## Original Article
# Use of segmental colorectal lavage cytology during surveillance colonoscopy for detecting dysplastic and cancer cells in patients with ulcerative colitis

Yoshitaka Torii[1*], Daisuke Sano[1*], Hiroki Ikeuchi[2], Nobuyuki Hida[3], Seiichi Hirota[1]

[1]Department of Surgical Pathology, Hyogo College of Medicine, Nishinomiya, Japan; [2]Department of Inflammatory Bowel Disease, Division of Surgery, Hyogo College of Medicine, Nishinomiya, Japan; [3]Inflammatory Bowel Disease Center, Division of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan. *Equal contributors.

**Abstract:** Purpose: To examine whether segmental colorectal lavage cytology (CRLC) during surveillance colonoscopy was useful for detecting dysplastic and cancer cells in ulcerative colitis (UC) patients. Methods: We examined whether CRLC can detect dysplastic and cancer cells in total colectomy materials of 39 UC patients. After washing the luminal surface of dissected colorectal tissues with saline, the fluid was collected. We also examined whether segmental CRLC during surveillance colonoscopy can detect dysplastic and cancer cells in 45 UC patients. Fluid was collected after washing segmental colorectum including the suspicious region. Cytological specimens were stained with Papanicolaou and immunocytochemically stained with anti-p53 antibody. Results: Although cancer and dysplastic cells were found by CRLC in 9 and 4 UC patients receiving total colectomy respectively, histology revealed 9 cancer lesions but only 2 dysplastic foci. In segmental CRLC, cancer cells were detected in 2 UC patients and dysplastic cells in 4 UC patients. Biopsy revealed 2 cases with colon cancer lesions but only 2 cases with dysplastic foci. Conclusions: Segmental CRLC is a less-invasive and effective method in detecting dysplastic and cancer cells in UC patients during surveillance colonoscopy. It could be used as a complementary method for colonoscopy-directed biopsy.

**Keywords:** Ulcerative colitis, dysplasia, colitic cancer, surveillance, colorectal lavage cytology

## Introduction

The increased risk for development of colorectal cancer is well documented in patients with chronic ulcerative colitis (UC). The risk of the colon cancer seems to be related directly to the duration of the colitis and the extent of the colonic involvement. The detection of dysplastic and early cancer lesions is extremely important for curative treatment of such neoplastic lesions in long-standing and extensive UC patients [1-4].

Since colonoscopy devices were poor in operability and resolution until 1980's, colonoscopy-directed biopsy was not usually applicable to the detection of colorectal cancer at that time. Therefore, colorectal lavage cytology (CRLC) was tried to detect colorectal cancers not only in non-UC patients but also in UC patients until the 1980's [2-11]. However, colonoscopy-directed biopsy became a major diagnostic method for detecting colorectal cancers, as devices of colonoscopy were improved. Consequently, CRLC has scarcely been applicable to detection of colorectal cancers lately.

Although colonoscopy and colonoscopy-directed biopsy are extremely useful for detection of usual colorectal cancers, they have some limitations in case of UC-associated colorectal cancers. For example, widespread inflammation in UC patients can obscure the colonoscopist's view from suspicious lesions. Moreover, endoscopic inspection of the mucosa may not reveal the flat infiltrating carcinoma that often occurs in UC patients. Even multiple biopsies could not cover dysplastic and cancer foci with patchy distribution. Consequently, many early and even advanced neoplastic lesions may escape

detection in the present routine methods, and in fact we sometimes experience far advanced colon cancers in UC patients in spite of periodic examination by colonoscopy. Thus, alternative or complementary methods are considered necessary for surveillance of UC patients. CRLC could take specimens from a wide surface and with minimal trauma to the patients, in contrast to colonoscopy-directed multiple biopsies.

In the present study, therefore, we developed a method of segmental CRLC and evaluated the usefulness of the method for surveillance of detecting dysplastic and cancer cells in UC patients. Preliminary, we examined whether CRLC could detect cancer cells in partially resected colorectal tissues of ordinary cancer patients without UC, and whether CRLC could detect dysplastic and cancer cells in resected whole colorectal tissues of uncontrolled UC patients. Finally, we examined whether segmental CRLC could be utilized for detection of dysplastic and cancer cells during surveillance colonoscopy for UC patients.

