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ORIGINAL ARTICLE

# Survivin expression in pituitary adenomas

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## Abstract

Survivin has received great attention due to its expression in many human tumours and its potential as a therapeutic target in cancer. Its expression is developmentally regulated: present during fetal development, it is undetectable in terminally differentiated normal adult tissue. Survivin expression has been described to be cell cycle-dependent and restricted to the G2-M checkpoint, where it inhibits apoptosis in proliferating cells.

**OBJECTIVES**: The aim of our study was to determine the survivin expression in different types of pituitary adenomas.

**METHODS**: Tissue samples were obtained during surgical removal of the tumour from 12 patients with diagnosed: acromegaly in seven cases, non-functioning pituitary tumours in four cases and prolactinoma in one case. Six patients with acromegaly received long-acting somatostatin analogues before tumour resection. After RNA extraction and cDNA synthesis, the amplification of specific survivin's gene fragment was performed.

**RESULTS**: In agreement with the current view that survivin is a tumor-associated antigen, highly expressed in various tumours, we found the presence of survivin expression as a characteristic feature of human pituitary adenomas. The findings of our study demonstrated the presence of an active survivin gene in all twelve analysed pituitary tumours.

**CONCLUSIONS**: Based on these findings, we conclude that the estimation of survivin expression in human pituitary tumours may help predict tumour growth and prognosis.

**Abbrevations**

| | |
|---|---|
| IAP – | inhibitor of apoptosis family |
| GH – | growth hormone |
| IGF-1 – | insulin-like growth factor |
| LAR – | long-acting release |
| HeLa – | cells of human cervical carcinoma |
| PTTG – | pituitary tumor transforming gene |
| PCR – | polymerase chain reaction |

## Introduction

Survivin is an unique member of the inhibitor of apoptosis protein family (IAP) [7]. The survivin gene is located on 17q25 chromosome and consists of 4 exons [4]. Survivin is present during fetal development, it is downregulated in normal adult tissues, however it becomes re-expressed in variety of cancers [6,21,2,15]. Survivin expres-

sion in tumours has been associated with increased aggressiveness and decreased patients survival [26,32,30,3,1,20]. It inhibits caspase-3 and -7 activity and is restricted to the G-2-M checkpoint [23,31,18]. Structurally it is characterised by a single beculovirus IAP repeat (BIR), an 70-amino-acid zinc-finger fold that is the hallmark of all IAPs and an extended C- terminus α-helical coiled-coil, but it lacks a carboxyl-terminal RING finger. [6]. Recently, survivin has received great attention as one of new targeting anti-cancer therapy since it could inhibit apoptosis induced by a variety of factors [8,11]. The frequency and prognostic significance of survivin expression in many human cancers is discussed worldwide, but there is no evidence of survivin expression in pituitary tumour tissues. Pituitary tumours constitute 10% of intracranial neoplasms and the most frequent are hormone-secreting pituitary adenomas. They are characterized by local malignancy due to their critical location and expanding size, however there are single reports about cancer of the pituitary gland [16,25]. The aims of the therapy of pituitary adenomas are to reduce the hypersecretion of pituitary hormones and to remove the tumour mass. Apart from the surgery (by means of transsphenoidal or transcranial approach), an effective treatment of adenomas involves radiotherapy and pharmacotherapy. Recently pharmacotherapy of GH-secreting pituitary adenomas has been improved by introducing long-acting forms of somatostatin analogues (octreotide, lanreotide). In a long-term therapy they induce disease control by reducing GH and IGF-1 levels and additionally causing tumour shrinkage, which facilitates surgical procedure [13,10,5]. Moreover, many current studies demonstrate the induction of apoptosis by somatostatin analogues in somatotropinoma type tumors [33,14]. The etiology of pituitary tumours is unresolved and presumably many initiating and promoting factors are involved. Inhibition of apoptosis is likely to play a major role in pituitary tumorogenesis and inhibition of apoptotic activity due to survivin may result in the oncogenic transformation in the pituitary and other tumours.

Therefore, we decided to investigate the expression of a novel member of IAP-family – survivin in different types of pituitary adenomas.

## Materials and methods.

*Tissue samples.* Pituitary tumour tissues were obtained from 12 patients treated with the surgery at the Department of Neurosurgery in Poznań University of Medical Sciences in 2003. The studied group consisted of 4 females and 8 males (mean age 49 years, range 24–71). Seven out of twelve patients have been diagnosed with GH-producing pituitary adenomas. Six of them had received long-term somatostatin analogues – octreotide LAR (Sandostatin) at the dose of 20 mg–30 mg–30 mg – in monthly intervals – before tumour resection. One of the studied patients was not treated with somatostatin analogues. Acromegaly was diagnosed on the basis of clinical findings and elevated level of GH and IGF-1. Four out of twelve patients have been diagnosed with non-functioning pituitary tumours and one patient with a prolactinoma. The presence of a pituitary adenoma was confirmed in magnetic resonance imaging in all twelve cases. The histopathological examination revealed adenoma acidophilicum in eight cases, I-st grade of malignancy, craniopharyngiomas in two cases and non-specific changes in remaining patients. The qualification of each tissue samples went through macroscopic pathologist examination. Tissue samples, obtained at the operation were immediately frozen in liquid nitrogen. As a control of the study the total RNA isolated from a tumour cell line – HeLa was used.

