Monoclonal Antibody Register

New Monoclonal Antibodies Recognizing p53 Protein Phosphorylated by Casein Kinase II at Serine 392

Š. POSPÍŠILOVÁ[1], K. KAŇKOVÁ[2], M. SVITÁKOVÁ[1], R. NENUTIL[2], B. VOJTĚŠEK[1]

[1]Department of Experimental Oncology, Masaryk Memorial Cancer Institute, Brno, Czech Republic
[2]Department of Pathology, Faculty Woman's Hospital Brno, Brno, Czech Republic

Background

The p53 tumour suppressor protein is a nuclear phosphoprotein which plays a key role in cell-cycle regulation. The p53 protein protects cells from undergoing tumorigenic alterations by inducing either the cell growth arrest or programmed cell death in response to a variety of cellular stress signals such as DNA damage, hypoxia, heat shock, oncogene activation or metabolic changes (Levine, 1997). One of the critical issues of p53 response to cellular stress or DNA damage is the way of its activation. It has been suggested that post-translational modification involving phosphorylation of the p53 protein is most likely the mechanism through which the activity of the p53 protein may be regulated. p53 is phosphorylated on several serine residues within the N- and C-terminal regions by several cellular kinases. The N-terminal part of p53 is phosphorylated using different protein kinases including casein kinase I (CKI) (Milne et al., 1992), DNA-PK (Lees-Miller et al., 1992), Chk1, Chk2, MAP kinase (Milne et al., 1994) and c-Jun kinase (Milne et al., 1995). There are also at least three phosphorylation sites on the C-terminus of human p53 at amino acids 315, 378 and 392. Serine^{315} of p53 is a target for cdk1 and cdk2 (Price et al., 1995). Serine^{378} is phosphorylated by PKC (Baudier et al., 1992). Serine^{392} is phosphorylated by purified casein kinase II (CKII) *in vitro* (Blaydes and Hupp, 1998). Phosphorylation of the human p53 at Ser^{392} has been shown to enhance p53 sequence-specific DNA binding *in vitro* (Hupp and Lane, 1994), which is responsible for transcriptional activation of the p53 protein. Recently, it has also been shown that phosphorylation of Ser^{392} is important for p53-mediated transcriptional activation *in vivo* (Hao et al., 1996).

Phosphorylation of different proteins in cells can be studied using a range of different methods, but the primary technique for determining phosphate incorporation into the specific sites in target proteins involves labelling of cells with [^{32}P] phosphate followed by phospho-amino-acid analysis or sequencing of the protein of interest (Van der Geer et al., 1993). The main problem associated with this technique is the incubation of cells with a radioactive precursor [^{32}P], which itself can activate growth arrest and stress-responsive signalling pathways, obviously perturbing protein phosphorylation (Yeargin and Haas, 1995; Dover et al., 1994). Preparation of monoclonal antibodies that are specific to either phosphorylated or non-phosphorylated epitopes within the target protein provides a powerful alternative to the above techniques.

Description of the antibodies S-P-1.1, S-P-2.1 and S-P-3.1

Production

The hybridoma cell lines producing S-P-1.1, S-P-2.1 and S-P-3.1 phospho-specific monoclonal antibodies were selected after immunization of BALB/c mice with the phosphopeptide SRHKKLMFKTEGPDS$^{(PO)}{}_3$D coupled to keyhole limpet haemocyanin (KHL). Mouse splenocytes were fused with non-producing myeloma cell line SP2/0 using polyethylene glycol as a fusogen. Dot-blot using the phosphorylated and non-phosphorylated forms of peptide coupled to bovine serum albumin (BSA) was used as a screening method (Fig. 1). Monoclonal antibody Bp53-6.1 (Bartek et al., 1993) was used as a positive control, as this antibody recognizes both forms of the peptide. Hybridoma cell lines were prepared using limiting-dilution cloning and recloning. Monoclonal antibodies were characterized more precisely on bacterially expressed p53 protein phosphorylated *in vitro* at Ser^{392} with CK II.

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Corresponding author: Bořivoj Vojtěšek, Masaryk Memorial Cancer Institute, Žlutý kopec 7, 656 53 Brno, Czech Republic. Fax: 420 (5) 43211696; e-mail: vojtesek@mou.cz.

