Int J Clin Exp Med 2020;13(7):5259-5266
www.ijcem.com /ISSN:1940-5901/IJCEM0112058

*Original Article*

# The roles of MMP-2, MMP-9, TIMP-1, and TIMP-2 in neuroblastoma

Wenfu Wang[1], Bo Song[2], Zhen Zhang[3]

*[1]Department of Neurosurgery, South Hospital of Jiaozhou People's Hospital, Jiaozhou 266300, Shandong, China; [2]Department of Neurology II, Qing Dao Center Hospital The Second Department of Neurology, 266041, Shandong, China; [3]Department of Neurosurgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Ji'nan 250012, Shandong, China*

Received April 7, 2020; Accepted May 24, 2020; Epub July 15, 2020; Published July 30, 2020

**Abstract:** Objective: This study aimed to analyze the levels and clinical significance of MMP-2, MMP-9, and the inhibitors TIMP-1 and TIMP-2 in patients with neuroblastoma (NB). Methods: The levels of MMP-2, MMP-9, TIMP-1, and TIMP-2 in paraffin pathological sections of NB tissues obtained by surgical resection or biopsy were determined using immunohistochemical methods. Results: (1) The positive, weak positive, and strong positive rates of the MMP-2 expressions were 82.86%, 27.59%, and 72.41%, and the corresponding rates of MMP-9 expression were 84.29%, 28.81%, and 71.19%; (2) The strong positive rates of the MMP-2 and MMP-9 expressions in patients with early stage NB were 16.00% and 32.00%, but in patients with advanced stage NB, the rates were 84.44% and 75.56% ($P$<0.05); (3) The positive, weak positive, and strong positive rates of TIMP-2 expression were 80.00%, 32.14%, and 67.86%. As for the TIMP-1 expression, the corresponding rates were 65.71%, 26.09% and 73.91%. In patients with advanced NB, the strong positive rates of the TIMP-2 and TIMP-1 expressions were 44.44% and 40%. There was a significant difference in the strong positive rates of TIMP-2 expression between the early and advanced stages ($P$<0.05), but the TIMP-1 expressions showed no significant differences. Conclusion: The positive expression levels of MMP-2 and MMP-9 and MMP-2 are correlated with NB progression and prognosis, demonstrating that these indicators can be regarded as biomarkers for the outcome of NB.

**Keywords:** Neuroblastoma, MMP-2, MMP-9, TIMP-1, TIMP-2

## Introduction

Neuroblastoma (NB) is the most common extracranial solid tumor and it has a high incidence in neonates and infants [1]. NB originates in the primitive neural crest tissues and it may be present everywhere in the sympathetic nervous system, and it mainly involves the sympathetic chain of the neck, chest, and abdomen [2]. Data show that more than half of all patients with NB are toddlers, and nearly 90% of patients are less than 5 years old, and patients over 10 years old are rare [3].

In NB patients, some lesions remain localized at their primary site, and the risk of metastasis is low. The cancer cells are well differentiated and can be removed radically by surgery [4]. However, most patients have infiltration and metastasis outside the primary site in the early stages of NB, leading to a poor prognosis. The liver, the intracranial area, the local lymph nodes, the bones, and the lungs are typical sites of distant metastasis [5]. The early diagnosis of NB significantly improves patients' clinical outcomes and prognoses.

Matrix metalloproteinases (MMPs) are proteolytic enzymes. A variety of tumor cells and stromal cells produce MMPs [6]. MMPs can degrade macromolecular substances in the extracellular matrix (ECM), effectively destroying the ECM and participating in various pathological and physiological processes [7]. Tissue inhibitor metallo proteinases (TIMPs) are tissue inhibitors specific to MMPs. They can bind noncovalently to MMPs and block their active site binding substrates, reducing the degradation of the ECM induced by MMPs [8]. In addition, TIMPs compete with the active sites of the MMPs and prevent their activation [9].

In this study, the MMP-2 and MMP-9 levels and the TIMP-1 and TIMP-2 inhibitor levels were determined using paraffin sections of NB tissue. The relationships among them were evaluated to provide guidance for the disease's clinical diagnosis, staging, treatment, and prognosis evaluation.

