

# RHAMM, A Potential Therapeutic Target, Is Over Expressed in Diffuse Intrinsic Pontine Gliomas

Zhigang Lan<sup>#</sup>, Meng Tian<sup>#</sup>, Yong Deng, Chenghong Wang and Yuekang Zhang\*

Departments of Neurosurgery, West China Hospital of Sichuan University, 37 Guoxue Alley, Chengdu, Sichuan Province, China

<sup>#</sup>Authors Contributed equally

\*Corresponding author: Yuekang Zhang, Department of Neurosurgery, West China Hospital of Sichuan University, 37 Guoxue Alley, Chengdu, Sichuan Province, China



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## ABSTRACT

**Objective:** Supratentorial glioma is enriched in receptors for glycosaminoglycan hyaluronan (HA), including CD44 and the Receptor for HA-Mediated Motility (RHAMM). They have been implicated in cell migration and tumor invasion. In the present study, we have investigated the expression of CD44 and RHAMM in diffuse intrinsic pontine glioma (DIPG), a disease entity distinct from supratentorial glioma.

**Methods:** Expression of CD44 and RHAMM mRNA in DIPG compared with non-diffused brainstem glioma (NDBS) was confirmed by real-time-PCR of 8 DIPG specimens and 6 NDBS specimens while Western blot of 15 DIPG specimens and 3 NDBS specimens was performed to detect the expression of CD44 and RHAMM protein in DIPG compared with NDBS.

**Results:** Results of qPCR and Western blot showed that difference in expression levels of CD44 between DIPG and NDBG were not significant while RHAMM expression levels in DIPG group is significantly higher than in NDBG group.

**Conclusion:** Expression levels of CD44 in DIPG and NDBG were similar while RHAMM was over-expressed in DIPG.

## Introduction

Diffuse intrinsic pontine glioma (DIPG) is a fatal brain cancer. Transient response to radiotherapy, ineffective chemotherapy and aggressive biology lead to rapid progression and a poor prognosis [1-3]. The glycosaminoglycan hyaluronan (HA) is a major component in extracellular matrices. CD44 and receptor for HA-mediated motility (RHAMM), two main cell surface HA-receptors, have been reported to play important roles in tumor invasion, proliferation, adhesion and migration [4-6]. Overexpression of CD44 and RHAMM has been detected in supratentorial gliomas [5,6]. However, the expression of CD44 and RHAMM in DIPG, a disease entity distinct from supratentorial gliomas [1,3], has received little attention. In this study, we are aiming to compare the expression levels of CD44 and RHAMM, in surgical samples of DIPG with those in non-diffused brainstem glioma (NDBS).

## Methods

Patients were included into DIPG group when preoperative MRI showed that the tumor was poorly demarcated and occupied >50% of the brainstem diameter [7], while NDBG group consisted of patients with focal or exophytic brainstem gliomas. The diagnosis of brainstem glioma was confirmed at pathology unit of West China Hospital. The samples were frozen in liquid nitrogen before the experiments. The study was approved by the Ethics Committee of West China Hospital, Sichuan University. Eighteen DIPG samples and 6 NDBG samples were used for qPCR analysis. Total RNA was isolated from the samples using TRIzol reagent (thermo fisher) according to the manufacturer's protocol. RNA integrity was checked by gel electrophoresis on a 1% (w/v) agarose gel RNA

and no signs of degrading were detected. RNA concentration was measured with a ScanDrop 100 Spectrophotometer. Subsequently, RNA was reversely transcribed into cDNA. Quantitative real-time PCR was performed on a CFX Connect™ Real-Time System qPCR instrument (Bio-Rad US). PCR conditions were as follows: 1 cycle of 95 °C for 3 min, followed by 40 cycles of a two-step cycling program (95 °C for 5 s; 60 °C for 30s). The mRNA expression of CD44 and RHAMM was normalized to the expression of GAPDH mRNA. Transcription abundance of CD44 and RHAMM was calculated by the  $2^{-\Delta\Delta Ct}$  method.

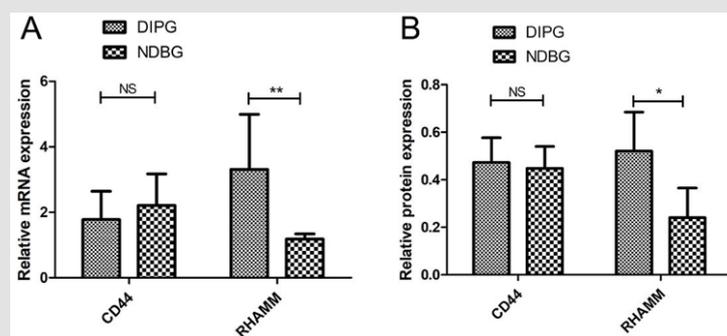
Western blot was performed to detect the expression of CD44 and RHAMM on the protein levels in DIPG and NDBG. Fifteen DIPG samples and 3 NDBG samples were lysed in a modified RIPA (Radio immunoprecipitation assay) buffer and then cleared by centrifugation. Proteins were extracted, separated on Tris-glycine SDS-PAGE gels, and then transferred to nitrocellulose membrane. CD44 and RHAMM were detected by the affinity purified anti-CD44 and RHAMM antibody, respectively. Actin served as a control. The t test was utilized to compare the continuous variables in the

two independent groups. Histogram was utilized to demonstrate graphical visualization of the continuous variables. All statistical analyses were performed using the SPSS software version 22.0. P values < 0.05 were considered statistically significant, and those < 0.01 highly significant.

