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.

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-

論文審査の結果の要旨			
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審査委員 Examiner	主査 Chief Examiner 南 康博 副査 Vice-examiner 中村俊一 副査 Vice-examiner 勾坂雄朗		

（要旨は１，０００字～２，０００字程度）

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. In order for transformed cells to progress to malignancy, they must acquire migration and invasion properties to metastasize to other tissues. 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.

In this work, we studied the interaction between NRK-52E Ras-transformed epithelial cells and surrounding normal cells. we showed that NRK-52E cells expressing RasV12 are extruded to the basal side of normal NRK-52E cells, formed protrusions and were actively motile only when surrounded by normal cells. This interesting finding makes this model perfect for the study of cell migration and cell invasion during the first stage of cancer initiation.

RESULTS

1. 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 (as a control) or RasV12 cells were mixed with normal NRK-52E cells and Time Lapse microscopy was performed. The results revealed that NRK-52E GFP-RasV12 cells were extremely motile only when surrounded by normal cells but not when cultured alone. These results indicate a non-cell-autonomous migration of RasV12 cells.

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

To check how RasV12-expressing NRK-52E cells were able to move around packed normal cells, a z-sectioning using confocal microscopy was performed. Interestingly, the results showed that most of the RasV12-expressing NRK-52E cells were found beneath the layer of normal NRK-52E cells. These results indicate that RasV12-expressing NRK-52E cells are basally extruded from the epithelial monolayer to become motile.

3. Involvement of PI3K pathway in the basal extrusion of RasV12-expressing cells

Both PI3K/Akt and MAPK function downstream of the Ras signaling pathway. To understand the molecular mechanism of basal protrusion by RasV12-expressing 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. To determine the identity of the pathway that was essential for basal extrusion, we treated the mixed culture cells with PI3K, JNK, p38 and MEK inhibitors. Interestingly, only treatment with the PI3K inhibitor LY294002 significantly reduced basal extrusion of RasV12 cells. These data suggest that the PI3K/Akt pathway is involved in the basal extrusion of RasV12-expressing NRK-52E cells.

4. 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 cells. Later, we aimed to identify the myosin isoform that was essential for basal extrusion. Therefore, myosin IIA and IIB isoforms were knocked down using siRNA. We found that RasV12 cells that are deficient in myosin IIA, but not IIB, showed a significant decrease in basal extrusion. 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 for 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.

The candidate, having completed studies on the mechanism of cancer cell motility, with a specialty in the non-cell-autonomous invasive migration towards the surrounding normal cells, and having advanced the field of knowledge in the area of cancer cell biology, is hereby recognized as having qualified for the degree of Ph.D. (Medicine).