—Case Reports—

Squamous Cell Carcinoma Arising from Recurrent Anal Fistula

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Abstract

Here, we report on a patient with squamous cell carcinoma (SCC) arising from recurrent anal fistula. The patient was a 57-year-old woman who had 32-year history of having a recurrent perianal abscesses that ruptured spontaneously. Six months before her admission to our hospital, anal pain developed. She had no history of inflammatory bowel disease. Physical examination revealed three external fistulous openings at the two o'clock position, 2 cm from the anal verge. One internal opening in the lower rectum was found with proctoscopy. The patient underwent fistulectomy. Microscopic examination showed SCC arising from the anal fistula, which was accompanied by vessel invasion. The tumor was observed to be continuous from the external opening but was not exposed to the internal opening of the rectal mucosa. Because human papillomavirus (HPV) infection was suspected, immunohistochemical analysis was performed, but showed no HPV infection. Two weeks after fistulectomy, abdominoperineal resection with lymph node dissection was performed. Histopathological examination revealed no remnant cancer tissue or lymph node metastasis. She was discharged after surgery without complications. Eight years after the operation, she complained of constant pain during micturition. Urological examination revealed urinary bladder cancer, and transurethral resection of the bladder tumor was performed. Histopathological examination revealed transitional cell carcinoma of the urinary bladder. Two years later, the patient died of metastatic urinary bladder cancer, without recurrence of the fistula cancer. Because the patient's mother had died of urinary bladder cancer and she herself had metachronous urinary bladder cancer in addition to fistula cancer, we investigated whether microsatellite instability (MSI) and chromosomal instability correlated with fistula cancer development. Immunohistochemical analysis of formalin-fixed, paraffin-embedded surgical tumor specimens for p53, MLH1, and MSH2 was performed. The tumor specimens showed no MLH1 expression but did show normal MSH2 expression. p53 was not expressed. Five microsatellite loci were examined using the tumor specimens to detect MSI, namely two loci with mononucleotide runs (i.e., BAT25 and BAT26) and three loci with dinucleotide repeats (i.e., APC, Mfd15, and D2S123). The tumor specimens showed alternations in the repeated sequences of two loci (i.e., BAT26 and D2S123). As a result, the tumor was classified as MSI-H (high) according to the

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Bethesda criteria. Our patient had MSI and one of the smallest reported SCCs arising from recurrent anal fistulae.
(J Nippon Med Sch 2007; 74: 319–324)

Key words: anal fistula, squamous cell carcinoma, microsatellite instability, chromosomal instability, human papillomavirus

Introduction

Squamous cell carcinoma (SCC) associated with chronic anal fistulae is rare in Japan[1]. There is a risk of the malignant transformation of recurrent anal fistulae, particularly for those persisting for more than 10 years[2,3]. There has been much debate about the origin of the tumor. Three hypotheses on the origin of the tumor are as follows: 1) congenital duplications of the anorectal mucosa[4], 2) focal adenomatous hyperplasia of the anal glands[5], and 3) cancerous transformation of rectal mucosal cells that have migrated into anal fistulae[6]. Recently, the incidences of anal carcinoma and its precursor lesions have increased, particularly among sexually active homosexual men. Human papillomavirus (HPV) is reported to be associated with anal squamous cell dysplasias and carcinomas. Although patients with anal fistulae and fissures or perianal abscesses present with mucosal lesions, which provide direct viral access to the basal epithelial layer, HPV infection alone is insufficient for the malignant transformation of these benign lesions[7]. Chromosomal instability (CI) and microsatellite instability (MSI) have been studied and were found to contribute to the development of anal SCC[8]. Here, we describe a patient with long-standing perianal fistula in whom an extremely small SCC developed. To determine whether HPV infection was present, CI and MSI were examined.