## Materials and methods

### *Specimens*

A total of 70 NB specimens were collected from our hospital by surgical resection or biopsy, including 43 males and 27 females. The patients ranged in age from 3 months to 10 years, with an average age of (4.62 ± 2.29) years, including 13 patients under one year old and 57 patients aged between 1-10 years old. The INSS stages I and II are defined as early stage and stages III and IV are classified as advance stages. In all, there were 12 cases in stage I, 13 in stage II, 21 in stage III, and 24 in stage IV.

Among the patients we studied, the NB occurred in the retroperitoneum (20 cases), the adrenal glands (17 cases), the neck (10 cases), the mediastinum (12 cases), and other organs (11 cases). All the specimens were fixed with formalin and embedded in paraffin. Inclusion criteria: the patient specimens were collected by surgery or biopsy; the patients were of either gender; and only patients with complete pathological and clinical data were included. All the patients signed a written informed consent, and the study was approved by the ethics committee of our hospital. Exclusion criteria: specimens from other sources, patients over 12 years old, and patients with other tumor diseases or other serious systemic diseases.

### *Methods*

Sectioning and staining: All the specimens were serially sectioned to 4 μm samples, which were affixed to clean glass slides treated with APES and baked in a 60°C oven for 2-3 hours. The sections were routinely dewaxed with xylene, and the gradient alcohol was hydrated with distilled water. The sections were placed in prepared 3% $H_2O_2$, and the endogenous peroxidase was inactivated at room temperature for 10 minutes. The sections were washed with distilled water 3 times, 2 minutes each time, and then washed with PBS solution 3 times. Each cycle lasted 2 minutes. Antigen retrieval: 0.01 mol/L citrate buffer solution (pH=6.0) was added into sections, heated to 95°C with a microwave oven, and then cooled at room temperature for half an hour, and washed with PBS solution 3 times. In the MMP-2 group, a digestive enzyme was added dropwise, incubated at 37°C in a constant-temperature water bath incubator for half an hour, and then washed with PBS solution 3 times, followed by the addition of normal goat serum, and left to stand for a quarter of an hour.

Normal goat serum was added to the remaining three groups, which were then allowed to stand for a quarter of an hour. Non-specific antigens were blocked and the excess fluid was wiped off. A mouse anti-human MMP-2 monoclonal antibody was added to the MMP-2 group, and a mouse anti-human MMP-9 monoclonal antibody was added to the MMP-2 group. A mouse anti-human TIMP-1 polyclonal antibody was added to the TIMP-1 group. A mouse anti-human TIMP-2 polyclonal antibody was added to the TIMP-2 group. The MMP-2 was incubated in a constant temperature water bath incubator at 37°C for 2 hours, and the remaining three groups were incubated in the water bath for 1-1.5 hours and washed with the PBS solution 3 times.

A biotinylated goat anti-mouse IgG was added dropwise to the TIMP-2 and TIMP-1 groups, and a biotinylated universal secondary antibody working solution was added dropwise to the MMP-2 and MMP-9 groups, which were incubated in a constant temperature water bath incubator at 37°C for one quarter of an hour and rinsed with PBS solution 3 times, 2 minutes each time. In the MMP-2 and MMP-9 groups, the horseradish enzyme-labeled streptavidin working solution was added dropwise, and in the TIMP-2 and TIMP-1 groups, the SABC reagent was added dropwise. The color was developed using 3,3'-diaminobenzidine (DAB), which was left standing for half an hour and then observed through a microscope. After being rinsed with distilled water for 3 minutes, hematoxylin was used for the counterstaining, and then the tissue was dehydrated and xylene was added to make it transparent. PBS was selected as the negative control instead of the primary antibody.

**Table 1.** The positive expression rates of MMP-2 and MMP-9

| Grouping | Positive cases | + | ++ | +++ |
|---|---|---|---|---|
| MMP-2 | 58 | 16 | 20 | 22 |
| MMP-9 | 59 | 17 | 21 | 21 |

Positive was defined as brown-yellow in color, with signs of cytoplasm or cell membrane staining. The staining intensity was determined according to the differences in the coloration depth and the number of positive cells: -: no tumor cells, negative staining; +: less than one-third of the tumor cells stained with a light color; ++: one-third to two-thirds of the cells stained with a dark color; +++: more than two thirds of the tumor cells stained with a dark color.

*Outcome measurement*

The following outcomes were measured: (1) The positive rate of the MMP-2 and MMP-9 expressions and (2) their relationship with the clinical stage and prognosis; (3) The positive rate of TIMP-2 and TIMP-1 expressions and (4) their relationship with the clinical stage and prognosis.