## Materials and methods

### Patients

Five colon cancer patients without UC who underwent partial colorectal resection, 39 UC patients who received total colorectal resection, and 45 UC patients who received surveillance colonoscopy were included in the present study. In UC patients, the diagnosis of the disease was established at two to thirty years before operation or surveillance colonoscopy.

### Methods of lavage and collection of fluids

In 5 colon cancer patients without UC, 500 ml of saline was rapidly infused through the funnel from one end of the resected lumen after ligation of the other end. Similarly in 39 patients with long-standing and extensive UC, 500 ml of saline was rapidly infused through the funnel from the ileocecal portion of totally resected colorectum after ligation of the rectal portion. The resected segments were rotated to allow the fluid to run across the entire canal, until the whole mucosa was constantly irrigated with the saline. The fluid was then allowed to run out and collected in the bottle. On the other hand, segmental colorectal lavage was done before

taking biopsy samples by spraying 20 ml of the saline on the suspicious mucosa during surveillance colonoscopy in 45 UC patients. The fluid was collected through the colonoscope using a bronchoalveolar lavage device.

### Collection of cells and detection of atypical cells

Centrifugation of the retrieved colorectal lavage fluids was performed at 1500 rpm for 15 minutes to collect the cells. The deposits were smeared on glass slides, fixed with ethanol and stained with Papanicolaou, PAS, and Alcian-blue methods. Immunocytochemical staining with monoclonal antibody against p53 (BP53-12-1, dilution 1:6, BioGenex, CA, USA) was also performed. The cytological specimens were investigated under the microscope.

### Histological examination

After lavage, dissected colorectal tissues were fixed with formalin. In non-UC patients with colon cancer, the representative specimens were cut from the tissues. In UC patients receiving resection of the total colorectum, the whole tissues were cut at 3.0 to 5.0 mm intervals. Seventy two to 139 pieces were made in each case. In UC patients receiving segmental colorectal lavage, biopsy specimens were obtained after lavage and fixed in formalin. All the fixed tissues were embedded in paraffin, cut 3 micrometers thick, and stained with hematoxylin and eosin.

## Results

### CRLC in colorectal cancer patients without UC

In colorectal cancers without UC, the CRLC procedure was sufficient to collect evaluable cells. Several types of cells including normal epithelial cells and inflammatory cells were identified, and we could detect cancer cells in all 5 patients (**Table 1**). The cancer cells appeared as individually scattered cells or loosely cohesive clusters (**Figure 1A**). The clusters often showed small, three-dimensional structure and irregularly frayed borders. They had large round nuclei showing hyperchromasia and prominent nucleoli. A necrotic background was often noted. Immunocytochemical staining showed that the cancer cells were strongly positive for p53 (**Figure 1B**). Histologically, all cases were well to

**Table 1.** Results of CRLC and histological examination

| UC or Non UC | Sample | No. of cases | CRLC | | Histology | |
|---|---|---|---|---|---|---|
| | | | No. of cases with | No. of cases with | No. of cases with | No. of cases with |
| | | examined | dysplastic cells (%) | cancer cells (%) | dysplasia (%) | cancer (%) |
| Non UC | partially resected colorectum | 5 | 0 (0%) | 5 (100%) | 0 (0%) | 5 (100%) |
| UC | totally resected colorectum | 39 | 4 (10%) | 9 (23%) | 2 (5%) | 9 (23%) |
| UC | segmental CRLC or biopsy specimens | 45 | 4 (8.9%) | 2 (4.4%) | 2 (4.4%) | 2 (4.4%) |
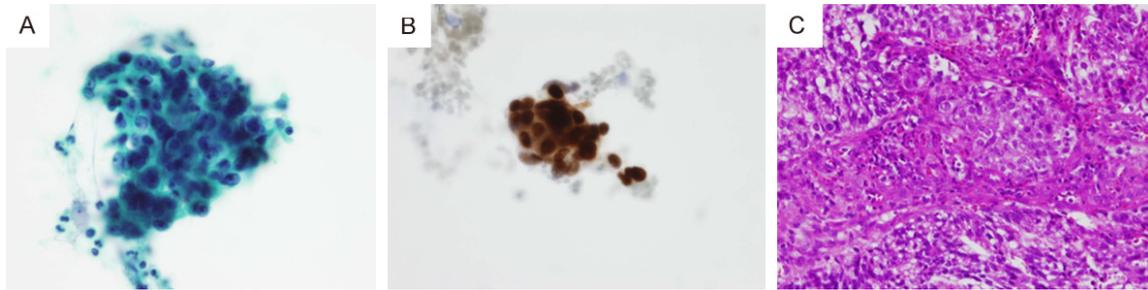


