Differential Expression of Folate Receptor 1 in Medulloblastoma and Its Relationship to Clinicopathological Characteristics and Targeted Therapy

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Introduction

Medulloblastoma is the most common malignant CNS tumor of childhood. High expression of folate receptor 1 (Folr1) was observed in some malignant epithelial tumors. However its expression and the role for clinicopathological significance and targeted therapeutic potential in MB still remain unclear.

Methods

In current study we have detected the differential expression of Folr1 in MB specimens and cells and identified its clinical, pathological and radiological values to be considered as a biomarker for diagnosis of MB. Then we have studied the targeted treatment of MB with Folr 1 targeted cytarabine (Folr1-Ara-C) both in vitro and in vivo.

Results

Folr1 protein and mRNA were overexpressed in MB specimens, while the expression level correlated with pathological subtypes. Folr1 expression was positively correlated with CSF spreading, Ki-67, MMP9, pathological subtypes and serum Folr1. Factors of age, CSF spreading, Ki-67, MMP9, strong Folr1 expression and pathological subtypes were found to be the independent prognostic values for patients with MB. Serum Folr1 presented increasing trends in turn with the different subgroups of MB, indicating that serum Folr1 showed rational sensitivity and specificity in demonstrating histological types. Folr1-Ara-C led to changes in cellular proliferation and invasion with down-regulation of MMPs proteins and activation of apoptosis in vitro. Using mouse xenograft models, Folr1-Ara-C suppressed tumor growth and improved survival of mouse by MRI and PET/CT. Immunohistochemical analysis of xenograft specimens showed decreased Ki-67 and MMP9 labelling index suggesting the effects on proliferation and invasion in vivo.

Conclusions

Folr1 may be considered as a molecular predictive candidate for histological types and serum Folr1 may be a novel noninvasive biomarker for diagnosis of MB. The application of Folr1-Ara-C contributed

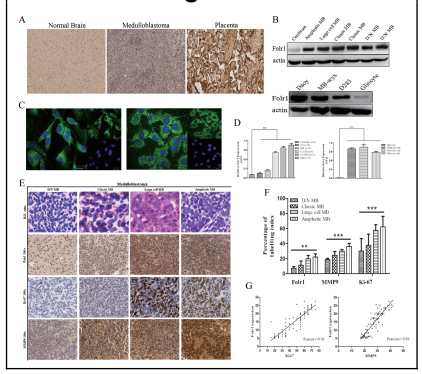
References

Available Upon Request

Learning Objectives

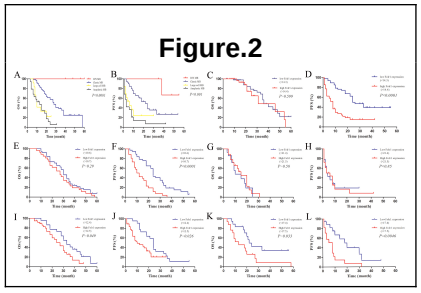
To discover the differential expression of Folr1 in MB and its relationship to clinical, pathological and radiological features as well as targeted therapy for MB indicating that Folr1 could be used as a potential candidate to diagnose and treat.

Figure.1



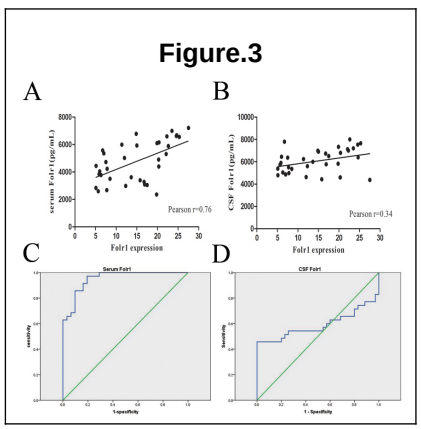
Folr1 expression in different tissues and cells. A. Representative IHC pictures of Folr1 expression in cerebrum, MB tumor specimens and placentas in low-magnifying fields (100×) and high-magnifying fields (200×). B. Immunoblotting analysis of the levels of Folr1 in human MB specimens (left) and MB cells (right). C. Representative confocal pictures of Folr1 expression in Daoy cells (left) and MB-wyx cells (right). D. The mRNA relative levels of folr1 in different tissues (above) and cells (below). Bars are mean ± SD from at least three independent experiments. ***P<0.001. E. Representative pictures of Folr1, Ki-67 and MMP9 expression in MB tumor specimens in low-magnifying fields (100×) and high-magnifying fields (200×). The first line showed morphology of four MB subtypes in high-magnifying fields (H.E. 200×). F. Statistical graph of Folr1, Ki-67 and MMP9 expression detected by IHC in four subtypes of MB. G. Positive correlation between Folr1 expression and Ki-67 (above) or MMP9 (below) was shown by Pearson analysis. **P<0.01, ***P<0.001.