*Statistical analysis*

SPSS 22.0 was used for the statistical analysis. The measurement data were expressed as the mean ± standard deviation (mean ± SD). The data that had a normal distribution were tested using independent sample t tests, and the data that did not have a normal distribution were tested using Mann-Whitney U tests. The comparisons within groups were examined using paired t tests, and the comparisons of the count data between groups were performed using $X^2$ tests. $P<0.05$ indicated statistical significance.

## Results

*The MMP-2 and MMP-9 expressions*

MMP-2 and MMP-9 were expressed in the tumor specimens. Positive staining was observed in the tumor cells, the vascular endothelial cells, and the stromal cells. The cell clusters were observed to be densely distributed in a focal and nested manner. There were differences in the positive expression levels of MMP-2 and MMP-9. There were 58 (82.86%) specimens with positive MMP-2 expressions, of which 16 were +, 20 were ++, and 22 were +++. Weak positives (+) accounted for 27.59% and strong positives (++, +++) accounted for 72.41%. The number of specimens with a positive MMP-9 expression was 59 (84.29%), including 17 (28.81%) that were weak positives (+) and 42 (71.19%) that were strong positives (++, +++) (**Table 1**; **Figures 1**, **2**).

*The relationships between the positive expressions of MMP-2, MMP-9, and prognosis*

There were 25 early NB cases and 45 advanced cases. The strong positive rates of MMP-2 and MMP-9 were 16.00% and 32.00% in the early NB patients, which were significantly different from the 84.44% and 75.56% in the patients with advanced NB ($P<0.05$) (**Table 2**; **Figure 3**).

*The positive expressions of TIMP-2, TIMP-1*

Vascular endothelial cells, stromal cells, and tumor cells all expressed TIMP-2 and TIMP-1. There were 56 (80%) specimens with a positive TIMP-2 expression. Among them, 18 (32.14%) were weak positive and 38 (67.86%) were strong positive. The number of specimens containing a TIMP-1 positive expression was 46 (65.71%), including 12 (26.09%) that were weak positives and 24 (73.91%) that were strong positives (**Table 3**; **Figures 1**, **4**).

*The relationships between TIMP-2, TIMP-1, positive expression, and prognosis*

The strong positive rate of TIMP-2 in the early-stage NB patients (72.00%) was significantly higher than it was in the advanced-stage NB patients (44.44%) ($P<0.05$), and the strong positive rate ofTIMP-1 in the early-stage NB patients (24.00%) was not significantly different from the rate in the advanced NB patients (40%) ($P>0.05$) (**Table 4**; **Figure 5**).

## Discussion

Among the causes of death in patients with malignant tumors, early metastasis to distant sites and the invasive growth of the tumor are the greatest threats [10]. The tumor metastasis involves multiple consecutive processes, and the destruction of the basement membrane