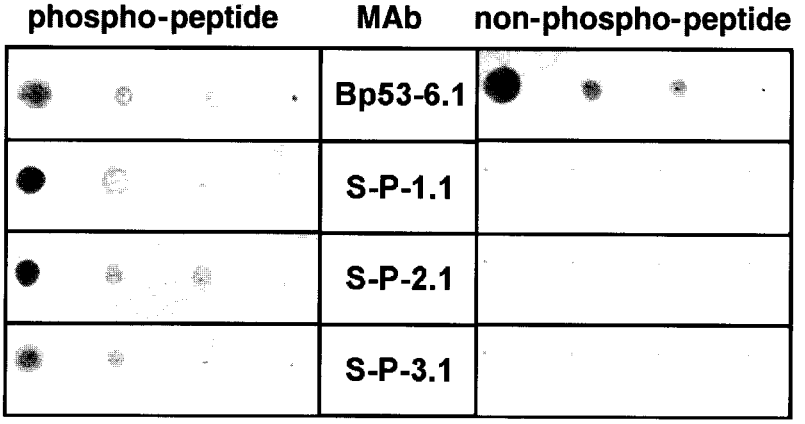


Fig. 1. Dot-blot analysis of phosphorylated and non-phosphorylated peptide SRHKKLM FKTEGPDS$^{(PO_3)}$D coupled to BSA using specified monoclonal antibodies (Bp53-6.1 – control MAb recognizing epitope ^{381}KKLMFKTEGP^{390}, S-P-1.1, S-P-2.1, S-P-3.1 – MAbs recognizing the p53 protein phosphorylated at Ser^{392}).

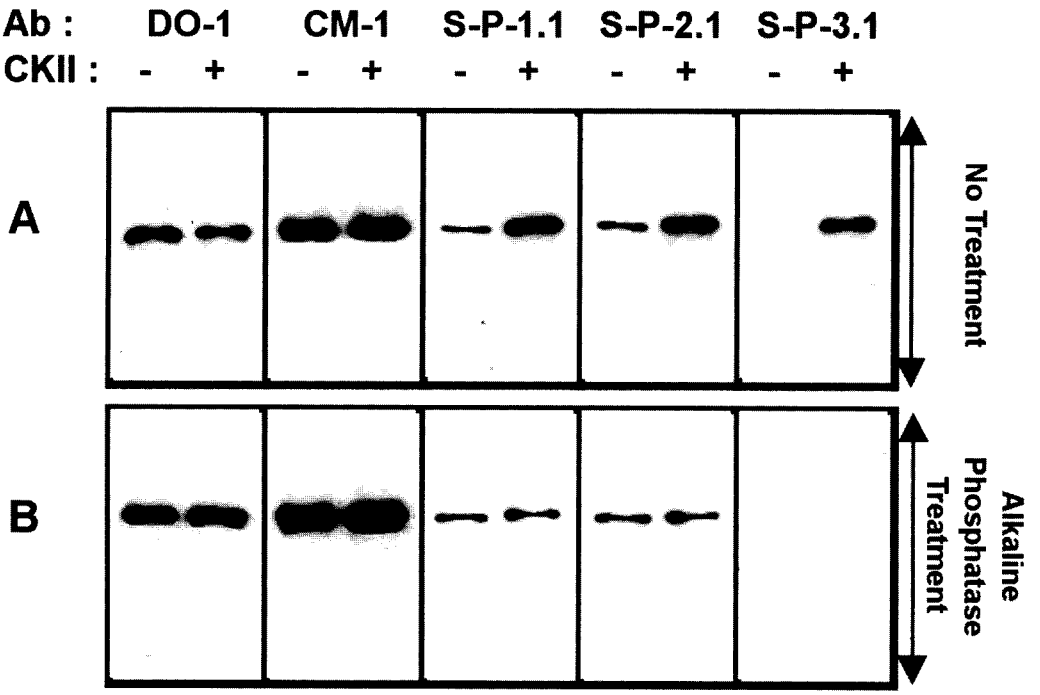
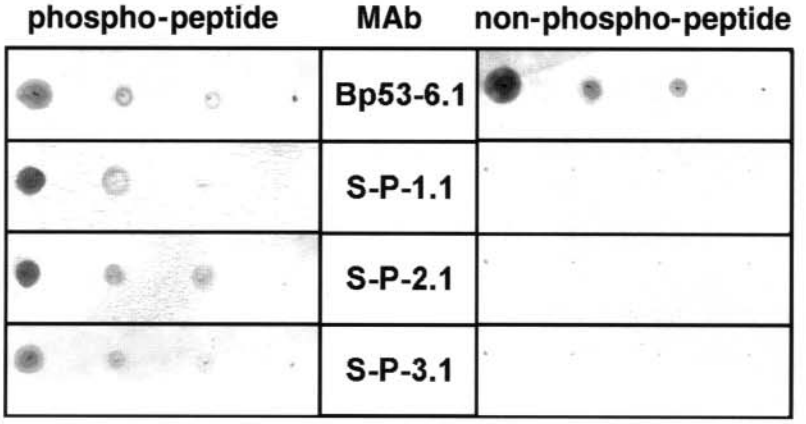