**Figure 1.** Cancer cells detected by CRLC in a colorectal cancer patient without UC. A. Clusters of cancer cells have enlarged and hyperchromatic nuclei. Papanicolaou staining, ×400. B. Immunocytochemically, cancer cells are positive for p53. p53 immunostaining, ×400. C. Histology shows moderately differentiated tubular adenocarcinoma. H&E staining, ×200.
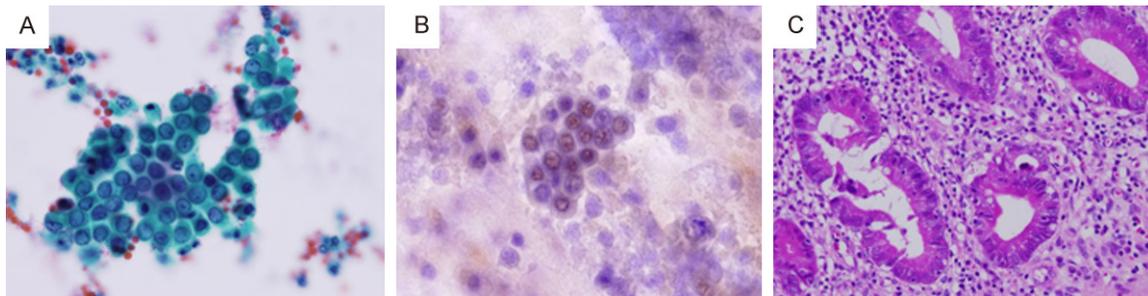


**Figure 2.** Dysplastic cells detected by CRLC in a UC patient receiving total colectomy. A. The smear shows cell clusters with nuclear enlargement, anisokaryosis, and irregular nuclear membrane. Papanicolaou staining, ×400. B. Dysplastic cells have positive staining for p53. p53 immunostaining, ×400. C. Resected tissue shows histology of low-grade dysplasia. H&E staining, ×200.

moderately differentiated adenocarcinomas (**Figure 1C**).

*CRLC in UC patients undergoing total colectomy*

CRLC in totally resected colorectums of 39 patients with long-standing UC was also satisfactory for cell collection and preservation. It revealed that large numbers of polymorphonuclear leukocytes, lymphocytes, macrophages and erythrocytes were intermingled. Twenty-six cases were negative for dysplastic and cancer cells. Four cases had dysplastic cells and 9

