Expression of galectin-3 correlates with apoptosis in pituitary adenoma cells

Chui-Xue HUANG¹, Yong-Hong HOU², Yun-Sheng LIU²

¹Department of Neurosurgery, Hainan Province People's Hospital, Haikou 570311, China

²Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha 410078, China

Abstract: Objective To investigate the expressions of Galectin-3 (Gal-3), Bcl-2 and Bax in human pituitary adenomas, and to explore the interrelation among them. **Methods** RT-PCR and immunohistochemistry were applied to detect the mRNA and protein expressions of Gal-3, Bcl-2 and Bax in surgically excised human pituitary adenoma tissues, including invasive and non-invasive pituitary adenomas, and the correlation analysis was performed. **Results** The Gal-3 and Bcl-2 expressions in the invasive pituitary group were significantly higher than those in the non-invasive group, and the expression of Bax had no significant difference between the two groups. Pearson's correlation analyses showed that the Gal-3 expression was positively correlated with Bcl-2, but was not correlated with Bax, which was inversely correlated with expression of Bcl-2. **Conclusion** Gal-3 may function through a cell death inhibition pathway involving Bcl-2 to enhance cell proliferation, which result in the invasive growth of pituitary adenoma. These results indicate that Gal-3 has an important role in pituitary tumor cell proliferation and may serves as a possible therapeutic target in treatment of pituitary tumors.

Keywords: pituitary neoplasma; galectin-3; apoptosis; invasiveness

1 Introduction

Galectin is a family of carbohydrate-binding proteins with a high affinity for β -galactoside. Among at least thirteen different members of the family which are associated with β -1,3,(4)-galactosylation, Gal-3, the only chimeric gene product, is one of the most frequently investigated members^[1]. Gal-3 is involved in many biological processes including cell growth and differentiation, cell adhesion, angiogenesis, tumor progression, apoptosis, and metastasis^[2,3]. Bcl-2 family plays an important role in the mitochondrial pathway of apoptosis^[4], in which Bcl-2 inhibits but Bax promotes the apoptosis. High proliferative activity is the basis of invasive growth for pituitary adenoma. Many researches have revealed that the invasiveness of pituitary adenoma is affected by adenoma cell proliferative activity^[5,6], but the effect of cell apoptotic

activity on invasiveness remains unknown. In this study, the expressions of Gal-3, Bcl-2 and Bax were measured in human pituitary adenoma to explore the interrelation and the effect of them to the invasiveness of pituitary adenomas.

2 Materials and methods

2.1 Specimens collection (1) Paraffin-embedded sections of surgically excised human pituitary tumor specimens from 78 patients [36 males and 42 females, 41.3 ± 6.2 years old (mean $\pm$ SD, ranging 10-71 years)] were collected for immunohistochemistry (IHC) analysis. (2) Twenty surgically excised human pituitary tumor tissues aliquots [12 male and 8 female, 39 ± 9 years old (mean $\pm$ SD, ranging 5.5-62 years)] were snap-frozen in liquid nitrogen for later RT-PCR.

2.2 Invasiveness determination We referred to Hardy-wilson's and Knosp's classification methods to determine the invasiveness of pituitary adenoma^[7,8]. Any item of the following conditions can be regarded as the criterion for invasive pituitary adenoma: 1, Hardy-Wilson grade III-V and stage C-E; 2, Knosp grade 3-4; 3, Destruction of bone in sellar region, cavernous sinus inner wall perforation can be found during the operation, and even the intracavernous

Corresponding author: Chui-Xue HUANG
Tel: 86-898-68642212
E-mail: ledongren@yahoo.com.cn
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structure was surrounded by tumor; 4, Histological examination showed that adenoma cells infiltrated sellae diaphragma.

In IHC group, 47 cases were diagnosed as invasive pituitary adenoma and 31 were non-invasive. There were 10 invasive and 10 non-invasive pituitary adenoma cases in RT-PCR group.

