Expression of survivin in invasive pituitary adenoma

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ABSTRACT

الأهداف: التحقق من العلاقة بين ظهور السورفيفان وورم الغدة النخامية الاقتحامي.

الطريقة: شملت الدراسة 66 مريضاً خضعوا لعملية جراحية عبر العظم الوتدي خلال الفترة ما بين يوليو 2006م وحتى مارس 2008م، بقسم جراحة المخ والأعصاب – مستشفى مقاطعة شاندونق – ومستشفى جينان المركزي – شاندونق – الصين. تم تقسيم كافة المرضى إلى المجموعة المقتحمة (عدد=39) مريضاً، والمجموعة الغير المقتحمة (عدد=27) مريضاً، بواسطة التقييم عقب العملية الجراحية باستعمال أشعة الرنين المغناطيسي والمعاينة أثناء العملية الجراحية. تم تحديد ظهور السورفيفان باستعمال كيمياء الأنسجة المناعية. تم إكمال التحليل الإحصائي لظهور السورفيفان بين المجموعتين باستعمال اختبار تشيكوير.

النتائج: تبين ظهور السورفيفان في 46 مريضاً (69.7%) من ورم الغدة النخامية الاقتحامي. كانت نتيجة صبغة ورم الغدة النخامية الاقتحامي بالسورفيفان موجبة في 35 مريضاً (89.7%)، كانت هنالك 11 عينة فقط (40.7%) موجبة بعدم ظهور السورفيفان في الأورام. اظهر اختبار تشيكوير الفرق الإحصائي الملحوظ لظهور السورفيفان بين ورم الغدة النخامية الاقتحامي وغير الاقتحامي (χ^2=14.309 – p=0.0002).

خاتمة: كان السورفيفان مصحوباً بصورة عالية مع ورم الغدة النخامية الاقتحامي وقد يكون الجين هدفاً فعالاً لعلاج جين ورم الغدة النخامية.

Objective: To investigate the relationship between survivin expression and invasiveness of pituitary adenoma.

Methods: A total of 66 patients, on whom trans sphenoidal surgery had been performed between July 2006 and March 2008, were enrolled in our study at the Department of Neurosurgery in Shandong Provincial Hospital and Jinan Central Hospital, Shandong, P. R. China. All patients were divided into the invasion group (n=39), and the non-invasion group (n=27) by assessment of preoperative MRI and intraoperative inspection. Survivin expression was determined by immunohistochemistry. Statistical analysis of survivin expression between the 2 sample groups was accomplished using the chi-square test.

Results: Survivin was expressed in 46 (69.7%) of the investigated pituitary adenomas. For invasive pituitary adenoma, survivin staining was positive in 35 (89.7%), only 11 (40.7%) specimens were positive in noninvasive tumors. The chi-square test demonstrated a statistically significant difference in survivin expression between invasive and noninvasive pituitary adenoma (χ^2=14.309, p=0.0002).

Conclusions: Survivin was highly associated with invasive pituitary adenoma, it is likely to serve as a useful tool for confirmation of invasive pituitary adenoma and the gene could be an effective target for pituitary adenoma gene therapy.

Saudi Med J 2008; Vol. 29 (11): 1589-1592

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Received 13th July 2008. Accepted 7th October 2008.

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Pituitary tumors constitute 10% of intracranial neoplasms. Although pituitary adenomas are regarded as benign, slowly growing brain tumors, some studies demonstrate tumor invasion into the adjacent tissues (bone, dura, or cavernous sinus) at initial diagnosis. The therapy of total resection of pituitary adenoma could be curative, but this strategy had so far been limited due to tumor invasion. It has been impossible to surgically resect tumor tissues within the cavernous sinus and hypophysis, due to the risk of damaging the cerebral nerves and hypophysial nucleus.

Disclosure. This study was supported by grants from the Program of Shandong Science and Technology Committee (2006GG3202013) and the National Natural Science Foundation of China (30801237).

In these cases, secondary tumors often grow from the resection margins. Therefore, invasiveness or infiltration of adjacent tissues might serve as an important factor adversely affecting the outcome of the tumor and compromising the disease free survival of patients.[1,2] To date, the mechanisms underlying this aggressive biological behavior had not yet been fully clarified.[3] Like other tumors, there was suggestion that a loss of balance between regulators of cells proliferation and apoptosis might have a role to play in invasiveness of pituitary tumors. Survivin, a 16.5 kDa protein, was a structurally unique member of the inhibitor of apoptosis protein (IAP) family.[4] The survivin gene was located on 17q25 and consists of 4 exons and was expressed during the G2/M phase of the cell cycle.[5,6] Survivin expression was present during fetal development and not found in nonneoplastic adult human tissue, and it was turned on in most common human cancers.[7,8] Survivin expression in tumors had been associated with increased invasiveness and decreased patients survival.[9-11] It was also reported that the survivin mRNA level was negative in non-tumorous anterior pituitary lobe tissues and high in pituitary tumors tissue.[12] We hypothesize that survivin expression is related to invasiveness of pituitary tumor tissues. Therefore, the aim of the present study is to investigate the expression of survivin in 66 patients with human pituitary adenomas by immunohistochemistry and determine its association with tumor invasion.

