The expression of ATF3, MMP-2 and maspin in tissue chip of glioma

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Abstract: This paper tested and analyzed the expression of ATF3 (activating transcription factor), MMP-2 (matrix metalloprotease) and maspin in tissue chip of glioma and its correlation with glioma advancement. Based on immunohistochemical staining, this paper selected 100 patients with glioma and 13 healthy persons to test the relative expression of ATF3, MMP-2 and maspin. The result witnessed 72.0% of ATF3 expression in glioma and 15.4% in healthy brain tissues with $P<0.05$; glioma had 76.0% of MMP-2 expression while healthy brain tissues only had 7.7% ($P<0.05$); but maspin expression with 53.0% in glioma was much lower than that with 100% in healthy tissues with $P<0.05$. If the pathological stage of glioma rose up, the expression of ATF3 and MMP-2 accordingly increased while maspin expression decreased. The correlation between ATF3 expression and MMP-2 expression was positive with $r=0.553$ and $p<0.01$; negative correlation between ATF3 expression and maspin expression was found with $r=-0.457$ and $p<0.01$; and the expression of MMP-2 and maspin were negatively related with $r=-0.551$ and $p<0.01$. According to the above results, it could be concluded that the expression of ATF3, MMP-2 and maspin did relate with each other. Besides, the high expression of ATF3 and MMP-2 as well as the low expression of maspin had great influence on glioma, playing a key role in glioma's occurrence, advancement, invasion and metastasis.

Keywords: ATF3; MMP-2; maspin; glioma; tissue chip.

INTRODUCTOIN

Glioma, a primary tumor often seen in nervous system, hits the public because of the invasion of glioma cells to surrounding tissues, incomplete excision with surgery and easy recurrence. Glioma invasion involves a complicated process, including the adherence and metastasis of tumor cell and extracellular matrix degradation (Wenjie *et al*., 2012). But glioma cannot be radical treated with traditional solutions, such as surgery, radiotherapy, chemotherapy, immuno-therapy and combined therapy. At present, bio-therapy based on molecular diagnosis is the hope for glioma treatment, which is according to the knowledge of incidence factors. In addition, gene therapy and molecular surgery are likely to cure glioma radically in clinical. The core of gene therapy is finding more and better target genes and realizing highly effective metastasis and expression. Bio-chip, gene chip and tissue chip in particular, enables the finding of genes related to glioma's occurrence, invasion and recurrence. Besides, clinical diagnosis and prognosis received more powerful approaches because of bio-chip technology (Xiaojing *et al*., 2014; Zengyi, 2014).

High expression of ATF3 has been reported recently in various malignant tumors. ATF3 induced cells into cell cycle from diastasis period to speed up cell reproduction, causing the invasion, metastasis and prognosis of a variety of tumors (Caiyun and Chunni, 2014). Researches indicated that during glioma invasion, MMP-2 advanced the formation of tumor vessels to accelerate glioma invasion and metastasis (Pengfei *et al.*, 2011). However, maspin, a anti-cancer gene, inhibited the born of blood vessels to enhance cell adherence, which made tumor metastasis impossible (Shengbing *et al*., 2012). This research adopted immunohistochemical staining to detect the expression of ATF3, MMP-2 and maspin in glioma, which aimed to analyze how the expressions affecting glioma's occurrence and development and how the expressions correlating with malignancy.