2.3 RT-PCR analysis RNA was extracted from approximately 100 mg of pituitary adenoma tissue by using Trizol (Invitrogen, Carlsbad, California) according to the manufacturer's protocol. Total RNA (2 µg) was subjected to cDNA synthesis using a first strand DNA synthesis kit (Bio-Rad, Hercules, CA). Oligonucleotide primers used for Gal-3 detection were forward 5'-TATTCCTGGTGTAGTCACCGG-3' and reverse 5'-TGCAACCTTGAA GTG GTCAG-3', producing a 302-bp fragment. Other primers included Bcl-2, forward 5'-GGACAACATCGCCTGTGT-3', reverse 5'-AGTCTTCAGACAGACAGCCAGGA-3', with a 137-bp product; Bax, forward 5'-ATC CAG GAT CGA GCA GGG CG-3', reverse 5'-ACT CGC TCA GCT TCT TGG TG-3', with a 94-bp product; and GAPDH, forward 5'-AAC TAAAC CTC CCT AGAGC-3'; reverse 5'-TGTGGT CATGAGTCC TTCA-3', with a 296-bp product. PCR amplification of cDNA was performed as described previously^[9].

2.4 Immunohistochemistry Briefly, the 4-µm sections were treated with 2% hydrogen peroxide to quench endogenous peroxide for 30 min and exposed to 5 µg/mL of proteinase K for 15 min at room temperature. The sections were washed extensively and exposed to equilibration buffer for 10 min. Each slide was then incubated with anti-Gal-3, anti-Bcl-2 and anti-Bax antibodies (Santa Cruz Biotechnology) at 4 °C overnight. The reaction was visualized with the avidin-biotin method and 3,3'-diaminobenzidine. A thyroid papillary carcinoma was used as positive control and normal serum substituted for the primary antibody was used as negative control.

2.5 Statistical analysis Data of RT-PCR are expressed as mean±SD. Statistical analysis was performed by Student's *t*-test. The percentage of immunopositive cells in IHC was evaluated by chi-square test. Correlation among expressions of the three proteins was performed with the Pearson rank sum test. $P < 0.05$ was considered statistical significant.

3 Results

3.1 The mRNA expression levels of Gal-3, Bcl-2 and Bax in human pituitary adenomas The expression levels of Gal-3 and Bcl-2 mRNA in invasive pituitary adenoma were significantly higher than that in non-invasive pituitary adenoma ($t = 5.813$, $P < 0.01$; $t = 4.921$, $P < 0.01$, respectively); while the expression of Bax mRNA had no significant difference between the two groups ($t = -0.668$, $P > 0.05$) (Tab.1, Fig.1).

Tab.1 Expression levels of Gal-3, Bcl-2 and Bax mRNA in human pituitary tumors (mean±SD)

Group	<i>n</i>	Gal-3	Bcl-2	Bax
Invasive	10	1.148±0.263*	0.664±0.196**	0.266±0.098
Noninvasive	10	0.364±0.060	0.182±0.016	0.307±0.076

* $P < 0.01$, ** $P < 0.01$ vs non-invasive group

Fig. 1 Expression of Gal-3, Bcl-2 and Bax mRNA in the pituitary adenomas. Lane 1-4: Non-invasive pituitary adenomas; lane 5-8: invasive pituitary adenomas.

3.2 The protein levels of Gal-3, Bcl-2 and Bax in human pituitary adenomas By immunohistochemical staining, the Gal-3, Bcl-2 and Bax positive cells were stained brown in cytoplasm or on cell membrane. The protein expression levels of Gal-3 and Bcl-2 in invasive pituitary group were significantly higher than those in non-invasive group ($\chi^2 = 6.30$, $P < 0.05$; $\chi^2 = 7.56$, $P < 0.01$, respectively); while Bax level showed no significant difference between the two groups ($\chi^2 = 0.30$, $P > 0.05$). (Tab. 2, Fig. 2)

3.3 Correlation analysis for the protein levels of Gal-3, Bcl-2 and Bax Rank correlation analysis indicated that the Gal-3 protein level was correlated positively with Bcl-2 ($r = 0.291$, $P < 0.05$) but not correlated with Bax ($r = -0.023$, $P > 0.05$); while Bcl-2 level had a negative correlation with Bax ($r = -0.267$, $P < 0.05$).

