·Basic Research·

Expression of EMMPRIN, MMPs and TIMP2 in retinoblastoma and normal retinal tissue

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Abstract

• AIM: To investigate the expression of EMMPRIN, MMP1, MMP9 and TIMP2 in retinoblastoma (RB) and normal retinal tissues and their clinicopathological significance and interrelationship.

• METHODS: Envision immunohistochemistry stainings of EMMPRIN, MMP1, MMP9 and TIMP2 were performed in 30 enucleated eyeballs with retinoblastoma and 15 specimens of normal retina tissue, which had been routinely imbedded with paraffin.

• RESULTS: Positive rate of EMMPRIN, MMP1, MMP9 expression was higher in RB tissue than in normal control ($P<0.01$), while TIMP2 expression was lower in RB than in normal retinal tissue ($P<0.01$). Samples from RB cases of clinical stage I, differentiated type, and life span ≥ 2 years had lower positive rate in expression of EMMPRIN, MMP1, MMP9 than those from RB cases of clinical stage III, undifferentiated type, and life span<2 years ($P<0.05$ or $P<0.01$), while samples from RB cases of differentiated type, optic nerve unaffected, and life span ≥ 2 years had markedly higher positive rate in expression of TIMP2 than those from RB cases of undifferentiated type, optic nerve involved and life span< 2 years ($P<0.05$ or $P<0.01$). In RB tissues, EMMPRIN, MMP1, MMP9 expressions were highly consistent ($P<0.05$), whereas TIMP2 expression is highly inconsistent with EMMPRIN,MMP1,MMP9 expression levels($P<0.05$).

• CONCLUSION: The expression level of EMMPRIN, MMP1, MMP9 and TIMP2 may be an important marker of RB progression, invasion and prognosis. There exist internally mutual regulation relations among them.

• KEYWORDS: retinoblastoma; EMMPRIN; MMP1; MMP9; TIMP2; immunohistochemistry

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INTRODUCTION

Tumor invasion is the critical step that could lead to metastasis of retinoblastoma (RB), the most common ocular malignancy in childhood [1]. The matrix metalloproteinases (MMPs) play a vital role in the invasion and metastasis of malignant tumors by degrading the extracellular matrix and breaking down the first barrier [2-5]. The expression and activities of MMPs are regulated by tissue inhibitors of metalloproteinases (TIMPs) [3,6-8], while extracellular matrix metalloproteinase inducer (EMMPRIN, also known as CD147) has the effect of inducing the production of MMPs [8-10]. Recent researches have proved that the expression of EMMPRIN, MMPs and TIMPs network is associated with the oncogenesis, progression, angiogenesis, metastasis, invasion and prognosis of malignant tumors [2-10]. In this study Envision™ immunohistochemistry method was applied to investigate the expressions of EMMPRIN, MMP1, MMP9 and TIMP2 in retinoblastoma and normal retinal tissues, and their clinicopathological significance and correlations were discussed.

MATERIALS AND METHODS

This study was reviewed and approved by the ethics committee at the Second Xiangya Hospital, and the committee deemed that it conformed to the generally accepted principles of research in accordance with the Helsinki Declaration.

Specimens and Clinicopathological Data Paraffin-embedded tissue sections from 30 patients with RB who were admitted into Department of Ophthalmology, the Second Xiangya Hospital of Central South University between January 1996 and October 2005 and underwent enucleated operation were retrieved. Clinical and pathologic information was obtained from medical records and surgical pathology reports. There were 18 male(60%) and 12 female(40%); age ranged from 4 months to 6 years, with 8 cases under 1

