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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	Glycoprotein nmb is exposed on the surface of dormant breast cancer cells and induces stem cell properties （GPNMB は休眠乳がん細胞において細胞表面に局在し、幹細胞性を誘導する）
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論文の内容の要旨
Abstract of thesis

（目的　Purpose）

Glycoprotein nmb (GPNMB) which is highly expressed in various aggressive cancers, including melanoma, glioma, and breast cancer, especially triple negative breast cancer (TNBC), is a poor prognostic factor for recurrence, metastasis-free and overall survival. Previous studies have shown that the enhanced expression of GPNMB confers epithelial-mesenchymal transition (EMT) and tumorigenic potential to mammary gland epithelial cells. EMT state is associated with cancer stem cells (CSCs) which are thought to be one of the root causes of the failure of cancer treatment. The applicant focused on studying the roles of GPNMB in breast cancer stem cells.

（対象と方法　Materials and Methods）

In this thesis, Quantitative real-time PCR (qPCR) was used to quantify mRNA expression levels. Immunoblot analysis was performed to examine the expression levels of proteins. Immunofluorescence staining was used to examine GPNMB protein expression and localization in breast cancer cells. Immunohistochemical staining was performed to examine MKI67 protein expression in spheres and xenograft tumor tissues of breast cancer. Fluorescence-activated cell sorting (FACS) analysis was used to quantify surface GPNMB amount and isolate surface $GPNMB^{low}$ and $GPNMB^{high}$ cell populations. Extreme Limiting Dilution Analysis (ELDA) and sphere formation assay were performed to examine stem cell frequency of different cell groups.

（結果　Results）

The mRNA expression of *GPNMB* in breast cancer cells was significantly higher in three-dimensional (3D) sphere culture condition than in two-dimensional (2D) monolayer culture condition, as well as known CSC markers such as *SOX2*, *NANOG*, and *FOXO3*. In addition, the expression of GPNMB and CSC marker genes is closely correlated with the ratio of MKI67-negative cells. Interestingly, GPNMB was exposed only on the surface of the growth-arrested breast cancer cells. Surface GPNMB^{high} cells showed higher stem cell frequency and expression of CSC marker genes than those of the surface GPNMB^{low} cells. Additionally, tumorigenic wild-type GPNMB induced expression of CSC marker genes in the 3D sphere culture condition, but the non-tumorigenic GPNMB mutant did not.

（考察　Discussion）

The transmembrane protein GPNMB usually localizes within endosomal or lysosomal compartments. However, the applicant showed that the cell surface expression of GPNMB was increased in dormant breast cancer cells, and the levels of stem cell genes and EMT-inducing transcription factors (EMT-TFs) were also enhanced. Breast cancer stem cells (BCSCs) stop proliferating and cause G0/G1 phase cell cycle arrest in serum starvation or 3D sphere culture condition, which is also an important stimulation to promote GPNMB trafficking to the cell surface, and subsequently regulates downstream signaling pathways or ensures the malignant characteristics of breast cancer cells. GPNMB contains a half immunoreceptor tyrosine-based activation motif (hemITAM) (YxxI) and a dileucine motif (D/ExxLL) in its cytoplasmic tail, these motifs are frequently found in transmembrane proteins and function as a sorting signals which associate with endocytosis or endosomal/lysosomal membrane trafficking. It has been demonstrated that hemITAM is essential for inducing EMT and tumorigenesis by phosphorylation of a tyrosine residue. Furthermore, in this study, hemITAM is essential to enhance the expression of CSC marker genes.

Considering the specific expression patterns and levels of GPNMB in CSCs, targeting GPNMB will be an effective therapeutic choice for cancer treatments. Similar to antibody-drug conjugate (ADC) for instant, CDX-011 (glembatumumab vedotin), cyclic peptide-drug conjugated (cPDC) which is cyclic peptide-based delivery vehicles specific to targeting cells, such as GPNMB-cPDC will offer good prospects for the treatment of GPNMB-expressing cancers.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The 3D sphere culture condition which is strongly correlated with slowly proliferative states of cancer cells, induces the expression of stem cell markers and GPNMB. GPNMB is exposed on the surface of dormant breast cancer cells. In addition, the tyrosine residue in hemITAM of GPNMB is essential for regulation of CSC marker genes. These findings suggest that surface GPNMB which is exposed on dormant breast cancer cells contributes to the acquisition of stem cell properties. These results are very interesting and will be used for new therapy.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on Dec 20, 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.

（結論　Conclusion）

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