第８１回 病態生化学セミナー

日時：平成２６年４月１１日（金曜日）午後６時００分～
場所：医学部 基礎研究棟６階 セミナー室
演題：The role of Dlg5 in the progression of human prostate cancer
演者：Kyoto University, Graduate School of Agriculture
Lucia Tomiyama 先生

Discs large homolog 5 (Dlg5) is a member of the membrane-associated guanylate kinase (MAGUK) family of proteins. The MAGUK proteins are composed of a catalytically inactive guanylate kinase domain, in addition to PDZ and SH3 domains. All these domains are assumed to be involved in protein-protein interactions, supporting the idea that Dlg5 is a multifunctional adaptor and scaffold protein. Dlg5 has been reported to participate in cancer progression. Previous studies demonstrated that Dlg5 expression is downregulated in breast and pancreatic cancers, leading to a more malignant phenotype. Prostate is one of the tissues expressing Dlg5 at the highest level, but the roles played by this molecule in prostate cancer are largely unknown. Therefore, understanding the function of Dlg5 in prostate cancer development and progression will provide insight into potential therapeutic targets to treat prostate cancer.

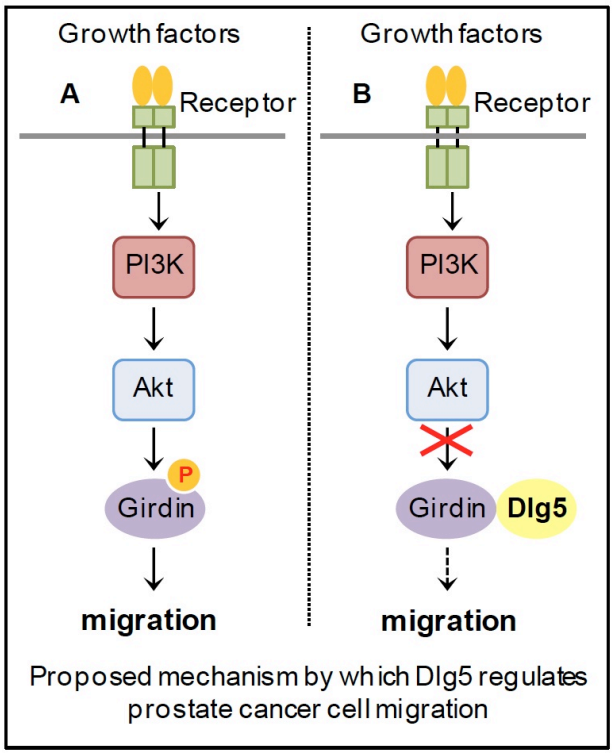
In our study, we found that Dlg5 is frequently downregulated in prostate cancer. Lentiviral-mediated stable knockdown of Dlg5 significantly enhanced migration and invasion of prostate cancer cells. Similar results were obtained upon siRNA-mediated transient Dlg5 knockdown, resulting in the significant increase in cell migration. These findings suggest that Dlg5 may act as a tumor suppressor in human prostate. The PI3K/Akt signaling pathway is required for cell migration induced by depletion of Dlg5, since wortmannin treatment, a specific inhibitor of PI3K, abrogated the effect of Dlg5 knockdown on cell migration. Importantly, we showed, for the first time, that Dlg5 interacts with Girdin, an actin-binding protein and downstream target of Akt. We found that levels of Akt-dependent Girdin phosphorylation (p-Girdin serine 1416) are increased in Dlg5-depleted cells. Knockdown of endogenous Girdin led to significant suppression of cell migration induced by Dlg5 depletion. Taken together, our findings demonstrate that Dlg5

interacts with Girdin and inhibits its activity, thereby suppressing the migration of prostate cancer cells.

In order to elucidate the mechanism by which Dlg5 inhibits Akt-mediated Girdin phosphorylation, we tried to map the domain necessary for Dlg5-Girdin interaction. The structure of Girdin can be divided into three different regions; an N-terminal region (NT), a central large coiled-coil domain and a C-terminal region (CT). The CT domain of Girdin is further divided into two regions: the halved C-terminal region named CT1 (including an Akt phosphorylation site) and another C-terminal region named CT2, which contains the actin-binding site. To determine the important region of Girdin for Dlg5 binding, GST pull-down assays were performed using lysates from cells stably expressing Dlg5 and transiently expressing each domain of Girdin. We found that Dlg5 binds preferentially to the CT1 domain of Girdin. It has been previously shown that the CT1 domain of Girdin contains the Akt phosphorylation site. Besides the Akt phosphorylation site, we demonstrate that the CT1 domain of Girdin has a binding site for Akt. These findings suggest that Dlg5 and Akt bind to the same region of Girdin, raising the possibility that Dlg5 competes with Akt for the binding site. This would explain how Dlg5 suppresses phosphorylation of Girdin.

In conclusion, Dlg5 regulates migration of prostate cancer cells *via* modulation of the PI3K/Akt/Girdin signaling pathway, and may, therefore, act as a potential target in prostate cancer metastasis.

【Lucia Tomiyama】



Proposed mechanism by which Dlg5 regulates prostate cancer cell migration

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