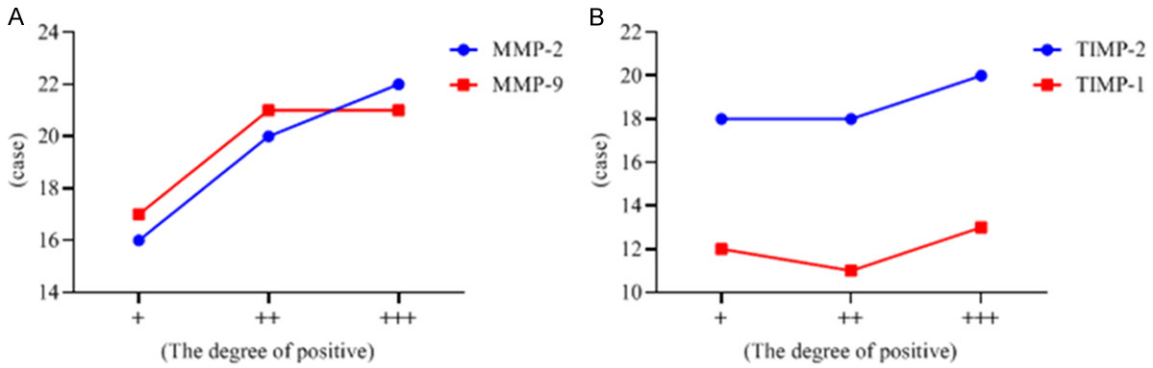


**Figure 1.** Positive expression rates. A. MMP-2 and MMP-9; B. TIMP-2 and TIMP-1.

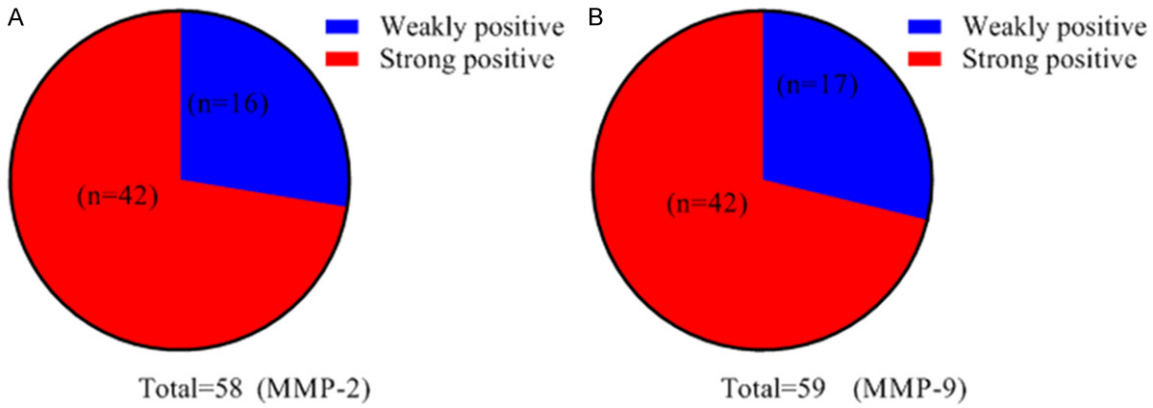


**Figure 2.** MMP-2, MMP-9 expressions. Compared with the weak positive rate, the strong positive rates of the MMP-2 and MMP-9 expressions were significantly higher ($P<0.05$).

**Table 2.** The relationship between the MMP-2 and MMP-9 positive expressions, staging, and prognosis

| Staging | MMP-2 | | MMP-9 | |
|---|---|---|---|---|
| | Negative/Weak positive | Strong positive | Negative/Weak positive | Strong positive |
| Early | 21 | 4 | 17 | 8 |
| Advanced | 7 | 38 | 11 | 34 |

and the degradation of the ECM are crucial factors. Multiple enzymes can degrade macromolecular proteins in the extracellular matrix, such as MMPs, cysteinase, and silk protease. MMPs are the most effective for degradation [11, 12].

The MMP family currently has at least 20 members. There are many studies on the relationship between MMP-2, MMP-9 and tumor invasion and metastasis, but few efforts have focused on NB [13, 14]. NB starts in certain very early forms of nerve cells, most commonly found in an embryo or fetus, and progresses rapidly with a poor prognosis. It is closely related to the early destruction of surrounding tissues and distant organ metastasis [15].

NB patients have a low, long-term survival rate due to the disease's high metastasis and infiltration [16]. MMP-2 contains hydroxyl terminals, amino acid terminals, and metal binding fragments. The total length of the structural gene is 27 kb, with 12 introns and 13 exons. The total length of the MMP-9 structural gene is 26-27 kbp, with 9 introns and 13 exons, and it can degrade and reshape the dynamic balance of the extracellular matrix. Previously, most studies on tumor specimens indicated that MMPs were secreted from tumor cells. In recent years, due to the gradual progress of biological technology, researchers have learned that MMPs are also secreted from a large number of cells in the mechanism tissues, and even the MMPs

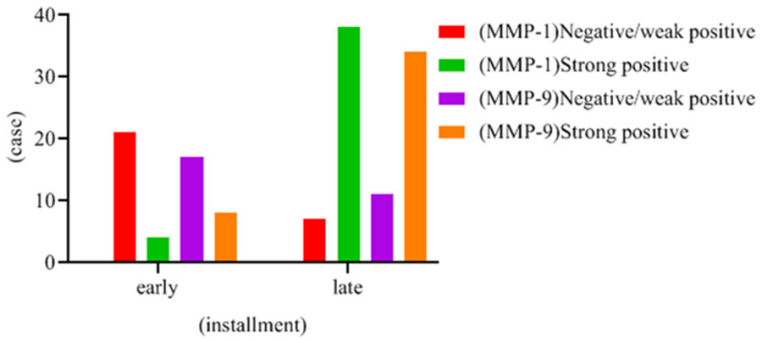


**Figure 3.** MMP-2, MMP-9 positive expressions, staging, and prognosis. Compared with the strong positive rate in early stages, the rate is significantly lower in the advanced stages ($P<0.05$). As for MMP-9, the strong positive rate is significantly higher ($P<0.05$).