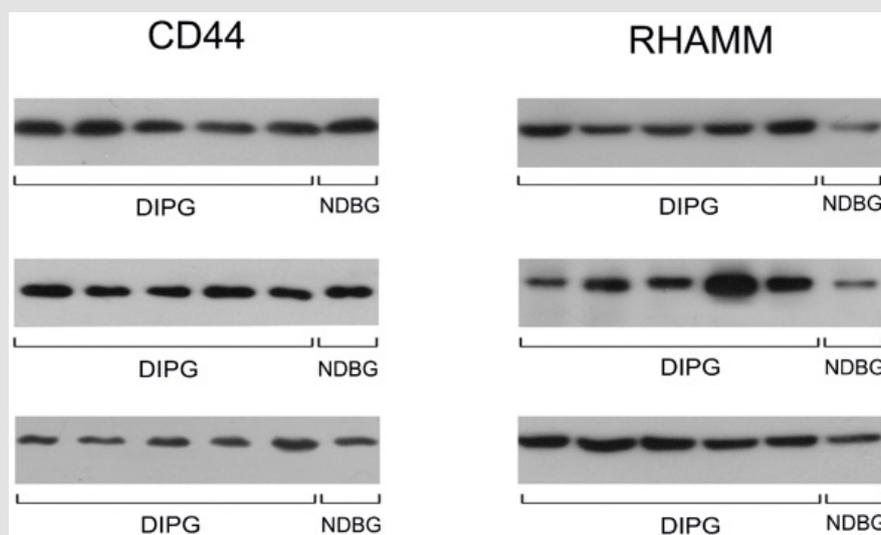
## Results

There was no statistically significant difference in expression values of CD44 mRNA between two groups ( $p = 0.391$ , t test). However, RHAMM mRNA expression levels in DIPG group is significantly higher than in NDBG group [mean  $\pm$  SD (standard deviation),  $3.31 \pm 1.68$  vs  $1.18 \pm 0.16$ , respectively,  $p = 0.009$ , t test] Figure 1A.

The relative expression of CD44 and RHAMM on the protein levels in DIPG and NDBG was examined by western blot. It was found that the expression levels of CD44 in DIPG and NDBG were similar ( $p = 0.708$ , t test) while DIPG had higher levels of RHAMM protein compared to NDBG (mean  $\pm$  SD,  $0.52 \pm 0.16$  vs  $0.24 \pm 0.13$ , respectively,  $p = 0.013$ , t test) Figures 1B and 2.



**Figure 1:** The expression of CD44 and RHAMM in DIPG and NDBG. (A) Quantitative RT-PCR analyses of CD44 and RHAMM mRNA in DIPG and NDBG. (B) Western blot analyses of CD44 and RHAMM protein in DIPG and NDBG. Data are expressed as mean  $\pm$  standard deviation. \*  $p < 0.05$  and \*\*  $p < 0.01$  indicate a significant difference while NS indicates a non-significant difference.



**Figure 2:** Western blot analysis of CD44 and RHAMM expression in specimens of DIPG ( $n = 15$ ) and NDBG ( $n = 3$ ). The expression of CD44 band did not change with the type of brainstem glioma while DIPG and NDBG exhibited strong and diminished expression of RHAMM band, respectively.

## Discussion and Conclusion

In this study, we found that the expression levels of CD44 in DIPG and those in NDBG were similar while RHAMM was overexpressed in DIPG. In contrast to NDBG, DIPG has diffuse growth pattern and can hardly be surgically removed [1,3], which contributes to the dismal prognosis. It has been reported that RHAMM may play a more important role in cell migration than CD44 and that blocking RHAMM-HA interactions results in an inhibition of tumor cell migration and proliferation [6]. Under homeostatic condition, the expression of RHAMM is very low, which makes it a suitable target for cancer therapy with low toxicity [4]. RHAMM-based therapies, including RHAMM-R3 peptides and the combination of RHAMM-R3 and cell-based strategies have shown efficacy and low toxicity in patients with cancer [4,8,9]. Our study showed that the expression levels of CD44 in DIPG and NDBG were similar while RHAMM was overexpressed in DIPG, which might give promise of a novel therapeutic target for the treatment of this fatal disease. Obviously, further studies should be carried out to investigate the RHAMM signaling in DIPG and assess the effect of RHAMM-based therapies on DIPG.

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Yuekang Zhang, Biomed J Sci & Tech Res



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