Non-cell-autonomous migration of RasV12-transformed cells towards the basal side of surrounding normal cells

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学位論文の内容要旨

Non-cell-autonomous migration of RasV12-transformed cells towards the basal side of surrounding normal cells

RasV12 形質転換細胞が示す周囲正常細胞の基底面への非細胞自律的な運動

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Introduction

The epithelium is one of the cellular compartments known for fast cell turnover in the body. Continuous rapid cell division increases the probability of mutations. Most human cancers arise from single epithelial cells that acquire mutations in oncogenes or tumor suppressor genes. For transformed cells to escape elimination and progress to malignancy, they must acquire migration and invasion properties to metastasize to other tissues. Metastasis of tumor cells within the human body is one of the most dangerous pathological events that leads to mortality. Therefore, extensive studies have been conducted to better understand the molecular mechanisms of cancer cell migration and invasion. Recent studies have focused on the role of interaction between transformed cells and the microenvironment comprising non-transformed cells.

One of the most known mutations that initiate cancer transformation is the mutation of the GTPase Ras. The activation of oncogenic Ras facilitates all aspects of malignancy, including cell proliferation and cell invasion. Studies on the interaction between Ras-transformed epithelial cells and surrounding normal cells showed that the majority of transformed cells were apically extruded from the epithelial monolayer in an apoptosis-independent manner. However, a minority of the transformed Ras cells were basally extruded. These Ras cells formed protrusions and showed a phenotype favoring cell invasion.

In this study, we show that Normal Rat Kidney-52E (NRK-52E) cells expressing RasV12 are extruded to the basal side of normal NRK-52E cells when co-cultured. The basally extruded Ras cells exhibit motility only when surrounded by normal NRK-52E cells, indicating a non-cell-autonomous mechanism. We also demonstrate that the knockdown of myosin IIA and the inhibition of PI3K suppress basal extrusion of Ras cells. Decreased motility of myosin IIA-depleted Ras cells is correlated with the apical extrusion of cancer cells from normal cells.

Results

The motility of RasV12-expressing NRK-52E cells is enhanced when surrounded by normal cells

In order to study the behavior of RasV12 cells surrounded by normal cells, NRK-52E cells expressing GFP or GFP-RasV12 in a doxycycline inducible manner were established. Time-lapse microscopy revealed that cell–cell contact was disrupted as GFP-RasV12 was expressed. Consistently, the protein level of E-cadherin was significantly decreased in RasV12 cells.

Next, the migratory behavior of RasV12 cells was assessed in the presence of normal cells surrounding them. To this end, GFP (as a control) or RasV12 cells were mixed with normal NRK-52E cells at a ratio of 1:100. Time Lapse microscopy revealed that when NRK-52E GFP-RasV12 cells are cultured alone, they didn't exhibit motility. However, when RasV12 cells were surrounded by WT cells, they exhibited movement which was concomitant with active membrane protrusions. The motility of RasV12 cells was observed both at low and high cell density. As a control, GFP cells that were cultured with normal NRK-52E cells did not show any abnormal morphology or behavior. These results indicate a non-cell-autonomous migration of RasV12 cells enhanced by the surrounding normal cells.

Fragmentation of NRK-52E RasV12 cells was also observed. To examine if this fragmentation occurred due to apoptosis, cells were mixed and then treated with the caspase inhibitor z-VAD. The treatment didn't block neither the fragmentation of RasV12 cells nor their motility, indicating that this phenomenon occurred in an apoptosis-independent manner.

RasV12-expressing NRK-52E cells are basally extruded from surrounding normal cells

Previous studies showed that RasV12-expressing Madin-Darby canine kidney (MDCK) cells are apically extruded when surrounded by normal MDCK cells. To check whether the motility of RasV12-expressing NRK-52E cells occurred beneath or above the epithelial monolayer, RasV12 cells were mixed with normal NRK-52E cells, and plated on collagen gel. Then xz images were obtained by confocal microscopy. Interestingly, most of the RasV12-expressing NRK-52E cells were found beneath

the layer of normal NRK-52E cells. When RasV12-expressing NRK-52E cells were plated alone, they formed a monolayer or a cluster of cells.

These results indicate that RasV12-expressing NRK-52E cells are basally extruded from the epithelial monolayer to become motile.

Involvement of PI3K pathway in the basal extrusion of RasV12-expressing NRK-52E cells

Both PI3K/Akt and MAPK function downstream of the Ras signaling pathway. To understand the molecular mechanism of basal protrusion by RasV12-expressing NRK-52E cells, western blot analyses were performed using phospho-specific antibodies. As a result, we found that the phosphorylation of Akt, JNK, and ERK1/2 was significantly increased when RasV12 was expressed. This indicated that either of these pathways might be involved in the basal extrusion and motility of RasV12-expressing cells.

To determine the identity of the pathway that was essential for basal extrusion, RasV12 cells were mixed with normal NRK-52E cells and then treated with PI3K, JNK, p38 and MEK inhibitors. Interestingly, only treatment with the PI3K inhibitor LY294002 significantly reduced basal extrusion and increased the apical extrusion of RasV12 cells. These data suggest that the PI3K/Akt pathway is involved in the basal extrusion of RasV12-expressing NRK-52E cells.

Myosin IIA is essential for basal extrusion of RasV12-expressing NRK-52E cells

Previous studies have demonstrated that myosin II is involved in the apical extrusion of RasV12-expressing MDCK cells. To check whether it was involved in the basal extrusion of RasV12-expressing NRK-52E cells, cells were treated with a myosin II inhibitor, blebbistatin. The treatment resulted in a significant increase in the apical extrusion of RasV12-expressing NRK-52E cells, indicating the involvement of myosin II in basal extrusion.

Myosin II has three different isoforms, namely IIA, IIB, and IIC, with the two most intensively studied isoforms being IIA and IIB. We aimed to identify the isoform in either normal or RasV12-

expressing NRK-52E cells that was essential for basal extrusion. To achieve this, myosin IIA and IIB were knocked down using siRNA either in normal cells or in RasV12 cells, and were then mixed. We found that RasV12 cells that are deficient in myosin IIA, but not IIB, showed a significant decrease in basal extrusion but an increase in apical extrusion, similar to the effect observed with blebbistatin. We also showed that myosin IIA deficient cells showed significantly reduced velocity when surrounded by normal cells.

These data demonstrate that myosin IIA is essential in RasV12-expressing NRK-52E cells RasV12 cells to exhibit efficient motility and move to the basal side of the epithelial monolayer.

Conclusion

In summary, we have demonstrated that the non-cell-autonomous motility of RasV12-expressing NRK-52E cells is induced by the surrounding normal cells. This study expands our knowledge of the mechanism of cancer invasion, depending on the local microenvironment comprising non-transformed epithelial cells.