·Basic Research·

Expression of MMP -2 and MMP -9 in retinoblastoma and their significance

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Abstract

• AIM: To investigate the expression of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) in retinoblastoma (Rb), and their relationships with tumor development stage.

• METHODS: Immunohistochemical technique was used to detect the expression of MMP-2 and MMP-9 in 41 cases of paraffin embedded Rb samples. Quantitative analysis of the expression of MMP-2 and MMP-9 was assessed by HMIAS-2000 Color Pathologic Analysis System. The differences of the expression of MMP-2 and MMP-9 in each clinical and pathological stage were analyzed statistically.

• RESULTS: In all the 41 Rb specimens, MMP-2 and MMP-9 expression was found in tumor cells. The expression of MMP-2 and MMP-9 was significantly higher in tumors with optic nerve invasion than in tumors without optic nerve invasion (P<0.05); the expression of MMP-2 and MMP-9 was significantly higher in tumors of extra-ocular stage than in tumors of glaucomatous stage or intra-ocular stage(P< 0.05).

• CONCLUSION: MMP-2 and MMP-9 exist in retinoblastoma cells. The level of MMP-2 and MMP-9 is related to optic nerve invasion and clinical stage of Rb, which suggests the expression of MMP-2 and MMP-9 could be connected to the invasion and development of tumor cells. Further research is needed for deeper understanding of the biological behavior and better evaluation of the prognosis of Rb.

• KEYWORDS: etinoblastoma; matrix metalloproteinase-2; matrix metalloproteinase-9; immunohistochemistry
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INTRODUCTION

Retinoblastoma (Rb) is most common, the hazardous biggest intraocular malignant tumor during the period of baby and infant. The newborn disease incidence rate is 1/15 000-1/28 000[1]. Usually to think that the prognosis of Rb has close relationship with optic nerve infiltration or not and whether there is the tumor distant place metastasis. Recently experimental study discover that matrix metalloproteinases (MMPs) is playing the vital role in the malignant tumor invasion and metastasis, and 72kDa matrix metalloproteinase-2 and 92kDa matrix metalloproteinase-9 belong to gelatinase class (Ⅳ-type gelatinase) of the MMPs family, they are excreted in the form of proenzyme, after the activation form Ⅳ-type gelatinases, then they degrade and destruct extracellular matrix near tumor surface, enable the tumor cell to infiltrate to acroteric tissue along the damaged basal membrane, at last to cause invasion and metastasis of tumor cell[2]. This article proposes to study that expression of MMP-2 and MMP-9 in retinoblastoma and their significance.

MATERIALS AND METHODS

Materials All paraffin wax embedding specimen in this experiment come from the Rb patient who accepts the eyeball excision operation during 1990-2005 years in the department of ophthalmology, Union hospital, Tongji medical college, Huazhong university of technology and science, altogether has 41 examples, in which male has 22 examples, the female has 19 examples. The patient age is in 4 months -10 years old, the average age is 3.9 years old. Clinical is by stages as follows: 12 examples in intra-ocular stage, 15 examples in glaucomatous stage, 14 examples in extra-ocular stage, 0 examples whole body metastatic stage, 15 examples with optic nerve invasion, 26 examples without optic nerve invasion. All cases were diagnosed by the pathology department of Union hospital.

Main reagents Mouse anti-person MMP-2 monoclonal antibody, the practical concentration is 1:50; Mouse anti-person MMP-9 monoclonal antibody, the practical concentration also is 1:50. Immunohistochemical sp kit, DAB developer, neutral gum and other reagents are all provided by the ZhongSan company.

Immunohistochemical staining Positive comparison adopted mammary adenocarcinoma tissue in which

expression of MMP-2 and MMP-9 are all positive, the negative comparison replaces primary antibody with PBS. The SP method was used to carry on the staining, detailed step was as follows: The paraffin wax specimens slice continuously 4μm thick, sections was dewaxed in xylene and rehydrated through graded alcohols. Endogenous peroxidase activity was blocked by placing sections in 3% hydrogen deionized water for 10 minutes. In order to detect MMP-2 and MMP-9 epitopes, Sections were placed in 0.01 mol/L citrate buffer (pH 6.0) at 92℃-98℃ for 20 minutes. To block nonspecific staining, slides were incubated in normal goat serum for 10 minutes. After three 5 minutes washed in PBS, sections were incubated overnight at 4℃ with primary antibodies at a dilution of 1:50. After three 5 minutes washed in PBS, sections were incubated sequentially with biotinylated rabbit anti-mouse IgG, followed by streptavidin combined in vitro with biotinylated horseradish peroxidase. After three 5 minutes washed in PBS, the reaction product was developed with diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin, dehydrated through graded alcohols, and mounted in resinous mountant.