MATERIALS AND METHODS

Specimen sources

A total of 100 specimens were taken from patients who have received resection operation of astrocytoma from July 2011 to July 2013 in the hospital. The specimens contained 58 females and 42 males, whose ages ranged from 18 to 66 with (42.3±3.1) in average. Pathological sections were interpreted by two experienced pathologists, and classified based on the standard of central nervous system tumors set by WHO (World Health Organization) in 2000. According to the standard, 15 cases were in stage I (pilocytic astrocytoma), 32 were in stage II (diffuse astrocytoma), 30 in stage III (anaplastic astrocytoma) and 23 in stage IV (gliobastona). For reference, the research selected 13 specimens of brain tissues that were pathologically proved healthy, from patients with craniocerebral trauma during July 2011 and July 2013. Ages of 8 males and 5 females, differed from 17 to 53 with (33.2±4.7) in average. During the experiment, firstly, keep two specimens of fresh and central tumor tissues without haemorrhage and necrosis in frozen reservation. Then both specimens were fixed with 10% neutral formalin and embedded with paraffin. Finally stain one specimen with HE staining and another with

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immunohistochemistry staining. This research was acquired and approved by patients, and authorized with the investigation by Medical Ethics Committee in the hospital.

Major reagents

Rabbit anti person ATF3 and MMP-2, and poly-clonal antibody of maspin applied in immunohistochemistry were from Santa Cruz Company in America, so was Chemiluminescence reagents. ZSGB-BIO Company in Beijing offered goat anti rabbit IgG antibody, DAB color kit and citrate buffer (0.01mol/L, PH 6.0). Others were analytical reagents.

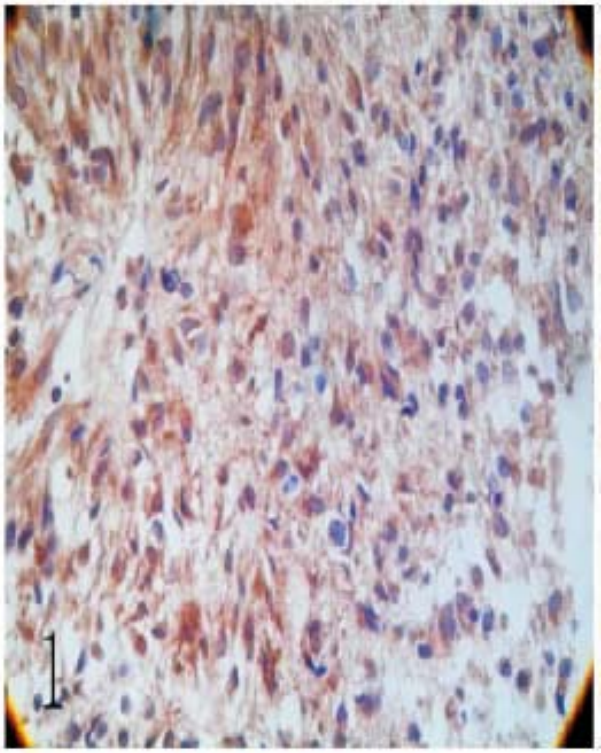

Fig. 1: the strong positive expression of ATF3 in stage IV glioma (IHC ×400)

Methods

The detection of the expression of ATF3, MMP-2 and maspin based on immunohistochemistry was put into practical strictly according to the instruction on kit. After normal dewaxing and hydration, microwave repair the tissue antigen and close specimens with 10% goat serum. With 1:200 working concentration, ATF3, MMP-2 and maspin were colored under DAB-H2O2 microscope, re-stained by Haematoxylin, normally hydrated and mounted by neutral balsam, respectively. Normal goat serum was taken as negative reference instead of primary antibody, while positive tissue section was positive reference. If yellow or brown particles showed up in nucleus or cytoplasm of ATF3, MMP-2 and maspin, positive staining could be defined. Then semi-quantitative determination of experimental results was carried out according to H-score (H =×P) systems, such as Gatalica. Staining intensity (I) was divided into 0 (colorless), 1(yellow), 2(brown) and 3(chocolate brown). Staining purview (P) was scored with the percent of stained cells; 0 referred to less than 0.05 positive cells, 1 was 0.06~0.25, 2 was 0.26~0.50 and 3 meant more than 0.50. The product of staining intensity and positive cell percent was regarded as the result of immunohistochemistry: 0 was evaluated as negative, 1~2 was (+), 3~4 (++), and 5~6 (+++); while (□) meant negative and (+,++,+++) positive. Observe cells in each section under 5 different high power fields (× 400) and account 100 cells in each field, finally calculate mean positive rate.

