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Functional analysis of adhesion GPCR latrophilin 2 in MDA-MB-231 human breast cancer cells

(ヒト乳癌細胞 MDA-MB-231 における adhesion GPCR latrophilin 2 の機能解析)

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Approved Digest

The tumor microenvironment strongly influences cancer cell growth, migration, invasiveness, and dynamics of gene expression. The microenvironment consists of non-tumor cells, active soluble factors, and extracellular matrix (ECM). ECM has essential factors to the cell such as acting as a physical support and has a signaling function that transduces signal to control cells through its receptor on the cell surface. Adhesion G protein-coupled receptors (GPCRs) are thought to be involved in cell-cell or cell-ECM interaction because of the presence of a long N-terminal extracellular domain (ECD) including multiple adhesive folds.

To elucidate the role of adhesion GPCRs in cancer invasion, we established three-dimensional (3D) cell culture system for MDA-MB-231 breast cancer cell line. Firstly, we found that MDA-MB-231 showed invasion-like protrusions when cells were grown in 3D culture system. By siRNA screening of 32 adhesion GPCRs in 3D culture of MDA-MB-231, we identified latrophilin 2 (LPHN2) as a potential candidate gene involved in breast cancer invasion. Furthermore, qPCR analysis showed that mRNA levels of LPHN2 in MDA-MB 231 cells were higher than other breast cancer and normal breast epithelial cell lines. Based on these findings, the cellular functions of LPHN2 in cell invasion, migration, and proliferation was investigated. The downstream signaling pathways from LPHN2 was also examined.

To understand the role of LPHN2 in specific cellular functions, LPHN2 knockdown (KD) in MDA-MB-231 cells was performed using two individual siRNAs against LPHN2. The KD efficiency of LPHN2 was confirmed by qRT-PCR and Western blot. In addition, we also generated LPHN2 knockout (KO) cells by using CRISPR-Cas9 genome editing system and obtained two KO cell lines. Then, we analyzed cell proliferation activity of LPHN2 KO cells. The cells showed less proliferative activity compared to wild type cells. This result suggested LPHN2 function on cancer cell proliferation. Then we analyzed the other LPHN2 function, including invasion-like structure formation, adherence to ECM, and migration activity.

As same with first screening result, knocking down of LPHN2 in MDA-MB 231 cells inhibited invasion-like formation in 3D culture. When wild type cells were cultured at 3 mg/ml collagen I, they exhibited extensive sprouting structures with a higher tendency to invade, while KD cells remained primarily spherical. Similar data were obtained from LPHN2 KO cells, showing inhibition of invasion-like formation compared with wild type.

The effects of LPHN2 depletion on the cell adhesion to ECMs-coated surface was further investigated. Both LPHN2 KD and KO cells showed that depleted LPHN2 decreased the adhesion of the cells to collagen I, but not to fibronectin.

Cell migration is highly associated with cell invasion. To examine the role of LPHN2 in cell migration, migration activity was measured and quantified by using the Boyden chamber assay and wound healing assay. The data showed that LPHN2 depletion inhibits MDA-MB-231 cells migration. Cell migration was also analyzed by measuring the total distance of single migratory cell using time-lapse imaging microscopy. Experiments using both KD and KO cells indicated that the LPHN2 depletion reduced cell migration activity.

LPHN2 possesses classical adhesion GPCR structure, with seven transmembrane helices (7TM) and a large N-terminal extracellular domain containing GAIN domain that harbors an autoproteolytic cleavage site (GPS site). In one model of adhesion GPCR activation mechanism, N-terminal fragment (NTF) is dissociated from C-terminal fragment (CTF) upon ligand binding. Reporter assay data showed that SRE-mediated gene transcription was most prominently activated by CTF. CRE and NF-kB activation were also found, albeit at a small increased. Interestingly, overexpression of CTF in LPHN2 KO cells restored migration activity.

We found the involvement of ROCK and PKA in LPHN2-induced cell motility on the extracellular matrix (ECM) gel in rescue experiments using KO cells and constitutively active mutant of LPHN2 (CTF). Rho and PKA are well known to be activated downstream Gα12/13 and Gαs, respectively. Moreover, knocking down of Gα12/Gα13 and Gαs was able to reduce cell migration in wild type and CTF-overexpressed in LPHN2 KO cells, supporting the coupling of LPHN2 with Gα12/13 and Gαs. This finding is the first report of the signaling of LPHN2 in mammalian cells that LPHN2 may stimulate cell migration through pathways mediated by Gα12/13-Rho and Gαs-PKA. Taken together, these findings in this study may provide evidence for the functional significance of LPHN2 in MDA-MB-231 breast cancer cells.