Tumour Suppressor PTEN Regulates Cell Cycle and Protein Kinase B/Akt Pathway in Breast Cancer Cells

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Abstract. Background: PTEN is a tumour suppressor protein with phosphatase activity frequently altered in several types of human cancers. Materials and Methods: The PTEN effect was studied on the cell cycle (by bromodeoxyuridine incorporation) and on the phosphatidylinositol-3-kinase/protein kinase B/Akt (PI3-K/PKB/Akt) pathway regulating proteins (by immunocytochemical, Western blot analysis and kinase assay) upon transfection of wild-type PTEN and its mutant H123Y in breast cancer cell lines. Results: The expression of the important proteins in the MCF-7 and BT-549 cells was characterised and the cellular localisation of PTEN was analysed. Transfection of H123Y led to the down-regulation of p27Kip1 and p21Waf1/Cip1 protein levels and the up-regulation of phosphorylated PKB/Akt. An overexpression of PTEN decreased cyclin E/cdk2 activity and inhibited S-phase entry in MCF-7. In BT-549 these changes were not observed, but overexpression of PTEN led to a diminution of PKB/Akt phosphorylation. Conclusion: PTEN function is mediated through the inhibition of the cell cycle and PKB/Akt phosphorylation in breast cancer cells.

PTEN (phosphatase and tensine homologue deleted on chromosome 10) / MMAC (mutated in multiple advanced cancers) was simultaneously identified by 2 groups as a tumour suppressor gene candidate located at chromosome 10q23 (1, 2). Another group identified the same gene during the search for new dual-specificity phosphatases and designated it as TEP-1 (TGF-β regulated and epithelial cell-enriched phosphatase) (3). Overall, PTEN is one of the most common targets of mutation in human cancers, with a mutation frequency approaching that of p53. PTEN mutations have been found in glioblastomas, malignant melanomas, carcinoma of the prostate, breast, kidney, urinary bladder, uterus and others, mostly in advanced stages of tumour progression (1, 4-6). Germline mutations of PTEN have been found in 3 inherited syndromes: Cowden’s syndrome, Lhermitte-Duclos syndrome and Bannayan-Riley-Ruvalcaba syndrome. The common feature of these diseases is a predisposition to hamartomas, benign tumours containing differentiated but disorganized cells (7, 8). The PTEN gene encodes a 403-amino acid peptide with a relative molecular mass of 55 kDa (1, 3, 9, 10). Immunohistological staining indicates an exclusively cytoplasmic staining of the PTEN protein in prostate tumour xenografts or surgical specimens (4, 11), whereas glioblastomas showed perinuclear cytoplasmic and nuclear staining (12). PTEN was also found in vascular endothelial cells and neurons (13).

The C-terminal domain of PTEN has the protein tyrosine phosphatase (PTP) motif. The phosphatase active site of PTEN is important for phosphatidyl-inositol-3, 4, 5-phosphate (Ptd-Ins-(3, 4, 5)-P3) binding (14). In the same domain, a PDZ binding motif (postsynaptic density protein/Drosophila/disc large tumour suppressor/tight junction protein ZO1) is also located, which can interact with PDZ domain-containing proteins (15). PTEN dephosphorylates phosphotyrosine, phosphoserine and/or phosphothreonine, and plays an important role in the modulation of the PI3-K pathway by catalysis of Ptd-Ins-(3,4,5)-P3 dephosphorylation at position 3 on the inositol ring. PTEN in vivo regulates the level of Ptd-Ins-(3,4,5)-P3 (15). Some authors have suggested a link between the PI3-K/Akt pathway and human cancers via defects in PTEN.
PTEN blocks S-phase entry by recruiting the cyclin-dependent kinase (cdk) inhibitor p27\(^{kip1}\) into the cyclin E/cdk2 complex and inhibiting cdk2 kinase activity in glioblastoma cell lines (18). The overexpression of wild-type PTEN in the MCF-7 breast cancer cell line leads to the suppression of cell growth through the blockade of cell cycle progression, accompanied by an increased abundance of p27\(^{kip1}\), a decrease in cyclin D\(_1\) and the inhibition of PKB/Akt phosphorylation (19). There is also a reduction in retinoblastoma protein (pRb) phosphorylation and G1 cell cycle arrest has been described (20). PTEN is capable of suppressing tumorigenicity and cell growth through G1 cell cycle arrest, or of inducing apoptosis, or both (9, 10, 21-24). PTEN-mediated growth suppression is probably dependent on the functional status of each particular signalling pathway that interacts with PTEN. Our previous experiments revealed an inhibition of S-phase entry following transfection of PTEN with the active protein phosphatase domain, whereas the lipid phosphatase domain was unessential. The cell cycle inhibition was detected only in cell lines carrying the wild-type tumour suppressor gene p53 and the retinoblastoma gene (Rb). In this study, the influences of transfected wild-type \(\text{PTEN}^{\text{pxmyc}}\) and its phosphatase defective-mutant H123Y on the cell cycle and on the PI3-K/PKB/Akt pathway regulating proteins were analysed.