year old(27%), 14 cases 1-3 years old(46%), 8 cases older than 3 years (27%). No bilateral case was included. The chief complaints to visit were as follows: 23 cases with leukocoria (77%), 9 with decreased vision (30%), 7 with eye pain (23%), 8 with exophthalmos (27%), 4 with elevated intraocular pressure(13%), 3 with strabismus (10%). The condition of the course of disease was as follows: 9 cases shorter than 1 month, 13 cases between 1 month and 3 months, 8 cases longer than 3 months. As for the clinical stage classification, the condition was as follows: 13 cases (43%) belonged to intraocular stage (stage I); 10 cases (33%) were at glaucoma stage (stage II); 7 cases (24%) belonged to extraocular stage (stage III). With regard to the pathological grouping, tumors were microscopically graded into two groups according to the predominant pattern of differentiation, but sometimes it was difficult to divide strictly. There were 11 cases (37%) of differentiated type and 19 cases of undifferentiated type (63%). The status of invasion and metastasis was as follows: the tumor invaded the optic nerve in 13 cases (43%), while in remained 17 cases (57%) the optic nerve was not invaded. In regard to the treatment and prognosis, enucleation combined with postoperative chemotherapy were performed in 4 cases (13%). In terms of survival time, during the follow-up of two years after the operation, 11 cases died of recurrence and matastasis, the remained 19 cases were still alive after two years. As for 15 samples of normal retinal tissues, 11 were from pathologically confirmed normal retina of the enucleated eyes with RB, and 4 were from normal adult cadaver eyeballs after penetrating keratoplasty. In brief, tissue specimens were conventionally embedded by paraffin and dissected into 4μm thick slices.

Antibodies and Reagents Rabbit anti-human EMMPRIN, MMP1, MMP9 and TIMP2 multiclonal antibodies were purchased from Boster Biotechnology Inc, Wuhan, China. Envision™ labelling kits were from Gene Biotechnology Inc, Shanghai, China.

Methods EMMPRIN, MMP1, MMP9 and TIMP2 immuno-histochemistry staining was performed by Envision™ method. All the steps strictly followed the specification of the kits. Positive slice provided by Boster Company was applied as positive control, and sections processed with 0.01mol/L phosphate buffer solution (PBS) instead of the first antibody were used as negative control. Cells whose cytoplasm contained brownish yellow particles were considered as positive cells. Randomly 10 tumor fields were scanned for protein expression under 40 magnification and average percentage of positive tumor cells was calculated from the 10 values for the entire slice. Sections with positive cells ≥20% were categorized as positive, whereas sections with positive cells<20% were considered as negative.

Statistical Analysis The SPSS 13.0 software package was employed to carry out statistical analysis. Chi square test and Fisher exact test were performed to process the data. *P* value<0.05 was considered significant.

RESULTS

Expression of EMMPRIN, MMP1, MMP9 and TIMP2 in Retinoblastoma and Normal Retinal Tissues The immunohistochemical reaction products were located in the cytoplasm, with the negative staining of nuclei (Figure 1-4). Among 30 cases of RB, the number of cases in which EMMPRIN, MMP1, MMP9 and TIMP2 had positive expres-sion was respectively 16(53%), 15(50%), 16(53%), 14 (47%). Among 15 samples of normal retinal tissues, the number of cases in which EMMPRIN, MMP1, MMP9 and TIMP2 had positive expression was respectively 1 (7%), 2 (13%), 1(7%), 13(87%). Positive expression rates of EMMPRIN, MMP1 and MMP9 were obviously higher in RB than in normal retinal tissue (P<0.01), while the positive expression rate of TIMP2 was significantly lower in RB than in normal retinal tissue (P<0.01).

Expression of Matrix Regulatory Protein and Its Relation with RB Clinicopathological Characteristics Tissues from the cases of clinical stage I, differentiated type in pathological classification, and life span ≥2 years had much lower positive expression rate of EMMPRIN, MMP1, MMP9 than those from the cases of clinical stage III, undifferentiated type, and life span<2 years (P<0.05 or P<0.01). Tissues from the RB cases of differentiated type, with optic nerve unaffected, and life span ≥2 years had higher positive expression rate of TIMP2 than those from the cases of undifferentiated type, with optic nerve involved, and life span <2 years (P<0.05 or P<0.01) (see Table 1). Expressions of EMMPRIN, MMP1, MMP9 and TIMP2 had no significant correlation with age, sex, course of disease, tumor location, clinical manifestation or treatment modality (P>0.05).

