論　文　概　要

○ 論 文 題 目

The roles of TMEPAI in Wnt signaling

(TMEPAIによるWntシグナル抑制機構の解明)

論文題目が外国語の場合には，（　　）書きで日本語訳を記入すること。

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目的：

[Purpose]

Transmembrane prostate androgen-induced protein (TMEPAI) is induced by androgen, TGF-β, PI3K/ ERK, and Wnt signaling. It is highly expressed in many types of cancer and associated with poor prognosis. Structurally, TMEPAI consists of a short extracellular domain, a transmembrane domain, and a Smad interaction motif (SIM) between two PPxY (PY) motifs. However, no solved protein structure has been reported. In this thesis, I performed evaluation of the predicted protein structure of TMEPAI. By structural analysis, I found a novel motif, Shisa-like motif, in TMEPAI. Shisa protein has been known as a suppressor of Wnt signaling. Hence, I further examined the role of TMEPAI in Wnt signaling pathway and its molecular mechanism.

対象と方法：

(Material and method)

I carried out computational prediction methods for TMEPAI protein structure. I used I-TASSER and RaptorX software to evaluate the secondary structure of human TMEPAI. Furthermore, I used I-TASSER and Phyre^2 to establish three-dimensional (3D) prediction structure of TMEPAI protein. I carried out molecular biological methods such as polymerase chain reaction (PCR), quantitative PCR (qPCR), western blotting, immunoprecipitation (IP), and nuclear fractionation to examine the expression, interactions, and localization of the proteins. I used luciferase-reporter assays to examine the transcriptional activity of intracellular signaling pathways. To understand the mechanism, I used TMEPAI knockout breast cancer cell line and TMEPAI knockdown breast cancer cell line that are established using CRISPR/ Cas9 system and small interference RNA (siRNA), respectively. I also performed sphere formation assays to examine oncogenic activities of TMEPAI in breast and colon cancer cells.

結果：

(Results)

The luciferase activity result showed that TMEPAI could suppress not only Wnt3A ligand-induced Wnt signaling but also LiCl-induced, and β-catenin overexpression-induced Wnt signaling. Targeted the interaction between TMEPAI and β-catenin, I found that TMEPAI binds to β-catenin and overexpression of TMEPAI prevents β-catenin nuclear translocation. TMEPAI

knockout in breast cancer cell lines, MDA-MB-231 and Hs578T cells, promotes β-catenin nuclear accumulation and increases the mRNA expression of Wnt target genes, *AXIN2* and *C-MYC*. Additionally, I examined the involvement of TGF-β signaling in TMEPAI-mediated suppression of Wnt signaling and I found that SB431542 inhibitor did not affect the enhanced mRNA expression of *AXIN2* in TMEPAI knockout cells and TMEPAI SIM mutant could suppress Wnt signaling as well as TMEPAI wild type.

考察：

[Disscussion]

I demonstrated that TMEPAI could interact with β-catenin and this interaction may be important for inhibition of β-catenin nuclear translocation and its transcriptional activity. Previous reports suggested that Smad3 and β-catenin interaction attenuated β-catenin degradation, facilitates β-catenin nuclear translocation, and transcriptional activity. Furthermore, TMEPAI is also known as a negative regulator of TGF-β signaling by binding to Smad3 and inhibiting its phosphorylation. However, the addition of TGF-β type I inhibitor did not affect the enhancement of Wnt target genes in TMEPAI knockout cells. These data indicated that TMEPAI directly suppressed Wnt signaling in a TGF-β-independent manner. Both TGF-β and Wnt signaling are involved in cell proliferation and stem cell maintenance and by regulating both signaling pathways, TMEPAI could play important roles in tumorigenesis.

結論：

[Conclusion]

TMEPAI could inhibit Wnt/ β-catenin signaling via its PY motifs and prevent β-catenin nuclear translocation and gene transcription. Furthermore, TMEPAI knockout in breast cancer cell line increased the mRNA level of Wnt target genes, *AXIN2* and *C-MYC*. I suggested a novel motif in the TMEPAI structure, Shisa-like motif, although I need to further investigate the role of it in TMEPAI functions.