**Materials and Methods**

**Plasmids and mutagenesis.** Human wild-type \(\text{PTEN}\) cDNA, kindly provided by Dr. P. A. Steck (University of Texas, Houston, USA), was excised from \(\text{PTEN}-\text{pBluescript}\) using XbaI and BamHI (New England Biolabs, Beverly, MA, USA), amplified by PCR using modified primers to create BamHI and Smal restriction sites and subcloned into the BamHI/Smal sites of the \(\text{pxmyc}\) vector, according to Lukas \etal\. (25). Mutagenesis of the \(\text{pxmycPTEN}\) carrying the wild-type sequence into the \(\text{PTEN}\) sequence encoding the mutant His123Tyr (H123Y) lacking lipid and protein phosphatase activity was performed with a Quick change site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). Mutations were verified by sequencing using the Thermo sequenase radiolabelled terminator cycle sequencing kit (Amersham-Pharmacia Biotech UK Ltd., Little Chalfont, UK).

**Cell culture and gene transfer.** The human breast cancer cell line MCF-7 was cultured in DME medium F12, while ZR-75, BT-549, MDA MB-468, MDA MB-453, MDA MB-231, T47D, SKBR-3 and the PMC-42 lines were cultured in DMEM medium (Invitrogen Life Technologies, Carlsbad, CA, USA). The human prostate cancer cell lines DU-145 and PC-3 were cultured in DMEM medium (Sigma-Aldrich). The percentage of S-phase was determined according to a formula described in detail in a previous publication (27). The induction of pRb in BT-549 cells was monitored by detection of protein using mouse monoclonal antibody against pRb (1F8, Novoceastra, Newcastle-upon-Tyne, UK).

**Evaluation of cell cycle effect.** The MCF-7 and BT-549 cells were transfected and cultured on glass coverslips or in dishes for an additional 36 or 48 h. BTA-pRb cells were transfected and pRb was induced by culturing the cells for 30 h. Twelve h before the fixation or harvesting of cells, 0.1 mM bromodeoxyuridine (BrDU, Sigma-Aldrich) was added to the culture medium. The cells were stained with mouse monoclonal antibody against myc (9E10), obtained from Dr. J. Bartek, BT-549 cells with the tetracycline-inducible stably-transfected Rb clone, BTA-pRb, obtained from Dr. J. Bartek, were cultured in DMEM medium with 10% foetal bovine serum, 2 mM glutamine, penicillin, streptomycin, tetracycline (1 \(\mu\)g/ml, Sigma-Aldrich). PTEN expression was induced by washing the cells 3 times in PBS and culturing them without tetracycline for the next 48 h. The induction of PTEN was checked by detection of a myc tag using mouse monoclonal antibody against myc (9E10), obtained from Dr. J. Bartek, BT-549 cells with the tetracycline-inducible stably-transfected Rb clone, BTA-pRb, obtained from Dr. J. Bartek, were cultured in DMEM medium with 10% foetal bovine serum, 2 mM glutamine, penicillin, streptomycin, tetracycline (1 \(\mu\)g/ml, Sigma-Aldrich). The cells were transfected by calcium phosphate precipitation with \(\text{PTENpxmyc}\) and \(\text{pxmyc}\) together with \(\text{pCMVCD-20}\). After 14 h of incubation with the precipitate, the cells were trypsinised, washed 3 times in PBS and pRb was induced by removing the tetracycline.

