Press Release

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Accumulation of Specific Gene Mutations Found in Patients with Ulcerative Colitis
Elucidating the mechanism of onset and exacerbation of intractable diseases

A research team led by Professor Toshiro Sato at the Keio University School of Medicine Sakaguchi Laboratory has discovered that specific gene mutations accumulate in the colonic epithelium of patients with ulcerative colitis.

In normal human colon epithelium, gene mutations accumulate with age and are known to cause carcinogenesis in the colon. Changes in the intestinal environment, such as dietary quality or chronic inflammation, can also increase the risk of carcinogenesis in the colon. However, it has remained unclear whether changes in the intestinal environment affect the accumulation of gene mutations in the colon epithelium.

In this study, the research team cultivated colon epithelium obtained from research participants and amplified colonic epithelial cells to efficiently analyze gene mutations. Results showed more gene mutations in colonic epithelial cells of patients with chronic ulcerative colitis than in the colons of healthy individuals. The team found that many of these gene mutations were related to chronic inflammation and were not those found in colorectal cancer.

The team identified the role of these gene mutations by using an organoid culture, a culture method for producing simplified versions of organs.

When an inflammatory molecule called IL-17 (interleukin-17)induces chronic inflammation in colonic epithelium, the stimulation damages the colon mucosa. The team found that the colonic epithelium of ulcerative colitis patients had acquired IL-17-related gene mutations that do not occur in healthy individuals, making it resistant to cell damage caused by chronic inflammation. In other words, the team clarified that in the colon of patients with ulcerative colitis, the number of epithelial cells with gene mutations that are likely to survive in an inflammatory environment increases selectively, replacing normal colonic epithelial cells.

While previous papers have stated that the human large intestine develops colorectal cancer through the accumulation of gene mutations (Fearon ER, et al., *Cell*, 1990), this study found that mutations also accumulate in order to adapt to changes such as chronic inflammation in the intestinal environment.

Future research is expected to reveal how the accumulation of colonic epithelial cells with gene mutations affects the pathogenesis and cancerization of ulcerative colitis.

The results of this research were published online in the online edition of the British journal *Nature* on December 18(GMT), 2019.

1. Research Background

Ulcerative colitis is an inflammatory bowel disease of unknown origin that causes chronic inflammation in the large intestine. In Japan, there are more than 170,000 people living with ulcerative colitis (according to the 2014 Report on Public Health Administration and Services by the Ministry of Health, Labor and Welfare) and that number is increasing. It has been reported that chronic ulcerative colitis leads to increasing incidence of colorectal cancer, but a clear reason why has yet to be clarified.

Many cancers result from the accumulation of gene mutations, which naturally increase with age. This is why it was expected that inflammation would affect this accumulation of gene mutations. To prove this hypothesis, it was necessary to analyze gene mutations in the colon tissue of ulcerative colitis patients, but technical limitations had until now made that elucidation difficult.

Many human tissues are maintained and repaired by cells known as stem cells[1]. Stem cells divide to produce offspring cells that maintain various organs throughout the body. Many offspring cells, however, cannot produce further progenies, so even if a gene mutation occurs in these offspring cells, the mutation will be removed as the cells age. On the other hand, if a gene mutation occurs in a stem cell, the mutation is passed on to the offspring cells as well as the descendant stem cells, meaning that gene mutation in the stem cells is required for carcinogenesis.

It is known that in the large intestine, 10–20 stem cells replace all single stem cells and their offspring cells in recesses called crypts[2] over a period of several months (all cells produced from a single stem cell are known as 'clones').

