Coexpression of survivin and PCNA in pituitary tumors and normal pituitary

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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 cells 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.
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. 1999).

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 survivin 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

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 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
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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 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**

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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 adrenocorticotropinomas. 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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