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Research Report

Identification of MMP-9 specific microRNA expression profile as potential targets of anti-invasion therapy in glioblastoma multiforme

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ABSTRACT

The poor prognosis of glioblastoma multiforme (GBM) is largely attributed to their highly invasive nature and MMP-9 plays a pivotal role in regulating invasiveness of malignant glioma cells. MicroRNAs (miRNAs) are small non-coding RNAs that have been shown to regulate a wide range of biological processes via targeting messenger RNA. Previous reports have shown many oncogenes regulate survival and invasion via targeting MMP-9 in GBM. But no literature indicates that miRNAs regulate glioma cell invasion through targeting MMP-9. Here, we show MMP-9 overexpression conferred a poor prognosis in 163 GBM patients. Furthermore, MMP-9 specific miRNA expression profile (14 positively and 31 negatively correlated miRNAs with MMP-9) was established via miRNA microarrays in 60 GBM samples. Among them, two miRNAs: miR-885-5p and miR-491-5p, were chosen for functional validation for their high positive correlation with MMP-9 expression. And upregulation of miR-885-5p and miR-491-5p were demonstrated to reduce the levels of MMP-9 expression and inhibit cellular invasion in U251 and U87 glioma cells. Furthermore, we found that miR-491-5p suppressed glioma cell invasion via targeting MMP-9 directly. To our knowledge, this is the first study to identify the MMP-9 specific microRNA signature which may provide potential targets for anti-invasion therapy in GBM.

1. Introduction

Glioblastoma multiforme (GBM) is the most lethal cancer in the central nervous system for which conventional therapies have not significantly improved patient outcome. A major challenge in patients with GBM is the propensity of the tumor to invade into adjacent normal brain tissue. Invasive tumor cells can escape surgical removal and are relatively resistant to radiation therapy and chemotherapy (Drappatz et al., 2009). It has been reported that multiple mediators of tumor invasion may provide

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important prognostic information in various cancers (Rich et al., 2005). And improved understanding of molecular determinants of glioma cell invasion will provide valuable insight into the underlying biological features of GBM and illuminate possible novel therapeutic targets (Nakada et al., 2007).

MicroRNAs (miRNAs) are a class of 21 to 25 nucleotide long, small non-coding RNAs that posttranscriptionally regulate the expression of target genes, thereby acting as both tumor suppressors and oncogenes (Zhang et al., 2007). The biological processes modulated by miRNAs include cell differentiation, proliferation, apoptosis and invasion. In recent years, miRNAs have been identified in the progression of various cancers and proposed as novel targets for anticancer therapies (Gabriely et al., 2008).

Members of the MMP family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in several diseases (Nagase et al., 2006; Surgucheva et al., 2010). Matrix metalloproteinase-9 (MMP-9) acts as an important oncogene that improves invasiveness of cancer cells (Egeblad and Werb, 2002). And majority of researches indicate that high level of MMP-9 confers a poor prognosis in various cancers (Folgueras et al., 2004; Liu et al., 2010). In GBM, the relationship between MMP-9 and prognosis remains unclear, and the molecular mechanism that underlies MMP-9 overexpression is not fully understood. In the present study, we identified that high level of MMP-9 was associated with a poor prognosis in GBM. MMP-9 specific miRNA expression profile (miRNAs that positively or negatively correlated with the level of MMP-9) was established via miRNA microarrays. Among them, two miRNAs: miR-885-5p and miR-491-5p, were chosen for functional validation for their high positive correlation with MMP-9 expression. And ectopic expressions of miR-885-5p and miR-491-5p were demonstrated to reduce the level of MMP-9 and inhibit cellular invasion in U251 and U87 glioma cells. To the best of our knowledge, our study firstly indicated that MMP-9 specific miRNAs might be used as targets for therapeutic intervention in GBM.

2. Results

2.1. *High level of MMP-9 was associated with a poor prognosis of GBMs*

In this study, the relationship of MMP-9 expression and patient prognosis was investigated. The expression of MMP-9 in 163 GBM specimens was evaluated by immunohistochemical staining. The mean progression-free survival time of GBM patients with high level of MMP-9 was 284.0 days, significantly shorter than that of patients with low level of MMP-9 (377.5 days; $p=0.0394$, log-rank test; Fig. 1A). And the mean overall survival time of GBM patients with high level of MMP-9 was 385.0 days, significantly shorter than that of patients with low level of MMP-9 (555.0 days; $p=0.0182$, log-rank test; Fig. 1B).

2.2. *miRNA expression profiling for identifying MMP-9 specific miRNA signature*

To evaluate the potential mechanism in aberrant expression of MMP-9, miRNA expression profiling was performed on 60 GBM specimens. The MMP-9 mRNA expression values of above 60 GBM specimens were obtained from respective messenger RNA microarrays. Pearson correlation was performed to analyze the relationships of MMP-9 and all miRNA values using matlab software. Only the miRNAs with high correlation with MMP-9 level ($r>0.4$ and $r<0.4$, $p<0.01$) were considered as MMP-9 specific miRNA signature. As shown in Table 1 and Fig. 2, the MMP-9 specific miRNA signature included 14 positively and 31 negatively correlated miRNAs with MMP-9 expression. Among them, two miRNAs: miR-885-5p and miR-491-5p, were chosen for functional validation in glioma cells for their high positive correlation with MMP-9 expression.

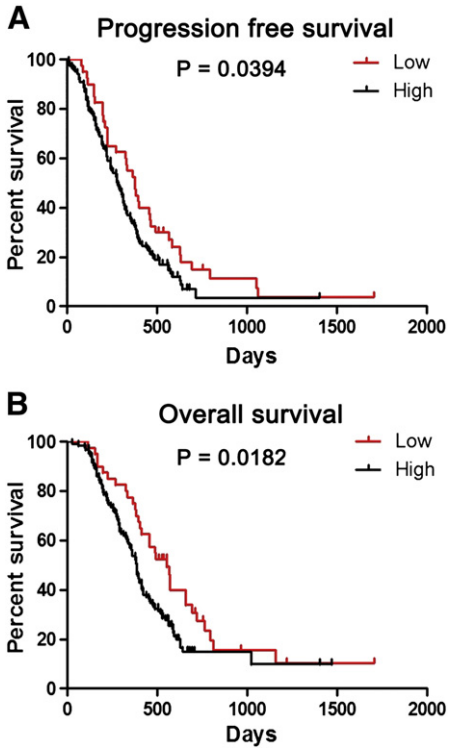

Fig. 1 – Kaplan-Meier plots of progression free and overall survival duration in patients with GBM. Kaplan–Meier survival analysis of progression free and overall survival duration in 163 GBM patients according to MMP-9 protein expression. The log-rank test was used to calculate p values.

2.3. *miR-885-5p and miR-491-5p inhibited cellular invasion and downregulated MMP-9 expression in glioma cells*

To investigate the anti-invasion effect of MMP-9 specific miRNAs, two miRNAs: miR-885-5p and miR-491-5p, were chosen for functional validation in glioma cells for their high positive correlation with MMP-9 expression. As shown in Fig. 3, ectopic expressions of miR-885-5p and miR-491-5p mimics could significantly suppress invasion of U251 and U87 cells in vitro (Fig. 3A). miR-885-5p overexpression led to a 61.89% reduction in invasion compared to the control in U87 cells and 63.78% in U251 cells. And miR-491-5p overexpression led to a 60.32% reduction in invasion compared to the control in U87 cells and 57.27% in U251 cells (Fig. 3B). Furthermore, western blot assays (Fig. 3C) showed that transfection of miR-