### Title

Studies on molecular mechanisms underlying 5-ALA mediated fluorescence-guided surgery and the prognostic significance of CD44 in grade II/III gliomas

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Studies on molecular mechanisms underlying 5-ALA mediated fluorescence-guided surgery and the prognostic significance of CD44 in grade II/III gliomas

Patients with glioma can benefit from the maximum safest resection. However, surgeons often have difficulty distinguishing tumor tissue from normal tissue and in recognizing infiltrating glioma cells in normal tissues adjacent to tumor tissue intraoperatively. 5-ALA mediated FGS appears to be a promising treatment for glioma. However, 5-ALA-mediated fluorescence cannot always be detected in the World Health Organization grade II/III gliomas. It was hypothesized that gene expression patterns in the Protoporphyrin IX (PpIX) synthesis pathway may be associated with the intraoperative fluorescence status of grade II/III gliomas, and the first part of this study attempted to identify the molecular mechanisms underlying 5-ALA-mediated fluorescence.

CD44 is a major cell surface receptor for hyaluronan (HA) and many other extracellular matrix components, and is implicated in cell adhesion, cell migration, and signaling. Overexpression of CD44 has been detected in many types of tumor tissues. Moreover, CD44 is recognized as a cancer stem cell marker for many cancers. However, the prognostic value of CD44 for glioma patients has not yet been clarified. We tried to explore the impact of CD44 expression on grade II/III glioma patients.
PEPT2, and UROS were significantly higher in the fluorescence-positive grade II/III gliomas than the fluorescence-negative grade II/III gliomas. Among the above candidate genes, the present study focused on PEPT2 because it is an upstream molecule in the PpIX synthesis pathway and PEPT2 plays an important role in the selective transportation of peptides, amino acids, and drugs of the cells in the central nervous system. The protein expression of PEPT2 was also significantly higher in the fluorescence-positive gliomas, which was confirmed by western blot analysis and immunofluorescence analysis. The siRNA-mediated downregulation of the mRNA and protein expression of PEPT2 led to decreased PpIX fluorescence intensity, as confirmed by fluorescence spectrum analysis. Through down-regulating the expression of PEPT2, the fluorescence intensity may be managed by neurosurgeons in the future.

(Studics on the prognostic significance of CD44 in grade II/III gliomas)

【Methods and materials】To assess the RNA expression levels of CD44 in glioma tissues and normal brain tissues, meta-analyses were conducted in the online Oncomine database. Then, the mRNA expression levels of CD44, CD44s, and CD44v2–v10 in 112 grade II/III glioma patients in Hokkaido University Hospital (HUH) were detected by qPCR. The RNA-seq data and clinical data of grade II/III glioma patients were obtained from The Cancer Genome Atlas (TCGA) and the Chinese Glioma Genome Atlas (CGGA) databases. The Kaplan-Meier survival curve analysis was performed to explore the association between CD44 gene expression and overall survival of glioma patients. Then we used univariate and multivariate Cox regression analyses to evaluate the utility of CD44 expression as an independent prognostic factor. Gene Set Enrichment Analysis (GSEA) was performed to explore the function of CD44 and its related signaling pathways base on the TCGA database.

【Results and discussion】In the study on the prognostic significance of CD44, based on the Oncomine database, CD44 has significantly high expression in glioma tissues as compared with normal tissues. Compared with mRNA expression level of CD44s, the mRNA expression levels of CD44v3, CD44v4, CD44v5, CD44v6, CD44v7, CD44v8, CD44v9, and CD44v10 were much lower. Then, we explored the clinical relevance of CD44 mRNA expression based on the HUH cohorts, the TCGA cohorts, and the CGGA cohorts. In survival analysis, high mRNA expression of CD44 was correlated with poor overall survival and poor progression-free survival in grade II/III glioma patients. Multivariate Cox regression analyses confirmed CD44 as an independent prognostic factor for grade II/III glioma patients. According to GSEA, gene sets of Toll-like receptors (TLRs) signaling pathway, cell adhesion molecules, regulation of actin cytoskeleton, and chemokine signaling pathway are differentially enriched in CD44 high expression phenotype.

【Conclusions】The study on key molecules in 5-ALA mediated FGS is the first, to the best of our knowledge, to demonstrate that PEPT2 is an important gene/protein in 5-ALA-mediated FGS in grade II/III glioma. The overexpression of PEPT2 was associated with a higher fluorescence intensity of PpIX in grade II/III gliomas. These results may provide clues to improve the surgical treatment of grade II/III gliomas in the future.

The study on the prognostic significance of CD44 demonstrated that overexpression of CD44 is correlated with a poor prognosis for grade II/III glioma patients. Our findings suggest that CD44 could play an important role as a useful prognostic biomarker for grade II/III glioma patients.