Coexpression of survivin and PCNA in pituitary tumors and normal pituitary

Ryszard Wasko [1], Joanna Waligorska-Stachura [1], Anna Jankowska [2], Jerzy B. Warchol [2], Wlodzimierz Liebert [3], Jerzy Sowinski [2]

1. Department of Endocrinology, Metabolism and Internal Diseases;
2. Department of Cell Biology;
3. Department of Neurosurgery and Neurotraumatology; University of Medical Sciences in Poznan, Poland.

Correspondence to: Ryszard Wasko, M.D., Ph.D., Professor of Endocrinology
University of Medical Sciences,
Deparment of Endocrinology, Metabolism and Internal Diseases
Przybyszewski street 49, 60-355 Poznan, POLAND
PHONE: +48 61 8691332; FAX: +48 61 8691682; E-MAIL: rwasko@amp.edu.pl

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Abstract

OBJECTIVES: The survivin is the protein involved in regulation of basic and cycle-specific functions of cells both in normal and cancer tissue. Recent studies present survivin as a factor having the leading role in the regulation of apoptosis and mitosis as well as a target of anticancer therapy. The employing of survivin in this therapy is based on its high expression level in most human cancers, as well as its association with the disease's progression. The aim of our study was to evaluate the expression and localization of survivin's gene product on the protein level in different types of pituitary tumors and normal pituitary. The coexpression of survivin and proliferating cell nuclear antigen – PCNA in pituitary was also examined.

DESIGN AND METHODS: The study was conducted on the postoperative pituitary tumors tissue taken during standard neurosurgical removal of tumor from 43 patients. The group of patients consists of 23 women and 20 men, aged from 27 to 71 years. As a control of the study three normal pituitary tissues obtained at the autopsy were used. Evaluation of survivin and PCNA expression was performed using immunohistochemical staining.

RESULTS: The study demonstrated the presence of survivin in all analyzed by us pituitary tumors. Survivin was present also in normal pituitary tissue. The protein was localized mainly in cell's nuclei, however the less intense immunostaining was observed also in the cytoplasm of pituitary tumors cells. Furthermore survivin was found in normal pituitary, but the positive immunostaining was limited to a single cells. The analysis of pituitary tumor cells proliferation index based on PCNA reactivity showed that survivin is coexpressed with PCNA, especially in invasive tumors.

CONCLUSIONS: The study documented the presence of survivin in different types of pituitary tumors as well as in normal pituitary. Additionally the coexpression of survivin and PCNA in tumor cells was shown. The expression of survivin in both normal and cancer pituitary cells suggests that it may play an important role in regulation of the gland's proliferation.

ORIGINAL ARTICLE

Abbrevations & Units:
IAP – inhibitor of apoptosis protein
BIR – Baculovarius IAP repeat
PCNA – proliferating cell nuclear antigen
RING – really interesting new gene
NFκB – nuclear factor kappa B

INTRODUCTION

Survivin (IAP 4) is one of the proteins with the ability to regulate cell cycle and programmed cell death. It belongs to the family of proteins inhibiting the apoptosis – IAP (inhibitor of apoptosis protein) (Holcik 2002). These proteins disturb transfer of apoptotic signal by creation of complex with other proteins, such as caspases end NFκB (Roy *et al.* 1997).

Survivin is a protein possessing a single baculovarius IAP repeat (BIR) domain and an extended α-helical coiled-coil C-terminus. Contrary to other members of the IAP family it does not contain a RING-finger domain (La Casse *et al.* 1998). Unique structure of survivin protein allows the protection of cell against apoptosis and the participation in regulation of mitosis through „stabilization" of mitotic apparatus (Li & Altieri 1999). The antiapoptotic action of survivin result from blocking the activity of caspase-9, but not the caspases 3 and 7 as it was postulated before (O'Connor *et al.* 2000, Ambrosini *et al.* 1998).

