論　文　概　要

○ 論 文 題 目

TMEPAI tumorigenic functions in breast cancer cells

(乳がん細胞における TMEPAI の腫瘍形成促進能)

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Abstract

Purpose

TMEPAI (transmembrane prostate androgen-induced protein) has been reported to be implicated in human malignancies by regulating multiple cellular signaling pathways, such as TGF-β, androgen, PI3K/AKT, and Wnt signaling pathways. However, the molecular mechanism of how TMEPAI regulates tumorigenesis is still not fully understood. Therefore, in this present study I aimed to investigate the molecular mechanism of TMEPAI functions in breast cancer cells, particularly by regulating TGF-β and PI3K/AKT signaling pathways.

Materials and methods

In this study, I generated CRISPR/Cas9-mediated TMEPAI knockout (KO) breast cancer cell lines and used a lentiviral expression system to complement each TMEPAI isoform and mutant individually in TMEPAI KO cells. General molecular biology experimental methods such as quantitative polymerase chain reaction (qPCR) and western blotting were carried out to examine the expressions of target genes at mRNA and protein levels. DNA sequencing was used to screen for TMEPAI KO clones. Cell proliferation assay, colony formation assay, and sphere formation assay were used to examine tumorigenic activities of cancer cells *in vitro*. Tumor xenograft assay was used to examine the tumorigenic activities of cancer cells *in vivo* by subcutaneous injection. Extreme Limiting Dilution Analysis (ELDA) was used to examine stem cell frequency of cells.

Results

Two TMEPAI KO clones were obtained from the Hs578T cell line: clones #5 and #18 and from BT-549 cell line: clones #22 and #29. These KO clones were used for the evaluation of TMEPAI's cellular functions. As a result, TMEPAI KO did not significantly affect monolayer cell proliferation in both Hs578T and BT-549, whereas TMEPAI KO significantly reduced colony and sphere formation in Hs578T and BT-549 cells. Then, I re-expressed TMEPAI isoforms and TMEPAI isoform a mutants into TMEPAI KO cells and found that all re-expression of TMEPAI isoforms a, b, and d, significantly rescued colony and sphere-forming abilities in TMEPAI KO clones compared to vector controls and there was no significant difference in rescued tumorigenic function among TMEPAI isoforms. While the rescue functions of TMEPAI double PY mutant and SIM mutant were significantly weaker compared to TMEPAI isoform a wild type and single PY mutants (each PY 1 and PY 2 mutant) in colony formation and sphere formation assays. Next, I also found that TMEPAI was implicated in cancer stemness induction. The expressions of TMEPAI and well-known cancer stem cells (CSC) marker genes such as CD44, NANOG, OCT4, and SOX2, were significantly increased in three-dimensional (3D) sphere culture than in two-dimensional (2D) monolayer culture. Interestingly, TMEPAI KO clones showed decreased stem cell frequency and expression of CSC marker genes in the 3D sphere culture condition together with the decreased level of AKT phosphorylation compared to the breast cancer parental cells.

Discussion

Some publications are suggesting the conflicting results of TMEPAI functions as a tumor promoter or suppressor in various cancer types. Therefore, I investigated whether these conflicting results are due to the difference of TMEPAI isoform-specific function. Using breast cancer as the experiment model, my study showed that TMEPAI isoforms had a similar function as a driver of sphere and colony formation and both TMEPAI double PY motifs and

a SIM where located on its intracellular domain, were essential for colony and sphere formation but not for monolayer cell proliferation. TMEPAI has been reported to be involved in tumorigenicity by regulating multiple intracellular signaling pathways including TGF-β, AR, PI3K/AKT, and Wnt via its SIM and double PY motifs. Thus, TMEPAI acts as a tumor suppressor or a tumor promoter depending on the context of the underlying oncogenic pathways activated in cancer cells. In the second part of my study, I found that TMEPAI knockout reduced stem cell frequency and the expression of stemness marker genes. My data also suggested that the activation of the PI3K/AKT/mTOR pathway was an essential signaling pathway to induce cancer stemness. Since TMEPAI via its PY motifs indirectly activated PI3K/AKT pathway by promoting PTEN degradation, TMEPAI enhanced tumorigenic activity by regulating oncogenic signaling pathways such as PI3K/AKT/mTOR resulting the induction and maintenance of cancer stemness for the persistent growth of cancer cells.

Conclusion

My study provides a novel insight into TMEPAI functions in breast cancer cells. The presence of TMEPAI via its PY motifs promotes PI3K/AKT signaling pathway-induced cell growth. The other highlight of my study is the importance of TMEPAI and PI3K/AKT/mTOR axis in cancer stemness induction, which may lead to the development of a new generation of cancer therapeutic agents targeting CSCs.