HOKKAIDO UNIVERSITY

Title	Studies on molecular mechanisms underlying 5-ALA mediated fluorescence-guided surgery and the prognostic significance of CD44 in grade II/III gliomas [an abstract of dissertation and a summary of dissertation review]
Author(s)	侯, 崇显
Description	配架番号：2527
Degree Grantor	北海道大学
Degree Name	博士(医学)
Dissertation Number	甲第14036号
Issue Date	2020-03-25
Doc URL	https://hdl.handle.net/2115/78167
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	doctoral thesis
File Information	Hou_Chongxian_abstract.pdf, 論文内容の要旨

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

学位論文内容の要旨

博士の専攻分野の名称　博士（医　学）　氏 名　侯　崇显

学 位 論 文 題 名

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

（グレードII/III神経膠腫における5-ALAを利用した蛍光誘導手術の分子機構とCD44の予後への重要性に関する研究）

Summary

【Background and Objectives】Glioma is the most frequent primary intra-axial brain tumor. Surgery, combined with chemotherapy and radiotherapy, is the standard treatment; however, glioma remains incurable due to its high recurrence rate and invasiveness. Thus, studies on new therapeutic approaches and targets for gliomas are of great importance. The present study mainly focused on two topics for glioma, The first topic is the study on molecular mechanisms underlying in 5-aminolevulinic acid (5-ALA) mediated fluorescence-guided surgery (FGS) in grade II/III gliomas and the second topic is the study on 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.

CD 44 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.

(Studies on molecular mechanisms underlying 5-ALA mediated fluorescence-guided surgery in grade II/III gliomas)

【Methods and materials】The present study first attempted to identify candidate genes with an effect on 5-ALA-mediated PpIX fluorescence intensity. The mRNA expression levels of genes (*ALAD*, *ALAS1*, *ABCG2*, *ABCB6*, *CPOX*, *FECH*, *HO-1*, *PEPT2* and *UROS*) in the PpIX synthesis pathway were compared among normal brain tissues, fluorescence-negative grade II/III gliomas, and fluorescence-positive grade II/III gliomas. The most likely candidate gene was selected and confirmed by protein expression analysis. To further investigate the exact function of the target gene, the mRNA and protein expression of the target gene was inhibited in a grade III glioma cell line and the PpIX fluorescence spectrum was detected.

【Results and discussion】The mRNA expression levels of *ALAD*, *ABCG2*, *ABCB6*, *CPOX*, *HO-1*,

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.

(Studies 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.