PTEN is required for the migration and invasion of Ras-transformed MDCK cells

Yan, Lu

(Degree)
博士（医学）

(Date of Degree)
2021-03-25

(Resource Type)
doctoral thesis

(Report Number)
甲第8100号

(URL)
https://hdl.handle.net/20.500.14094/D1008100

学位論文の内容要旨

PTEN is required for the migration and invasion of Ras-transformed MDCK cells

Ras で形質転換された MDCK 細胞の遊走および浸潤における PTEN の役割

神戸大学大学院医学研究科医科学専攻

膜生物学

指導教員：伊藤俊樹　教授

LU YAN

INTRODUCTION

Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is one of the most crucial tumor suppressor genes involved in the regulation of phosphatidylinositol 3-kinase (PI3K) mediated signal transduction. It plays a vital role in the induction of cell polarity in motile cells, including the single-celled organism *Dictyostelium discoideum*, and mammalian immune cells such as neutrophils. The segregated localization of PI3K and PTEN which correlate with $PI(3,4,5)P_3$ and $PI(4,5)P_2$ at the plasma membrane has been observed to modulate cell polarity, migration and epithelial-mesenchymal transition. Ras is a small GTPase protein that is frequently mutated in patients with cancer. At the cellular level, it regulates numerous tumor-promoting processes, including enhancement of cell proliferation, suppression of cell death, cell metastasis, and modulation of the tumor microenvironment. One of the most studied pathways downstream of Ras is the PI3K pathway, which regulates actin reorganization to induce membrane ruffling through the Rac family of small GTPases for cell motility.

In this study, we examined the role of PTEN in the migration and invasion of cancer cells expressing oncogenic Ras (RasV12). Homozygous knockout (KO) of the PTEN gene in RasV12-transformed MDCK epithelial cells resulted in almost complete suppression of 2D migration, as well as 3D invasion through Matrigel. Here, we propose a migration mechanism for epithelial cancer cells mediated by the balance between Ras and PTEN activities.

RESULTS

1. Synergistic activation of the PI3K pathway in PTEN KO MDCK cell line expressing RasV12

To investigate the role of PTEN in epithelial cancer migration and invasion, we used MDCK cells expressing the active form of Ras, RasV12, in a doxycycline (Dox)-inducible manner (RasV12 cells), and established PTEN homozygous KO cells using the CRISPR/Cas9 system. Two independent cell lines, targeted with distinct sgRNAs, were obtained from normal MDCK or RasV12 cells. we analyzed AKT phosphorylation in PTEN KO cell lines to evaluate their $PI(3,4,5)P_3$ levels. AKT was highly phosphorylated in PTEN KO cell lines. Consistent with the role of Ras in the PI3K pathway, RasV12 (+Dox) cells also showed elevated AKT phosphorylation, which was further enhanced by the loss of PTEN. These results indicate that RasV12 and the loss of PTEN synergistically activate the PI3K signaling pathway.

2. Migratory morphology of RasV12-transformed MDCK cells depends on PTEN

The wild-type MDCK cells had a typical epithelial morphology and formed island-like cell aggregates. Consistent with its roles in cancer migration, the induction of RasV12 expression transformed MDCK cells to a laterally polarized appearance. Dimensional measurements revealed a higher length/width ratio and a smaller shape factor for RasV12 (+Dox) cells, reflecting their enhanced ability for directional migration. Smaller cell heights and larger areas also supported their migratory morphology compared to wild-type MDCK cells. Interestingly, further deletion of the PTEN gene in RasV12 (+Dox) cells resulted in an evenly spread shape. The average length/width ratio and shape factor returned to approximately one, indicating a loss of directionality of migration. These data suggest that PTEN plays an essential role in the directional migration of RasV12-transformed MDCK cells, thus facilitating cancer migration and invasion.

