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審査研究科	人間総合科学研究科
学位論文題目	The roles of TMEPAI in Wnt signaling (TMEPAI による Wnt シグナル抑制機構の解明)

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論文の内容の要旨
Abstract of thesis

【背景　Background】

TMEPAI (Transmembrane prostate androgen-induced protein) is involved in intracellular signaling pathways such as androgen, TGF-β, PI3K/ERK, and Wnt signaling. In the present study, the applicant elucidated TMEPAI's function in Wnt signaling. TOP-Flash reporter luciferase assays showed that TMEPAI suppressed Wnt signaling induced by treatment of Wnt3A, and LiCl, and β-catenin overexpression. Moreover, the applicant found that TMEPAI inhibits Wnt signaling by binding to β-catenin and preventing β-catenin nuclear translocation, the mRNA levels of Wnt-target genes, *AXIN2* and *C-MYC*, were increased in TMEPAI knockout breast cancer cell lines. Furthermore, SB431542, a TGF-β signaling inhibitor, did not change *AXIN2* level in TEMEPAI knockout cells. Overall, the applicant suggests that TMEPAI might directly interfere Wnt signaling.

【目的　Purpose】

Expression of Transmembrane prostate androgen-induced protein (TMEPAI) is induced by androgen, TGF-β, PI3K/ ERK, and Wnt signaling. Structurally, TMEPAI consists of a short extracellular domain, a transmembrane domain, and a Smad interaction motif (SIM) between two PPxY (PY) motifs; however, protein structure has not been precisely clarified. In this thesis, the applicant investigated predicted protein structure of TMEPAI and found a novel motif, Shisa-like motif, in TMEPAI. Since Shisa protein has been known as a suppressor of Wnt signaling, the applicant examined the role and molecular mechanism of of TMEPAI in Wnt signaling pathway.

【対象と方法　Materials and methods】

Computational prediction methods for TMEPAI protein structure were performed by using I-TASSER and

RaptorX software and I-TASSER and Phyre[2]. To understand the mechanism, the applicant used TMEPAI knockout breast cancer cell line and TMEPAI knockdown breast cancer cell lines established using CRISPR/ Cas9 system and small interference RNA (siRNA), respectively. Sphere formation assays were done to examine oncogenic activities of TMEPAI in breast and colon cancer cells.

【結果　Results】

The luciferase activity showed that TMEPAI could suppress not only Wnt3A ligand-induced Wnt signaling but also LiCl-induced, and β-catenin overexpression-induced Wnt signaling. In addition, the applicant found that PY motifs of TMEPAI are involved in the β-catenin-dependent Wnt signaling. Therefore, the applicant examined the molecular mechanism and found that TMEPAI binds to β-catenin and prevent β-catenin nuclear translocation. In contrast, nuclear accumulation of β-catenin and mRNA expression of Wnt target genes, *AXIN2* and *C-MYC*, were enhanced in TMEPAI knockout cancer cell lines, MDAMB-231 and Hs578T cells. In addition, the applicant examined the involvement of TGF-β signaling in TMEPAI-mediated suppression of Wnt signaling and 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　Discussion】

The applicant demonstrated that TMEPAI could interact with β-catenin and this interaction might be important for inhibition of β-catenin nuclear translocation and its transcriptional activity. TGF-β type I inhibitor did not affect the enhancement of Wnt target genes in TMEPAI knockout cells, suggesting that TMEPAI directly suppressed Wnt signaling in TGF-β-independent manner. Since both TGF-β and Wnt signaling might be involved in cell proliferation and stem cell maintenance, TMEPAI could play important roles in tumorigenesis by regulating both signaling.

【結果　Conclusion】

TMEPAI could inhibit Wnt/ β-catenin signaling via its PY motifs and prevent β-catenin nuclear translocation and gene transcription.

審査の結果の要旨
Abstract of assessment result

【批評　General Comments】

The doctoral thesis of Ms. Riezki Amalia elucidated that TMEPAI directly interfered Wnt signaling. The applicant also suggested a novel motif in the TMEPAI structure, Shisa-like motif and 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. Although further study is required to investigate the role in TMEPAI functions, these original findings constitute valuable contribution to this field of research.

【最終試験の結果　Assessment】

The final examination committee conducted a meeting as a final examination on June 15, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結果　Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.