Case Report

A 57-year-old woman with anal fistulae was admitted to our hospital in June 1995. She had a 32-year history of suffering from a recurrent perianal abscesses that ruptured spontaneously. Six months before admission, anal pain developed. She had no history of inflammatory bowel disease. Her mother had died of urinary bladder cancer. Physical examination revealed three external fistulous openings at the two o'clock position, 2 cm from the anal verge. An internal opening in the lower rectum was found with proctoscopy. There was an induration in the left anterolateral position of the anus. She showed no abnormal laboratory findings on admission. Barium enema and colonoscopic examinations revealed no colonic disease. Anal intersphincter fistulae were diagnosed, and the patient underwent a fistulectomy on June 18. The resected fistula was 2.5 cm long. Microscopic examination revealed SCC arising from anal fistulae, which were accompanied by vessel invasion **(Fig. 1 a)**. The tumor was observed to be continuous from the external opening but was not exposed to the internal opening of the rectal mucosa and remained in the fistula. Some of the superficial cells of the epithelium showed densely stained nuclei with perinuclear haloes similar to HPV-associated lesions **(Fig. 1b)**. Immunohistochemical analysis with monoclonal antibody against HPV showed the absence of HPV. On July 3, because vessel invasion was observed in the resected specimen, abdominoperineal resection with lymph node dissection was performed. Histopathological examination revealed no remnant cancer tissue in the specimen. There was no metastasis of cancer cells to regional lymph nodes. The patient was discharged 30 days after the surgery without complications. Eight years after the operation, the patient complained of constant pain during micturition. Urological examination revealed urinary bladder cancer, and transurethral resection of the urinary bladder tumor was performed on March 16, 2004. Histopathogical examination revealed transitional cell carcinoma of the urinary bladder. Two years later diagnosis, the patient died of

