氏　　名	RUDY
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学位論文題目	The role of GPNMB ectodomain in breast cancer development （乳がんの発生・進展における GPNMB 細胞外領域の役割）
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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Mr. RUDY examined the biological functions of two ectodomains of glycoprotein nmb (GPNMB):Kringle-like domain (KLD) and C-mannosylation motif, in breast cancer development. GPNMB is a type IA transmembrane protein highly expressed in breast cancer and is associated with poor prognosis. However, the precise role and contribution of the KLD and C-mannosylation motif of GPNMB in breast cancer development have not been fully elucidated. Therefore, Mr. RUDY examined the roles and mechanism by which thesetwo ectodomains confer tumorigenicity in vitro and in vivo. He found that cells with a deletion of either ectodomain significantly reduced the tumorigenic activity by suppressing epithelial to mesenchymal transformation (EMT) and cell proliferation. The information obtained from this research will serve as a platform to develop anti-tumor agents by targeting KLD or C-mannosylation motifessential for tumor formation. The content is summarized as follows:

（目的　Purpose）

The purpose of this research is to elucidate the role of GPNMB ectodomains, specifically the KLD and

the C-mannosylation motif, in breast cancer development.

（対象と方法　Materials and Methods）

(1) KLD-project

The author first took an in vitro approach to evaluate the function of KLD by generating expression vectors encoding the wild-type (WT) and mutant GPNMB lacking KLD (ΔKLD). Stable cell lines expressingthese constructs were established using 293T and NMuMG cells, and various biochemical and cell biological experiments were performed, including immunofluorescence staining, Western blotting, migration assays, proliferation assays, sphere formation assays, EMT assays,to examine the effects of overexpressing WT GPNMB or ΔKLD mutants. Second, the author performed in vivo tumor formation assays by subcutaneous injection of NMuMG cells (WT and ΔKLD mutant) into nude mice and tumor burden, cellular polarity, proliferation, and ultrastructure of tumors were evaluated.

(2) C-mannosylation project

The authorused the information on the consensus sequence of C-mannosylation (four tryptophan residues W69, W73, W168 and W171) in GPNMBand C-mannosylation was evaluated by mass spectrometry. Stable cell lines expressing WT, $GPNMB^{W69H}$ and $GPNMB^{W168H}$mutants were generated and the cells were assessed for tumorigenicity in sphere assays,as well as by subcutaneous injection into nude mice. Lastly, the author generated monoclonal antibodies against C-mannosylated GPNMB by preparing the fusion protein expressing a part of the GPNMB sequence containing the C-mannosylation motif and Fc region of mIgG2. Mass spectrometry and ELISA were performed to evaluate C-mannosylation and titers of the antibodies.

（結果　Results）

(1) KLD of GPNMB was well-conserved across the species. The deletion of KLD did not affect the physiological properties of GPNMB, including surface expression, subcellular localization, Src-induced tyrosine phosphorylation, and homodimerization. However, ΔKLD mutant cells showed a significant decrease in sphere-forming activity and developed smaller tumors followingtumor xenograft into mice.Both WT and ΔKLD mutants exhibited EMT phenotype as shown by downregulation of E-cadherin.Ultrastructure of the tumorsrevealed that WT, as well as ΔKLDNMuMG-injected tumors,did not form tight junctions; however, ΔKLD tumors exhibited more tight-like junctions. Although ΔKLD suppressed E-cadherin expression, cellular migration and activation of Wnt/β-catenin signaling were decreased in ΔKLDtumors.

(2) C-mannosylation at W69 and W168 of GPNMB was confirmed by mass spectrometry. Mutant NMuMG cells expressing $GPNMB^{W69H}$ and $GPNMB^{W168H}$ lost the sphere-forming ability and $GPNMB^{W168H}$NMuMGcells formed significantly smaller tumors compared to WT cells by tumor xenograft assays.

（考察　Discussion）

The deletion of KLD impaired GPNMB-induced tumorigenesis without affecting the physiological properties of GPNMB.The mechanism by whichGPNMB induces tumorigenesisismediated by KLD and C-mannosylation motif since the deletion of these motifs significantly reduced the sphere-forming ability and tumor development in vivo by suppressing EMT. Although the molecular mechanism of KLD or C-mannosylation is still unknown; i.e. identity of binding partners or signal transduction mediated by the motifs, it is clear that KLD and C-mannosylation are crucial for breast cancer development. The aurthor is currently in the process of generating monoclonal antibodies against C-mannoslyated GPNMB peptide, which may be a useful tool fortargeting all C-mannosylation sites. The motifs within the ectodomain can be efficient and effective targetsfor drug development and this study provided a potential new direction for breast cancer treatment.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

This study revealed two functionally essential domains of GPNMB, KLD and C-mannosylation motif, in the development of breast cancer. The author first performed a series of in vitro studies to examine the effects of loss of KLD and C-mannosylation motifin cellular functions,and successfully characterized the phenotype of mutant cells. These results led to the next stage of experiments using anin vivo xenograft model. The comparison between in vitro and in vivo for assessing the function of the ectodomains was well-performed and the author obtained significant amounts of information leading to the pathological insights on the tumorigenesis induced by GPNMB. This study provided a basis for the development of new anti-breast cancer therapy.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on July 1, 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.

（結論　Conclusion）

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