学　位　論　文（要約）

Functional analysis of MAP17 in glioblastoma-initiating cells
（膠芽腫幹細胞における MAP17 の機能解析）

2024 年 3 月

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Summary

Background and Purpose:

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), and radiotherapies (Ostermann et al, 2004; Stupp 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: hGICs have been shown to possess strong tumorigenic abilities. 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 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, we found that the expression of many genes increased and decreased in GICRs. After arranging gene expression changes from largest to smallest, we picked up 30 genes that increased in GICRs, and two genes were selected as candidates, MAP17 (Membrane-associated protein 17, also known as PDZK1IP1) and Wnt7B (Wingless Type MMTV Integration Site Family, Member 7B). However, co-culturing hGICs with Wnt7B for 1 week did not affect TMZ resistance of hGICs. For that reason, I focused on MAP17 which is upregulated in GICRs and have been shown to be involved in malignant tumors (Guijarro et al, 2007a; Wang et al, 2012).

MAP17 is a small, 17 kDa membrane protein located in the Golgi apparatus and plasma membranes (Rivero et al, 2018). MAP17 protein sequence contains two transmembrane regions and a PDZ-binding domain, by its PDZ-binding domain, it can interact with PDZK1 protein and act as a carrier from the Golgi to the cell membrane (Lanaspa et al, 2007). In normal human cells, its expression is restricted to specific epithelial cells from the kidney (proximal tubular cells) and epidermal keratinocytes (Kocher et al, 1996; Jaeger et al, 2000). Ectopic expression of MAP17 in tumor was reported in most human carcinomas, and in other non-epithelial neoplasia, such as GBM. Its increased expression is linked with tumor progression and malignancy (Carnero et al, 2012). Various research has already confirmed that overexpression of MAP17 could increase tumorigenesis, specifically, overexpression in tumor cells would inhibit cell apoptosis and increase cell growth. Although MAP17 has no enzymatic or

transcriptional activity, it can act as a cell signaling pathway regulator to generate described roles. Previously, MAP17 has already been identified as a target of a highly conserved transcription factor TWIST1 (Twist Family bHLH Transcription Factor 1) (Di Maro et al, 2014). TWIST1 overexpression is also associated with many aggressive tumors and their poor prognosis (Ansieau et al, 2010). It also was identified as a main EMT (Epithelial-Mesenchymal Transition) regulator, which can promote tumor invasion and chemotherapy resistance (Sánchez-Tilló et al, 2012; De Craene et al, 2013).

In this study, I identified that MAP17 and TWIST1 expression can be induced by TMZ treatment, and their expression level also elevated in GICRs. Further analysis revealed that MAP17 was highly expressed in GBM patients, which was related with cell proliferation and chemoresistance. *In vivo* results also demonstrated that MAP17 overexpression could significantly promote tumorigenesis of hGICs. After analysis of RNA-seq data, I found two main genes that were involved in chemoresistance of hGICs, TWIST1 and BCL2. And there was a positive feedback-loop formed between TWIST1 and MAP17 which would induce the expression of BCL2 and further increase the chemoresistance of hGICs. Our finding suggested that MAP17 might serve as a potential target for GBM recurrences.

Subjects and Methods:

My subject is to characterize MAP17's function 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 up regulated in GBM patients and GICRs, indicating that it was involved in TMZ resistance of GBM. MAP17 overexpression could not only increase TMZ resistance abilities of hGICs but also increase their proliferation abilities *in vitro* and tumorigenic abilities *in vivo*. These data suggested that MAP17 plays an important role in tumor progression. After further analyzing the expression profile of MAP17 overexpressing hGICs, Prrx1 (Paired Related Homeobox 1), TWIST1 and BCL2 were significantly altered between control hGICs and MAP17-

overexpressing hGICs. Using qPCR analysis, we confirmed that Prrx1, TWIST1 and BCL2 expression were significantly increased in MAP17-overexpressing hGICs. Furthermore, by using TMZ resistance analysis, TWIST1/BCL2-overexpressing hGICs were found to be TMZ resistant. Further analysis unraveled that there was a feedback-loop between TWIST1 and MAP17 expression, leading to induce chemoresistance of hGICs. And Prrx1 can also induce the expression of BCL2 which further increased resistance to TMZ. The finding suggested that MAP17 may serve as a potential target for GBM treatment.

Discussion:

In this research, the main focus was on the changes of gene expression, however, the protein levels and related pathways were not mentioned. 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 et al, 1996). Recently, there are several articles indicated that MAP17 sequestrated NUMB, leading to Notch pathway activation (Garcia-Heredia et al, 2017), and even more, MAP17 increased the exosomes in tumor cells, where MAP17 was released as cargo protein (García-Heredia et al, 2020). Then, the localization of MAP17 protein would be both on the cell membrane and in the cytoplasm.

However, connected with my results, 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. This might shed a light on the direction of future research. In the following research, protein-protein interaction would be the key to finding pathway ways involved in TMZ resistance of hGICs. And finding the interaction protein of MAP17 protein can also unravel the mechanisms of MAP17 protein localization and its movement in hGICs. The present results and future research may provide a new perspective and understanding of MAP17 function in brain tumor progression and tumor recurrences. And the functional analysis of MAP17 will clear its role specifically in GBM and provide an answer to TMZ resistance that has plagued GBM for a long time.

Current finding suggests that MAP17, which is originally a transmembrane protein, may be a co-activator of gene expression. This will provide more possibilities for future research and finding useful therapeutic tool for improving the prognosis of patients with GBM. Furthermore, future studies of 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 TWIST1 and MAP17 expression and higher level of MAP17 will confer hGICs TMZ resistance abilities by upregulating TWIST1 and Prrx1 expression. As a transcription factor, TWIST1 can also promote MAP17 expression, forming a positive feedback loop and both Prrx1 and TWIST1 can positively induce BCL2 expression, which is the key regulator of TMZ resistance.