Because there are countless crypts in even a small area of the large intestine, conventional methods can only use samples that are a mixture of mutated stem cells and their clones, and it is not possible to examine how gene mutations accumulate in individual stem cells. (See Fig. 1)

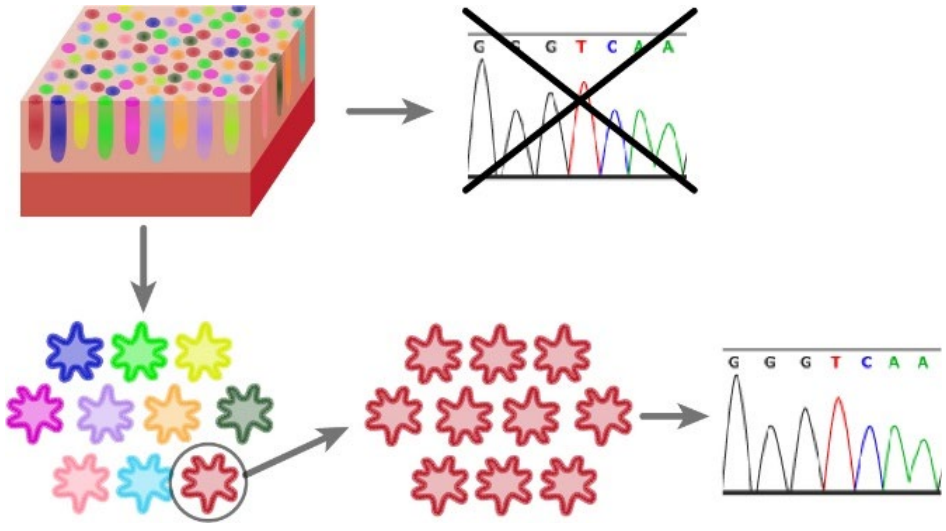

Fig. 1: Gene mutation analysis of stem cells using cloning technology

By using cloning technology in our organoid culture, it was possible to proliferate only individual stem cell clones and perform detailed genomic analysis.

In recent years, through the application of an organoid culture method (Sato T, et al., *Nature*, 2009; Sato T, et al., *Gastroenterology*, 2011)[3], a single stem cell clone can be cultured and proliferated, resulting in the development of a method for analyzing individual stem cells. (See Fig. 1) As a result, it has been reported that gene mutations accumulate in healthy colonic epithelial stem cell in proportion to age (Blokzijl F, et al., *Nature*, 2016). Those results show that the accumulation of gene mutations varies between individuals.

In this study, we considered that the intestinal environment may play a role in these individual differences. And so we chose to focus on a type of ulcerative colitis called left-sided colitis where inflammation only occurs on the left side of the colon (the side of the large intestine closer to the anal canal), and not on the right side (the side closer to the mouth of the large intestine). By conducting detailed genetic analysis of individual stem cell clones, we succeeded in elucidating how an intestinal environment like chronic inflammation affects the accumulation of gene mutations in stem cells.

In addition, by using organoids, we clarified what gene mutations occur in the inflammatory environment of ulcerative colitis as well as what roles gene mutations play in that inflammation.

2. Research Significance and Future Development

In this study, we used an organoid culture method to perform in vitro cultures on large intestine tissue from both healthy individuals and patients with ulcerative colitis and clarified how gene mutations occur in each stem cell.

Results show a higher frequency of gene mutations accumulating in the colon epithelium of patients with ulcerative colitis than in healthy individuals. At the same time, increase in the frequency of gene mutations due to inflammation was negligible.

As a result of focusing on the qualitative change of which genes had accumulated mutations instead of the quantitative change of mutation, we discovered that, in colonic epithelial stem cells of patients with ulcerative colitis, there are relatively few gene mutations that contribute to the development of cancer, while there are various gene mutations along the IL-17 (interleukin 17) inflammatory signaling pathway, which is one of the cytokines that causes inflammation. (See Fig. 2)