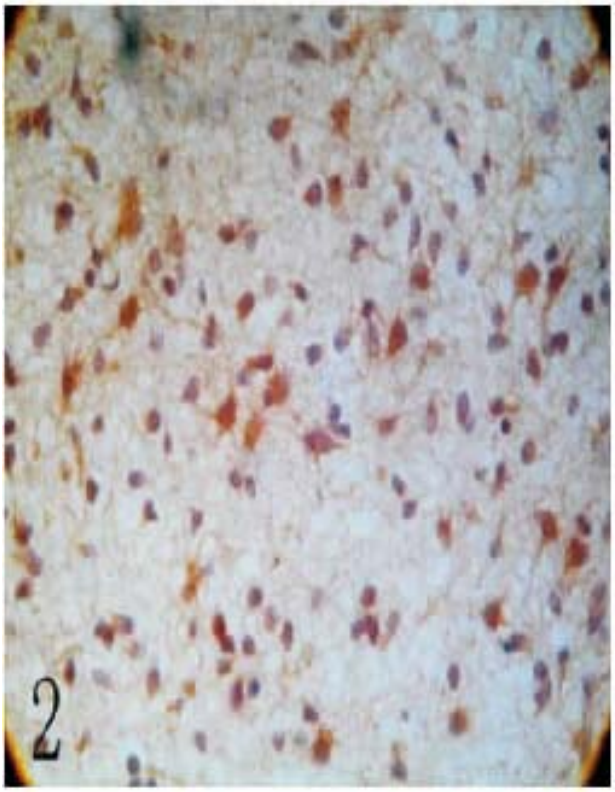

Fig. 2: the strong positive expression of maspin in stage I glioma (IHC ×400)

STATISTICAL ANALYSIS

SPSS 19.0 was performed in data processing. Ranked data comparison adopted rank test, bi-specimen comparison of ranked data adopted Mann-Whitney U test and multi-specimen adopted Kruskal-Wallis H. Spearman correlation analysis was performed in the expression of ATF3, MMP-2 and maspin in glioma with different stages and the correlation among the expression of ATF3, MMP-2 and maspin. As for the mean of multi-specimen, the comparison applied Oneway-ANOVA. If $P<0.05$, the difference was considered to have statistic significance.

RESULTS

As shown in table 1, positive expression of protein ATF3 occupied 15.4% (2/13) in healthy brain tissues and 72.0%

(72/100) in glioma cells, and the difference were significant in statistics. Positive ATF3 was performed as brown particles mainly in nucleus and few in cytoplasm. Diffusely distributed in glioma cells in low or high stages, ATF3 did not express or weekly express in healthy brain tissues. The difference of ATF3 positive expression between stage I glioma and healthy brain tissues was not significant in statistics with P>0.05; but the differences between glioma with higher stages and healthy brain tissues were all considered statistically significant. Spearman indicated that ATF3 expression positively related to glioma stage with r=0.735 and p<0.01.