Transcription of the gene is controlled by specific promoter sequences and increases in G1 phase of the cell cycle, however the highest accumulation of the protein was noted in the G2-M phase (Altieri 2003, Marusawa *et al.* 2003, Tamm *et al.* 1999).

In a cell survivin was found into two separate immunohistochemical areas (O'Connor *et al.* 2000a). One includes cellular core of kinetochores of metaphase chromosome and mitotic spindle during anaphase. In the cytosole the majorities of surivivn is observed in a complex with interphase centrosomes and in the microtubules of mitotic spindle during metaphase and anaphase.

Survivin is expressed especially in fetal and embryonic cells but it also present in mature tissues with high proliferation potential such as: cells of placenta, thymus, endothelium, and CD34 + hematopoietic progenitor cells (Deguchi *et al.* 2002, Chiou *et al.* 2003). Besides its occurrence was revealed in many human neoplasms. The presence of survivin in both normal and neoplastic tissues suggests, that the protein may play a regulative role during proliferation tissues (Wanget *et al.* 2004, Hassounah *et al.* 2005, Jankowska *et al.* 2008).

Taking into account the fact, that survivin is detected in many human tumors, it can be assumed that it may also be a key factor of pituitary carcinogenesis (Hengartner 1998, Sasaki *et al.* 2002). The result of the present study expands our previous research showing survivin expression in pituitary adenomas (Fukuda *et al.* 2006). Our current studies were designed to localize survivin in pituitary tumors and to correlate its expression with the ability of the cells to proliferate.

MATERIALS AND METHODS

The study was performed on pituitary adenomas tissue received from 43 patients. Among the patients undergoing pituitary tumor removal were 23 women and 20 men, aged from 27 to 71 years (mean 52.5). Immunohistochemical evaluation of survivin expression was conducted in 22 somatotropinomas, 16 nonfunctioning pituitary tumors, 4 prolactinomas and 1 adrenocorticotropin–producing pituitary tumor. The tumors after surgery were subjected to routine operational histolopathological assessment at the Department of Patomorphology in Poznan University of Medical Science.

As a control for the research normal pituitary tissue (n=3) obtained at the autopsy was used. Directly after surgical removal of the tumor, tissue was fixed in 4% paraformaldehyde.

The study was approved by the ethics reviewed board of Poznan University of Medical Sciences; all patients participated after written consent.

MATERIALS AND METHODS

Immunohistichemistry

Paraffin sections of the analysed tissue were used for immunohistochemical detection of survivin. The antigens were retrieved by microwave activation in citrate buffer (10 mM, pH 6.0). After being blocked in a TBS-T blocking buffer, pH 7.5 (containing 100 mM TRIS-HCl, 0.9% NaCl, 0.05% Tween-20 (TBS-T) and 3% BSA) the sections were incubated overnight at 4°C in humid chamber with primary polyclonal antibodies against survivin (BIOTREND Chemikalien GmbH) diluted 1:200. After washing 3 × 15 minutes in TBS-T buffer the EnVision+ System (DAKO A/S) was applied for detection. Control experiments included reactions carried out under identical conditions except the primary antibodies were replaced by non-immune serum. Detection of antigen-antibodies complex was visualized employing light microscope (Zeiss, Axioskop 2).

RESULTS

Immunohistochemical assessment of survivin expression in pituitary tumors

A positive result of immunohistochemical reaction confirmed the presence of survivin in analyzed by us pituitary tumors. Survivin was detected in a number of cells both invasive (Fig.1) and non-invasive tumors (Fig.2). Positive results of the reactions were observed mainly in the nuclei of tumor cells, however a weak immunohistochemical signal was seen also in the cytoplasm.