Methods. *Tissues.* Pituitary adenoma tissues were obtained by trans sphenoidal surgery from 66 patients at the Department of Neurosurgery in Shandong Provincial Hospital and Jinan Central Hospital, Shandong, P. R. China from July 2006 to March 2008. The study complied with ethical principles, and permission was obtained from the 2 hospitals. The diagnosis of pituitary adenoma was confirmed by pathology. All patients who had undergone pituitary surgery or underwent previous anti-tumor treatment were excluded. The subjects included 29 women (mean age 65 years, range 29-77 years) and 37 men (mean age 68 years, range 34-82years). None of the patients with acromegaly received octreotide, and none of the patients with prolactinoma received preoperative dopamine agonists. Parasellar invasiveness of pituitary adenomas was evaluated by preoperative MRI,[13] and verified by intraoperative inspection of the medial wall of the cavernous sinus using the mirror technique:[14] 39 cases were found to be invasive, and 27 tumors were noninvasive. Tissue samples were fixed in formalin (4%) and paraffin-embedded and stained with hematoxylin-eosin (HE). Immunohistochemistry was performed with antibodies for survivin. All pituitary adenomas were evaluated histologically according to the World Health Organization classification.[15,16]

Immunohistochemical staining procedure. Primary rabbit monoclonal antibodies to human survivin (71G4, 1:100 Cell Signaling Technology, United States) and Secondary anti-rabbit antibody (ZB-2301, 1:500 Zhongshan Goldenbridge Biotechnology Co. Ltd, P. R. China) were used. The immunohistochemical staining procedure was carried out according to the Cell Signaling Technology's protocol. A breast cancer served as positive control. Citrate buffer instead of primary antibody served as the negative control.

Analysis of immunohistochemical results. Survivin immunoreactivity was evaluated semiquantitatively based on the intensity of staining. The percentage of positive tumor cells was evaluated in 5 areas at ×400. The slides were analyzed independently by 4 observers blinded for expression of survivin. There was general agreement between the observers in most cases. For the discrepancies a second evaluation course was run to reach agreement. The survivin expression was scored as: strong (+++) if more than 50% of cells were positive or a strong diffuse reaction was seen; moderate (++) if less than 50% of cells were positive, or a moderate diffuse reaction was observed; slightly positive (+) if immunoreactions were found in less than 10% of tumour cells or the diffuse reaction was weak; and negative (-) if no survivin was stained.

Statistical analysis. Statistical analysis of survivin expression between the 2 sample groups was accomplished using the chi-square test. The SPSS Version 13.0 was used for statistical analyses. *P*-value less than 0.01 was considered to be statistically significant.

Results. In the pituitary adenomas, a cytoplasmic survivin reaction predominated and only scattered cells exhibited nuclear reaction (**Figure 1**). Survivin was expressed in 46 (69.7%) of the investigated pituitary adenomas. Therefore, there was high expression of survivin in pituitary adenoma. For invasive pituitary adenomas, survivin staining was positive in 35 (89.7%). Only 11 (40.7%) specimens were positive in noninvasive tumors. The chi-square test demonstrated a statistically significant difference in survivin expression between invasive and noninvasive pituitary adenomas ($\chi 2=14.309$, $p=0.0002$). Furthermore, the strong and moderate were the majority of positive invasive pituitary adenomas, but most of positive noninvasive pituitary adenomas were slight. So, the level of its expression was much higher in invasive pituitary adenomas than in noninvasive ones (**Table 1**).