**Immunocytochemical analysis of endogenous PTEN in MCF-7 and BT-549 cells.** Exponentially-growing MCF-7 and BT-549 cells were...
cultured on glass coverslips, fixed, washed in PBS and incubated with mouse monoclonal antibody against PTEN (26H9, Cell Signaling, Beverly, MA, USA), washed and incubated with secondary antibody (Alexa colours 594, Molecular Probes, Eugene, OR, USA), washed again, counterstained with Hoechst 33258 and mounted.

**Gel electrophoresis and Western blot analysis.** The cells were harvested and lysed in cold protein extraction (PЕX) buffer: 50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 2.5 mM EGTA, 10% glycerol, 0.1% Tween 20, with protease and phosphatase inhibitors: 2.5 μg/ml aprotenin, 2.5 μg/ml leupeptin, 25 μg/ml phenylmethanesulfonyl fluoride (PMSF), 10 mM β-glycerolphosphate, 1 mM natrium fluoride, 0.1 mM natrium vanadate and 1 mM diethiotreitol. The protein concentration was measured in the supernatant by the Bradford assay-Protein assay dye reagent (Bio-Rad, Hercules, CA, USA). γ-globulin was used as a standard (Bio-Rad) and the supernatant was mixed with 4 x concentrated Laemmli sample buffer (26). The proteins (20-50 μg/sample) were electrophoretically separated on 8%, 10% and 12% polyacrylamide gels in the presence of SDS (SDS-PAGE). Separated proteins were blotted into nitrocellulose membranes (ECL, Amersham-Pharmacia Biotech UK Ltd.). The immunodetection was carried out using mouse monoclonal antibodies against: p21Waf1/Cip1 (clones DCS-61, DCS-62, 118), p27Kip1 (Ab-3, NeoMarkers, Westhinghouse, CA, USA, SX 53G8, DakoCytomation), cyclins D1,2 (5D-4), cyclin D3 (DCS-28), cyclin E (HE-12), cyclin B1 (GSN1, Santa Cruz Biotechnology, CA, USA), phosphospecific pRb for serin 795 (Rb-10), total pRb (14001A, Pharmingen, San Diego, CA, USA), cyclin-dependent kinase cdk2 (D-12, Santa Cruz Biotechnology), p53 (DO-1), proliferating cell nuclear antigen PCNA (PC-10), phosphospecific PKB/Akt for serin 473 (4E2, Cell Signaling), mcm-7 (DCS-141.1) and myc (9E10); polyclonal rabbit sera to: cdk4 (C-22, Santa Cruz Biotechnology), cdk6 (C-21, Santa Cruz Biotechnology), cyclin A (H-432, Santa Cruz Biotechnology), Pi3-K 85α (Z8, Santa Cruz Biotechnology) and total PKB/Akt (Cell Signaling). For detection, rabbit anti-mouse (P0260, DakoCytomation) and goat anti-rabbit (PI-1000, Vector Laboratories, Burlingame, CA, USA) secondary antibodies and a chemoluminescens system (Pierce Biotechnology, Rockford, IL, USA) were employed. The antibody against mcm-7 was used as a control of equal loading and the antibody against myc was used as a control to verify the expression of transfected DNA constructs. The antibodies used against p21Waf1/Cip1 (clones DCS-61, DCS-62), cyclins D1,2,3, cyclin E, phosphospecific pRb, mcm-7 and myc were kindly provided by Dr. J. Bartek, and the antibodies against p27Kip1/Cip1 (clone 118), p53 and PCNA were kindly provided by Dr. B. Vojtesek, Masaryk Memorial Cancer Institute, Brno, Czech Republic. The experiment was repeated at least 3 times.