Positive reactions with antibodies directed against survivin were also noted in normal pituitary. In this

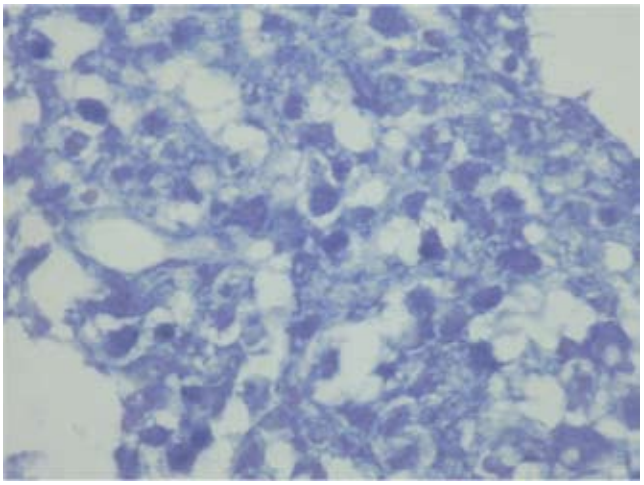

Figure 1. Localization of survivin in invasive pituitary tumor. Immunohistochemistry was performed using the antibodies against survivin as it was described in the Material and methods section. The positive staining was observed mainly in the nuclei of pituitary cells; some cytoplasmic staining was detected in a single cells. Original magnification x1000.

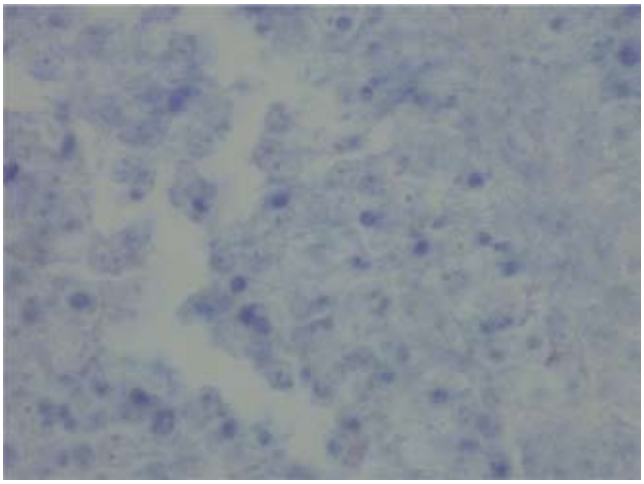

Figure 2. Immunohistochemical detection of survivin in non-invasive pituitary tumor. Immunoreactive survivin was noticed in the nuclei of study cells. Original magnification x400.

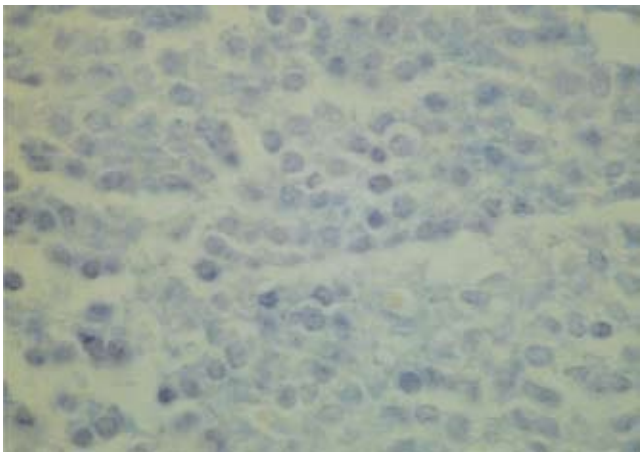

Figure 3. Survivin expression in normal pituitary. A single cells producing survivin were observed in normal tissue. Original magnification x400.

