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学位論文題目	The Role of PMEPA1/TMEPAI in Lung Metastatic Colonization of MDA-MB-231 Cells （MDA-MB-231 細胞の肺転移における PMEPA1/TMEPAI の役割）
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論文の内容の要旨

Abstract of Thesis

In this dissertation, Mohammed Abdelaziz describes the role of PMEPA1/ TMEPAI in lung metastatic colonization of MDA-MB-231 cells. The summary is as follows.

(Purpose): PMEPA1/TMEPAI, a type Ib transmembrane protein whose transcription is upregulated by androgens, TGF-β signaling, Wnt signaling and EGF signaling, is implicated in regulation of important signaling pathways that are modified in oncogenic transformation including suppression of TGF-β/Smad and androgen signaling, as well as activation of PI3K/AKT signaling through negative regulation of PTEN. For its role in metastasis, reports are conflicting likely reflective of the molecular context in which PMEPA1 works. In this study, the author experimentally investigated the role of PMEPA1 in metastasis of the human breast cancer cell line MDA-MB-231 to the lung.

(Materials and Methods): The author used the CRISPR-Cas9 system to establish PMEPA1 knockout (KO) MDA-MB-231 cell clones. Two cell clones (PMEPA1 KO 1-3 and PMEPA1 KO 3-5) were selected and expanded after screening for TGF-β-induced PMEPA1 protein expression levels. Luciferase (Luc) - or Green Fluorescence Protein (GFP) - expressing PMEPA1 KO or parental MDAMB231 cells were established using a lentivirus Luc- or GFP-

expressing vector (CSII-CMV-MCS-IRES2-Bsd-Luc/GFP), respectively. PMEPA1 KO and parental MDA-MB-231 cell lines were used for investigation of PMEPA1's role in cell proliferation *in vivo* by subcutaneous injection of tumor cells into the left flank of female NOD/ShiJic-scid mice and *in vitro* both at high seeding density by cell proliferation assay and at low density by colony formation assay. Gene set enrichment analysis (GSEA) was used to identify clinical association between altered expression of PMEPA1 and changes in PMEPA1-related signaling pathways in The Cancer Genome Atlas (TCGA) invasive breast cancer cases (BRCA). Statistical analysis was performed using Student t-test in Excel, One-way/ Welch's ANOVA and the appropriate Post Hoc test in SPSS (IBM, version 24) or Fishers exact test in GraphPad Prism 7.

(Results): 1) Knockout of PMEPA1 imparted no significant difference on the *in vitro* cell proliferation of MDA-MB-231 cells in monolayer cell culture condition. However, the author noted that PMEPA1 KO cell lines had less *in vivo* tumor growth as indicated by significantl smaller volumes of surgically resected tumor xenografts derived from PMEPA1KO cell lines than those derived from parental cell lines. Next, the author examined the expression level of angiogenesis-related genes such as *IL8, FGF2* and *VEGFA*. Interestingly, PMEPA1KO cell lines showed a marked reduction of both *VEGFA* and *IL8* compared with parental cells. 2) In addition, PMEPA1 KO MDA-MB-231 cells tended to have smaller metastatic lung lesions than those of parental MDA-MB-231 cells in lung metastatic colonization assay after tail vein injection of tumor cells. 3) Although Knockout of PMEPA1 imparted no significant difference in cell growth of MDA-MB-231 cells in monolayer adherent cell culture condition, it markedly reduced the number of colonies larger than 2 mm in the colony formation assay that indicates the capacity of the tumor cells to initiate tumor formation at low seeding density. 4) At high seeding density, GFP-expressing PMEPA1 KO MDA-MB-231 cells had a stronger response than that GFP-expressing parental cells to the growth signals provided at high seeding density by the surrounding Non-GFP-expressing population of either PMEPA1 KO or parental cell, respectively. Yet, GFP-expressing PMEPA1 KO MDA-MB-231 cells kept showing less capacity than parental cells to proliferate from single cells even on switching the surrounding Non-GFP-expressing population either to parental or to PMEPA1 KO MDA-MB-231 cells, respectively 5) LY294002 (PI3K/AKT inhibitor) treatment resulted in dramatic slowing down of cell proliferation that colonies of neither parental MDA-MB231s nor Knockout MDA-MDA-MB231 cells at low seeding density.

(Discussion and Conclusion): Impaired growth of PMEPA1 KO cell line-derived tumor xenografts is consistent with several reports that dominantly indicate a tumorigenic function of PMEPA1. The author argued that PMEPA1 KO-mediated induction of TGF-β signaling alone could not sufficiently explain this *in vivo* phenotype since MDA-MB-231 cell growth is poorly inhibited by TGF-β. Rather, the author considered that discrepancy between *in vivo* tumor growth and metastasis on the one hand and *in vitro* cell proliferation in monolayer adherent cell culture condition of PMEPA1 KO cell lines at high seeding density on the other hand partially reflects the marked impact of PMEPA1 KO-mediated suppression of critical proangiogenic mediators, including *IL8* and *VEGFA,* and therefore impaired angiogenesis on *in vivo* tumor growth and on metastasis which is negligible on *in vitro* proliferating cells that evenly receive nutrients from enriched medium. On the other hand, PMEPA1 supports metastasis not only through maintaining transcription of important proangiogenic mediators and promotion of angiogenesis, but also

probably through initiation of proliferation from single cells early during distant organ colonization. Both parental and PMEPA1 KO MDA-MB-231 cells could comparably provide growth signals enough to further drive cell proliferation at high seeding density suggesting insignificant impact of PMEPA1 knockout on supportive growth signals provided by MDA-MB-231 cells at high seeding density. The data attest a critical role of PI3K/AKT signaling in the proliferation of both parental and PMEPA1 KO MDA-MB-231 cells at low seeding density. Therefore, the author proposed that PMEPA1 KO-mediated attenuation of PI3K/AKT signaling probably contributes to the reduction of proliferation from single cells at low seeding density in PMEPA1 KO MDA-MB-231 cells.

審査の要旨

Abstract of assessment result

（General Comments）

This study focused on the function of PMEPA1 in MDA0MB-231 cell line. The results are very interesting and the author found various differences of in vitro and in vivo experiments, such as cell growth activity and tumor growth activity between parental MDA-MB-231 cell and knockout cells. The author also indicated that pro-angiogenic mediators, including *IL8* and *VEGFA* might be associated with the difference and insisted a critical role of PI3K/AKT signaling in the proliferation of both parental and PMEPA1 KO MDA-MB-231 cells at low seeding density. However, the definite molecular mechanism of the PMEPA1 is still unclear and more detailed analysis should be performed in the future.

（Assessment Result）

The final examination committee conducted a meeting as a final examination on April 26th, 2019. 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.

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