Methods Sections were observed by light microscopy, and surveyed the average optical density value of MMP-2 and MMP-9 in the normal retina tissue and the Rb cell by use of the HMIAS-2000 completely automatic medicine color image analysis system at 400 time of light microscope. Each section selects 5 fields of vision.

Statistical Analysis Data were expressed as the mean ± SEM, and carried on statistical processing using the SPSS 11.0 software. The differences of the expression were analyzed with a independent-samples test with $P<0.05$ set as the level of significance.

RESULTS

Expression of MMP-2 and MMP-9 in Normal Retina Tissue and Tumor Tissue In all the 41 Rb specimens, MMP-2 and MMP-9 positive expression was found in tumor cells, the endochylema and cell membrane was stained with the yellowish brown color (Figure 1, 2). The average optical density value of MMP-2 in tumor cell was 0.1711±0.0525 and 0.0855±0.0091 in normal retina, the differences of two group expression have the extremely remarkable statistics significance by use of T test ($P<0.001$). The average optical density value of MMP-9 in tumor cell and in normal retina respectively are 0.2157±0.0704, but was 0.0769±0.0087, their differences have the extremely remarkable statistics significance ($P<0.001$).

Relationship between Expression Level of MMP-2 and MMP-9 and Optic Nerve Invasion The average optical density value of MMP-2 in Rb with optic nerve invasion and in Rb without optic nerve invasion respectively are 0.2347±0.0257 and 0.1343±0.0145, expression differences between two groups have remarkable statistics significance ($P<0.01$); The average optical density value of MMP-9 in Rb with optic nerve invasion and in Rb without optic nerve invasion respectively are 0.2977±0.0469 and 0.1682±0.0173, expression differences between two groups have remarkable statistics significance too($P<0.01$, Table 1).

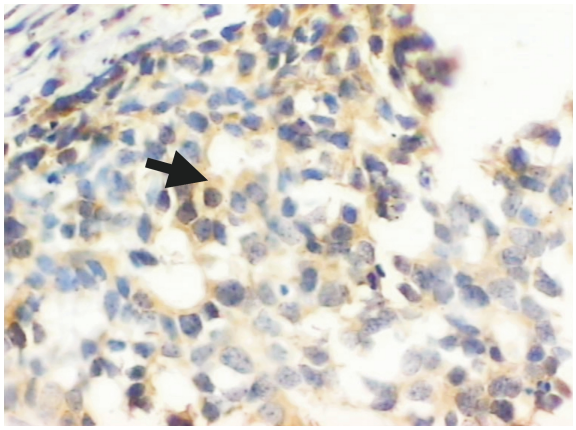

Figure 1 Positive expression of MMP-2 in retinoblastoma (extra-ocular stage)

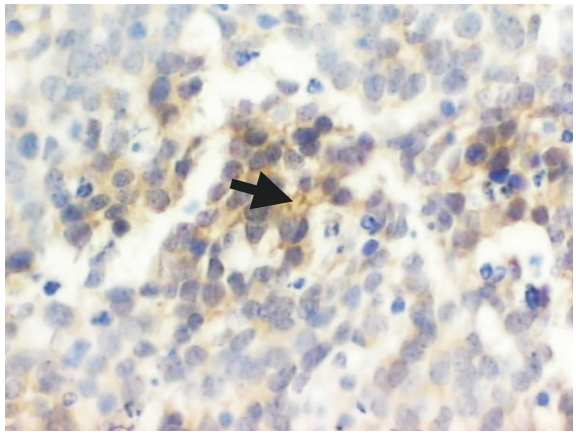

Figure 2 Positive expression of MMP-9 in retinoblastoma (extra-ocular stage)

Relationship between Expression Level of MMP-2 and MMP-9 and Clinical Stage The average optical density values of MMP-2 and MMP-9 in extra-ocular stage respectively are 0.2347±0.0257 and 0.2977±0.0469, they respectively are 0.1303±0.0169 and 0.1651±0.0122 in intra-ocular stage, they respectively are 0.1397±0.0136 and 0.1733±0.0222 in glaucomatous stage. Statistial analysis result is as follows: the expression of MMP-2 and MMP-9 was significantly higher in tumors of extra-ocular stage than in tumors of glaucomatous stage or intra-ocular stage ($P<0.01$); the expression differences of MMP-2 and MMP-9 between intra-ocular stage and glaucomatous stage have no obvious statistical significance ($P>0.05$, Table 2).