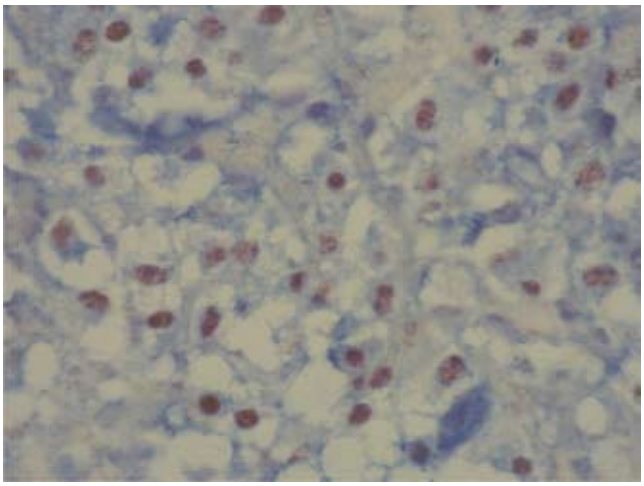

Figure 4. Immunolocalization of survivin and PCNA in invasive pituitary tumor. Coexpression of survivin (blue) and PCNA (brown color) visible as a purple staining in the nuclei of dividing cells was noted. Original magnification x400.

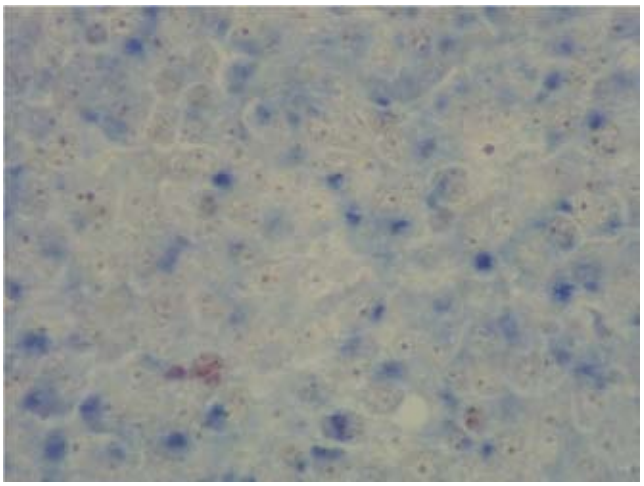

Figure 5. Coexpression of survivin and PCNA in non-inwasive pituitary tumor. Most nuclei showed only the expression of PCNA (light brown color) or survivin (blue). Both antigens (purple) were present in a small number of cells. Original magnification x400.

case immunohistochemical staining was significantly weaker and was limited to single cells of the tissue (Fig. 3).

Evaluation of proliferation in pituitary tumors characterized by survivin expression

In order to assess the proliferation status of pituitary tumor cells, in which the presence of survivin was detected the tumors' section were tested for the presence of proliferation antigen – PCNA. Cross-reactivity of antibodies against PCNA and survivin showed that survivin is expressed only in part of the proliferating tumor cells tested. The colocalization of survivin and PCNA characterized larger number of cells especially in the case of invasive pituitary tumors. Almost all the tumor cells showing the expression of PCNA were

characterized by the presence of survivin (Fig.4). In non-invasive tumor both proteins were observed only in individual cell's nuclei (Fig.5).

DISCUSSION

The process of carcinogenesis within pituitary gland is very complex. The multi-stage and pathogenesis of pituitary tumors is not fully known yet. Current research focuses mainly on the identification of prognostic markers that would be helpful in predicting the behavior of pituitary tumors. Among the potential markers of pituitary tumors is survivin. Numerous studies confirmed its participation in carcinogenesis, which is associated with inhibition of apoptosis mainly. In the majority of cancers in humans, survivin expression correlates with high proliferation index, low apoptotic rate, resistance to chemio- and radiotherapy and increased percentage of tumor relapse. Therefore, survivin is called an independent prognostic marker and treated as a good target of therapeutic approaches.

Our previous research showed the expression of survivin in pituitary tumors as well as in normal pituitary, nevertheless we demonstrated that the level of this protein expression was significantly higher in tumors compared with normal pituitary (Hassounah *et al.* 2005, Jankowska *et al.* 2008). The results of the current study are consistent with these previous observations, since they confirmed the presence of survivin protein in all studied by us tumors. The expression of survivin is characteristic feature of both non-invasive and invasive tumors, nonfunctional pituitary tumors, somatotropinomas, prolaktinoma-type tumors and adrenocorticotropinoma. Additionally we revealed that survivin expression correlates with the cells proliferation.