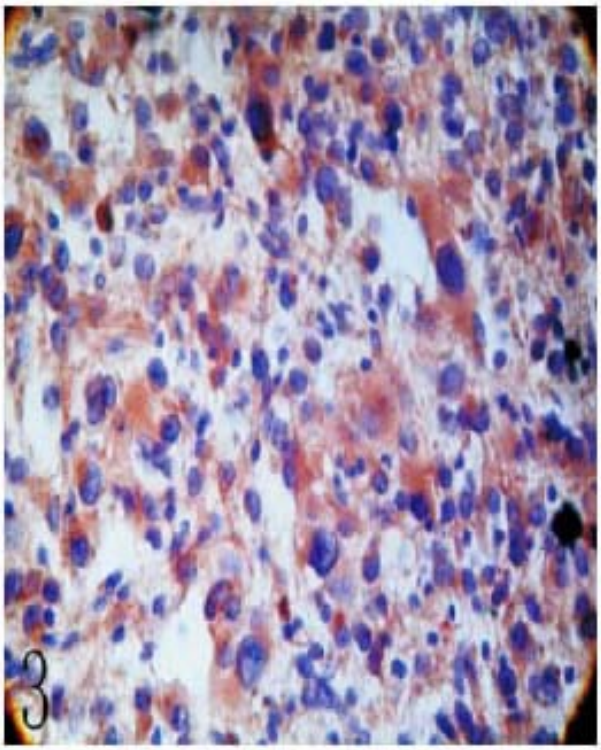

Fig. 3: the strong positive expression of MMP-2 in stage IV glioma (IHC ×400)

The difference between 100.0% (13/13) of positive expression in healthy brain tissues and 53.0% (53/100) in glioma tissues meant statistical significance with P<0.05 (table 1). As brown or light yellow particles in cytoplasm and (or) nucleus, the positive expression of maspin strongly and diffusely distributed in healthy brain tissues (fig. 2). Fig. 2 also showed that with diffused distributions, maspin expressed positively in low stage glioma as brown particles and high stage glioma as light yellow particles. The difference of maspin expression between healthy brain tissues and glioma in any stage had statistical meaning with P<0.05 (table 1). Negative relationship between maspin expression and glioma stage was performed in Spearman with r=-0.542 and p<0.01.

In healthy brain tissues, the positive expression of MMP-2 was 4.7% (1/13), which was much lower than 76.0% (76/100) in glioma tissues, and the difference was significant in statistics. The positive expression of MMP-2 was performed as brown particles mainly in cytoplasm and few in nucleus; while diffusely distributed in glioma with different stages, MMP-2 did not express or weekly express in healthy brain tissues (fig. 3). As presented in table 1, the positive expression of MMP-2 differed from stage I glioma to healthy brain tissues without statistical meaning (P>0.05), but with significance from healthy brain tissues to glioma in higher stages. With the analysis by Spearman, MMP-2 expression was in a positive relation with glioma stage with r=0.446 and P<0.01.

On the basis of analysis on the expression correlation among ATF3, MMP-2 and maspin, it was showed that ATF3 and MMP-2 were in positive relation with r=0.553 and p<0.01, ATF 3 and maspin in negative with r=-0.457 and p<0.01, and maspin and MMP-2 in negative with r=-0.551 and p<0.01.

DISCUSSION

Basic leucine zipper has a subfamily containing transcription factors (ATF/CREB), and ATF3 is a member of that subfamily. ATF3 has been reported to regulate the expression of its target genes via complicated mechanism. As a key regulatory factor for transcriptional control, cell apoptosis, cell division and survival, ATF3 functions importantly in tumor invasion and metastasis (Yiqun *et al*., 2014). Researchers found high expression of ATF3 in the high metastatic sub-line of B16 melanoma cell, but did not find any expression in parent B16 cell strains. Yet further studies fig. out that the transfection of ATF3 turned low metastatic cells of B16 into high metastatic cells (Wanfu *et al*., 2013). Another study suggested that antisense nucleotide of ATF3 could slow down the ectopic growth of HT29 (colon carcinoma cell line) in mice; and experiment in vitro found the control of anti-sense nucleotide of ATF3 on the adherence and invasion of HT29 (Junwen *et al*., 2014). All mentioned researches indicated that ATF3 promoted the occurrence, development and invasion of tumor. In various experiments, ATF3 was regarded as a stress-induced gene.