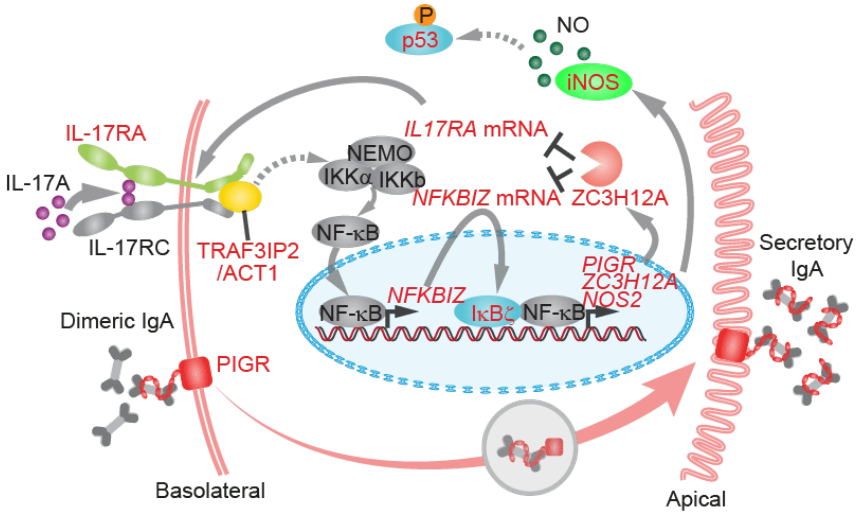

Fig. 2: Inflammation resistance gene mutations that accumulate in colonic epithelial stem cells of patients with ulcerative colitis
Gene mutation on the IL-17 pathway found by the research team. (Red) When IL-17A binds to its receptor, ACT1[4] and NFKBIZ[5] are activated downstream, after which the expression of iNOS[6] activates p53[7], leading to cell death.

To investigate the clonal expansion of these gene mutations, we examined a larger set of patient-derived samples to understand the extent to which these gene mutations occurred in all colonic stem cells in the area sampled using biopsy forceps[8].

Of the 45 patients with ulcerative colitis that we examined, we confirmed that 27 patients (60%) had these mutations, and more than 75% of patients with colorectal cancer had gene mutations in non-cancerous colorectal epithelial cells. We also confirmed an increasing number of stem cell clones with these gene mutations.
The colonic epithelial cells of patients with active ulcerative colitis are chronically damaged by inflammation, and IL-17 is known to play an important role as one of the substances that causes this kind of inflammation. We compared the cytotoxicity of normal cells and cells with these gene mutations when stimulated by IL-17.

Results showed that normal colonic epithelial cells undergo programmed cell death (apoptosis)[9], while colonic epithelial cells with gene mutations are resistant to apoptosis and survive even in the presence of IL-17 (inflammation via IL-17).

From these results we see that because the cells with the gene mutations that we have identified avoid damage and continue to survive, as opposed to healthy cells that are damaged and die under normal inflammation, it is possible that clonal expansion of mutated cells may continue. We have also successfully demonstrated the mechanism of cell proliferation in clones of colorectal epithelial cells with specific gene mutations.

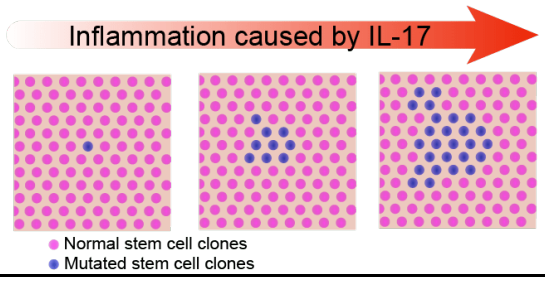

Fig. 3: Clonal expansion of mutant stem cells under inflammation
Under inflammation caused by IL-17, the regions of stem cell clones (blue) where we identified gene mutations have become resistant to inflammation damage, and these regions of gene mutation will expand under chronic inflammation.

In this study, we found that in the colon epithelium of many patients with ulcerative colitis, gene mutations that acquire resistance to inflammation (i.e. inflammatory resistance gene mutations) accumulate in stem cells in order to adapt to the inflammatory environment, which leads to further clonal expansion. Since these gene mutations are more common in ulcerative colitis patients who have colorectal cancer, they are expected to be potential biomarkers for ulcerative colitis patients who are at high risk of developing colorectal cancer.

On the other hand, because these inflammation resistance gene mutations are not observed in the cancer itself, it is possible that the cancer originated from epithelium that had no mutations and that inflammation resistance gene mutation may help to suppress the cancerization of cells. (See Fig. 4)

Interestingly, reports of studies using mice have shown that when the gene mutations that we found in our study spread to the epithelium of the entire large intestine, the intestinal environment changes and inflammation worsens. So while inflammation resistance gene mutations suppress the cancerization of cells in human ulcerative colitis, the clonal expansion of mutant cells may exacerbate inflammation of the large intestine and may cause carcinogenesis in surrounding cells that do not have the gene mutation.

