論　文　概　要 (Thesis Abstract)

○ 論 文 題 目
(Theme)

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)

○ 指 導 教 員
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RUDY

Purpose (目的)

To elucidate the role of GPNMB ectodomain, especially the Kringle-like domain (KLD) and the C-mannosylation motif, in breast cancer development

Material and Method (対象と方法)

1. Polymerase chain reaction

To construct the mammalian expressing vector of mGPNMB$^{\Delta KLD}$, mGPNMB^{W69H}, mGPNMB^{W168H}, and hGPNMBΔC mutants.

2. FACS analysis

To analyze the surface expression of GPNMB-WT and GPNMB$^{\Delta KLD}$ protein.

3. Immunofluorescence assay

To analyse the subcellular localization of GPNMB-WT and GPNMB$^{\Delta KLD}$ protein.

4. Western blotting

To detect the protein expression in cell lysate or immunoprecipitated protein.

5. Transwell migration assay

To evaluate the migratory ability of the cell lines.

6. 2D proliferation assay

To evaluate the monolayer culture growth of the cell lines.

7. Sphere formation assay

To evaluate the sphere forming ability as an in vitro tumorigenic assay of the cell lines.

8. In vivo tumor formation assay

To evaluate the in vivo tumorigenic ability of the cell lines by injecting the cells subcutaneously to nude mice.

9. Immunohistochemical staining

To analyse the histological features of the tumors.

10. Electron microscopy

To analyse the cell junctions formed in the tumors.

11. Mass spectrometry

To analyse the C-mannosylation status of GPNMB-WT and GPp-Fc proteins.

12. ELISA

To analyse the antibodies produced by the mouse injected by GPp-Fc antigen.

13. Statistical analysis

To show the significance of the results.

Result (結果)

1. GPNMB has conserved Kringle-like domain across species

KLD of GPNMB is well conserved in across species, including the cysteines which are important in disulfide bond formation.

2. Deletion of KLD does not affect GPNMB physiological properties

The deletion of KLD does not affect the surface expression, subcellular location, Src-induced tyrosine phosphorylation, and homodimerization of GPNMB.

3. KLD is important in GPNMB-induced tumor formation

ΔKLD mutant expressing cells showed significantly lower sphere forming activity and had significantly smaller tumors with less incidence compared to those of WT.

4. ΔKLD mutants have less disruption of cell polarity than WT

ΔKLD mutants could form epithelial tubular structure in tumors, meaning that they can partially maintain cellular polarity.

5. GPNMB-WT and GPNMB-ΔKLD mutants do not form tight junction

The formation of tight junction was observed in mock, whereas none could be observed from WT and ΔKLD mutants; however, ΔKLD mutants were observed to have more tight-like junction compared to those of WT.

6. Deletion of KLD suppresses E-cadherin expression but impairs cellular migration ability

Both WT and ΔKLD mutants had EMT phenotype as shown by the suppression of E-cadherin, however through immunofluorescence staining we observed that WT formed more stress fibers while ΔKLD mutants maintained cortical actin fibers. Moreover, ΔKLD7 mutant expressing cells had significantly lower migration ability compared to those of WT expressing cells

7. Conservation profile of the C-mannosylation motif of GPNMB

hGPNMB has a WXXXW motif from amino acid 69 to 73, which is conserved among species and a WXXW motif, which is conserved in mouse and monkey.

8. GPNMB is C-mannosylated

All tryptophan residues in both C-mannosylation motifs were shown to be mannosylated through GC-MS/MS analysis.

9. Tryptophan residue in the C-mannosylation consensus motif is essential in tumorigenesis

Point mutation of the tryptophan residue in any C-mannosylation motifs could impair GPNMB-impaired tumorigenesis.

Discussion (考察)

Here, we provide the first experimental evidence that KLD and C-mannosylation motif of GPNMB are important in breast cancer development. The deletion of KLD, while not affecting GPNMB physiological properties, can impair GPNMB-induced tumorigenesis. Although the deletion of KLD suppresses E-cadherin expression, it somehow impairs the GPNMB-induced disruption of cellular polarity, hence reducing its tumorigenic activity. On another note, the mutation of the tryptophan residue at the C-mannosylation motif, which was shown to be uniquely mannosylated on both tryptophan residues, causes significant loss of tumorigenic activity induced by GPNMB. Taken together, these results suggest that both KLD and C-

mannosylation motif of GPNMB, which are located at the ectodomain, could be potential targets for future breast cancer therapy in patients overexpressing GPNMB.

Conclusion (結論)

KLD and C-mannosylation motif of GPNMB are essential in breast cancer development and can be potential targets for future therapy.