学位論文内容の要旨
（Summary of dissertation）

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学 位 論 文 題 名
（Title of dissertation）
Functional analysis of MAP17 in glioblastoma-initiating cells

（膠芽腫幹細胞における MAP17 の機能解析）

【Background and Objectives】 Glioblastoma (GBM), WHO grade IV brain tumor, is the most common malignant brain tumor in adults with a median survival rate of approximately 15 months, even after multimodal treatment using surgeries, chemotherapies (e.g., temozolomide (TMZ), and radiotherapies (Stupp R et al, 2005)). Since the overall survival rate for GBM has not improved over the past decades, there is a strong demand for developing new therapeutic methods.

The discovery of human GBM-initiating cells (hGICs) has affected the direction of GBM research, as hGICs have been shown to possess strong tumorigenic ability. They are resistant to irradiation and chemotherapy including TMZ, suggesting that hGICs are the cell-of-origin of recurrence. Thus, it is important to characterize hGICs and to find novel therapeutic targets. We have previously established hGICs from the patients and their TMZ-resistant lines (GICRs) (Yamashita et al, 2015; Tsukamoto et al, 2016). By comparing the expression profiles of hGICs and GICRs, I focused on membrane-associated protein 17 (MAP17, also known as PDZK1IP1), which is upregulated in GICRs and has been implicated in malignant tumors.

MAP17 is a small, 17 kDa membrane protein located in the Golgi apparatus and plasma membranes (Rivero M et al, 2003). MAP17 contains two transmembrane regions and a PDZ-binding domain. Through PDZ domain, MAP17 can interact with PDZK1 and act as a carrier from the Golgi to the cell membrane (Lanaspa et al, 2007). Increased expression of MAP17 has been reported in most human carcinomas and in other non-epithelial neoplasia, such as glioblastomas. Notably, high MAP17 level is associated with tumor progression and malignancy (Carnero A et al, 2012). Several research have already confirmed that overexpression of MAP17 could increase tumorigenesis with reduced apoptosis and increased cell growth in tumors. Although MAP17 has no enzymatic or transcriptional activity, it can act as a cell signaling pathway regulator. Previously, MAP17 has been identified as a target of a highly conserved transcription factor Twist family BHLH transcription factor 1 (Twist1) (Di Maro G et al, 2014). Twist1 overexpression is also associated with many aggressive tumors and their poor prognosis (Ansieau S et al, 2010). It is also identified as a main regulator of epithelial to mesenchymal transition (EMT), which can promote tumor invasion and chemotherapy resistance (Sánchez-Tilló E et al, 2012; De Craene B et al, 2013).

The main objective of this research is to unravel the function of MAP17 in GBM recurrences and its role in chemoresistance of hGICs. Furthermore, we want to verify whether increased expression of

MAP17 would change the tumorigenesis of hGICs *in vivo* and *in vitro*, and we also aim to revel the mechanisms of MAP17-induced TMZ resistance.

【Subjects and Methods】 My subject is to characterize the function of MAP17 in hGICs and GBM in order to unravel the mechanisms of chemoresistance and tumor recurrences of GBM. Bioinformatics, Western blotting and immunohistochemistry were used to analyze the expression of MAP17 in GICRs and hGICs. Transfection was used to construct overexpression cell lines. The gene expression level was measured by qPCR. Cell viability and TMZ resistance analysis were determined by MTT assay. Wound healing assay were used to measure the migration of cells. The effect of MAP17 on tumor growth was determined in animal experiments.

【Results】 The results showed that MAP17 was upregulated in GBM patients and GICRs, indicating that MAP17 is involved in TMZ resistance of GBM. MAP17-overexpressed hGICs showed increased TMZ resistance and proliferation abilities *in vitro,* and also enhanced tumorigenesis abilities *in vivo*. These data suggested that MAP17 plays an important role in tumor progression. Gene expression profiling analysis showed that Prrx1, Twist1 and BCL2 are significantly increased in MAP17-overexpressing hGICs compared to control hGICs. Using qPCR analysis, we also confirmed that Prrx1, Twist1 and BCL2 expression are significantly increased in MAP17-overexpressing hGICs. Furthermore, by using TMZ resistance analysis, we found that Twist1/BCL2-overexpressing hGICs are TMZ resistant. There was a feedback-loop between Twist1 and MAP17 expression to induce chemoresistance of hGICs. Prrx1 can also induce the expression of BCL2 which is important for TMZ resistance. These findings suggested that MAP17 may serve as a potential target for GBM treatment.

【Discussion】 Despite the relative lack of publications, MAP17 overexpression has been linked to numerous different effects. The current mainstream view is that MAP17 is mainly a transmembrane protein which is involved in cell-cell interactions (Kocher O et al, 1996). Recently, there are several articles indicated that MAP17 sequestrated NUMB, leading to Notch pathway activation (Garcia-Heredia JM et al, 2017), and even more, MAP17 increased the exosomes in tumor cells, where MAP17 was released as cargo protein (García-Heredia, J.M. et al, 2020). Then, the localization of MAP17 protein would be both on the cell membrane and in the cytoplasm.

However, in the confocal fluorescent images of human GBM sections, there were MAP17 signals overlapped with nucleus or around nucleus. Both fluorescent images of control and MAP17-overexpressing hGICs showed the same results. Connected with current study, MAP17 can promote two transcription factor gene expression, and MAP17 protein localization are mainly in the nucleus. These results make me doubt MAP17 could be a co-activator of gene transcriptions. The present results may provide a new perspective and understanding of MAP17 in tumor progression and tumor recurrences. And this might shed a light on the direction of future research.

Our current finding suggests that MAP17, which is originally a transmembrane protein, may be function as a co-activator of gene expression. This will provide more possibilities for future research and finding useful therapeutic tools to improve the prognosis of GBM patients. Furthermore, future studies to define the functional significance of MAP17 target molecules, including Twist1 and BCL2, might provide a greater understanding of the complex mechanisms of GBM progression and recurrence.

【Conclusion】

By the construction of MAP17-overexpressing hGICs, the relationship between MAP17 and TMZ resistance was revealed. TMZ can induce MAP17 expression in hGICs, and higher level of MAP17 will confer TMZ resistance by upregulating Twist1 and Prrx1 expression. As a transcription factor, Twist1 can also promote MAP17 expression by forming a positive feedback loop, and both Prrx1 and Twist1 can positively induce BCL2 expression, which is the key regulator of TMZ resistance.