**Kinase assay.** Transfected MCF-7 cells with PTENpxmyc, H123Ypxmyc and pxmyc vector were cultured for an additional 36 h. The cells were harvested and lysed in cold PEX buffer with protease and phosphatase inhibitors, as described above. Lysates (200-500 μg) from transfected cells were immunoprecipitated with mouse monoclonal anti-cyclin E antibody (HE-172, kindly provided by Dr. J. Bartek) or rabbit polyclonal anti-cyclin A antibody (H-432, Santa Cruz Biotechnology) and protein G-sepharose or protein A-sepharose beads (Amersham-Pharmacia Biotech UK Ltd.) were used. The kinase reaction was performed using 20 μM cold ATP (Roche Diagnostics GmbH, Mannheim, Germany), 10 μCi

\[ ^{32}P-\gamma-ATP \text{(Amersham-Pharmacia Biotech UK Ltd.) or } ^{33}P-\gamma-ATP \text{(ICN, Irvine, CA, USA), 2 μg histone H1 (Roche Diagnostics GmbH) as a substrate, for 30 min at 30°C. The samples were separated on 10% polyacrylamide gel, transferred into a nitrocellulose membrane and the radiation of phosphorylated histone H1 was quantified with a Phosphoimager (Fuji, FLA 3000, Japan). The kinase assays were repeated twice. The cdk2 total level was quantified by measurement of the integral optical density using image analysis software ACC version 5.0 (Sofo, Czech Republic).**

**Results**

**Effect of PTEN on the S-phase entry to the cell cycle.** In this study, the effect of PTEN on asynchronously transiently-transfected MCF-7 and BT-549 cells was demonstrated. PTEN-mediated S-phase inhibition was observed in the MCF-7 cell line which carries wild-type p53, pRb and PTEN. BT-549 represents a cell line with defects in PTEN, p53 and pRb. We failed to show any influence of overexpressed PTEN on the inhibition of the S-phase entry in this cell line (Figure 1). With the aim of documenting the potential importance of the pRb tumour suppressor for PTEN inhibitory activities, BT-549 cells with induced pRb (BTA-pRb) were transfected by PTENpxmyc and control vector pxmyc. PTEN-mediated S-phase inhibition was observed in 35-40% of cells, which represents half of the value observed in the MCF-7 cell line (60-70%). This partial effect of PTEN on S-phase inhibition in BTA-pRb cells indicated that pRb is necessary for PTEN function, but that wild-type p53 also plays an important role.

**Analysis of protein expression regulating the cell cycle and the P13-K/PKB/Akt pathway in MCF-7 and BT-499 cells.** The levels of the cyclins D1,2,3, p21Waf1/Cip1 and p27Kip1 were higher in the MCF-7 cells than in BT-549, where the expression of D-type cyclins and p21Waf1/Cip1 were almost undetectable and the level of p27Kip1 was much lower. Cyclins E, A, B, cdk2, 4 and 6 were present in both cell lines at similar levels (Figure 2a). The 85α subunit of PI3-K was detected in both cell lines, but a higher level was observed in the MCF-7 cells. The total PKB/Akt was the same in both cell lines, but its phosphorylated form was much higher in BT-549. PTEN was detected in the MCF-7 cells only, but a higher level was observed in the MCF-7 cell lines. The total PKB/Akt was the same in both cell lines, but its phosphorylated form was much higher in BT-549. PTEN was detected in the MCF-7 cells only (Figure 2a). An inverse relationship between PTEN and phosphorylated PKB/Akt on serin 473 in the other breast cancer cell lines (MDA MB-468, ZR-75, MDA MB-453, MDA MB-231, T47D, SKBR-3, PMC-42) and prostate cancer cell lines (LNCaP, DU-145, PC-3) was also found (Figure 2b). The cancer cell lines, which carried mutations of PTEN and showed no expression of PTEN protein on Western blot (BT-549, MDA MB-468, ZR-75 and LNCaP), did not show protein expression as a consequence of a defect other than mutation (PC-3 cells), highly expressed the phosphorylated form of PKB/Akt. The cell lines with
wild-type PTEN or which expressed some level of PTEN protein (MCF-7, MDA MB-453, MDA MB-231, T47D, SKBR-3, PMC-42 and DU-145) showed low expression of the phosphorylated PKB/Akt form or PKB/Akt phosphorylation on serin 473 was not detected. The total PKB/Akt level was very similar in all the demonstrated cell lines. The immunocytochemical detection of endogenous PTEN revealed cytoplasmic and, mainly, nuclear staining with a granular character in the MCF-7 cells. In the BT-549 cells, the PTEN protein was not detected or was below detectable levels using our method (Figure 3).