were positive for cancer cells (**Table 1**). The dysplastic cells appeared as cohesive sheets or small clusters (**Figure 2A**). Their cytoplasm was often abundant. The nuclei were enlarged and pale in some cases and slightly hyperchromatic in others. They also showed irregular borders but little variation in size. The nuclear chromatin was often dispersed, coarse, and condensed. Compared to the dysplastic cells, the cancer cells were usually more abundant and easily recognized. They were observed as single cells or loosely cohesive small clusters (**Figure 3A**). Nuclear variation in size, irregularly thickened nuclear membranes, chromatin
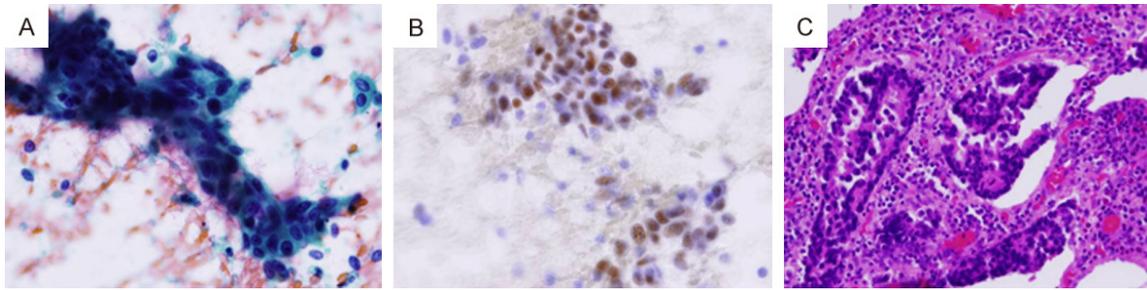
**Figure 3.** Cancer cells detected by CRLC in a UC patient receiving total colectomy. A. Nuclear hyperchromasia is prominent in cancer cell clusters. Papanicolaou staining, ×400. B. Immunocytologically, positive staining for p53 is observed. p53 immunostaining, ×400. C. Histological examination reveals well differentiated tubular adenocarcinoma. H&E staining, ×200.
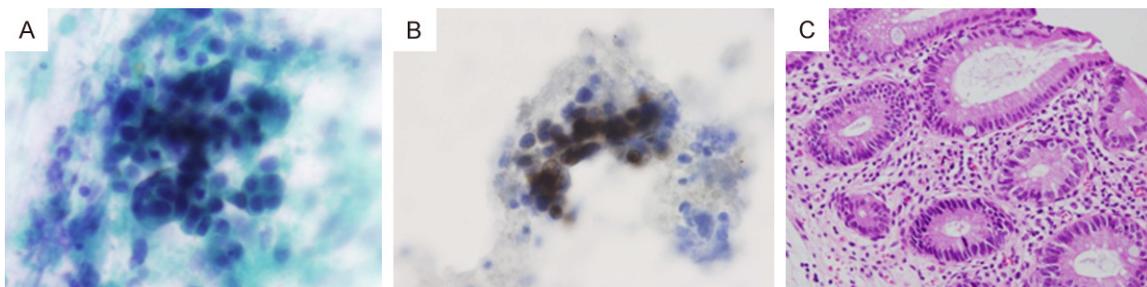


**Figure 4.** Dysplastic cells detected by segmental CRLC during surveillance colonoscopy in a UC patient. A. Cell clusters show nuclear enlargement, anisokaryosis, nuclear pleomorphism, and irregular nuclear membrane. Papanicolaou staining, ×400. B. Dysplastic cells show positive staining for p53. p53 immunostaining, ×400. C. Biopsy specimen includes glands showing low-grade dysplasia. H&E staining, ×200.

clumping, and eosinophilic macronucleoli were observed. Thus, the harvested cells showed the characteristics of well to moderately differentiated adenocarcinoma. When both dysplastic and cancer cells collected by CRLC were examined immunocytochemically, all cases were positive for p53 (**Figures 2B** and **3B**). In all 9 patients proven to have cancer cells by CRLC, histological examination revealed well to moderately differentiated adenocarcinoma in the colorectum (**Figure 3C** and **Table 1**). Dysplastic lesions were histologically recognized in 2 patients (**Figure 2C** and **Table 1**). Therefore, in 2 patients dysplastic cells were detected only by CRLC (**Table 1**).

*Segmental CRLC during colonoscopy in UC patients*

The segmental CRLC performed during surveillance colonoscopy in 45 patients with long-standing UC was also satisfactory to collect and preserve various types of cells including many polymorphonuclear leukocytes. The specimens of 39 patients were negative for dysplastic or cancer cells, but we could detect those cells in 6 cases (**Table 1**). Four cases were classified as having dysplastic cells (**Figure 4A**), and 2 were positive for cancer cells (**Figure 5A** and **Table 1**). Cytologic features of dysplastic cells and cancer cells observed were similar to those in CRLC specimens of UC patients receiving total colectomy. Immunocytochemistry of p53 showed positive reaction in both cell types (**Figures 4B** and **5B**). Histologic examination of biopsy specimens revealed that 2 cases had cancer lesions of moderately differentiated adenocarcinoma (**Figure 5C**) and 2 cases had dysplastic foci (**Figure 4C** and **Table 1**). In 2 cases, therefore, dysplastic cells were not detected by histologic examination (**Table 1**).