3. PTEN is required for the 2D migration of RasV12-transformed MDCK cells

To examine whether the loss of PTEN had an effect on epithelial cancer migration, we performed a wound-healing assay using RasV12 (+Dox) or RasV12 (+Dox) +PTEN KO cells. RasV12 (+Dox) +PTEN KO cells showed reduced migration compared to RasV12 (+Dox) cells. Quantification of the gap area showed that RasV12 (+Dox) cells moved faster than RasV12 (+Dox) +PTEN KO cells. To analyze single-cell speed, we tracked individual cells at the leading edge and found that the loss of PTEN suppressed the speed of movement of RasV12 (+Dox) cells. The effect of PTEN KO was also investigated in normal (non-transformed) MDCK cells. We found that PTEN KO cells promoted gap closure as well as single-cell migration. These results demonstrate a unique requirement of the PTEN gene for the migration of RasV12 (+Dox) cells.

4. PTEN gene is necessary for invasion of RasV12-transformed MDCK cells through Matrigel

A series of confocal images and subsequent three-dimensional reconstitution, showed that wild-type MDCK cells formed a relatively globular cluster with a smooth surface. In contrast, RasV12 (+Dox) cells exhibited extended protrusions, thereby supporting their invasive features. Importantly, RasV12 (+Dox) +PTEN KO cells showed less aggressive features without forming invasive cell extensions. These data suggest that loss of PTEN also affects the invasiveness of RasV12-transformed MDCK cells in 3D.

Next, we performed a Matrigel invasion assay using wild-type, RasV12 (+Dox), PTEN KO, and RasV12 (+Dox) +PTEN KO MDCK cells. While the wild-type and PTEN KO cells did not migrate through Matrigel, RasV12 (+Dox) cells showed a significantly higher degree of invasiveness, which was suppressed by KO of PTEN. A 3D migration assay without Matrigel showed similar results.

RasV12-promoted invasion through Matrigel was recovered by expressing full-length PTEN. Transfection of PTEN (C124A), a catalytically dead mutant, or PTEN (ΔPho),

in which the entire phosphatase domain was deleted, failed to rescue the phenotype. A mutant lacking the C2 domain could not recover the invasion ability of RasV12 (+Dox) +PTEN KO cells. Consistently, deletion of the C-terminal tail, which is known to induce an open conformation that exposes the C2 domain for membrane binding, rescued the invasive phenotype. Deletion of PDZ-binding motif, also rescued the invasion of RasV12 cells, indicating that PDZ binding is dispensable. Finally, a constitutive membrane-associated mutant of PTEN, PTEN (Myr), efficiently rescued this phenotype. These results collectively demonstrate that membrane recruitment of PTEN is an essential step for its function in epithelial cancer cell invasion driven by RasV12.

5. Localization of PTEN at the cell rear during cancer invasion

To explore the localization of PTEN in RasV12 cells, we expressed mCherry-tagged wild-type PTEN in RasV12 (+Dox) +PTEN KO cells, and performed an invasion assay. Three-dimensional imaging showed that mCherry-PTEN preferentially accumulated at the rear side of cells, penetrating through the membrane pores. We verified our observation by quantifying mCherry-PTEN fluorescence intensity at the cell rear relative to the front. While the fluorescence of GFP-RasV12 was uniformly distributed throughout the plasma membrane, mCherry-PTEN showed a significant preference for the cell rear.

CONCLUSION

We have shown an unexpected role for PTEN in supporting epithelial cancer cell migration. The mechanism by which PTEN functions to create a balance between $PI(4,5)P_2$ and $PI(3,4,5)P_3$ at the plasma membrane underpins RasV12-driven cancer cell migration and invasion. This study provides important insights into the common mechanisms of cell migration shared between immune cells and metastatic cancer cells.

論文審査の結果の要旨			
受付番号	甲 第3076号	氏　名	閻　露
論文題目 Title of Dissertation	PTEN is required for the migration and invasion of Ras-transformed MDCK cells Rasで形質転換されたMDCK細胞の遊走および浸潤におけるPTENの役割		
審査委員 Examiner	主　査 Chief Examiner 吉屋敦裕之 副　査 Vice-examiner 鈴木聡 副　査 Vice-examiner 上山健彦		

（要旨は１，０００字～２，０００字程度）

INTRODUCTION

Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is one of the most crucial tumor suppressor genes involved in regulating phosphatidylinositol 3-kinase (PI3K) mediated signal transduction. The segregated localization of PI3K and PTEN, which correlate with that of $PI(3,4,5)P_3$ and $PI(4,5)P_2$ at the plasma membrane, has been observed to modulate cell polarity, migration, and epithelial-mesenchymal transition. Ras is a small GTPase protein that is frequently mutated in patients with cancer. One of the most studied pathways downstream of Ras is the PI3K pathway, which regulates actin reorganization to induce membrane ruffling through the Rac family of small GTPases for cell motility.