Effect of overexpressed PTEN on the cell cycle and the PI3-K/PKB/Akt pathway regulating proteins. Transfected PTEN mutant H123Y, defective in protein and phospholipid phosphatase activity, caused a decrease in total p21Waf1/Cip1 and p27Kip1 levels, especially 36 h after transfection. In the growth deprivation condition (5% foetal bovine serum in the culture medium), the down-regulation of p27Kip1 caused by H123Y transfection was more intense. The expressions of cyclins D1,2,3, A, B and E, cdk2, 4, 6, p53 and PCNA were unchanged. The total pRb level and its phosphorylation on serin 795 were also not affected. In the BT-549 cells, no changes in expression of the monitored cell cycle regulating proteins were found. The decrease in p21Waf1/Cip1 and p27Kip1 protein levels after H123Y transfection were accompanied by a slight increase in PKB/Akt phosphorylation on serin 473, (48 h after transfection) in the MCF-7 cells. The transfection of PTEN into BT-549 cells did not inhibit S-phase entry and did not affect the protein expression of the cdk inhibitors p21Waf1/Cip1, and p27Kip1, but did lead to down-regulation of PKB/Akt phosphorylation on serin 473 (Figure 4).

Effect of PTEN on cdk2 kinase activity. The kinase activity of the cyclin E/cdk2 complex in MCF-7 cells transfected with wild-type PTEN was markedly suppressed (50%, SD±4.5) in comparison with the control vector pmyc and the H123Y mutant (Figure 5). No significant changes in cyclin E/cdk2 activity were found in the BT-549 cells and no changes in the kinase activity of the cyclin A/cdk2 complex were observed in the MCF-7 cell line.
In this study, a mechanism which exerts the growth-suppressive effects of overexpressed PTEN in MCF-7 and BT-549 cell lines was reported. Two cell lines derived from breast cancer were used, characterised by different conditions. PTEN regulates cell cycle and PKB/Akt pathway. Figure 2. a) Western blot analysis, in exponentially-growing MCF-7 and BT-549 cells, documents the endogenous expression of proteins involved in cell cycle regulation: p53 (DO-1), cyclins D1,2 (5D-4), cyclin D3 (DCS-28), cyclin E (HE-12), cyclin A (H-432), cyclin B1 (GNS5), cdk4 (C-22), cdk6 (C-21), cdk2 (D-12), total pRb (14001A), its phosphorylated form on serine 795, P pRb (Rb-10), p27Kip1 (Ab-3), p21Waf1/Cip1 (DCS-61+62) and proteins involved in regulation of the PI3-K/PKB/Akt pathway: PTEN (26H9), total PKB/Akt, its phosphorylated form on serine 473, P PKB/Akt (4E2), and 85· subunit of PI3-K (Z8). Mcm-7 (DCS-141.1) was used as control of equal loading. Twenty to 50 µg of total proteins per sample were used. Proteins with significant differences of expression are marked by asterisk. b) Western blot analysis, in exponentially-growing breast (MCF-7, BT-549, MDA MB-468, ZR-75, MDA MB-453, MDA MB-231, T47D, SKBR-3 and PMC-42) and prostate cancer cell lines (LNCaP, DU-145 and PC-3) showed the endogenous expression of PTEN (26H9), serine 473 phosphorylation of PKB/Akt (4E2) and total (tot) PKB/Akt. The result, demonstrating an inverse relationship between PTEN and the phosphorylated form of PKB/Akt. Mcm-7 (DCS-141.1), was used as a marker of equal loading.

