学位論文内容の要旨
（Summary of dissertation）

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Plectin is a novel regulator for apical extrusion of RasV12-transformed cells

（プレクチンは Ras 変異細胞の頂端側への逸脱に関わる新規制御因子である）

【Background and Objectives】

In multicellular organisms, oncogenic mutations occur within the epithelial tissues at the initial stage of carcinogenesis, though the fate of the transformed cells remained enigmatic. Recent studies have revealed that the newly emerging transformed cells are often eliminated from the epithelium. During this process, normal and transformed epithelial cells compete with each other for survival, a process called cell competition. However, underlying mechanisms of this process still remain to be explored. Plectin is a versatile cytoskeleton cross-linker. Here, I aim to clarify a role of plectin in cell competition between normal and RasV12-transformed cells.

【Methods and Results】

In this study, normal Madin-Darby canine kidney (MDCK) cells and MDCK-pTR GFP-RasV12-transfromed (hereafter RasV12) cells were mainly used for the experimental cell culture system. Under three different cell culture conditions (normal MDCK cells alone, RasV12 cells alone and their mixture at 1:1 ratio) I first used anti-phospho-tyrosine antibody and performed immunoprecipitation. I found that amount of precipitated plectin increased under the mix culture condition, compared with normal or RasV12 cells alone. By western blotting, I demonstrated that plectin was not tyrosine-phosphorylated. Next, I examined the subcellular localization of plectin by immunofluorescence analysis. I mixed RasV12 cells with normal MDCK cells at a ratio of 1:50. I found that plectin was non-cell-autonomously accumulated in RasV12 cells surrounded by normal cells. To understand the functional role of plectin in apical extrusion, I knocked down plectin in RasV12 cells. Plectin knockdown significantly suppressed apical extrusion of RasV12 cells, indicating that plectin is a positive regulator for apical extrusion.

Plectin is a cytoskeletons organizer. I thus examined the correlation between

plectin and microtubules or keratin5+8. Microtubules and keratin5+8 were accumulated in RasV12-transformed cells and partially co-localized with plectin. And disruption of microtubules by nocodazole suppressed accumulation of plectin and keratin5+8. In addition, nocodazole suppressed apical extrusion of RasV12 cells. Conversely, plectin-knockdown suppressed accumulation of microtubules and keratin5+8. These findings indicate that plectin and microtubules were mutually accumulated to co-regulate apical extrusion of RasV12 cells.

In our previous studies, EPLIN was identified as a crucial regulator in RasV12 cells for apical extrusion. I performed immunoprecipitation with anti-EPLIN antibody to examine the relevance between EPLIN and plectin. I found the increased interaction between EPLIN and plectin under the mix culture condition. In addition, knockdown of plectin or knockdown of EPLIN diminished EPLIN accumulation or plectin accumulation in RasV12 cells, respectively. Similarly, EPLIN-knockdown suppressed microtubule accumulation, and nocodazole treatment diminished EPLIN accumulation. These findings indicate that plectin, EEPLIN, and microtubules mutually affect their accumulation in a complex in RasV12 cells surrounded by normal cells.

In the previous study, caveolin-1 (Cav-1) and PKA were identified as downstream of EPLIN in RasV12 cells surrounded by normal cells. I found that plectin-knockdown diminished Cav-1 accumulation, whereas knockdown of Cav-1 in RasV12 cells did not affect accumulation of plectin. Furthermore, plectin-knockdown profoundly suppressed the non-cell-autonomous activation of PKA. These data suggest that in concert with EPLIN and microtubules, plectin regulates these downstream regulators for apical extrusion of RasV12-transformed cells.

【Discussion】

In this study, we have revealed that plectin is a new player in apical elimination of RasV12-transformed cells from the epithelium. Plectin is co-accumulated with EPLIN and microtubules in RasV12 cells surrounded by normal cells to regulate downstream signaling molecules Cav-1 and PKA, thereby promoting apical extrusion. In this multiple-molecular complex, plectin seems to act as a key adaptor that interlinks these cytoskeletal proteins. There still remains a question to be addressed: what are the upstream and downstream regulators of the plectin-EPLIN-microtubules complex?

【Conclusion】

Apical extrusion of transformed cells can be regarded as a cancer preventive process, which is supposed to occur at the initial stage of carcinogenesis. Thus, the plectin-EPLIN-microtubules complex could be a potential drug target; the activation of the plectin complex is expected to enhance the eradication of newly emerging and/or remaining transformed cells from epithelia. Further development of this study would open a new avenue for cancer preventive medicine.