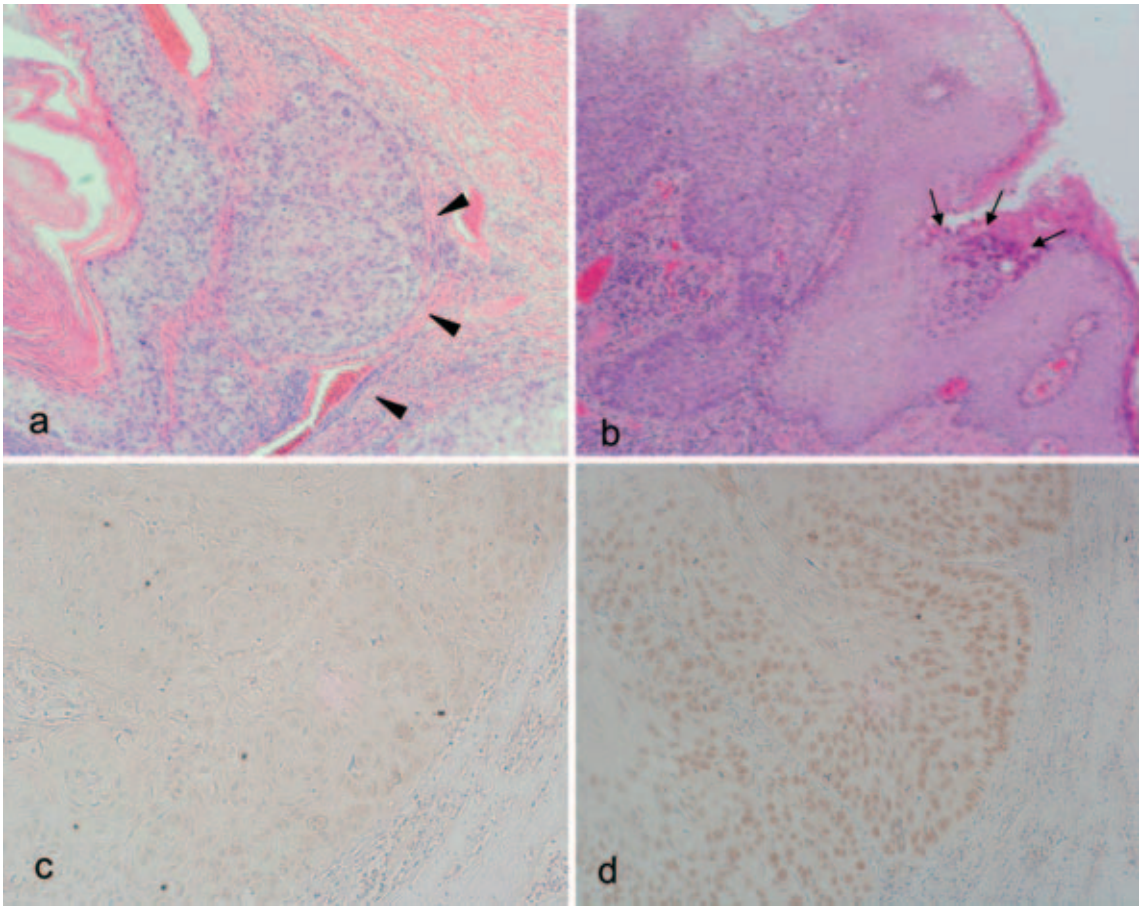

Fig. 1 **a:** Histological appearance of SCC arising from an anal fistula. SCC remains within the fistula, but vessel invasion **(arrow heads)** was detected. **b:** Superficial koilocytes with dense "India ink" nuclei, perinuclear haloes **(arrows)** in SCC (H&E, ×60). **c:** Loss of MLH1 expression in tumor. **d:** Normal expression of MSH2 in tumor.

Table 1 Used Primer sequence

gene name	Accession ID	Sequence 5' → 3'	5' Position *
BAT25	L04143	Forward: TCGCCTCCAAGAATGTAAGT	6839
		Reverse: TCTGCTTTTAACTATGGCTC	6961
BAT26	AY601851	Forward: TGACTACTTTTGACTTCAGCC	12786
		Reverse: AACCATTCAACATTTTTAACC	12907
APC	AC008536	Forward: AGCAGATAAGACAGTATTACTAGTT	18248
		Reverse: ACTCACTCTAGTGATAAATCG	18362
Mfd15	AC006441	Forward: GGAAGAATCAAATAGACAAT	29299
		Reverse: GCTGGCCATATATATATTTAAACC	29450
D2S123	Z16551	Forward: AAACAGGATGCCTGCCTTTA	98
		Reverse: GGACTTTCCACCTATGGGAC	324

urinary bladder cancer dissemination, without the recurrence of fistula cancer. Because the patient's mother had died of urinary bladder cancer and she herself had metachronous bladder cancer in addition to fistula cancer, we investigated whether MSI and CI correlated with fistula cancer development. This study was carried out in accordance with the principles of the Declaration of Helsinki 1975.

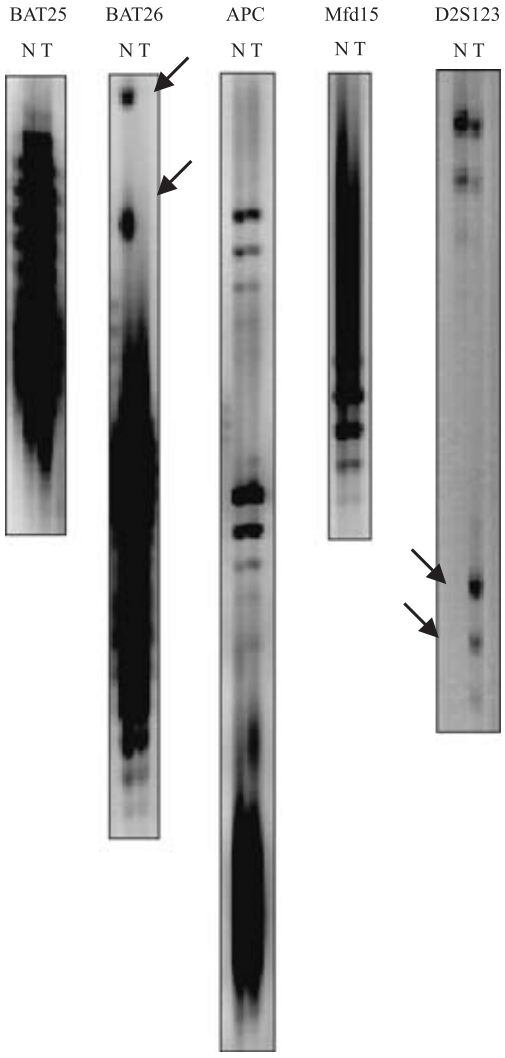

Fig. 2 Microsatellite analysis of SCC arising from an anal fistula.
Arrows show MSI in BAT26 and D2S123.