Discussion

In this study, a mechanism which exerts the growth-suppressive effects of overexpressed PTEN in MCF-7 and BT-549 cell lines was reported. Two cell lines derived from breast cancer were used, characterised by different conditions. PTEN regulates cell cycle and PKB/Akt pathway. Figure 2. a) Western blot analysis, in exponentially-growing MCF-7 and BT-549 cells, documents the endogenous expression of proteins involved in cell cycle regulation: p53 (DO-1), cyclins D1,2 (5D-4), cyclin D3 (DCS-28), cyclin E (HE-12), cyclin A (H-432), cyclin B1 (GNS5), cdk4 (C-22), cdk6 (C-21), cdk2 (D-12), total pRb (14001A), its phosphorylated form on serine 795, P pRb (Rb-10), p27Kip1 (Ab-3), p21Waf1/Cip1 (DCS-61+62) and proteins involved in regulation of the PI3-K/PKB/Akt pathway: PTEN (26H9), total PKB/Akt, its phosphorylated form on serine 473, P PKB/Akt (4E2), and 85· subunit of PI3-K (Z8). Mcm-7 (DCS-141.1) was used as control of equal loading. Twenty to 50 µg of total proteins per sample were used. Proteins with significant differences of expression are marked by asterisk. b) Western blot analysis, in exponentially-growing breast (MCF-7, BT-549, MDA MB-468, ZR-75, MDA MB-453, MDA MB-231, T47D, SKBR-3 and PMC-42) and prostate cancer cell lines (LNCaP, DU-145 and PC-3) showed the endogenous expression of PTEN (26H9), serine 473 phosphorylation of PKB/Akt (4E2) and total (tot) PKB/Akt. The result, demonstrating an inverse relationship between PTEN and the phosphorylated form of PKB/Akt. Mcm-7 (DCS-141.1), was used as a marker of equal loading.

Figure 3. The immunoflourescence detection of endogenous PTEN by using the 26H9 clone antibody. The MCF-7 cells revealed a slight cytoplasmic and mainly nuclear expression of PTEN with a granular character of staining. In comparison, endogenous PTEN was not detected in the BT-549 cells, where only the background can be seen. The counterstaining of nuclei by Hoechst was used to visualise the total cell number. Magnification 1000x.

Figure 4. The effect of transfected PTEN, control vector pxmyc and H123Y, mutant with phospholipid and protein phosphatase activity defects. Western blot analysis showed that H123Y transfection caused the decrease of p27Kip1 and p21Waf1/Cip1 protein levels after 36 h and a slight increase of phosphorylated PKB/Akt on serine 473 after 48 h in MCF-7 cells (left panel). PTEN transfection caused a decrease of PKB/Akt phosphorylation on serine 473 after 48 h in BT-549 cells (right panel). In both cell lines, equal levels of mc-myc demonstrated the same amount of total proteins in the samples and myc detection showed comparable levels of PTEN and H123Y mutant overexpression in cells.
expressions of the cell cycle and PI3-K/PKB/Akt pathway-regulating proteins. The MCF-7 cells expressed wild-type p53, pRb, PTEN, high levels of the cdk inhibitors p21\textsuperscript{Waf1/Cip1} and p27\textsuperscript{Kip1}, high levels of phosphorylated PKB/Akt, low PI3-K levels and did not express PTEN due to a hemizygous point mutation (Trp274 deletion of G) in exon 8 (27). The results from the Western blot analysis of the endogenously-expressed cell cycle and PI3-K/PKB/Akt pathway-regulating proteins in non-transfected cells demonstrated an inverse relationship between PTEN and phosphorylated PKB/Akt in the group of breast and prostate cancer cell lines. The immunocytochemical analysis showed slight cytoplasmic and, especially, nuclear expression of PTEN in MCF-7 cells and no expression in BT-549 cells. The MCF-7 and BT-549 cell lines contain different levels of proteins regulating the cell cycle and major differences were presented in the p53 and pRb status. We believe that these tumour suppressors are prerequisites for the PTEN inhibitory function in the tested breast cancer cell lines. Partial S-phase inhibition after PTEN transfection in BT-549 cells with stably-transfected Rb (BTA-pRb) demonstrated the important role of Rb, but also that of p53.