Discussion. In the study, we demonstrated that the positive rate and intensity of survivin were much higher in invasive pituitary adenomas than in

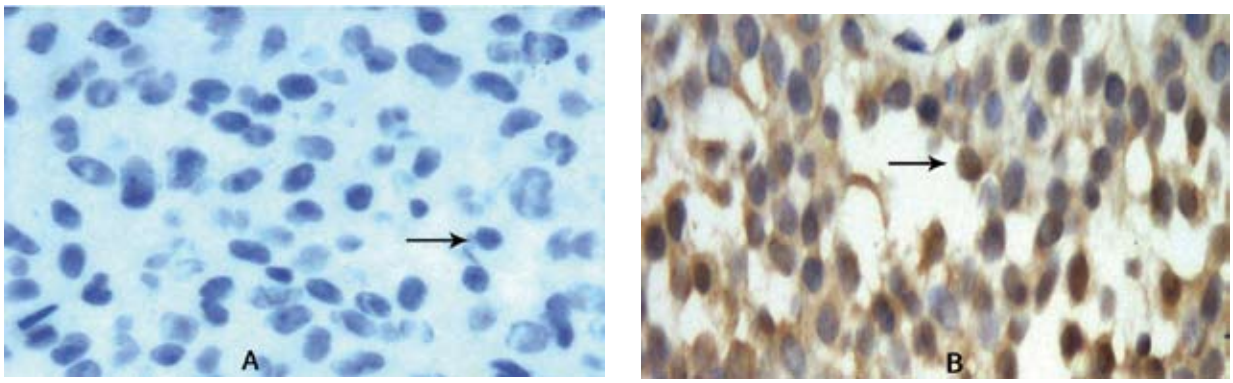

Figure 1 - Expression of survivin in human pituitary adenomas showing; **a**) negative control. Arrow shows nuclear negative. **b**) diffuse expression was classified as strong (+++), a cytoplasmic survivin reaction predominated and only scattered cells exhibited nuclear reaction. Arrow shows nuclear reaction.

Table 1 - Expression of survivin in human pituitary adenomas with respect to invasiveness of the tumor.

Pituitary tumor	N	+++	++	+	Positive, n (%)
Invasive	39	7	18	11	35 (89.7)
Noninvasive	27	0	3	8	11 (40.7)
Total	**66**	7	**21**	**19**	**46 (69.7)**

The distinguishing criteria: +++/++/+ were pooled as "positive", +++ - strong, ++ - moderate, + - slightly positive

noninvasive ones. Also, we also found there was high expression of survivin in pituitary adenoma (69.7%) by immunohistochemistry. We investigated survivin expression of pituitary tumors at the protein level, and this is in accord with survivin expression of pituitary tumors at the mRNA level.[12] Therefore, it suggests that survivin is closely associated with invasiveness and tumorigenesis of pituitary tumors.

Like other tumors, tumorigenesis of pituitary tumors involves a loss of balance between regulators of cells proliferation and apoptosis. Activation of apoptosis has been shown to be important in tumorigenesis and contributes to tumor invasion and metastasis. Survivin is a structurally unique member of the IAP family.[4] The survivin gene is located on 17q25 and consists of 4 exons. It is expressed in the G2/M phase of the cell cycle and at the beginning of mitosis, where survivin associates with microtubules of the mitotic spindle.[5,6] The mechanism whereby it blocks apoptosis is assumed to be via an effect on caspase-9, which is activated through extrinsic and intrinsic pathways.[17,18] In the intrinsic cell death pathway, upstream stimuli induced expression of proapoptotic bcl-2 family proteins such as bax. The membrane-permeabilizing effects of bax and other proapoptotic proteins were inhibited by antiapoptotic proteins, such as bcl-2 and bcl-XL, which also might inactivate caspase-9 by binding and inactivating the adapter protein Apaf-1.[19,20] When, as a result, caspase-3 was not activated, apoptosis was inhibited.[11,21] Much of the available data described that survivin expression in tumors had been associated with increased invasiveness and decreased patients survival.[9-11] Considering the results of the study, it seems that survivin was a reliable and effective diagnostic molecular marker predicting invasiveness of pituitary adenomas. Moreover, survivin acts as a suppressor of apoptosis and plays a central role in cell division. Its expression is present during fetal development and is not found in nonneoplastic adult human tissue, it could be attractive for cancer treatment.[7,8,22] Many data have demonstrated that downregulation of survivin gene expression or function, accomplished by various strategies, reduces tumor growth potential, increases the apoptotic rate, and sensitizes tumor cells to chemotherapeutic drugs and radiation in different human tumor models.[23-25] The data presented here shows that there was high expression of survivin in pituitary adenoma. Thus, the survivin gene also could be an effective target for pituitary adenoma therapy.

In conclusion, survivin was highly associated with invasive pituitary adenomas, it is likely to serve as a useful tool for confirmation of invasive pituitary adenoma and the gene could be an effective target for pituitary adenoma gene therapy. However, only 66 patients were involved in our study, and further research in a larger number of patients is needed to confirm the relation between survivin expression and invasion of pituitary adenoma.

Acknowledgment. *We thank the supporting medical staff for making this study possible.*

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