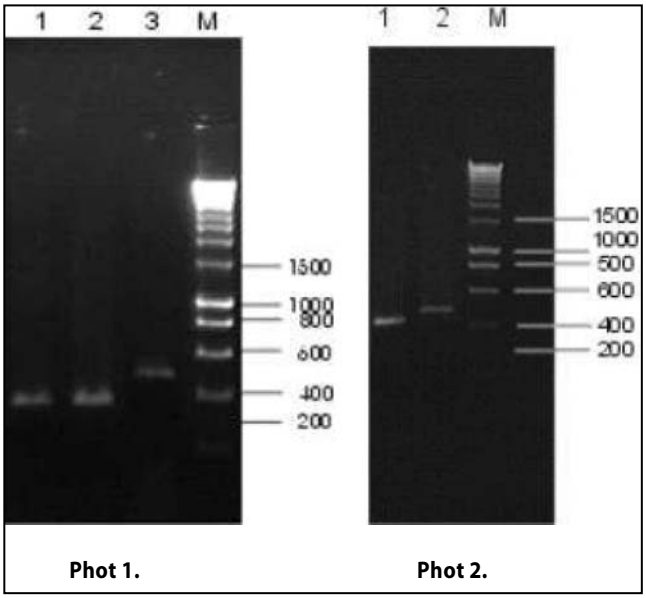
The study was approved by the Local Ethics Committee.

*RNA extraction and cDNA synthesis.* Total RNA was isolated from the tumor tissue and from HeLa cells with TRIZOL Reagent (GIBCO BRL, Grand Island, New York, USA), according to the manufacture protocol. About 1μg of RNA (DNase treated) was employed individually for one reverse transcription reaction in a mixture containing: 50 pmoles of survivin sequence specific primer 5'- GGC CTC AAT CCA TGG CAG C -3' (nucleotides 438-457, PubMed – AC: AF077350) or 100 pmoles Oligo $(dT)_{10}$ primer, 5U/μl Expand Reverse Transcriptase, 1x Expand Reverse Transcriptase buffer, 1mM of dNTPs, 20U RNase Inhibitor, 10 mM DDT. The reaction mixture was incubated at 42°C for 60 min, and the reaction was stopped by putting on ice. All compounds used for cDNA synthesis was obtained from Roche Molecular Biochemicals, Mannheim, Germany.

*PCR amplification.* A 424 bp fragment of *survivin* was amplified from cDNA using following primers: sense 5'-CAT GGG TGC CCC GAC GTT-3' (nucleotides 34-51, PubMed – AC: AF077350) and antisense 5'- GGC CTC AAT CCA TGG CAG C -3' (nucleotides 438-457, PubMed – AC: AF077350). Primers were designed to be complementary to the splice junction.

Additionally, as a control, a 509 bp specific fragment of house-keeping gene-*ß-actin* was amplified from cDNA amplified with universal primers using RNA isolated from the study tissue. In PCR the following primers: sense 5'-CATGTACGTTGCTATCCAGGC-3' (nucleotides 434-454, PubMed – AC: X00351) and antisense: 5'-CAGACAGCACTGCTGTGTTGGC-3' (nucleotides 924-942, PubMed AC: X00351) were used.

The amplification was performed in a reaction mixture containing: 1x Taq DNA polymerase buffer, 2.5mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM of each primer and 1 unit of Taq DNA polymerase, with thermal profile as followed: 5 min at 95°C, 1 min at 95°C, 45 sec at temperature specific for the primer's set, 1 min at 72°C for 30 cycles. All compounds used for DNA synthesis was obtained from Bioline, London, UK. The amplified products (half of the reaction mixture) were electrophoresed on 1% agarose gel (FMC BioProducts, Rockland, Maine, USA).




Phot 1. Phot 2.

**Phot 1.** Expression of survin gene in pituitarty tumours. RNA was isolated from the pituitary tumors and reverse transcribed. From the resulting cDNA a 424 bp fragment of survivin (lane 1-2), and a 509 bp fragment of β-actin (lane 3) was amplified. Molecular size marker is given alongside.

**Phot 2.** Expression of survin gene in HeLa cel line. RNA was isolated from the HeLa cell line cultured in vitro and reverse transcribed. From the resulting cDNA a 424 bp fragment of survivin (lane 1), and a 509 bp fragment of β-actin (lane 2) was amplified. Molecular size marker is given alongside.