**Table 3.** The positive expression rates of TIMP-2 and TIMP-1

| Staging | Positive cases | + | ++ | +++ |
|---|---|---|---|---|
| TIMP-2 | 56 | 18 | 18 | 20 |
| TIMP-1 | 46 | 12 | 11 | 13 |

they produce play a significant role. Later studies also found that MMP-2 and MMP-9 can be found in NB tumor cells, peripheral stromal cells, and vascular endothelial cells, among which the positive expression rate of MMP-2 in the peripheral stromal cells is more than 80%, and the rate of MMP-9 is more than 70%, indicating higher expression levels than the levels in the tumor cells. Studies on pancreatic cancer found that MMP-2 and MMP-9 were detected in tumor cells and adjacent mesenchymal cells, were flaky or focal, and were basically located near the lumens and at the edge of the nests. The results of this study showed that the strong positive rates of MMP-2 and MMP-9 in advanced NB are significantly higher than those in the early stages. The strong positive rate of the MMP-2 expression increased from 16.00% to 84.44%, and the MMP-9 rate increased from 32.00% to 75.56%, suggesting that the positive expressions of MMP-2 and MMP-9 are correlated with tumor stage. Webb et al. [17] measured the MMP-2 and MMP-9 levels in NB specimens and tumor cells and found that MMP-2 exists as an inactive precursor, but MMP-9 is expressed as an active precursor. However, the underlying mechanism has not been fully elucidated. We speculated that the reason may be the lack of the MMP-2 activating factor membrane type 1-matrix metalloproteinase (MT1-MMP) and the high expression of the inhibitor TIMP-2. In addition, Agnieszka et al. [18] failed to detect the MT1-MMP mRNA expression using the northern-blot method, but in the case of inhibiting TIMP-2 activity, 90% of the incubated cells were activated after one day.

TIMPs are specific MMP inhibitors. In the regulation of ECM metabolism, TIMPs are negative regulators corresponding to MMPs. TIMP-1 selectively inhibits MMP-9, and TIMP-2 selectively inhibits MMP-2. The four maintain a dynamic equilibrium. If one of them were destroyed, it would cause changes in the other three levels. Studies have found that when MMPs are overexpressed, TIMPs are also highly expressed, but the degree of MMPs is greater than that of TIMPs, so the ratio of MMPs to TIMPs will be imbalanced, and the ratio will be higher, resulting in a faster degradation rate of ECM than that of synthesis. Studies of bladder cancer have shown that the levels of TIMPs in the blood increase as the disease progresses. Studies on ovarian tumors have shown that TIMP-l has a high-intensity color band in normal ovarian tissue, and the positive result of the band in tumor specimens is enhanced. We also found that the positive rate of TIMP-2 expression was 80.00%, and the positive rate of TIMP-1 expression was 65.71%. Combined with the MMP-2 analysis, there seems to be a negative correlation between the TIMP-2 positive rate and the MMP-2 positive rate, suggesting that TIMP-2 plays an important role in the degradation process of ECM and can inhibit the formation of tumor metastasis. However, there is no significant difference in the positive expression of TIMP-1 from early to advanced, so its effects on NB have not been fully determined, and its specificity in terms of tumor metastasis and invasion may not be as strong as that of TIMP-2. Early studies have suggested MMPs are mainly secreted by tumor cells. However, cells in stromal tissue can also produce MMPs [19, 20]. In this study, it was found that the positive staining of MMP-2, MMP-9, TIMP-2, and TIMP-1 all existed in stromal cells and tumor cells, especially in the vicinity of blood vessels and lumens. Studies [21] showed that stromal cells

