Regenerative medicine for inflammatory bowel disease

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Q You are well along in research involving colon epithelial stem cells. Please explain briefly.

A: We succeeded in establishing our own method (called the TMDU-method) to maintain and efficiently expand mice colonic epithelial stem cells *in vitro*. By using the TMDU-method, we also succeeded in preparing a large number of colonic epithelial stem cells starting from a single cell, and regenerated damaged colonic mucosa by *in vivo* transplantation of those *ex vivo* expanded stem cells. We are now ready to expand *human* colonic epithelial stem cells *in vitro* by using endoscopic biopsy specimens as the starting material.

Q What brought you to this research? Did it have anything to do with TMDU's perceived strengths?

A: "Mucosal healing" (MH) has recently become the most important goal of treatment of inflammatory bowel disease (IBD). However, up to 40% of IBD patients currently fail to achieve MH. Therefore, a new therapeutic approach—such as regenerative medicine—is urgently needed. TMDU has long been the world's leading institution in the treatment of IBD, and enteroscopy techniques. In 2012, we started running the Advanced Clinical Center for Inflammatory Bowel Diseases (ACCIBD), which is dedicated solely to the treatment of IBD. Since then, an increasing number of newly diagnosed IBD patients are rushing to our clinical center. Through the treatment of these patients, we have become quite sure that a new therapeutic approach is needed to improve the prognosis and treatment of refractory patients.

Dr. Watanabe completed his medical and graduate school at Keio University, where he received his MD and PhD. He performed postdoctoral research at Harvard Medical School in Boston, Massachusetts. He became an Instructor at Keio University Hospital in 1992, then an Associate Professor in 1996. He joined TMDU as a Professor and Chairman of the Department of Gastroenterology and Hepatology in 2000. He became the Vice President of TMDU in 2016.

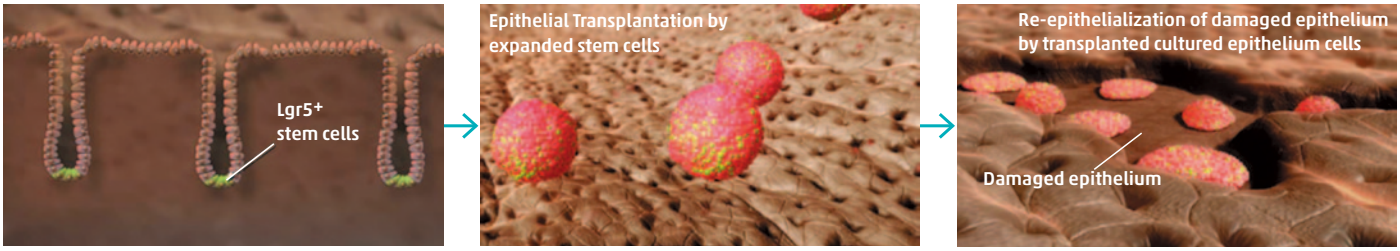
Q Stem cells, like the iPS and ES, have made headlines in regenerative medicine in recent years. Meanwhile, you are focused on epithelial cells. Why?

A: We always start thinking from the bedside, not from the bench. We know that pluripotent cells, such as iPS cells and ES cells, surely have great scientific, as well as therapeutic, potential. However, to use those cells in clinical applications, we may need to clear various technical as well as ethical problems, which may take many years to solve. In the meantime, why not use adult tissue stem cells? They have low risk of tumorigenicity and do not require time for differentiation. Moreover, we already have a method for preparing the required number of donor stem cells from a small piece of mucosal tissue. This will make it possible to establish and provide stem cell-based regenerative therapy for IBD patients in a minimum period of time.

Q You have been successful in culturing small intestine epithelial cells *in vitro* and transplanting them to the colon, too. In this case, transplanted cells showed the properties of small intestine cells even months later. What might this accomplishment mean?

A: Firstly, it provides an important scientific message that adult somatic stem cells can maintain their identities even in an orthotopic environment. This indicates that those stem cell identities are tightly programmed in the cell - intrinsically - and may not be easily affected by extrinsic signals. Future studies may reveal the mechanism behind how such a tight identity can be maintained in each stem cell. Secondly, from a therapeutic point of view, the data guarantees the therapeutic potential of *ex vivo* cultured small intestinal stem cells. Thus, the data open a way to use stem cell transplantation to treat refractory small intestinal ulcers that may appear in Crohn's disease, Behcet's disease, radiation enteritis or NSAID-induced enteropathy.

Colonic stem cell culture and stem cell transplantation to damaged epithelium



Q How do you expect your research to develop in the future? What about the possibility of a joint study with international researchers?

A: Currently, we are doing our best to apply our stem cell culture method and transplantation technique to treat refractory ulcers of IBD patients. There still remain plenty of technical problems and regulatory issues that need to be solved, but we now feel quite confident that we are very close to reaching our goal. Also, we are running several basic research projects to find the answer to questions raised in such areas as stem cell biology, mucosal immunology and gastrointestinal oncology. We definitely welcome joint studies with international researchers to share both scientific and therapeutic interests in various areas.

Functional engraftment of colon epithelium expanded *in vitro* from a single adult Lgr5+ stem cell, *Nat. Med.*, doi: 10.1038/nm.2695.
Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury, *Cell Stem Cell*, doi: 10.1016/j.stem.2013.09.015.
Small intestinal stem cell identity is maintained with functional Paneth cells in heterotopically grafted epithelium onto the colon, *Genes Dev.*, doi: 10.1101/gad.245233.114.

Innovative Researchers

Stem cell aging study for tissue regeneration

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Diffuse hair loss radially extends with aging. Shown is a 28-month-old wild-type mouse (C57BL/6N).

In aging societies, it is crucial to understand the mechanisms of physiological aging and the basis for aging-associated diseases. Stem cell systems play fundamental roles in the homeostatic maintenance of many tissues and organs. I did my dermatology residency at Kyoto University Hospital and earned my Ph.D. at Kyoto University, studying melanocyte (pigment cell) development. We subsequently identified melanocyte stem cells as reservoirs for melanocytes in skin and hair follicles (*Nature*, doi: 10.1038/416854a), and then revealed that aging-associated depletion of the population results in graying hair (*Science*, doi: 10.1126/science. 1099593). The study clearly indicated that the aging of stem cells is a key to understanding tissue and organ aging.

Since establishing my own research team as a Professor at Kanazawa University in 2006 and then at TMDU in 2009, we started working on the niche microenvironment of stem cells and have revealed that hair follicle stem cells function as "niche cells" for melanocyte stem cells (*Cell Stem Cell*, doi: 10.1016/j.stem.2010.11.029). We also have found the existence of a "stemness checkpoint", which determines the fate of somatic stem cells, namely whether stem cells stay in an immature state as stem cells or commit to differentiation, during aging and under genomic stress (*Cell*, doi: 10.1016/j.cell. 2009.03.037).

More recently, we have been focusing on the *in vivo* cellular dynamics and exact changes that occur in somatic stem cells during aging and the eventual fate of those stem cells in tissues and organs. Our approach has revealed the existence of a "stem cell-centric aging program" that governs tissue aging characterized by miniaturization of hair follicle, the mini-organ to grow hair and hair loss (senescent alopecia) (*Science*, doi: 10.1126/science.aad4395). This helps explain why and how organs in our bodies become smaller (miniaturize) and/or thinner, with associated functional decline, due to aging. We are devising new strategies for tissue regeneration and rejuvenation by focusing on somatic stem cells. We welcome international collaboration to realize the therapeutic potential in this area.