The results of our research are in good agreement with already published dates demonstrating survivin expression in primary tumors of the nervous system, meningiomas and benign peripheral nerve tumors (Sasaki *et al.* 2002).

In our study positive results of reactions with antibodies directed against survivin were also detected in normal pituitary. The accumulation of survivin in healthy tissue was very low and was limited to single cells. Survivin expression in both healthy and pathologically changed tissue of the pituitary could characterized stem cells capable to proliferate. This hypothesis appears to be confirmed by the recent studies of Fukuda and coworkers showing the survivin presence and describing its role in the differentiation of the hematopoietic stem cells (Fukuda *et al.* 2006).

The detail analysis of pituitary tumors performed in this study showed the presence of survivin mainly in the cells' nuclei. Still some weak immunohistochemical staining was observed in the cytoplasm of several cells. The nuclear localization of survivin in pituitary tumors as well as in normal pituitary noted by us imply that survivin plays an important role during proliferation both normal and neoplastic pituitary cells. However, the increased accumulation of survivin in tumor cells suggests that the protein is one of factors involved in neoplastic transformation of pituitary. To date many researches showed that the nuclear localization of survivin in cancer (eg. in esophagus cancer, liver, non-smallcell lung cancer, ovarian cancer, endometrial and lymphomas) is an adverse prognostic factor (Li *et al.* 2005, Fields *et al.* 2004, Shinohara *et al.* 2005). At the other hand some other articles illustrate that survivin accumulation in cell nucleus is linked with favorable course of disease (Li *et al.* 2005).

In our study we did not observed any significant differences in the expression level or localization of the protein between invasive and non-invasive tumors; in both cases the nuclear localization of survivin was observed.

During our study in addition to the evaluation of survivin expression in pituitary tumors and normal pituitary the proliferation status of the tissue was assess. The examination of proliferation status was carried out using the proliferation marker – PCNA. The increased expression of proliferating cell nuclear antigen, particularly in invasive tumors was established. This expression correlated with the occurrence of survivin in the analyzed tumors. Almost all the invasive tumor cells showing the expression of PCNA were also characterized by the presence of survivin. In the non-invasive tumors both proteins were observed only in individual cells.

The results concerning the correlation of survivin and PCNA coexpression in various pituitary tumors are consistent with other authors reports, which have shown that survivin expression in cancer cells is closely associated with high proliferation index, low apoptotic rate, resistance to chemio- and radiotherapy and increased percentage of cancer relapse (Kawasaki *et al.* 1998, Tanaka *et al.* 2000, Zaffaroni *et al.* 2002, Tran *et al.* 2002, Swana *et al.* 1999). High PCNA index in invasive pituitary tumors compared with non-invasive tumors was noted previously (Pawlikowski *et al.* 2006, Hsu *et al.* 1993). Also significantly higher concentration of survivin and Ki76 in brain tumors type meningioma with a more advanced degree of malignancy was observed (Kayaselcuk *et al.* 2004). Thus, coexpression of survivin and PCNA in pituitary tumors demonstrated in this study suggests that survivin may be a factor regulating pituitary cells proliferation.

CONCLUDING REMARKS

The results of the study showed the expression of survivin in pituitary tumors and normal pituitary. In addition, increased expression of PCNA correlated with

proliferation of pituitary cells, particularly in invasive tumors was confirmed. The expression of PCNA was associated with survivin occurrence.