Table 1 Expressions of MMP-2 and MMP-9 in retinoblastoma with optic nerve invasion and without invasion

	Cases	MMP-2	MMP-9
With optic nerve invasion	26	0.1343±0.0145	0.1682±0.0173
Without optic nerve invasion	15	0.2347±0.0257	0.2977±0.0469
t		16.057	10.296
P		0.000	0.000

Table 2 Expressions of MMP-2 and MMP-9 in retinoblastoma in different clinical stage

	Cases	MMP-2	MMP-9
Intraocular stage	12	0.1303±0.0169[b]	0.1651±0.0122[b]
Glaucoma stage	14	0.1397±0.0136[b]	0.1733±0.0222[b]
Extra-ocular stage	15	0.2347±0.0257	0.2977±0.0469

Note: with extra-ocular stage group comparison: [b]P<0.01

DISCUSSION

Partial invasion and migration of malignant tumor must pass through following several processes: (1) the tumor cells detach from the cancer nest; (2) induce and excrete many kinds of proteinases which can degrade extracellular matrix; (3) tumor cells invade vascular and lymphatic; (4) they arrive the local normal tissue, then migrate across vascular and lymphatic; (5) the migration of cancer cells adhere to local normal tissue, where the cells proliferate, induce angiogenesis, and form metastatic tumors [3]. MMPs are the main proteinases which can degrade the extracellular matrix, they belong to the highly conservative incision proteinase family dependent to the zinc ion and can degrade majority protein in basal membrane and extracellular matrix. Among them, gelatinase A (MMP-2) and gelatinase B (MMP-9) belonging to gelatinase mainly degrade gelatin and IV, V, VII, X-type basal membrane collagen, and they play a vital role in the adherency between cells and matrix [4,5]. Some documents proved that the ability of tumor cell generating MMP-2 and MMP-9 had a close positive correlation with potential of tumor invasion and metastasis. MMP-2 and MMP-9 not only participated in extracellular matrix degeneration, but also had influences to adherency ability and motility of tumor cell [6]. The expression of MMP-2 and MMP-9 remarkably increased in many kinds of malignant tumor, such as gastric cancer, pancreatic carcinoma, colon carcinoma and so on [4,5,7]. In ophthalmology, MMPs also participate in the many kinds of the pathology physiological process of some ophthalmopathy, for example, glaucoma, proliferative vitreoretinopathy, senile macular degeneration[8,9]. There still has no related research report about relation between Rb and expression of MMP-2 and MMP-9.

This experiment discovered by immunohistochemistry method, the expression of MMP-2 and MMP-9 was significantly higher in Rb tissue than in normal retina (P< 0.001), the expression of MMP-2 and MMP-9 was significantly higher in tumors with optic nerve invasion than in tumors without optic nerve invasion (P<0.01); the expression of MMP-2 and MMP-9 was significantly higher in tumors of extra-ocular stage than in tumors of glaucomatous stage or intra-ocular stage (P<0.01). These results suggest that the level of MMP-2 and MMP-9 is related to optic nerve invasion and clinical stage of Rb. In other kinds of tumor originating to neuroectoderm the same as Rb, expression of MMPs are similar to this experiment results. For instance, the expression of MMP-2 and MMP-9 were related to pathological grades in spongiocytoma, the expression of MMP-2 and MMP-9 was significantly higher in tumors of third grade and forth grade than in tumors of first grade and second grade[10,11].

Usually thought that prognosis of Rb is significantly related to optic nerve invasion or not and clinical stage. Our experiment showed that level of MMP-2 and MMP-9 has significantly relation with optic nerve invasion and clinical stage of Rb. Further studying the expression and adjustment of MMP-2 and MMP-9 in Rb is helpful to deeper understanding of the biological behavior and better evaluation of the prognosis of Rb. Inhibiting the expression of MMP-2 and MMP-9 and blocking their activity could have some inhibition to optic nerve invasion and metastasis, degrade malignant degree of tumor and strive for the more clinical treatment time.

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