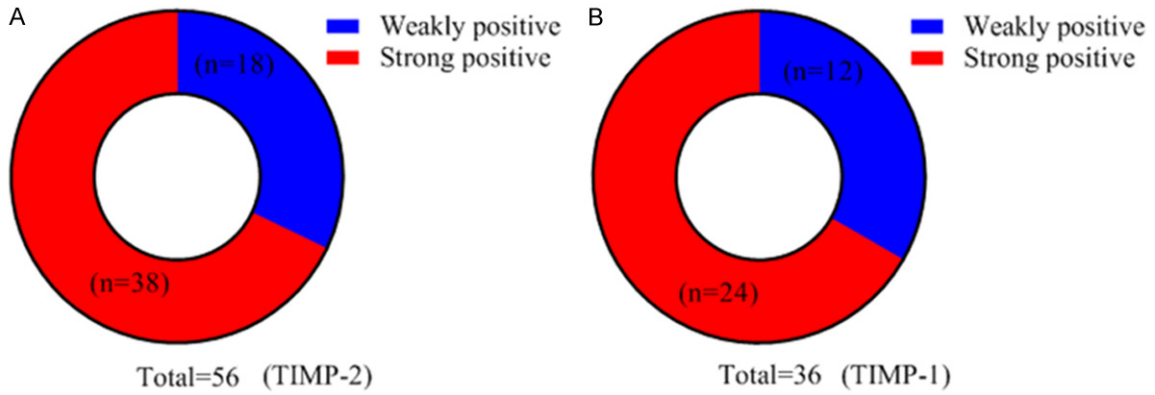


**Figure 4.** TIMP-2, TIMP-1 expressions. Compared with the weak positive rate, the strong positive rates of the TIMP-2, TIMP-1 expressions are significantly higher ($P<0.05$).

**Table 4.** The relationship between the TIMP-2 and TIMP-1 positive expressions and prognosis

| Grouping | TIMP-2 | | TIMP-1 | |
|---|---|---|---|---|
| | Negative/Weak positive | Strong positive | Negative/Weak positive | Strong positive |
| Early | 7 | 18 | 19 | 6 |
| Advanced | 25 | 20 | 27 | 18 |

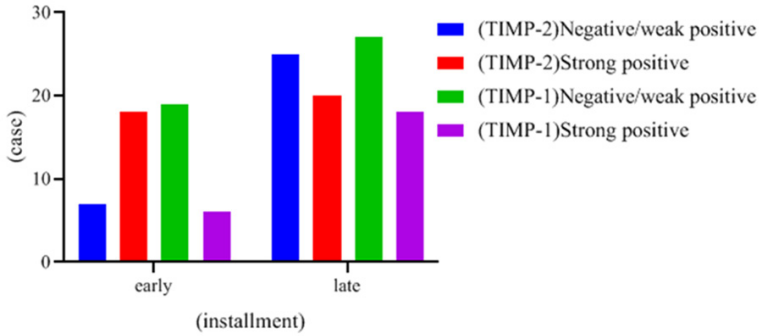


**Figure 5.** TIMP-2, TIMP-1 positive expressions and staging, prognosis. The strong positive rate of TIMP-2 in the early stages was not significantly different from the rate at the advanced stages ($P>0.05$). The strong positive rate of TIMP-1 in the advanced stages was significantly higher than it was at the early stages ($P<0.05$).

and vascular endothelial cells surrounding tumor cells were immunostained with MMP-2 and MMP-9, and the positive expression rates of MMP-2 and MMP-9 were higher in them than they were in tumor cells.

In the research of Joergensen et al. on pancreatic cancer, it was found [22] that tumor cells and peripheral interstitial cells expressed MMP-2, MMP-9, and TIMP-1, and they showed a patch-like, focal-shaped distribution at the edge of the cancer nest or near the lumen. Research by Johanns et al. [23] found that TIMP-2 can form a complex with pro-MMP-2 to prevent the formation of zymogen activation and can non-covalently bind to active MMP-2, which can be regarded as a metastasis repressor gene.

Nissi et al. [24] found that the loss of the MMP-2 and TIMP-2 ratio balance may be related to tumor invasion and metastasis. MMP-2/TIMP-2=1.0 indicates a low risk of tumor metastasis, and >1.0 indicates that the dynamic balance between ECM degradation and reconstruction is distorted, which significantly increases the risk of tumor metastasis [25]. It can be seen that measuring the ratio of the two indicators is helpful in predicting the risk of tumor metastasis.

In summary, the positive expression levels of MMP-2 and MMP-9 are correlated with the progression of NB and the poor prognoses of NB patients, indicating that these indicators can be considered useful biomarkers for NB outcomes.