Fig.2 Expression of Gal-3, Bcl-2 and Bax protein in the invasive pituitary adenomas by immunohistochemistry. Scale bar, 80 μ m in A, 135 μ m in B and C.

Tab.2 Protein levels of Gal-3, Bcl-2 and Bax in human pituitary tumors

Group	n	Gal-3		Positive (%)	Bcl-2		Positive (%)	Bax		Positive (%)
		-	+		-	+		-	+	
Invasive	47	10	37	78.7*	20	27	57.4**	18	29	61.7 [#]
Noninvasive	31	15	16	51.6	23	8	25.8	10	21	66.7
Total	78	25	53	67.9	43	35	44.9	28	50	64.1

* $P < 0.05$ vs non-invasive, $\chi^2 = 6.30$; ** $P < 0.01$ vs non-invasive, $\chi^2 = 7.56$; [#] $P > 0.05$ vs non-invasive, $\chi^2 = 0.30$

4 Discussion

Human Gal-3 is a 31-kDa protein with three distinct structural domains, including a 12-amino acid NH_2 -terminal domain that controls cellular targeting; a collagen-like sequence rich in glycine, tyrosine, and proline, functioning as a substrate for matrix metalloproteinases; and a COOH-terminal domain similar to Gal-1, with a globular structure that contains a single carbohydrate-binding site^[9].

Gal-3 may inhibit the apoptosis of epithelial cells induced by staurosporine, cis-diamminedichloroplatinum (DDP), genistein and anoikis^[10]. In some human cancers, Gal-3 level shows a direct correlation with the progress of cancers. Raz *et al.* firstly reported that the expression of Gal-3 was related to the malignant transformation and metastasis of tumors. Expression of recombinant Gal-3 in nude mice's fibrosarcoma, which has low ability of metastases, may give rise to the high rate of pulmonary metastases^[11]. The Gal-3 expressed in colon carcinoma was related to the transformation and metastases of cancer. Down-regulation of Gal-3 in colon carcinoma cell will decrease its ability of settling down in the liver, and conversely enhance the metastases potency of the tumor cells^[12,13]. In primary gastric carcinoma and its metastases, Gal-3 level is higher than that in the adjacent

normal mucosa^[14]. Estrogen and progesterin in sexual hormone sensitive breast carcinoma are likely to serve as the specific coordinate regulatory agents to promote the up-regulation of Gal-3 expression with a result of metastasis of carcinoma cells^[15]. Nangia-Makker *et al.* discovered that Gal-3 was related to oncogenesis and metastasis of human breast carcinoma^[16].

In pituitary adenoma, Riss *et al.* used immunohistochemistry and Western blot to investigate the role of Gal-3 in the development and progress of pituitary tumors. The results showed that only lactotroph (PRL) and corticotroph (ACTH) hormone-producing cells and tumors expressed Gal-3. Gal-3 was present in 24 of 38 (63.2%) PRL adenomas, 5 of 6 (83.3%) PRL carcinomas, 19 of 41 (46.3%) ACTH adenomas, and 7 of 8 (87.5%) ACTH carcinomas, but not in 112 other pituitary adenomas and carcinomas^[7]. Wang *et al.* evaluated expressions of Gal-3 by immunohistochemistry in 16 invasive prolactinomas and 16 prolactinomas. The results showed that Gal-3 was expressed in both invasive prolactinomas and noninvasive prolactinomas while significantly higher in the invasive prolactinomas^[18]. Jin *et al.* discovered that Gal-3 was associated with functioning ACTH and PRL tumors and expressed infrequently in silent ACTH adenomas, suggesting that Gal-3 protein and/or gene is altered in non-function-

