Study on Epithelial Defense against Cancer (EDAC) for Elimination of RasV12-transformed Cells from Epithelia

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Chapter 1 General Introduction and aim of this study

Cell transformation arises from the activation of oncoproteins and/or inactivation of tumor suppressor proteins. At the initial stage of multi-step carcinogenesis, a transformed cell comes out in the normal epithelium. Using cell culture systems that Fujita group originally established, previously his group has revealed that when RasV12-transformed cells are surrounded by normal cells, the transformed cells are apically extruded from a monolayer of normal epithelial cells. Further studies have shown that the neighboring normal cells have abilities to sense and eliminate the transformed cells, called Epithelial Defense against Cancer (EDAC). Although it comes to be known that surrounding normal cells actively eliminate the neighboring transformed cells via the EDAC process, the molecular mechanism now still remains unclear.

The general goal of this study is to gain a better understanding of the process of extrusion of RasV12-transformed cells from a monolayer of normal epithelial cells. I aimed to clarify the molecular mechanisms involved in EDAC, thus I performed a candidate approach and protein screening. As a candidate approach, I focused on the S1P-S1PR2 pathway and examined a role of the S1P-S1PR2 pathway in EDAC by a collaboration with a former lab member, Sayaka Yamamoto. As screens for novel regulatory proteins that are involved in the EDAC process, I performed quantitative mass spectrometric analysis by SILAC and quantitative gene expression analysis by microarray.

Chapter 2 A role for the S1P-S1PR2 pathway in EDAC

Sphingosine-1-phosphate (S1P) is a lipid mediator. S1P is secreted from various cell types and

secreted S1P binds to a family of specific G-protein-coupled receptors (GPCRs), S1P receptor (S1PR) 1-S1PR5. S1P-S1PRs regulate various cellular processes, such as cell growth, cell survival, cell migration and cell adhesion. Gu *et al*. showed that the S1P-S1PR2 pathway is involved in apical extrusion of apoptotic cells from the epithelial monolayer. At the early phase of apoptosis, dying cells produce S1P, and the secreted S1P binds to S1PR2 in the neighboring normal cells. Then S1PR2 activates the downstream Rho–Rho kinase pathway, leading to the formation of actin–myosin rings that squeeze out apoptotic cells.

In this chapter, I examined whether the S1P–S1PR2 pathway was also involved in the EDAC process, with Sayaka Yamamoto. Using a S1PR2 inhibitor or S1PR2-knock down cells, we showed that S1PR2 in the surrounding normal cells is important for elimination of the RasV12-transformed cells. Importantly, not endogenous S1P but exogenous S1P plays a major role in this process. Using a FRET biosensor, we demonstrated that S1P–S1PR2 mediates Rho activation in normal cells neighboring RasV12-transformed cells. Furthermore, S1P-S1PR2 regulates filamin accumulation via Rho-Rho kinase, thereby promoting apical extrusion of RasV12-transformed cells. These data demonstrate that the S1P–S1PR2 pathway is a crucial regulator of EDAC and that cell competition can be substantially influenced by factors from the outer environment.

Chapter 3 A role for ADAMDEC1 in EDAC

Several studies in *Dorosophila* reported that soluble factors can regulate cell competition. Portela *et al*. reported that dSPARC, the *Drosophila* homologue of the Sparc/Osteonectin protein family, is transcriptionally upregulated in loser cells during cell competition, and acts as a self-protective signal for loser cells. Previously we reported that RasV12 cells are apically extruded as the result of cell competition, and filamin, an actin-binding protein, in normal cells is a crucial regulator for epithelial defense against cancer (EDAC). However, it still remains unclear whether soluble proteins function as a mediator for EDAC.

ADAM-like Decysin-1 (ADAMDEC1) is a secreted-type of ADAM family proteins. ADAMDEC1 has metalloprotease activity at least *in vitro* and glutamate 353 is important for its proteolytic activity in human. However, physiological roles of ADAMDEC1 still remain unknown.

In this chapter, I examined involvement of ADAMDEC1 for elimination of RasV12-transformed cells. By using SILAC analyses, I identified ADAMDEC1 as a protein that increased in mix-culture of normal and RasV12-transformed cells. ADAMDEC1 upregulated in normal cells neighboring RasV12-transformed cells. Knock-down of ADAMDEC1 suppressed

filamin accumulation in normal cells that were adjacent to RasV12 cells and down-regulated apical extrusion of the RasV12 cells. Further analyses showed that wild-type of ADAMDEC1 rescued the apical extrusion of RasV12-transformed cells surrounded by ADAMDEC1-knockdown cells, but proteolytic activity lacked mutant were not, suggesting that ADAMDEC1 proteolytic activity does not contribute this process. These data indicate that ADAMDEC1 is a positive regulator in EDAC and cell competition at the initial stage of carcinogenesis could be regulated by soluble protein.

Chapter 4 A role for COX-2 in EDAC

We have reported that RasV12-transformed cells are apically extruded from a monolayer of the normal epithelium and filamin is a key regulator for EDAC. However, a part of RasV12-transformed cells is not extruded and remains in the epithelial monolayer, suggesting that not only positive regulators but also negative regulators involve in this process.

Prostaglandin-endoperoxide synthase 2 (PTGS2), also known as cyclooxygenase-2 (COX-2), is an enzyme that inductively expresses during inflammation, and synthases prostaglandin H_2 (PGH_2) form arachidonic acid.

In this chapter, I tried to identify the proteins that specifically upregulate in normal cells neighboring transformed cells and examined whether these proteins regulate the EDAC process. I performed gene expression profiling and I identified COX-2 as a protein that upregulated at the expression level in normal cells neighboring transformed cells. Further analyses showed that COX-2 promoted PGE_2 production and secreted PGE_2 nagatively regulated apical extrusion of RasV12-transformed cells by suppressing filamin accumulation. By using lumiracoxib or ibuprofen, selective and non-selective COX-2 inhibitor, we showed that suppression of prostaglandin production promoted apical extrusion of RasV12-transformed cells. These results indicate that COX-2 is a negative regulator of EDAC and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as ibuprofen and lumiracoxib could be preventive medicines for cancer.

Chapter 5 Conclusion

In this thesis, I identified two novel EDAC regulators that are expressed in normal cells neighboring transformed cells. One is ADAMDEC1. This is the first report to reveal that a soluble protein is upregulated at the expression level and positively regulates EDAC. Another is COX-2. This is the first report to clarify that a EDAC negative regulator is upregulated at the

expression level in normal cells neighboring transformed cells, and this is also the first report to demonstrate the possibility that general and cheaper medicines, for example ibuprofen, promote EDAC and could be a prophylactic for cancer. Collectively with S1P-S1PR2 pathway, these results suggest that elimination of transformed cells by EDAC is the outcome of balance between positive and negative regulators not only from intracellular expression regulation but also from outer environment.