However, this study did not find a strong correlation between TIMP-1 and NB staging, so further research is needed to explore the relationship between TIMP-1 and NB staging and prognosis.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Zhen Zhang, Department of Neurosurgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324, Jingwuwei 7th Road, Ji'nan 250012, Shandong, China. Tel: +86-0531-68776352; E-mail: enangzzz@163.com

## References

[1] Tsubota S and Kadomatsu K. Origin and initiation mechanisms of neuroblastoma. Cell Tissue Res 2018; 372: 211-221.

[2] Janoueix-Lerosey I, Novikov E, Monteiro M, Gruel N, Schleiermacher G, Loriod B, Nguyen C and Delattre O. Gene expression profiling of 1p35-36 genes in neuroblastoma. Oncogene 2004; 23: 5912-5922.

[3] Coburn J, Harris J, Zakharov AD, Poirier J, Ikegaki N, Kajdacsy-Balla A, Pilichowska M, Lyubimov AV, Shimada H, Kaplan DL and Chiu B. Implantable chemotherapy-loaded silk protein materials for neuroblastoma treatment. Int J Cancer 2017; 140: 726-735.

[4] Bosse KR, Raman P, Zhu Z, Lane M, Martinez D, Heitzeneder S, Rathi KS, Kendsersky NM, Randall M, Donovan L, Morrissy S, Sussman RT, Zhelev DV, Feng Y, Wang Y, Hwang J, Lopez G, Harenza JL, Wei JS, Pawel B, Bhatti T, Santi M, Ganguly A, Khan J, Marra MA, Taylor MD, Dimitrov DS, Mackall CL and Maris JM. Identification of GPC2 as an oncoprotein and candidate immunotherapeutic target in high-risk neuroblastoma. Cancer Cell 2017; 32: 295-309, e212.

[5] Heczey A, Louis CU, Savoldo B, Dakhova O, Durett A, Grilley B, Liu H, Wu MF, Mei Z, Gee A, Mehta B, Zhang H, Mahmood N, Tashiro H, Heslop HE, Dotti G, Rooney CM and Brenner MK. CAR T cells administered in combination with lymphodepletion and PD-1 inhibition to patients with neuroblastoma. Mol Ther 2017; 25: 2214-2224.

[6] Barillari G, Monini P, Sgadari C and Ensoli B. The impact of human papilloma viruses, matrix metallo-proteinases and HIV protease inhibitors on the onset and progression of uterine cervix epithelial tumors: a review of preclinical and clinical studies. Int J Mol Sci 2018; 19: 1418.

[7] Zaman MH, Matsudaira P and Lauffenburger DA. Understanding effects of matrix protease and matrix organization on directional persistence and translational speed in three-dimensional cell migration. Ann Biomed Eng 2007; 35: 91-100.

[8] Singer CF, Marbaix E, Lemoine P, Courtoy PJ and Eeckhout Y. Local cytokines induce differential expression of matrix metalloproteinases but not their tissue inhibitors in human endometrial fibroblasts. Eur J Biochem 1999; 259: 40-45.

[9] Patruno A, Pesce M, Marrone A, Speranza L, Grilli A, De Lutiis MA, Felaco M and Reale M. Activity of matrix metallo proteinases (MMPs) and the tissue inhibitor of MMP (TIMP)-1 in electromagnetic field-exposed THP-1 cells. J Cell Physiol 2012; 227: 2767-2774.

[10] Xie YX, Zhao JH and Cheng AB. Died in patients with malignant tumor blood infection risk factor analysis. Zhong Liu Fang Zhi Za Zhi 2014; 21: 1405-1407.

[11] Roomi MW, Monterrey JC, Kalinovsky T, Rath M and Niedzwiecki A. Comparative effects of EGCG, green tea and a nutrient mixture on the patterns of MMP-2 and MMP-9 expression in cancer cell lines. Oncol Rep 2010; 24: 747-757.

[12] Syed I, Rathod J, Parmar M, Corcoran GB and Ray SD. Matrix metalloproteinase-9, -10, and -12, MDM2 and p53 expression in mouse liver during dimethylnitrosamine-induced oxidative stress and genomic injury. Mol Cell Biochem 2012; 365: 351-361.

[13] Liu Q, Pan D, Cheng C, Zhang A, Ma C, Wang L, Zhang D, Liu H, Jiang H, Wang T, Xu Y, Yang R, Chen F, Yang M and Zuo C. Targeting of MMP2 activity in malignant tumors with a 68Ga-labeled gelatinase inhibitor cyclic peptide. Nucl Med Biol 2015; 42: 939-944.