ing ACTH tumors^[19]. Thodou *et al.* recently showed that Gal-3 was highly expressed in the functioning corticotroph adenomas of pituitary gland, while silent adenomas exhibited very focal to null expression of Gal-3. This observation can be used in pathological diagnosis to separate functioning from silent corticotroph adenomas of the pituitary^[20]. Inhibition of Gal-3 gene expression by RNA interference decreased HP75 cell proliferation and increased apoptosis. These results indicate that Gal-3 has an important role in pituitary cell proliferation and tumor progression^[17].

There is a NWGR primitive structure at the carboxyl terminus of Gal-3 that plays an important role in inhibiting apoptosis. NWGR is highly conserved in Bcl-2 family, and Gal-3 may interact with Bcl-2 through a kind of lactose^[21]. The homologous sequence in Gal-3 and Bcl-2 is less than 30%, so Gal-3 does not belong to Bcl-2 family. Interaction of Gal-3 and Bcl-2 may block the change in mitochondrial membrane which has potential to decrease the production of reactive oxidative product, and so to inhibit the cell apoptosis^[22].

We used RT-PCR and immunohistochemical staining to detect the mRNA and protein expressions of Gal-3, Bcl-2 and Bax in human pituitary adenomas in this study. Gal-3 and Bcl-2 were expressed highly in invasive pituitary adenoma and showed a significant difference from non-invasive pituitary adenoma. Correlation analysis indicated that the expression of Gal-3 protein was correlated positively with Bcl-2 protein but not correlated with Bax. These data suggest Gal-3 exerts its anti-apoptosis effect only by the way of synergistic action with Bcl-2, which promotes the multiplication of pituitary adenoma cells, resulting in their invasive growth. The above results suggest that Gal-3 may serve as a possible therapeutic target for treatment of pituitary tumors.

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垂体腺瘤中半乳糖凝集素-3的表达及其与细胞凋亡关系

黄垂学¹, 侯永宏², 刘运生²

¹海南省人民医院神经外科, 海口 570311

²中南大学湘雅医院神经外科, 长沙 410078

摘要: 目的 探讨垂体腺瘤中半乳糖凝集素-3(Galectin-3, Gal-3)和凋亡相关基因 Bcl-2、Bax 的内在联系。**方法** RT-PCR 检测 20 例垂体腺瘤 Gal-3、Bcl-2 和 Bax mRNA 的表达; 免疫组化染色检测 78 例垂体腺瘤中 Gal-3、Bcl-2 和 Bax 蛋白表达, 分析侵袭性和非侵袭性垂体腺瘤中 Gal-3、Bcl-2 和 Bax 表达的差异及其相关关系。**结果** 侵袭组垂体腺瘤 Gal-3 和 Bcl-2 mRNA 和蛋白的表达水平较非侵袭组显著增高, 而 Bax mRNA 和蛋白在两组中的表达水平无显著差异。等级相关分析显示 Gal-3 蛋白与 Bcl-2 蛋白之间有正直线相关关系($r = 0.291$, $P = 0.01$); Gal-3 蛋白与 Bax 蛋白之间无直线相关关系($r = -0.023$, $P > 0.05$); Bcl-2 蛋白与 Bax 蛋白表达之间的 Pearson 相关系数为 $r = -0.267$, $P < 0.05$ (双侧), 呈负相关关系。**结论** Gal-3 可能主要是通过与 Bcl-2 协同作用, 发挥其抗凋亡效应, 相对促进垂体腺瘤细胞增殖, 导致垂体腺瘤的侵袭性生长。这些结果提示 Gal-3 在垂体肿瘤细胞增殖过程中发挥重要作用, 有望作为垂体腺瘤的一个治疗靶点。

关键词: 垂体腺瘤; 半乳糖凝集素-3; 凋亡; 侵袭性