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研究課題名（和文）Identifying the Role of Cell Polarity in 'Real-Time' during Epithelial Homeostasis

研究課題名（英文）Identifying the Role of Cell Polarity in 'Real-Time' during Epithelial Homeostasis

研究代表者

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研究成果の概要（和文）：本研究によって、上皮細胞の恒常性維持に重要な因子を、細胞極性調節因子のノックダウンスクリーニングによって、候補因子を同定しました。興味深いことに、細胞死の際に細胞間接着部位に機能するような遺伝子グループを同定した。興味深いことに、これらの遺伝子をノックダウンすると、上皮の一過性の増加が観察された。このように、下流の分子標的を同定することができ、上皮の恒常性の維持において、上皮がどのようにバリア機能を維持するかについての分子メカニズムがわかってきた。

研究成果の概要（英文）：To understand how epithelia maintain homeostasis, in FY2016 I have screened knockdown lines using a candidate approach of known cell polarity regulators in MDCK cells. I have identified and verified several candidates that resulted in phenotypes during epithelial homeostasis. Some of the phenotypes observed included overproliferation, excessive cell death and epithelial barrier malfunction.

In FY2017, I characterized the candidates identified from the knockdown screen of cell polarity regulators during epithelial homeostasis. I identified a group of genes that resulted in defects at cell-cell contact sites specifically during cell death. Interestingly, this was accompanied by a transient increases in the integrity of the epithelium. I was able to identify the downstream molecular targets, thereby giving insights into the molecular mechanism of how epithelia maintain barrier function during during homeostasis.

研究分野：Cell Polarity

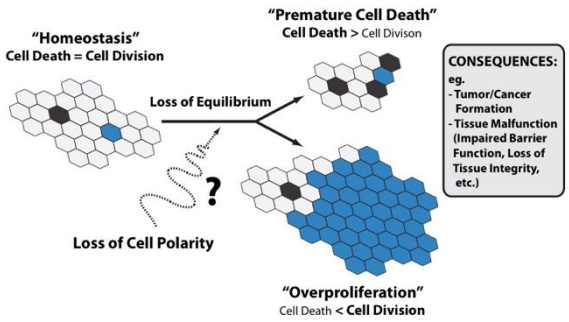
キーワード：Cell Polarity　Epithelial Homeostasis　Cell Death　Barrier Malfunction

1. 研究開始当初の背景

Throughout the lifespan of an organism, there is constant cellular turnover, a process whereby dead or dying cells are replaced by new-born cells. When an equilibrium in this process is achieved, this is known as homeostasis. If the balance between cell proliferation and cell death is perturbed, this can lead to detrimental consequences such as overproliferation, that may lead to tumor formation or alternatively, to premature cell death, that may lead to tissue defects and malfunction (Figure 1).

Epithelia, typically found encasing various organs and tissues in organisms, consist of a single or multi-layered structure, which forms a protective barrier against the external environment, while maintaining the capability to absorb nutrients and allow ion exchange. Epithelial-derived tissues exhibit some of the highest levels of cellular turnover, with epithelia such as in the intestinal tract undergoing complete self-renewal within a week (Blanplain et al., 2007. Cell). Furthermore, as most tumors originate from epithelia, known as carcinomas (Lim & Thiery, 2012. Development), it appears that maintaining homeostasis in high turnover tissues is a complex process. Therefore, a fundamental question is how is cellular turnover maintained during epithelial homeostasis?

