducing either cell cycle arrest or programmed cell death in response to a variety of cellular stress signals (Ko and Prives, 1996; Levine, 1997) and also plays an important role in maintaining the integrity of the genome (Lane, 1992). Attempts to find *p53* homologues analogous to the *pRb* family of tumour suppressors were successful and a new gene, *p73*, has been identified on the short arm of chromosome 1 in a region frequently deleted in neuroblastoma (Kaghad et al., 1997; Yang et al., 1998). This gene encodes several proteins with structural and functional homology to p53 (De Laurenzi et al., 1998) that can activate transcription of the $p21^{WAF1}$ gene responsible for cell-cycle arrest, and can also induce apoptosis when overexpressed (Jost et al., 1997; Kaghad et al., 1997). There are several differentially spliced variants of mRNAs, which are translated into different p73 proteins. This splicing occurs at the 3' end of the sequence and translated proteins have differing C-termini, in which p73α is a full-length protein and the β and δ isoforms are truncated forms of this protein. The δ isoform lacking the major part of the C-terminal region is most similar to p53. The γ isoform contains a different 75-residue C-terminus compared to the α isoform due to a long alternative reading frame in this region (Kaghad et al., 1997; De Laurenzi et al., 1998; De Laurenzi et al., 1999; Ueda et al., 1999). p73 as well as p53 contains a transactivation domain, a DNA-binding domain and an oligomerization domain. The highest level of homology is exhibited in the DNA-binding domain (63% identity between the p53 and p73), and this finding suggests that both these proteins can bind to the same DNA sequences and transactivate the same target genes. The conservation of high homology in the oligomerization domain suggests that the members of this family could form mixed oligomers. Although the existence of these mixed oligomers *in vivo* is still open to question, the availability of specific antibodies could help to address this problem.

The identification of a family of p53-related transcription factors that can be potentially redundant in their ability to activate the same cellular responses (i.e. cell-cycle arrest and apoptosis) has encouraged a study into the basis for their similarities and differences in terms of their physical and functional interactions with one another, their mechanism of activation and their regulation. Monoclonal antibodies specific to different forms of the p73 protein therefore provide a powerful tool to study these proteins both *in vivo* and *in vitro*.

Description of the antibodies p73-1.1 and p73α-1.1

Production

The hybridoma cell lines producing p73-1.1 and p73α-1.1 monoclonal antibodies recognizing the p73α protein were selected after immunization of BALB/c mice with either the purified p73α protein expressed in bacteria for development of antibody p73-1.1 or peptide QDLKQGHDYSTAQQ coupled to keyhole limpet haemocyanin (KLH) for development of antibody

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p73α-1.1. The mouse splenocytes were fused with non-producing myeloma cell line Sp2/0 using polyethylene glycol as a fusogen. The dot-blot using specific and non-specific peptides coupled to bovine serum albumin (BSA) was used as a screening method for a panel of antibodies raised against the peptide derived from the p73α protein sequence. Monoclonal antibodies developed against the p73α protein were screened by Western blotting of the partially purified p73α protein expressed in bacteria. Hybridoma cell lines were prepared using limiting-dilution cloning and re-cloning (Harlow and Lane, 1988). Monoclonal antibodies were more precisely characterized using different isoforms of the p73 protein over-expressed in a cell line transfected with plasmids carrying the cDNA coding the p73 isoforms α, β, γ and δ.

Specificity

Initially the specificity of p73-1.1 and p73α-1.1 MAbs was tested by Western blotting against p73α expressed in *E. coli* performed under denaturing conditions. To test the specificity of MAbs to different isoforms of the p73 protein, human lung carcinoma cell line H1299 expressing detectable levels of only the γ isoform of endogenous p73 protein was transfected with the plasmid pcDNA3 carrying the cDNA for *p73α, p73β, p73γ* and *p73δ* using Effectene™ Transfection Reagent (Qiagen, Hilden, Germany). Non-transfected H1299 cells were used as a negative control. To prove the high levels of p73 isoform expression in transfected cells, the extracts were immunoblotted with the p73 (H-79) rabbit polyclonal sera (Santa Cruz Biotechnology, Santa Cruz, CA) specific to the N-terminus (amino acids 1–80) of p73, and recognizing all known p73 protein isoforms (data not shown). Western blot analysis employing p73-1.1 and p73α-1.1 MAbs detected the purified p73α protein from the bacteria as well as the transfected p73α protein in H1299 cells (Figs. 1 and 2). The p73-1.1antibody also binds the γ isoform with