## Results

Malignant transformation of cells is accompanied by multiple genetic abnormalities with atypical expression of genes. Using the reverse transcriptase polymerase chain reaction we have analyzed the *survivin* gene expression in pituitary tumours.

### Tissue expression of survivin mRNA

*Pituitary tumours group*. To verify at molecular level the presence of *survivin* in studied pituitary tumours, total RNA was isolated from the tissues and reversely transcribed. A 424 bp fragment corresponding to *survivin* nucleotides 34-457 [PubMed, AC: AF077350] was amplified from cDNA library (Phot. 1, line 1-2). Amplification of a 509 bp fragment of the *ß-actin* [PubMed AC: X00351] served as control (Phot. 1, line 3). In both cases the amplification yielded a single band of the predicted size

*Control group.*PCR with cDNA synthesized from total RNA isolated from HeLa cell lines, run as positive control show the presence of *survivin* transcripts (Phot.2, line 1).

## Discussion

There are many reports on survivin in various human cancers, describing the correlation between the degree of survivin expression and tumour aggressiveness and disease progression [26,2,32,30,3,15]. On the base of previous findings, we concluded that survivin could be detectable in pituitary tumours and the level of its expression could correlate with local malignancy of pituitary neoplasms. Our present study was performed to determinate the expression of the *survivin* gene in pituitary tumours. The findings indicate that mRNA of the gene is present in pituitary secreting adenomas as well as in non-functioning pituitary tumours. We observed that the level of survivin expression was much lower in pituitary tumours than in HeLa group, but there were no significant difference in survivin expression between pituitary tumours groups. This is, to our knowledge, the first report, which directly confirms the production of survivin in pituitary tumours tissue. Although pituitary adenomas are mostly benign, monoclonal, derived from single mutant cells, their continued slow growth in the confined sellar and suprasellar areas causes serious neurologic damage. The pathogenesis of pituitary adenomas proceeds through a series of genetic changes involving the activation of oncogenes and loss of tumour suppressor genes. Previous studies investigated the role of p53 family, p27, which were thought to play a major role in the pituitary tumorogenesis [27]. They also focused on the role of the GH/IGF-1 system in the process of tumorogenesis [36]. The Mib-1 has been also proposed as the most reliable marker of proliferation in pituitary tumours [29]. An important role in pituitary tumorogenesis is attributed nowadays to the pituitary tumour transforming gene (PTTG) oncogene family [28]. PTTG is expressed in most pituitary tumours and other neoplasms, activates p53 and causes p53-dependent and – independent apoptosis [35]. Current studies suggest that targeted inhibition of PTTG1 action may be a potential tool for the therapy of prolactin-secreting adenomas [17]. Survivin – a new member of IAP-family proteins has received great attention as a new targeting anti-cancer therapy [8,11,19,9]. It can inhibit apoptosis induced by a variety of factors. Ambrosini et al. first proposed survivin as a potential new target for the apoptosis-based therapy in cancer and lymphoma [6]. Further reports focus on cancers of lung, colon, skin, prostate, breast, leukaemia and lymphomas and indicate that survivin expression is an important predictive factor of poor outcome in these neoplasms [26,2,32,30,3,15]. Importantly, there seems to be a survivin implication in resistance to different apoptotic stimuli including chemiotherapy [24]. The newest study identified and characterized a novel survivin isoform, designed survivin 2α. Survivin 2α can alter the anti-apoptotic functions of survivin in malignant cells and may be useful in chemoresistant tumor cells therapy [12]. Previous research showed a rising incidence of survivin expression from 9% in low-grade adenomatous hyperplasia of the lung to 100% in bro-

chioloalveolar carcinoma and also no immunoreactivity of survivin in normal colorectal mucosas. They also showed a gradual increase of survivin level from adenomas with low grade dysplasia through high grade dysplasia to carcinomas [25, 13]. Because of the fact that survivin may play an important role in ocogenesis and progression of most cancers, many data suggest survivin as an attractive target for cancer therapy [26,8,15,19]. No research concerning survivin expression in pituitary adenomas has been performed so far, there were, however, few reports in other neuroendocrine neoplasms [22]. The survivin expression was described as a neuroendocrine marker of pheochromocytoma and was overexpressed in the neuroendocrine cells in human prostate cancer [22,34]. Considering the results of the study it is seems that estimation of *survivin* gene transcripts may have relevant application in the prognostic and therapeutic assessment of the cancer as well as of the pituitary tumours.

In conclusion, we found an active *survivin* gene to be present in pituitary tumours tissue.

There was no significant difference in survivin expression between pituitary tumours groups. We also observed its significantly higher expression in HeLa cells. Furthermore quantity research is needed to establish the level of expression in studied tissue.

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