Figure.2



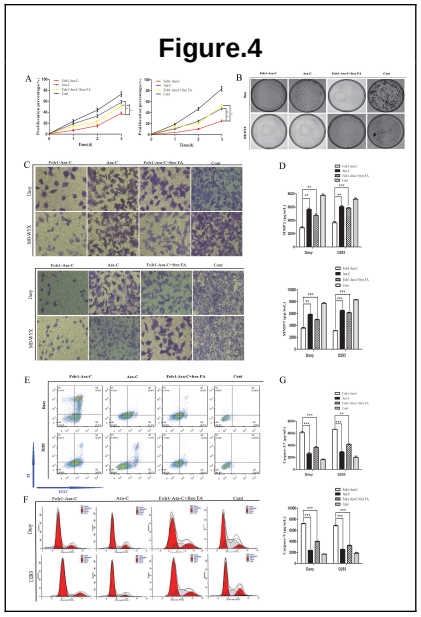
The relationship between prognosis and Folr1 expression in MB patients. A-B. The relationship between OS (A) or PFS (B) and pathological subtypes in MB patients. C-D. The relationship between OS (C) or PFS (D) and the expression level of Folr1 in all the MB patients. E-H. The relationship between OS or PFS and the expression level of Folr1 in the first (E, F) or second (G, H) group of MB patients. I-J. The relationship between OS (I) or PFS (J) and the expression level of Folr1 in the average-risk group of MB patients. K-L. The relationship between OS (K) or PFS (L) and the expression level of Folr1 in the high-risk group of MB patients. Patients with Folr1 expression levels higher or lower than average expression are considered as high or low, respectively.

Figure.3



Results of serum and CSF Folr1 in MB patients. A-B. Statistical graph of correlation between Folr1 expression and serum (A) or CSF (B) Folr1 by Pearson analysis. C-D. ROC curve of serum (C) and CSF (D) Folr1 for evaluating the biomarker of MB.

Figure.4



Effects of Folr1-Ara-C on on the proliferation, mobility and apoptosis of MB cells. A. Daoy (left) and MB-wyx (right) cells were treated with the indicated concentration of Folr-Ara-C, Ara-C, Folr1-Ara-C+free FA and control for 8 h, respectively. Then cell proliferation was measured by using MTS assay. B. Colony formation assay of Daoy and MB-wyx cells was performed in the presence of Folr-Ara-C, Ara-C, Folr1-Ara-C+free FA and control for 8 h and cells were stained with 0.1% crystal violet. C. Cell migration and invasion was evaluated by the transwell chamber assay without or within the membrane coated with Matrigel. D. Secretion of MMP2 (above) and MMP9 (below) after 8 h of incubation of indicated agents was analyzed by ELISA. E. Representative pictures of cell apoptosis by FCM assay was shown. F. Impact of Folr1-Ara-C on Daoy and D283 cell cycle was analyzed by measuring the PI binding activity. G. Cell apoptosis was evaluated by Caspase-3/7 (above) and -9 (below) activity, respectively. Bars are mean ± SD from at least three independent experiments. *P<0.05, **P<0.01, ***P<0.001.

Molecular mechanisms of Folr1-Ara-C functions. A. Targeted effects of Folr1-Ara-C on the inhibition of Erk, pErk and pStat3 in Daoy and MB-wyx cells after 8 h incubation. Lysates were analyzed for pErk/Erk and pStat3/Stat3 antibodies using immunoblot. ß-actin was used as the

Figure.5

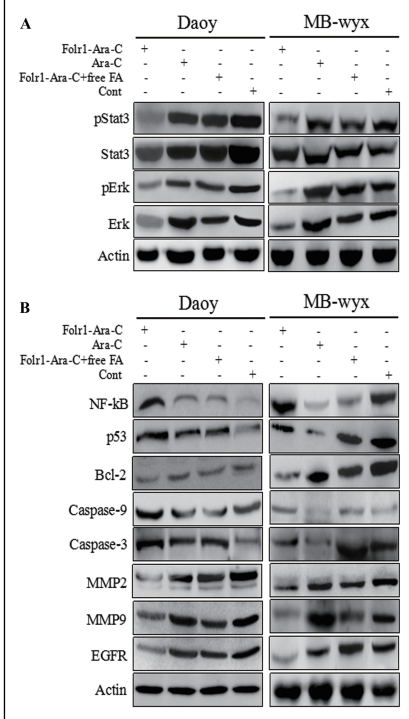


Figure.6