Figure 1



Epithelia have an inherent apical-basal axis of polarity. The so-called 'apico-basal' polarity in epithelial cells arises from cell-cell mediated contacts, which leads to the formation and maturation of distinct domains along the plasma membrane (Ohno et al., 2015. Cell Polarity 1). These domains can be divided into the apical domain consisting of the Crumbs-PALS-PatJ complex, the apico-lateral domain consisting of the PAR3-PAR6-aPKC (PAR/aPKC) complex and the baso-lateral domain consisting of the Dlg-Lgl-Scribble group (Suzuki & Ohno. 2006. J Cell Sci). Disrupting the localization of these proteins has been associated with various processes such as epithelial-mesenchymal transition (EMT), tumor overgrowth and even cancer (Aranda et al., 2008. Oncogene; Lee & Vasioukhin. 2008. J Cell Sci). Indeed, some of these proteins, such as ones of the Dlg-Lgl-Scribble group are known tumor-supressors, although often in a context-dependent manner (Martin-Belmonte & Perez-Moreno. 2012. Nat Rev Cancer). Many of the links that have been associated between cell polarity and homeostasis are based on data from invertebrates such as Drosophila. Previously, I have demonstrated that the PAR/aPKC complex, through its asymmetric localization, is required for the proper differentiation of stem cells in the adult Drosophila intestinal tract (Goulas et al., 2012. Cell Stem Cell). When this is perturbed, such as by the loss-of-function of the PAR/aPKC complex, it leads to the overproliferation of intestinal stem cells and to tumor-like formation. A similar mechanism appears to exist also in the mammalian epidermis (Niessen et al., 2013. J Cell Biol). Taken together, epithelial polarity appears to have a fundamental role not only in epithelial function but also in maintaining epithelial homeostasis.

Loss of polarity has been described as one of the hallmarks of cancer (Aranda et al., 2008. Oncogene; Lee & Vasioukhin. 2008. J Cell Sci), yet how its loss leads to disease progression is unknown. Progress, especially in vivo, has been limited as it is difficult to completely exclude the contribution of external factors, such as the surrounding microenvironment, as well as to identify and monitor the initial events that lead to homeostatic defects. Therefore, to address the exact role of cell polarity during homeostasis, it is necessary to investigate this process from the early 'trigger' stages to late stages and to do so in a relatively simple and isolated system.

2. 研究の目的

Cell polarity has been described to be essential during development in a variety of contexts, including body-axis establishment, apical-basal polarity and cell migration (Ohno et al., 2015. Cell Polarity). However, its roles during homeostasis are less clear.

As homeostasis is a fundamental process required during the lifespan of any organism, visualizing it in 'real-time' and identifying its molecular mechanism, will provide insights into how homeostatic defects arise and how this progresses to diseases such as cancer. Furthermore, the identified proteins are also likely to serve as novel therapeutic targets.

3. 研究の方法

In order to establish the functional role of cell polarity during epithelial homeostasis, my initial plan was to use the Nikon Biostation CT, which is equipped for automated live cell imaging. I selected to use mammalian epithelial MDCK cells, which upon confluency, form highly polarized epithelial monolayers, to assay for the function of the three core polarity complexes, namely the Crumbs-PALS-PatJ, PAR3-PAR6-aPKC complexes and the Dlg-Lgl-Scribble group, with small interference (si)RNA oligonucleotides. To determine the effects of knockdown on cell morphology and cellular state, I generated a cell line stably expressing fluorescently tagged membranes (Lyn-Venus) and chromatin (Histone H2B-Cherry). From long-term timelapse movies, I was planning to construct cell fate maps, indicating all changes in cell-cell junctions as well as cells that underwent cell division and cell death upon knockdown. However, due to unexpected technical difficulties of setting up this long-term live imaging system, I was unable to proceed and obtain reliable results. As these issues could not be resolved, I opted for a 'non-live' imaging approach using short hairpin (sh)RNA stable knockdown of the key polarity modules to circumvent this problem.

By using confocal immunofluorescence microscopy and staining for the tight junction marker ZO-1, the cell death marker cleaved-caspase 3 and the cell proliferation marker Ki67 (or phosphohistone H3), I screened the effects of loss of cell polarity in homeostatic epithelia. Based on the phenotypes observed, they were categorized broadly into 3 different groups, namely overproliferation, excessive cell death and cell-cell contact defects.

Due to time restrictions, I focused mainly on the group of genes that caused cell-cell contact defects during epithelial homeostasis. Using a combination of classical molecular and biochemical approaches, in addition to assays to measure epithelial integrity and 3D super-resolution STED microscopy, I was able to characterize these defects and also identify the molecular downstream targets of these cell polarity regulators.

4. 研究成果

From the candidate shRNA knockdown screen in fully-polarized MDCK epithelial monolayers, I identified phenotypes that could be broadly categorized into 3 groups: 1) overproliferation 2) excessive cell death and 3) cell-cell contact defects. Interestingly, from the key polarity modules screened, I identified that the PAR/aPKC complex was the only ternary complex that caused defects at cell-cell contact sites during homeostasis. As these defects appeared specifically only at sites of cell death and epithelia often form the first line of defense against pathogens, I decided to focus on the role of the PAR/aPKC complex during epithelial cell death.