Correlations among Matrix Regulatory Protein Expressions in RB In 16 EMPPRIN positive cases, the number of MMP1, MMP9 and TIMP2 positive cases were 11, 12 and 6 respectively. EMPPRIN expression showed highly consistency with MMP1 and MMP9 (P value was 0.033 and 0.014 respectively), but it had no obvious correlation with TIMP2 expression (P=0.23). In 15 MMP1 positive cases, there were 11 MMP9 positive cases and 3 TIMP2 positive cases.

Table 1 EMMPRIN, MMP1, MMP9 & TIMP2 expression and their correlation with principal clinicopathological features of RB

Clinicopathological features	Case No.	EMMPRIN positive(%)	MMP1 positive(%)	MMP9 positive(%)	TIMP2 positive(%)
Clinical stage					
Stage I	13	3(23)	3(23)	3(23)	8(62)
Stage II	10	7(70)	6(60)	7(70)	4(40)
Stage III	7	6(86)[a]	6(86)	6(86)	2(29)
Pathological grouping					
differentiated	11	4(36)	4(36)	4(36)	9(82)
undifferentiated	19	12(63)[b]	11(58)[a]	12(63)[b]	5(26)[b]
Optic nerve involvement					
uninvovled	17	7(41)	6(35)	7(41)	11(65)
involved	13	9(69)[a]	9(69)[a]	9(69)[a]	3(23)[b]
Life span					
<2 years	11	9(82)	9(82)	10(90)	4(36)
≥2 years	19	7(37)[a]	6(32)[b]	6(32)b	10(53)

Compared with clinical stage I, differentiated type, optic nerve uninvolved and life span <2 years: [a]$P<0.05$ [b]$P<0.01$

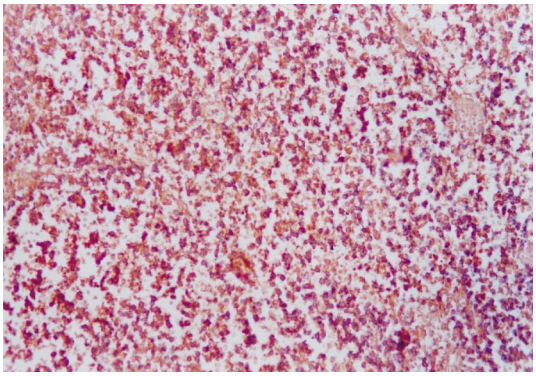

Figure 1 EMMPRIN positive expression, undifferentiated RB, Envision immunohistochemistry staining×200

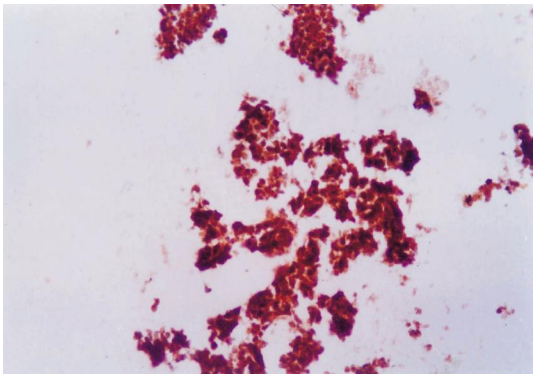

Figure 2 MMP1 positive expression, undifferentiated RB, Envision immunohistochemistry staining×200

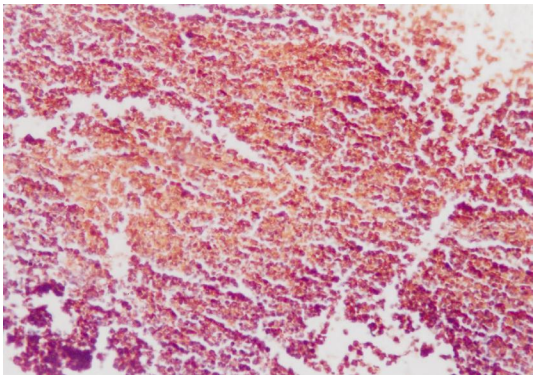

Figure 3 MMP9 positive expression, undifferentiated RB, Envision immunohistochemistry staining×200