**Discussion**

Patients with long-standing and extensive UC have a high risk for developing neoplastic changes such as dysplasia and cancer [12, 13]. However, macroscopic detection of the foci is difficult because of both UC-associated inflam-
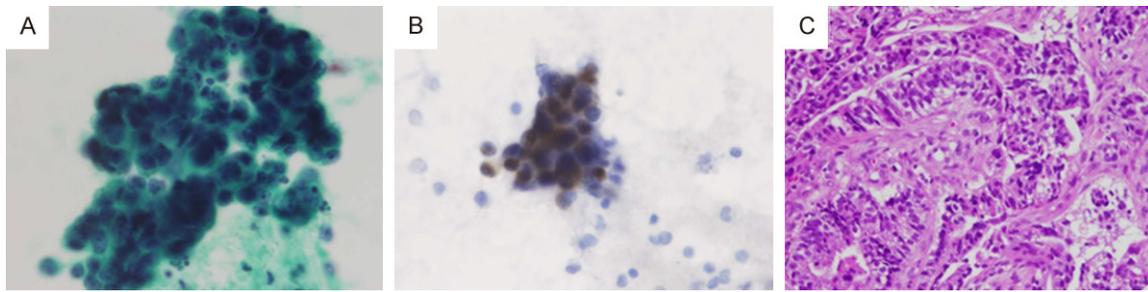
**Figure 5.** Cancer cells detected by segmental CRLC during surveillance colonoscopy in a UC patient. A. Cytological specimen shows cell clusters with nuclear enlargement and condensed chromatin, indicating cancer cells. Papanicolaou staining, ×400. B. Positive staining for p53 is observed in cancer cells. p53 immunostaining, ×400. C. Biopsy sample shows histology of moderately differentiated tubular adenocarcinoma. H&E staining, ×200.

matory change masking the lesions and multiplicity of the lesions [14-16]. Even in cancer lesions, foci are not easy to detect by endoscopy since macroscopic findings are often quite different from those in ordinary colorectal cancers without UC. Thus, colonoscopy-directed biopsy, the common diagnostic method of ordinary colon cancers, is not sufficient for surveillance of UC-associated neoplastic lesions. Alternative or complementary techniques are necessary for detection of such lesions. In the present study, we developed the method of segmental CRLC during surveillance colonoscopy and evaluated whether the method could detect dysplastic and cancer cells in UC patients.

In partially resected colorectum of non-UC patients with colorectal cancer, CRLC could detect cancer cells in all 5 cases examined. The successful result indicated that CRLC could be an appropriate method for detection of neoplastic lesions. Moreover, we could detect dysplastic and cancer cells by CRLC in totally resected colorectum of UC patients in spite of associated severe inflammatory change. The result suggested that segmental CRLC might be used to detect such cells in surveillance of UC patients. In fact, colonoscopy-directed segmental CRLC could detect dysplastic and cancer cells in UC patients during surveillance colonoscopy.

In the present study, we could similarly detect cancer cells or cancer lesions not only by histological examination but also CRLC in non-UC colon cancer patients and UC patients. On the other hand, the number of cases with dysplastic cells detected by CRLC was larger than that of cases with dysplastic foci detected by histo-

logical examination in UC patients. We speculate that this result might be due to successful cell collection from a wide surface area including the suspicious lesion in CRLC. Histological examination might only reflect the result of pinpoint samples even if multiple biopsies are done.

Differential diagnosis among regenerative atypia, UC-associated dysplasia, and cancer by histological examination is often difficult since histological findings of UC-associated neoplastic lesions are rather different from those of ordinary neoplastic lesions. For example, UC-associated neoplastic lesions often do not show densely packed atypical glands as observed in ordinary neoplastic lesions. Although cytological examination also may have difficulty in distinction among regenerative atypical cells, UC-associated dysplastic cells, and cancer cells, we consider that the increased opportunities to examine those cells make it possible for us to distinguish those cells because pure cytological assessment might be more amenable to differentiate those cells than histological assessment.

