学位論文内容の要旨
（Summary of Dissertation）

博士の専攻分野の名称　博士（医　学）　氏 名　陳　策文
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学 位 論 文 題 名（Dissertation Title）

Exploration of the molecular mechanism and physiological significance of fetal reprogramming in the intestinal epithelium
(腸上皮における胎児様リプログラミングの生理的意義とメカニズムの探求)

Background and Purpose:

The small intestinal epithelium stands out as the most rapidly renewing tissue in adult mammals. Emerging evidence suggests that intestinal epithelial cells (IECs) exhibit the previously unexpected plasticity to be reprogrammed into a fetal intestine-like state in certain situations. Although such plasticity has been implicated in intestinal regeneration and tumorigenesis, the underlying molecular mechanisms and pathophysiological significance need further investigation.

In this study, we aimed to address two technical limitations that hamper progress in this field. At first, to accelerate studies on molecular mechanisms, we aimed for the establishment of a novel culture system that recapitulates the reprogramming of IECs in vitro using synthetic hydrogels (Chapter 1). This approach aims to provide a more controlled and versatile platform for studying IEC biology, potentially advancing our understanding of intestinal epithelial plasticity and reprogramming. Second, we addressed the establishment of a reliable method to analyze functions of the reprogrammed IECs in vivo (Chapter 2). Here, we aimed to establish a robust protocol to orthotopically transplant intestinal cells to the intestinal epithelium. For the initial development and refinement of the protocol, we used colorectal cancer (CRC) cells that have higher survival ability than normal cells and developed a novel orthotopic CRC model that recapitulates the entire spectrum of cancer initiation and progression within an pathologically relevant microenvironment. The developed method will be applied to the study of in vivo functional analysis of the reprogrammed IECs in future studies. Overall, the objective of this study is to establish a new technological basis that will contribute to the elucidation of the molecular mechanisms and pathophysiological significance of IEC reprogramming.

Subjects and Methods:

IECs isolated from the mouse small intestine were cultured under the standard intestinal organoid culture conditions. For the development of novel culture systems (Chapter 1), hydrogels were synthesized by copolymerization of both cationic and anionic monomers, with varying ratios of these monomers resulting in different physical properties of the hydrogel. IECs were seeded on these hydrogels to examine their capability to support IEC adhesion, survival, and proliferation. Upon selecting the hydrogel suitable for the cultivation of IECs, its effects on the IEC reprogramming were analyzed by quantitative RT-PCR, immunoblotting and RNA-sequencing analyses.

To develop novel transplantation procedures (Chapter 2), Caco-2 CRC cells were transplanted into the cecum epithelium of immunodeficient mice. To achieve high engraftment efficiency, all procedures for the transplantation were successfully visualized by exteriorizing the cecum epithelium and utilizing appropriate staining techniques. Upon the engraftment and growth of xenografts, the cecum was isolated and subjected to the subsequent histological and immunofluorescence analyses.

Results:

In Chapter 1, we first found that the C3A1 hydrogel efficiently supports IEC survival and proliferation, outperforming other hydrogel formulations. IECs cultured on C3A1 hydrogel underwent reprogramming to a fetal

intestine-like state, as evidenced by gene expression profiling using RT-PCR and RNA-sequencing. Mechanistically, the reprogramming process was mediated by activation of the Integrin/Fak/Src, which in turn activated Yap/Taz and Erk5 signaling pathways to induce fetal intestinal gene expression. Notably, long-term culture on the C3A1 hydrogel induced persistently reprogrammed IECs (persistent fetal intestine-like cells, PFILCs) that maintain their fetal intestine-like characteristics even after re-cultivation under standard organoid culture conditions. PFILCs exhibited unique properties, including fetal IEC-like spheroid forming ability and the reduced growth factor dependency. Moreover, ectopic transplantation of PFILCs into mice demonstrated their regenerative potential, forming intestinal tube-like structures, albeit with incomplete maturation.

In Chapter 2, we successfully established a straightforward and robust protocol to transplant cells into the cecum epithelium. Soon after transplantation, Caco-2 CRC cells efficiently engrafted into the epithelial layer of the cecum, where tumors progressed from a flat monolayer epithelium to thickened aberrant crypt foci, and then to protruding polyps, aided by stromal cells infiltrating the tumors to form a stalk region, and eventually to large tumors invading the submucosa. Throughout this process, Caco-2 cells retained stem cell and fetal intestinal signatures, regardless of their location within the tumors or their proliferative status. Histopathological analysis further suggested that interactions between the transplanted Caco-2 cells and the surrounding normal epithelial and stromal cells play critical roles in tumor development and in the elimination of normal epithelial cells from the tumor in this model.

Discussion:

In this study, we aimed to establish two important methodologies that would lead to significant advancements in both in vitro reprogramming of IECs and the examination of in vivo functions of the reprogrammed cells. In Chapter 1, we successfully established a novel synthetic hydrogel-based culture system that induces rapid reprogramming of adult IECs to a fetal intestine-like state. The observed reprogramming effects surpassed those of the previously reported inducer of fetal reprogramming, Tgfb1. This highlights the superiority of this method over existing methods. Mechanistic investigations then revealed the crucial roles of integrin/Src, Yap/Taz, and Erk5 signaling pathways in mediating the reprogramming of IECs, demonstrating the usefulness of this method in elucidating molecular mechanisms of the IEC reprogramming. Notably, long-term culture on the C3A1 hydrogel induced PFILCs harboring unique properties, including reduced growth factor requirements and enhanced autocrine signaling. Given their high tissue-regenerating potential shown in transplantation experiments, PFILCs might serve as an ideal cell source for the transplantation therapy of the damaged intestinal epithelium.

In Chapter 2, we established a novel method to transplant cells into the mouse cecum epithelium. This method was then used to establish a novel orthotopic CRC model that closely mimics natural CRC development within the mouse cecal epithelium. The model captures important aspects of tumor heterogeneity, including the presence of quiescent cancer stem cells and the competition between CRC cells and normal epithelial cells during early tumorigenesis. The development of more pathologically relevant orthotopic CRC model would offer a valuable tool for evaluating novel therapeutic strategies. Furthermore, this novel transplantation method should enable us to evaluate the regenerative capacity of IECs reprogrammed using the C3A1 hydrogel system. We are thus thinking that this transplantation method, together with the C3A1 hydrogel-based reprogramming system, will provide important technical bases to elucidate molecular mechanisms and significance of cell plasticity in the intestine.

Conclusion:

In conclusion, this study presents two innovative methodologies, a synthetic hydrogel-based culture system that recapitulates reprogramming of IECs in vitro and an efficient cell transplantation method to the intestinal epithelium. These two methodologies may contribute to advance research on both intestinal regeneration and tumorigenesis by offering novel tools for investigating the mechanisms of IEC reprogramming and the in vivo functions of reprogrammed cells via orthotopic transplantation. We believe that our novel methods will serve as key technical bases to deepen our understanding of intestinal homeostasis and pathogenesis.