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REFERENCES

1 Holcik M. (2002). The IAP proteins. Trends Genet **18**; 537-538
2 Roy N *et al.* (1997). The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. EMBO J. **16**; 6914-6925
3 La Casse EC *et al.* (1998). The inhibitors of apoptosis (IAPs) amd their emerging role in cancer. Oncogene. **17**; 3247-59
4 Altieri DC. (2003). Validating survivin as a cancer therapeutic target. Nat Rev Cancer. **3**;46-54
5 Marusawa H *et al.* (2003). HBXIP functions as a cofactor of survivin in apoptosis suppression. EMBO J. **22**; 2729-40
6 Tamm I *et al.* (1998). IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax caspases, and anticancer drugs. Cancer Res. **58**; 5315-20
7 Li F, Altieri DC. (1999). The cancer anti-apoptosis mouse survivin gene: characerization of locus and transcriptional requirements of basal and cell cycle-dependent expression. Cancer Res. **59**; 3143-51
8 O'Connor DS et at. (2000a). Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. Proc Natl Acad Sci USA **97**; 13103-07
9 O'Connor DS *et al.* (2000). Control of apoptosis during angiogenesis by survivin expression in endothelial cells. Am J Pathol. **156**; 393-8
10 Ambrosini G *et al.* (1998). Induction of apoptosis and inhibition of cell proliferation by survivin gene targeting. J Biol Chem. **273**; 11177-82
11 Deguchi M *et al.* (2002). Expression of survivin during liver regeneration. Biochem Biophys Res Commun. **297**; 59-64
12 Chiou SK *et al.* (2003). Survivin expression in the stomach: implications for mucosal intergrity and protection. Biochem Biophys Res Commun. **305**; 374-379
13 Wang Y *et al.* (2004). Survivin expression in rat testis is up-regulated by stem-cell factor. Mol Cell Endocrinol. **218**; 165-174
14 Hassounah M *et al.* (2005). Benign tumors from the human nervous system express high levels of survivin and are resistant to spontaneous and radiation-induced apoptosis. J NeuroOncol. **72**; 203-208
15 Jankowska A *et al.* (2008). Survivin products in pituitary tumors. Neuroendocrinol Lett. **6**; 807
16 Hengartner MO. (1998). Nature death cycle and swiss army knives. Nature. **391**; 441-442
17 Sasaki T *et al.* (2002). Expression of survivin, an inhibitor of apoptosis protein, in tumors of the nervous system. Acta Neuropathol. **104**; 105-9
18 Fukuda S *et al.* (2006). Survivin, a cancer target with an emerging role in normal adult tissues. Mol Cancer Ther. **5**; 1087-1098
19 Li F *et al.* (2005). Nuclear or cytoplasmic expression of survivin: what is the significance? Int J Cancer. **114**; 5-9-12
20 Fields *et al.* (2004). Survivin expression in hepatocellular carcinoma: correlation with proliferation, prognostic parameters, and outcome. Mod Pathol. **17**; 1378-85
21 Shinohara *et al.* (2005). Nuclear survivin predicts recurrence and poor survival in patients with resected nonsmall cell lung carcinoma. Cancer. **103**; 1685-92
22 Kawasaki H *et al.* (1998). Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. Cancer Res. **58**; 5071-4
23 Tanaka K *et al.* (2000). Expression of survivivin and its relationship to loss of apoptosis in breast carcinomas. Clin Cancer Res. **6**; 127-34
24 Zaffaroni N *et al.* (2002). Survivin expression and resistance to anticancer treatments: perspectives for new therapeutic interventions. Drug Resist Updat. **5**; 65-72
25 Tran J *et al.* (2002). A role for survivin in chemoresistance of endothelial cells mediated by VEGF. Proc Natl Acad Sci USA **99**; 4349-54
26 Swana HS *et al.* (1999). Tumor content of the antiapoptosis molecule survivin and recurrence of bladder cancer. N Engl J Med. **341**; 452-3
27 Pawlikowski M *et al.* (2006). Proliferatin cell nuclear antygen (PCNA) expression in pituitary adenomas: relationship to the endocrine phenotype of adenoma. Folia Histochem Cytobiol. **44**; 37-41
28 Hsu DW *et al.* (1993). Significance of proliferating cell nulear antygen index in predicting pituiary recurrence. J Neurosurg. **78**; 753-761
29 Kayaselcuk F *et al.* (2004). The expression of survivin and Ki-67 in meningiomas: correlation with grade and clinical outcome. J Neuro Oncol. **67**; 209-214