The transfection of the H123Y mutant with defective lipid and protein phosphatase activity caused a decrease in p27\textsuperscript{Kip1} and p21\textsuperscript{Waf1/Cip1} and a slight increase in phosphorylated PKB/Akt in the MCF-7 cells. In the BT-549 cells, no changes in p27\textsuperscript{Kip1} and p21\textsuperscript{Waf1/Cip1} expressions were detected after transient transfection of H123Y, but PTEN transfection caused a decrease in PKB/Akt phosphorylation. Moreover, PTEN transfection led to a decrease of cyclin E/cdk2 activity in MCF-7 cells. These PTEN effects were accompanied by a reduction of the cell number reaching the S-phase of the cell cycle. Inhibition of S-phase entry caused by PTEN was observed in the MCF-7 cells containing the wild-type of endogenous PTEN, p53 and pRb and in the breast cancer cell lines ZR-75 and MCF-10a, as published previously (27). The ZR-75 cell line contains wild-type p53 and pRb and mutated PTEN and the MCF-10a cell line contains the wild-type of all 3 tumour suppressors. It is likely that p27\textsuperscript{Kip1} could be a candidate as a mediator of the PTEN cell cycle inhibitory effect in the analysed cell lines. PTEN-induced S-phase inhibition was supported by the first results from the M8-tTA6 cells, MCF-7 cells with tetracycline-inducible stably-transfected PTEN clone. Protein levels of p27\textsuperscript{Kip1} and those of p21\textsuperscript{Waf1/Cip1}, to a lesser degree, were increased after 48 h of PTEN induction, as detected by Western blotting.

However, the influence of PTEN on PKB/Akt phosphorylation was manifested in both cell lines, MCF-7 and BT-549, independently of p53, Rb and endogenous PTEN status. Weng et al. (19) published similar results in MCF-7/Toff cells. The expression of the PTEN stably-transfected clone led to an increase in p27\textsuperscript{Kip1} and to a decrease in phosphorylated PKB/Akt and cyclin D1, whereas
the expression of the phosphatase dead mutant C124S had
the opposite effect. The cell cycle arrest in G1-phase, the up-
regulation of p27\textsuperscript{Kip1} in complex with cyclin E and cdk2 and
and the reduction of cyclin E/cdk2 activity mediated by
transfected PTEN were observed in glioblastoma cell lines
with defective endogenous PTEN, but not in cell lines with
wild-type PTEN (18, 21). Cheney \textit{et al.} (18) and Paramio \textit{et al.}
(20) also described a down-regulation of pRb phosphorylation by PTEN in glioblastoma cells, as well as in
normal and transformed human/mouse fibroblasts and
keratinocytes. This effect was conditioned by defective
endogenous PTEN. In our experiment, the expression of
D-type cyclins and the total level and phosphorylation of
p27\textsuperscript{Kip1} could be cell type-specific (32-34). Our results
indicated that PTEN is involved in the PI3-K/PKB/Akt signalling pathway is probably
independent of the regulation of the G\textsubscript{1}-S transition in the
cell lines used. Some findings regarding the connection of
the PI3-K/PKB/Akt pathway and cell cycle proteins have been
published (28-31). Liang \textit{et al.} (29) showed that transfection of
PKB/Akt led to p27\textsuperscript{Kip1} phosphorylation, its transfer from
the nucleus to the cytoplasm and to an inability to induce G\textsubscript{1}
arrest. In human breast cancer, the cytoplasmic localisation of
p27\textsuperscript{Kip1} was associated with PKB/Akt activation, loss of
differentiation and also with poor patient outcome (29).

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Acknowledgements

We thank Mrs. E. Pimrova, Mrs. J. Holinkova, Mrs. L. Popova and
Mr. O. Błaha for their excellent technical assistance concerning the
experiments and documentation. Our special thanks go to Dr. J.
Bartek, Dr. J. Lukas, Dr. P. Guldberg and Dr. M. Jäätelä from the
Danish Cancer Society (Copenhagen, Denmark), and Dr. B.
Vojtesek from the Masaryk Memorial Cancer Institute (Brno,
Czech Republic), for providing some of the antibodies and cell
lines used as well as for their help with mutation analysis.

This work was supported by grants GACR 204/01/0488, IGA
MZ CR NC 6779-3, MSM 151100001 and MSM 6198959216.