Normally, ATF3 expresses lowly in most cells; unless cells are exposed to some pathological and physiological stimulation, ATF3 and ATF3 mRNA speed up their growth and productions. ATF3 is the first transcription factor appearing in cell stress response (Minhua *et al*., 2014). This research showed low expression of ATF3 in glia cells in healthy brain tissues, and higher expression in glioma with 72% (72/100) positive rate. It was indicated that ATF3 involved in the malignant transformation of glia cell. The theory was that some mechanism activated and ascended ATF3 expression to cause some chain reactions, including the activation of downstream oncogene and the inactivation of anti-oncogene, resulting

Table 1: The expression of ATF3, MMP-2and maspin in healthy brain tissues and glioma tissues (case/%)

WHO Classification	Cases	ATF3	MMP-2	Maspin
Healthy brain tissues	13	2(15.4)	1(7.7)	13(100.0)
Glioma	100	72 (72.0)[a]	76 (76.0)[a]	53 (53.0)[a]
WHO stages				
–	15	4(26.7)	4(26.7)	12 (80.0)[a]
α	32	18 (56.3)[a]	20(62.5) [a]	22 (78.1)[a]
β	30	28 (93.3)[a]	29(90.6) [a]	13 (43.3)[a]
χ	23	22 (95.7)[a]	23(100.0)	6 (26.1)[a]

PS: compared to healthy brain tissues, [a] $P<0.05$

in cells' infinite hyperplasia and malignant transformation. Experiments by Pelzer detected over-high expression of ATF3 in most cell strains of prostatic carcinoma, which promoted the reproduction of prostatic carcinoma cells and pushed cells in stage S into stage G1. So ATF3 expression was positively related to pathological stage of glioma. While IHC in this research witnessed the increasing expression of ATF3 in the advancement of glioma stage, respectively, 26.7% in stage I, 56.3% in stage II, 93.3% in stage III and 95.7% in stage IV. Besides, the positive correlation between ATF3 expression and glioma stage was performed in Spearman; this meant that if the stage went up, ATF3 expressed much higher. Thus it could be maintained that high expression of ATF3 was bound up with the development and malignancy of glioma.

MMP-2, a cancer-trigger gene, plays a key role in the formation of tumor new vessels, the invasion of tumor cells and the process of metastasis (Yujue *et al*., 2014; Xun *et al*., 2011). In the research, MMP-2 expressed higher in glioma than in healthy brain tissues and the expression went up with the advancement of gliom stage. Spearman found the positive relationship between MMP-2 expression and glioma stage.

Found in 1994, maspin is an anti-cancer gene, which can control tumor growth, enhance cell adherence, reduce cell movement and invasion, and inhibit tumor angiogenesis (Qingjun and Yiran, 2014). Maspin expression is higher in healthy brain tissues and turns down in tumor progress. In a relation with worse malignancy, larger tumor and higher histological stage, maspin expression also has an impact on lymphatic metastasis, local recurrence or tumor progress and short survival. This research found maspin expression in nucleus and cytoplasm and high expression in healthy brain tissues. In addition, maspin expression went down when glioma reached higher stage, which indicated negative relation between m s found negatively relating to ATF3 expression and MMP-2 expression, while the later two was positively related to each other. The similarity between results of this study and results of liver cancer research conducted by Maowu Xu gave a hint that ATF3 pushed glioma move forward. Besides, some regulatory mechanism played effect among ATF3, MMP-2 and maspin. However, other researches were ended up with different results (Maowu *et al*., 2009). It was reported that ATF3 lowered MMP-2 expression via its combination with MMP-2 promoter; but another study reported that ATF3 could control MMP-2 expression because it interfered the activation of p53 on MMP-2. Different results could be caused by various experimental conditions and subjects. For another thing, ATF3 and MMP-2 in different tissues were likely to regulate each other through different signal pathways, which also resulted in various experimental figs.

CONCLUSION

This research figs. out correlations among the expression of ATF3, MMP-2 and maspin. Expect that, the expression of ATF3, MMP-2 and maspin does have an effect on glioma malignancy; but the details of how the expression influencing the occurrence and advancement of glioma are remained to be analyzed and discussed.

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