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| 学 位 の 種 類 | 博士（ 医学 ） |
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| 審 査 研 究 科 | 人間総合科学研究科 |

学位論文題目　PMEPA1/TMEPAI is an Independent Tumorigenic Coactivator of AKT Working Through Proteasomal Degradation of PHLPP1 in Triple Negative Breast Cancer Cells（PMEPA1/TMEPAI は、トリプルネガティブ乳がん細胞において PHLPP1 のプロテアソームによる分解を介して作用する AKT の独立した腫瘍形成コアクチベーターである）

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# 論文の内容の要旨
# Abstract of thesis


In this doctoral dissertation, MD. ANWARUL HAQUE describes the role of PMEPA1/TMEPAI in triple negative breast cancer cells. The summary is as follows:

（目的　Purpose）

Transmembrane prostate androgen-induced protein (TMEPAI) is a transforming growth factor-β (TGF-β) induced oncoprotein. It is highly expressed in various types of cancer and activates multiple oncogenic pathways. However, there is no specific study regarding how and which motifs of TMEPAI have such oncogenic functions in triple-negative breast cancer (TNBC). Therefore, the author studied the oncogenic function of TMEPAI in triple negative breast cancer cells and aimed to elucidate the molecular mechanism behind such activities.

（対象と方法　Materials and Methods）

The author used well-controlled and established methods of molecular biology to elucidate the oncogenic functions and molecular mechanisms of TMEPAI in TNBC progression. Clustered regularly interspaced short

palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) gene-editing technique, small interfering RNA, and short hairpin RNA systems were used to knockout (KO) or knockdown (KD) of target proteins. Colony formation, cell migration, scratch wound healing, tumorsphere formation, and xenograft tumor formation assays were performed to investigate the oncogenic functions of TMEPAI and PHLPP1. Polymerase chain reaction, quantitative polymerase chain reaction, and Western blotting were used to examine the expression of targeted genes and proteins. Co-immunoprecipitations were performed to detect the protein-protein interaction. BALB/cAJcl-nu/nu mice were used to evaluate the xenograft tumor formation. Additionally, the cancer genome atlas (TCGA) database of invasive breast cancer (BRCA) patients was analyzed to find the correlation between TMEPAI and phosphoinositide 3-kinase (PI3K)/AK strain transforming (AKT) signaling in the patients.

（結果　Results）

TMEPAI expression is inversely correlated with patients' survival rate. The author found that TMEPAI KO cells remarkably reduced colony formation, cell migration, and tumorsphere formation. TCGA clinical data set analysis showed a positive correlation between TMEPAI and PI3K/AKT signaling activity especially in AKT phosphorylation on S473. Western blot data also demonstrated that TMEPAI enhanced S473 phosphorylation of AKT by suppressing PHLPP1. The author also found that KD of PHLPP1 from both parental and TMEPAI KO cells enhanced colony formation, cell migration and tumor sphere formation by promoting PI3K/AKT signaling. This correlation was further confirmed by in vivo experiments. To investigate which motifs of TMEPAI downregulates PHLPP1, the author performed TMEPAI re-expression experiments in TMEPAI KO cells using lentivirus expression system. The results clearly showed that a PPxY motifs (PY motifs) mutant could not suppress PHLPP1 unlike TMEPAI wild type and a Smad interacting motif (SIM) mutant, indicating the PHLPP1 suppressive role of TMEPAI through its PY motifs. Colony formation assay and Western blotting results also corroborated the role of TMEPAI PY motifs for the regulation of colony formation and promotion of PI3K/AKT signaling. Additionally, the author also found that TMEPAI binds NEDD4-2, a E3 ubiquitin ligase, through its PY motifs, enhanced the complex formation of NEDD4-2 with PHLPP1, and hence promotes PHLPP1 polyubiquitination and proteasomal degradation. Moreover, the author found the KD of NEDD4- 2 recovered PHLPP1 protein amount, suggesting a cumulative role of TMEPAI and NEDD4-2 is essential for the proteasomal degradation of PHLPP1 in TNBC cells.

（考察　Discussion）

Since the overactivation of PI3K and/or inactivation of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a negative regulator of PI3K/AKT signaling, are frequently observed in cancer cells, activation of PI3K/AKT signaling is considered as one of the main drivers of cancer initiation and progression. In this study, the author identified a unique oncogenic role of TMEPAI in TNBC cells. The author found that TMEPAI promotes colony formation, migration, and tumorsphere formation in breast cancer cells. Mechanistically, PY motifs of TMEPAI activates PI3K/AKT signaling specially by enhancing the phosphorylation at the serine 473 site of AKT through the downregulation of PHLPP1. These findings are in line with a clinical data (TCGA) analysis from invasive BRCA patients. PHLPP1 is one of the negative regulators of PI3K/AKT signaling and has a tumor-suppressive role which was proved by various in vitro and in vivo studies. The author also elucidated that PHLPP1 dephosphorylates serine 473 of AKT and suppresses colony formation, cell migration, tumor sphere formation and in vivo tumor formation. These results indicate that activation of PI3K and inactivation of PTEN are not enough for the oncogenic activation of AKT. Degradation of PHLPP1 by TGF-β-induced TMEPAI is essential for the tumorigenic AKT activation. To clarify the TMEPAI-mediated regulation of PHLPP1, the author observed that

PHLPP1 ubiquitination was enhanced in the presence of TMEPAI by facilitating complex formation with NEDD4-2. These data suggest a putative oncogenic function of TMEPAI in TNBC progression via activation of PI3K/AKT signaling.

## 審査の結果の要旨
## Abstract of assessment result

（批評　General Comments）

In the present study, the author revealed a novel oncogenic role of TMEPAI via the activation of PI3K/AKT signaling through the downregulation of PHLPP1. Therefore, TGF-β-induced TMEPAI is considered as an essential oncogenic coactivator of AKT working together with genomic mutations of PI3K and PTEN, and an independent biomarker and a promising target to establish molecular targeted therapy for the management and treatment of TNBC patients.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on 1 June 2021. The applicant provided an overview of the dissertation, addressed questions and comments raised during the Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

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