Epithelia undergo cell death through a mechanism known as cell extrusion, whereby dead or dying cells are removed from the epithelium while maintaining epithelial integrity, leading to a characteristic rosette-like cellular structure (Rosenblatt et al., 2001. Curr Biol). To characterize the potential role of the PAR/aPKC complex during this process, I first analyzed the localization of the PAR/aPKC complex. The PAR/aPKC complex was found to be enriched at the interface between the dying cell and the surrounding cells.

To further characterize the cell-cell contact defects observed upon the loss of the PAR/aPKC

complex, I carefully analyzed the formation of these so-called 'rosette junctions' during cell death upon stable knockdown. Upon knockdown of the PAR/aPKC complex, I found that the junctions were not properly closing at these sites, suggesting that they may be epithelial barrier defects.

To determine if defects in rosette formation affect epithelial barrier function, I artificially induced cell death using UV irradiation. By inducing excessive cell death, it would be expected that barrier function would decrease upon the loss of the PAR/aPKC complex due to defects in rosette formation. Surprisingly, when measuring the Transepithelial Electrical Resistance (TER), which is a measure of epithelial integrity, I found a transient increase in the resistance before it rapidly decreased. This indicates that epithelial integrity increased, albeit briefly, upon the loss of the PAR/aPKC complex, suggesting a potential mechanism that may compensate for the defects in rosette junction formation.

Next, to see whether a compensatory mechanism may be in place within epithelia to maintain its integrity upon defects in rosette formation, I analyzed the localization and activity of ROCK, as it is known to be involved during cell death for the cell extrusion process and it is also a known target of aPKC activity. I found that ROCK localized ectopically to the apicolateral domain of cells forming the rosette junction upon the loss of the PAR/aPKC complex and upon the inhibition of its activity, this dramatically increased the number of rosette malformations. Therefore, this indicates that ROCK is able to compensate, at least in part, for the loss of the PAR/aPKC complex during cell death in epithelial homeostasis.

In conclusion, I identified a novel role of the PAR/aPKC complex in regulating tensional force at sites of cell death during cell extrusion to maintain epithelial barrier function, providing insights into how epithelia may have evolved to prioritize its barrier integrity over other critical functions.

As for future directions, it remains unclear how the activity of the PAR/aPKC is spatio-temporally regulated in coordination with ROCK activity during rosette formation. Furthermore, as this work was done primarily using MDCK cells, it still remains unclear whether a similar cellular machinery is required in a more physiological context *in vivo*.

5. 主な発表論文等

〔**雑誌論文**〕（計 1 **件**）

Yazaki, M., Ito, Y., Yamada, M. Goulas, S., Teramoto, S., Nakaya, M., Ohno, S. and Yamaguchi, K. (2017) Oral ingestion of Collagen Hydrolysate Leads to the Transportation of Highly Concentrated Gly-Pro-Hyp and Its Hydrolyzed Form of Pro-Hyp into the Bloodstream and Skin. J Agric Food Chem *65*, 2315-2322 (Peer Reviewed)

〔**学会発表**〕（計 3 **件**）

Invited Talks

1) Goulas, S., Kishikawa, Y., Aono, S., Nakaya, M. A., Ohno, S. The Role of Cell Polarity in Regulating Tensional Integrity during Epithelial Barrier Homeostasis. The 1968th Biological Symposium. National Institute of Genetics (NIG). December 13th, 2017. Mishima, Japan

Poster Presentations

2) Goulas, S., Kishikawa, Y., Aono, S., Nakaya, M. A., Ohno, S. The PAR/aPKC Complex modulates the Tensional Integrity of Cell Junctions during Cell Death to Maintain Epithelial Homeostasis. ConBio 2017. December 6-9, 2017. Kobe, Japan

3) Goulas, S., Izumi, Y., Nakaya, M., Ohno, S. The PAR/aPKC Complex modulates the Tensional Integrity of Cell Junctions during Cell Death to Maintain Epithelial Homeostasis. Gordon Conference: Cell Contact and Adhesion. June 18 - 23, 2017. Andover, New Hampshire, USA

〔**図書**〕

該当ありません

〔産業財産権〕

該当ありません

〔その他〕

6. 研究組織

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