In this study, we examined the role of PTEN in the migration and invasion of cancer cells expressing oncogenic Ras (RasV12). We propose that the balance between Ras and PTEN activities regulates the migration of epithelial cancer cells.

RESULTS

1. Synergistic activation of the PI3K pathway in PTEN KO MDCK cell line expressing RasV12

We analyzed AKT phosphorylation in PTEN KO cell lines to evaluate their $PI(3,4,5)P_3$ levels. AKT was highly phosphorylated in PTEN KO cell lines. Consistent with the role of Ras in the PI3K pathway, RasV12 (+Dox) cells also showed elevated AKT phosphorylation, which was further enhanced by the loss of PTEN. These results indicate that RasV12 and the loss of PTEN synergistically activate the PI3K signaling pathway.

2. Migratory morphology of RasV12-transformed MDCK cells depends on PTEN

The wild-type MDCK cells had a typical epithelial morphology and formed island-like cell aggregates. RasV12 expression transformed MDCK cells to a laterally polarized appearance. Dimensional measurements, including length/width ratio, shape factor, smaller heights, and larger areas, suggested that PTEN plays an essential role in the directional migration of RasV12-transformed MDCK cells, thus facilitating cancer migration and invasion.

3. PTEN is required for the 2D migration of RasV12-transformed MDCK cells

RasV12 (+Dox) +PTEN KO cells showed reduced migration compared to RasV12 (+Dox) cells. Quantification of the gap area showed that RasV12 (+Dox) cells moved faster than RasV12 (+Dox) +PTEN KO cells. Loss of PTEN suppressed the speed of RasV12 (+Dox) cells. These results demonstrate the requirement of the PTEN gene for the migration of RasV12 (+Dox) cells.

4. PTEN gene is necessary for invasion of RasV12-transformed MDCK cells through Matrigel

Wild-type MDCK cells formed a relatively globular cluster with a smooth surface, while RasV12 (+Dox) cells exhibited extended protrusions, which was suppressed by the KO of PTEN. While the wild-type and PTEN KO cells did not migrate through Matrigel, RasV12 (+Dox) cells showed significantly higher invasiveness, which was suppressed by the KO of PTEN.

RasV12-promoted invasion through Matrigel was recovered by expressing full-length PTEN, but not by PTEN (C124A), a catalytically dead mutant, PTEN (ΔPho) lacking the phosphatase domain, or a PTEN mutant lacking the C2 domain. Consistently, deletion of the C-terminal tail, known to expose the C2 domain for membrane binding, rescued the invasive phenotype. Deletion of the PDZ-binding motif is dispensable. PTEN (Myr), a constitutive membrane-associated mutant, efficiently rescued this phenotype. These results collectively demonstrate that membrane recruitment of PTEN is essential for its function in epithelial cancer cell invasion driven by RasV12.

5. Localization of PTEN at the cell rear during cancer invasion

Three-dimensional imaging showed that mCherry-PTEN preferentially accumulated at the rear side of cells, penetrating through the membrane pores. We verified our observation by quantifying mCherry-PTEN fluorescence intensity at the cell rear relative to the front. While the fluorescence of GFP-RasV12 was uniformly distributed throughout the plasma membrane, mCherry-PTEN showed a significant preference to the cell rear.

CONCLUSION

We have shown an unexpected role for PTEN in supporting epithelial cancer cell migration. The mechanism by which PTEN creates the balance between PI(4,5)P2 and PI(3,4,5)P3 at the plasma membrane underpins RasV12-driven cancer cell migration and invasion. This study provides important insights into common mechanisms of cell migration for immune cells and metastatic cancer cells.

The candidate, having completed studies on the mechanism of cancer cell migration and invasion, with a specialty in the role of PTEN functions in RasV12-driven cancer cell, and having advanced the field of knowledge in the area of cell biology, is hereby recognized as having qualified for the degree of Ph.D. (Medicine).