[14] Jinga DC, Blidaru A, Condrea I, Ardeleanu C, Dragomir C, Szegli G, Stefanescu M and Matache C. MMP-9 and MMP-2 gelatinases and TIMP-1 and TIMP-2 inhibitors in breast cancer: correlations with prognostic factors. J Cell Mol Med 2006; 10: 499-510.

[15] Nakagawara A, Ikeda K, Tsuneyoshi M, Daimaru Y and Enjoji M. Malignant pheochromocytoma with ganglioneuroblastoma elements in a patient with von Recklinghausen's disease. Cancer 1985; 55: 2794-2798.

[16] Di Cataldo A, Agodi A, Balaguer J, Garaventa A, Barchitta M, Segura V, Bianchi M, Castel V, Castellano A, Cesaro S, Couselo JM, Cruz O, D'Angelo P, De Bernardi B, Donat J, de Andoin NG, Hernandez MI, La Spina M, Lillo M, Lopez-Almaraz R, Luksch R, Mastrangelo S, Mateos E, Molina J, Moscheo C, Mura R, Porta F, Russo G, Tondo A, Torrent M, Vetrella S, Villegas JA, Viscardi E, Zanazzo GA and Cañete A. Metastatic neuroblastoma in infants: are survival rates excellent only within the stringent framework of clinical trials? Clin Transl Oncol 2017; 19: 76-83.

[17] Webb AH, Gao BT, Goldsmith ZK, Irvine AS, Saleh N, Lee RP, Lendermon JB, Bheemreddy

R, Zhang Q, Brennan RC, Johnson D, Steinle JJ, Wilson MW and Morales-Tirado VM. Inhibition of MMP-2 and MMP-9 decreases cellular migration, and angiogenesis in in vitro models of retinoblastoma. BMC Cancer 2017; 17: 434.

[18] Juchniewicz A, Kowalczuk O, Milewski R, Laudański W, Dzięgielewski P, Kozłowski M and Nikliński J. MMP-10, MMP-7, TIMP-1 and TIMP-2 mRNA expression in esophageal cancer. Acta Biochim Pol 2017; 64: 295-299.

[19] Roomi MW, Kalinovsky T, Niedzwiecki A and Rath M. Modulation of MMP-2 and -9 secretion by cytokines, inducers and inhibitors in human melanoma A-2058 cells. Oncol Rep 2017; 37: 3681-3687.

[20] Riani M, Le Jan S, Plée J, Durlach A, Le Naour R, Haegeman G, Bernard P and Antonicelli F. Bullous pemphigoid outcome is associated with CXCL10-induced matrix metalloproteinase 9 secretion from monocytes and neutrophils but not lymphocytes. J Allergy Clin Immunol 2017; 139: 863-872, e863.

[21] Yang L, Song X, Zhu J, Li M, Ji Y, Wu F, Chen Y, Cui X, Hu J, Wang L, Cao Y, Wei Y, Zhang W and Li F. Tumor suppressor microRNA-34a inhibits cell migration and invasion by targeting MMP-2/MMP-9/FNDC3B in esophageal squamous cell carcinoma. Int J Oncol 2017; 51: 378-388.

[22] Joergensen MT, Brünner N and De Muckadell OB. Comparison of circulating MMP-9, TIMP-1 and CA19-9 in the detection of pancreatic cancer. Anticancer Res 2010; 30: 587-592.

[23] Johanns M, Lemoine P, Janssens V, Grieco G, Moestrup SK, Nielsen R, Christensen EI, Courtoy PJ, Emonard H, Marbaix E and Henriet P. Cellular uptake of proMMP-2: TIMP-2 complexes by the endocytic receptor megalin/LRP-2. Sci Rep 2017; 7: 4328.

[24] Nissi R, Talvensaari-Mattila A, Kotila V, Niinimäki M, Järvelä I and Turpeenniemi-Hujanen T. Circulating matrix metalloproteinase MMP-9 and MMP-2/TIMP-2 complex are associated with spontaneous early pregnancy failure. Reprod Biol Endocrinol 2013; 11: 2.

[25] Lu LC, Yang CW, Hsieh WY, Chuang WH, Lin YC and Lin CS. Decreases in plasma MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios in uremic patients during hemodialysis. Clin Exp Nephrol 2016; 20: 934-942.