In summary, the colonoscopy-directed segmental CRLC is a simple, less-invasive and useful method to detect early neoplastic lesions in UC patients. The method could be used as a complementary technique for colonoscopy-directed biopsies.

**Disclosure of conflict of interest**

None.

**Address correspondence to:** Dr. Seiichi Hirota, Department of Surgical Pathology, Hyogo College of

Medicine at Hyogo, 1-1 Mukogawa-cho, Nishinomiya 663-8501, Hyogo, Japan. Tel: +81-798-45-6666; Fax: +81-798-45-6671; E-mail: hiros@hyo-med.ac.jp

## References

[1] Riddell RH, Goldman H, Ransohoff DF, Appelman HD, Fenoglio CM, Haggitt RC, Ahren C, Correa P, Hamilton SR, Morson BC, Sommers SC and Jardley JH. Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. Hum Pathol 1983; 14: 931-968.

[2] Galambos JT, Massey BW, Klayman MI and Kirsner JB. Exfoliative cytology in chronic ulcerative colitis. Cancer 1956; 9: 152-159.

[3] Melville DM, Richman PI, Shepherd NA, Williams CB and Lennard-Jones JE. Brush cytology of the colon and rectum in ulcerative colitis: an aid to cancer diagnosis. J Clin Pathol 1988; 41: 1180-1186.

[4] Kline TS and Yum KK. Fiberoptic coloscopy and cytology. Cancer 1976; 37: 2553-2556.

[5] Festa VI, Hajdu SI and Winawer SJ. Colorectal cytology in chronic ulcerative colitis. Acta Cytol 1985; 29: 262-268.

[6] Thabet RJ and Macfarlane EW. Cytological field patterns and nuclear morphology in the diagnosis of colon pathology. Acta Cytol 1962; 6: 325-331.

[7] Granqvist S, Granberg-Ohman I and Sundelin P. Colonoscopic biopsies and cytological examination in chronic ulcerative colitis. Scand J Gastroent 1980; 15: 289-296.

[8] Bernstein CN. Ulcerative colitis with low-grade dysplasia. Gastroenterology 2004; 127: 950-956.

[9] Friedman S and Blumberg RS. Disorders of the gastrointestinal system: inflammatory bowel disease. In: Fauci AS and Longo DL, editors. Harrison's principles of internal medicine. 17th edition. New York, McGraw-Hill; 2008. pp. 1886-1899.

[10] Rosenberg IL and Giles GR. The value of colonic exfoliative cytology in the diagnosis of carcinoma of the large intestine. Dis Colon Rectum 1977; 20: 1-10.

[11] Katz S, Katzka I, Platt N, Hajdu EO and Bassett E. Cancer in chronic ulcerative colitis: diagnostic role of segmental colonic lavage. Am J Dig Dis 1977; 22: 355-364.

[12] Rozen P, Tobi M, Darmon E and Kaufman L. Exfoliative colonic cytology: a simplified method of collection and initial results. Acta Cytol 1990; 34: 627-631.

[13] Deery ARS. Alimentary system: small and large intestine. In: Gray W and McKee GT, editors. Diagnostic Cytopathology. 2nd edition. London, Churchill Livingstone; 2003. pp. 354-359.

[14] Geisinger KR, Raab SS, Stanley MW, Silverman JF and Abati A. Gastrointestinal system: large intestine. In: Geisinger KR, editor. Modern cytopathology. Philadelphia, Churchill Livingstone; 2004. pp. 482-487.

[15] Rosai J and Ackerman LV. Gastrointedtinal tract. In: Rosai J, editor. Surgical Pathology. New York, Mosby; 2004. pp. 782-787.

[16] Liu C and Crawford JM. The Gastrointestinal Tract. In: Kumar V, editor. Pathologic Basis of Disease. Philadelphia, Elsevier Saunders; 2005. pp. 846-851.