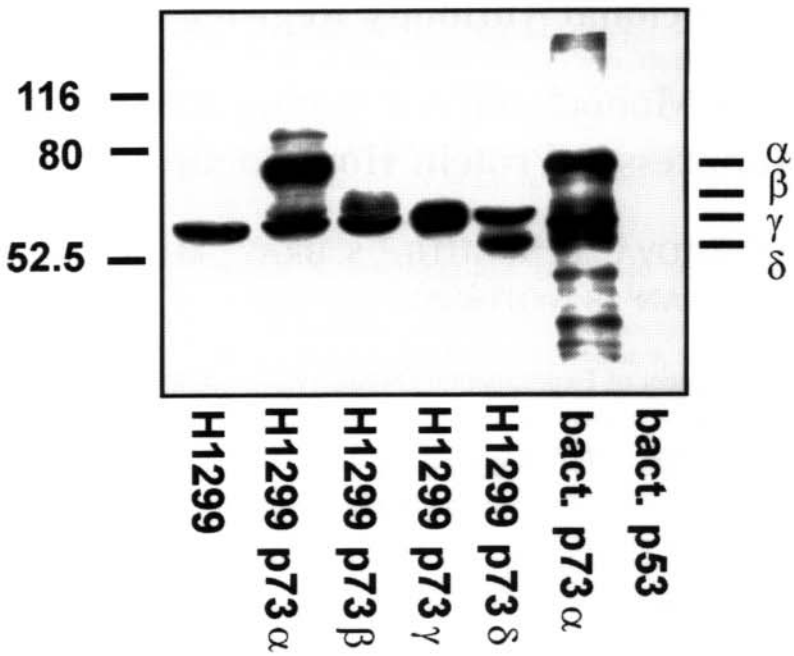

Fig. 1. Characterization of p73-1.1 MAb specificity using Western blot analysis. The H1299 cells were transfected with pcDNA3 plasmid vectors carrying *p73α, β, γ,* or *δ* isoform cDNA using Effectene™ Transfection Reagent (Qiagen). Non-transfected cells as well as purified p53 protein expressed in bacteria were used as negative controls. Partially purified p73 protein expressed in bacteria served as a positive control, antibody concentration was 1 μg/ml.

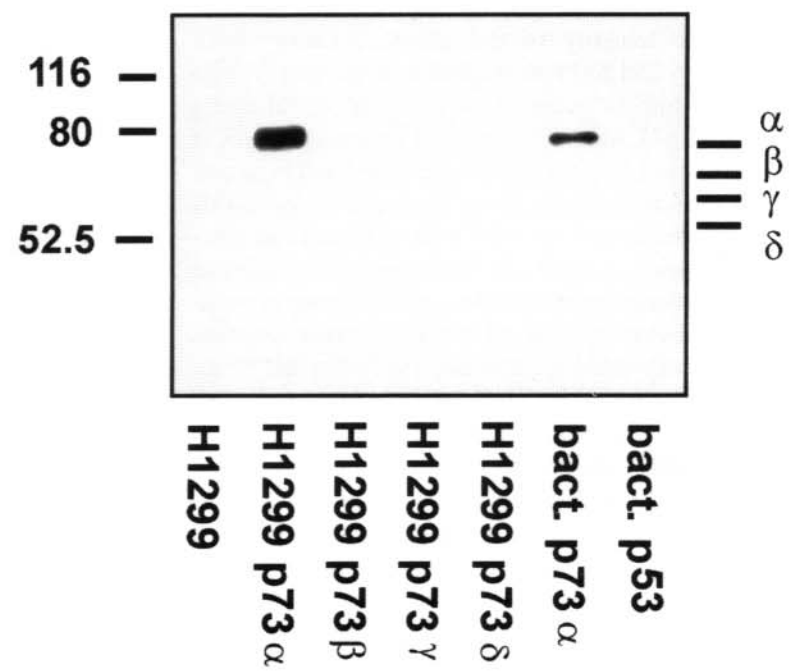

Fig. 2. Characterization of p73α-1.1 MAb specificity using Western blot analysis. The H1299 cells were transfected with pcDNA3 plasmid vectors carrying *p73α, β, γ,* or *δ* isoform cDNA using Effectene™ Transfection Reagent (Qiagen). Non-transfected cells as well as purified p53 protein expressed in bacteria were used as negative controls. Partially purified p73 protein expressed in bacteria served as a positive control, antibody concentration was 1 μg/ml.