The results of this study may provide important clues to help elucidate the currently unknown causes of ulcerative colitis, its exacerbation, and the treatment difficulties for chronic inflammation.

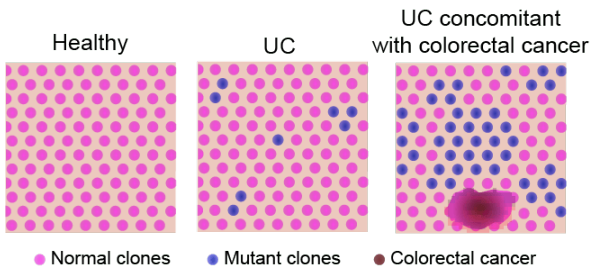

Fig. 4: Relationship between inflammation resistance gene mutations and colorectal cancer complications and carcinogenesis
An increased rate of clonal expansion in mutated stem cell is observed in ulcerative colitis patients who have colorectal cancer. Colorectal cancer, however, arises from colonic epithelial cells with no gene mutation.

3. Notes

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● Project for Cancer Research and Therapeutic Evolution（P-CREATE）
"Development of advanced drug discovery system based on understanding of cancer multi-level phenotype"
●Project for Elucidating and Controlling Mechanisms of Aging and Longevity, Elucidating Fundamental Mechanisms of Systemic and Organ /Tissue Aging
"Understanding changes in aging traits aimed at controlling the onset of gastrointestinal diseases"
●Advanced Research & Development Programs for Medical Innovation (AMED-CREST "Tissue Adaptation & Repair")
"Dissecting intestinal fibrogenic diseases by a newly developed 4D disease model system"

4. Research Paper

Title Somatic inflammatory gene mutations in human ulcerative colitis epithelium

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Publication *Nature*

[Glossary]

[1]Stem Cell
Stem cells are cells with the ability to self-replicate and develop into many different cell types, an ability known as pluripotency. Because differentiated cells do not produce offspring cells, the only gene mutations that accumulate without being eventually removed are the ones occurring in these self-replicating stem cells.

[2]Crypt
Crypts are the countless recesses formed by epithelial cells in the mucus membrane of the colon. Colonic stem cells reside at the bottom of these crypts.

[3]Organoid Culture Method
A culture method that allows stem cells to proliferate over a long period of time as a three-dimensional structure by embedding them in a gel that serves as a scaffold for growth and supplementing necessary cell growth factors. In this study, detailed genome analysis was made possible by proliferating a single stem cell, and further clarification of the function of the gene was obtained using an organoid with gene mutation.

[4] ACT1
ACT1 is an adapter protein involved in signaling interleukin-17 receptor (IL-17A). In this study, mutations were detected in a colon with ulcerative colitis. In cells in which the gene encoding a protein is mutated (in this case, TRAF3IP2), there was an impairment in inflammatory signaling by IL-17, and apoptosis (described below) did not occur.

[5] NFKBIZ
NFKBIZ is a molecule that exists downstream of IL-17A signaling, localized in the nucleus. In this study, mutations were detected in a colon with ulcerative colitis. Like ACT1, apoptosis (described below) in IL-17 did not occur in cells with mutations.

[6] iNOS
iNOS (inducible nitric oxide synthase) is a molecule that is induced by inflammation among enzymes involved in the production of nitric oxide (NO), a form of nitrogen oxide. NO is one of the signaling substances that causes apoptosis (described below).

[7] p53
p53 is one of the genes that controls apoptosis and functions as a tumor suppressor. It is considered to play a crucial role in the carcinogenesis of ulcerative colitis.

[8] Biopsy forceps
A biopsy forceps is an instrument used for collecting tissue during an endoscopy. Standard size biopsy forceps can collect 1-2 mm of tissue.

[9] Apoptosis
Apoptosis is a form of programmed cell death. In order to maintain the homeostasis of organs and other bodies, the enzyme caspase works to eliminate abnormal cells by leading them to 'cellular suicide.'

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