Fig. 2. Western-blot analysis of bacterially expressed p53 protein phosphorylated *in vitro* with casein kinase II at Ser^{392}. The kinase reaction was performed in the reaction mixture containing 20 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 1 mM $CaCl_2$, 100 μg/ml phosphatidylserine, 20 μg/ml dioleoyl-sn-glycerol, 1 mM 3-[(-cholamidopropyl) dimethylammonio]-1-propanesulphonate (CHAPS) and 0.2 mM ATP at 30°C for 30 min and the same samples were loaded to both A and B gels. After the blotting the B membranes were incubated with calf intestinal alkaline phosphatase at room temperature for 60 min while gently shaking and then all the membranes were blocked in PBS with 5% low-fat milk and incubated with indicated antibodies for 2.5 h at room temperature. Control polyclonal antibody CM-1 recognized several epitopes of human p53 protein, monoclonal antibody DO-1 recognized the epitope ^{20}SDLWKL^{25}, and monoclonal antibodies S-P-1.1, S-P-2.1, S-P-3.1 recognized the C-terminus of the p53 protein phosphorylated at Ser^{392}.



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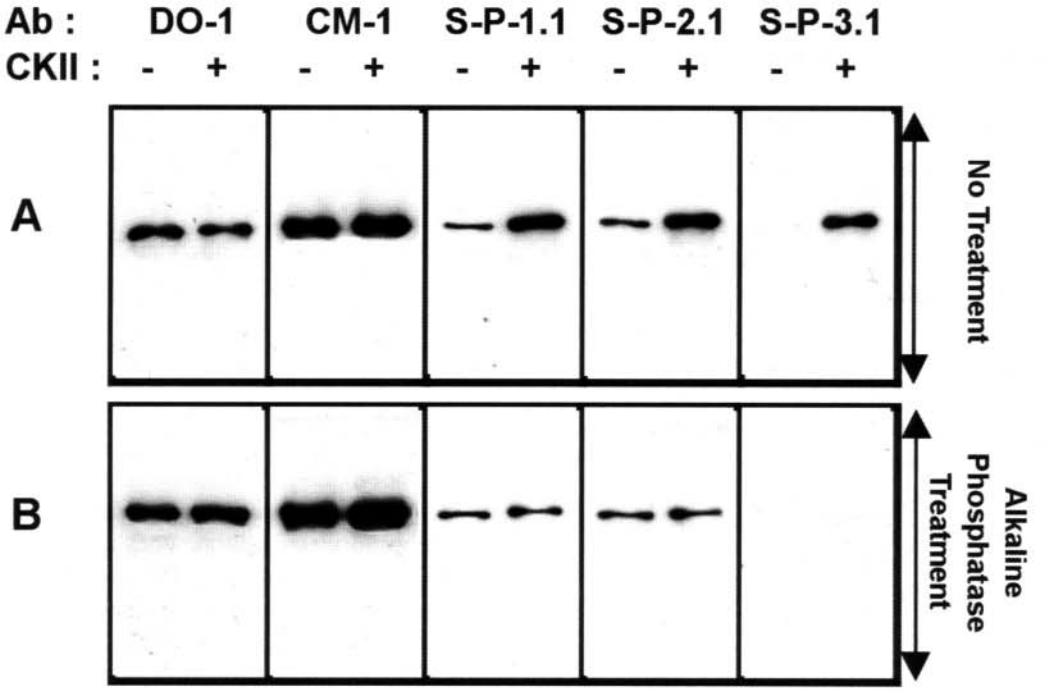

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