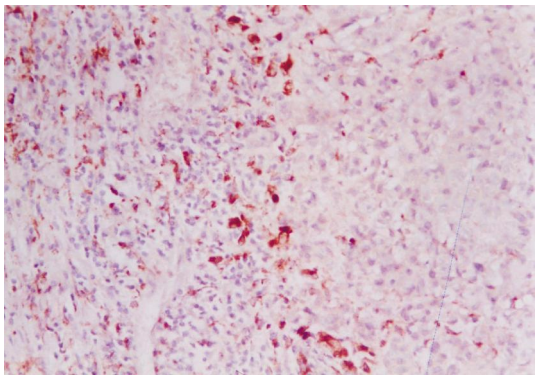

Figure 4 TIMP2 positive expression, differentiated RB, Envision immunohistochemistry staining×200

Therefore the expressions of MMP1 and MMP9 were highly consistent (P=0.013), while the expression of MMP1 is highly inconsistent with that of TIMP2 (P=0.005). In 16 MMP9 positive cases, there were 4 TIMP2 positive cases, which was highly inconsistent(P=0.014).

DISCUSSION

Retinoblastoma, a common malignant tumor in infancy, is one of the biggest challenges in ophthalmology. Its invasion and metastasis are closely related to its prognosis. In recent years, the matrix protein and its regulating enzymes are known as a key factor in invasion potential and metastasis of malignant tumor. The synthesis, secretion and balance of extracellular matrix (ECM) have a close relationship with matrix modulating proteinases[2-10]. EMMPRIN is a protein composed of 248 amino acids, which can induce biological synthesis of MMPs such as MMP1 and MMP2[8-10]. MMPs are a group of various enzymes which have many common biochemical properties and can degrade the extracellular matrix (basement membrane). They play a vital role in invasion, metastasis, progression, angiogenesis and prognosis of malignant tumor[2-8]. At least 14 MMPs have been identified, in which MMP1 and MMP9 are the most conspicuous

members. The synthesis and biological activity of MMPs can specifically be inhibited by TIMPs. More than 4 kinds of TIMPs have been cloned by now. TIMP1 and TIMP2 have been well defined as inhibitors of synthesis and biological activity of MMP1, but whether it can suppress the biological activity and synthesis of MMP9 has not been reported yet. Recent researches have shown that malignant tumors with higher expression level of TIMPs have a slower progression, lower invasive capability and less chance of metastasis, with better prognosis[3,6-8]. Adithi *et al*[8] found that the expression of matrix modulating proteins had a close relationship with RB clinical staging, pathological grouping and invasive capability. Our present study showed that the positive expression rate of EMMPRIN, MMP1 and MMP9 was much higher in RB tissue than in normal retina, while TIMP2 was on the contrary. RB cases of earlier clinical stage (stage I), differentiated type, with optic nerve unaffected and longer life span (>2 years) had obviously lower positive expression rates of EMMPRIN, MMP1 and MMP9, compared with cases of later clinical stage (stage III), undifferentiated type, with optic nerve involved and shorter life span (<2 years). Cases of pahthologically differentiated, with optic nerve unaffected and longer life span had a higher positive expression rate of TIMP2. Expressions of EMMPRIN, MMP1 and MMP9 have displayed high consistency in RB tissue, while TIMP2 expression shows high inconsisitency with expressions of MMP1, MMP9. The results above indicate that matrix modulating proteins could be important biomarkers of RB occurrence, progression, invasion and prognosis. EMMPRIN possibly induces the biosynthesis of MMP1 and MMP9, while TIMPs could inhibit the synthesis of MMP1 and MMP9. Its clear causal relation and the exact mechanism need further studies.

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