Immunohistochemical analysis for p53 was performed with formalin-fixed, paraffin-embedded surgical tumor specimens. Mismatch repair proteins (MMR) (MLH1 and MSH2) was performed. The tumor showed the loss of MLH1 expression and normal immunoreactivity for MSH2 (**Fig. 1c, d**). p53 was not expressed. Five microsatellite loci were used in the tumor to detect MSI, namely, two loci with mononucleotide runs (i.e., BAT25 and BAT26) and three loci with dinucleotide repeats (i.e., APC, Mfd15, and D2S123) (**Table 1**). The tumor showed alternations in the repeated sequences of two loci (**Fig. 2**). The tumor was classified as MSI-H (high) according to the Bethesda criteria[9,10].

Discussion

In 1931, Rossier first described six cases of chronic fistula in ano with the subsequent development of cancer[11]. For the diagnosis of carcinoma arising from an anal fistula, Rossier proposed the following criteria in 1931: 1) the fistula has been present for a long time, 2) there is no tumor in the luminal surface of the anorectum, and 3) the intestinal opening does not lie in malignant tissue.

The histogenesis of carcinoma in a preexisting anal fistula remains unclear. From the observation that in a significant number of patients, fistulous tracks are lined by a normal rectal mucosa, Dukes and Galvin suggested that such tumors arise via an unusual duplication of the anorectal mucosa[12]. On the other hand, Zimberg and Kay, as well as Nielsen and Koch, showed that the most common site of anal extramucosal glandular tumors is the anal gland, even for tumors arising from anal fistulae[13,14]. Getz et al. believed that the focal adenomatous hyperplasia of anal glands in anal fistulae developed well-differentiated adenocarcinoma[15]. Taniguchi et al. speculated that these tumors originate from the rectal mucosal cells that migrated into the anal canal, as shown by the positive staining of the carcinoma arising from an anal fistula by O-acetylated sialic acid[6]. Our case is in agreement with the criteria for diagnosing cancer arising from a long-standing anal fistula. However, the histogenesis of carcinoma was not clarified. In the surgical specimens, koilocytic changes, such as a perinuclear clear halo with the condensation of the cytoplasm, were observed. Because HPV infection was suspected, immunohistochemical analysis for HPV was performed, but showed the absence of HPV infection.

There is an increasing incidence of SCC in the anal canal with a known association of this carcinoma with HPV infection[16,17]. In European, Asian, and US populations, 86% to 100% of anal carcinoma biopsy specimens contain HPV DNA, and such DNA has never been found in normal anal mucosa or rectal adenocarcinoma[18–20]. HPV may play a role in the development of SCC in the anal canal.

However, there is not sufficient evidence that HPV infection promotes the progression of anogenital intraepithelial neoplasia to invasive carcinoma. Gervaz et al. suggested that in human immunodeficiency virus (HIV)-positive individuals, MSI rather than CI appears to be the prevalent factor for the rapid progression of intraepithelial neoplasia to invasive carcinoma and that in HIV-negative individuals, CI is the predominant feature in anal cancer[8]. As stated above, our patient was neither HIV-positive nor HPV-positive. However, she had metachronous urinary bladder cancer and her mother died of urinary bladder cancer; thus, we examined the tumor for the presence of MSI and CI. The amplification of five microsatellite loci (i.e., BAT25, BAT26, APC, Mfd15, and D2S123), and the expression of MMRs (i.e., MLH1 and MSH2), APC, and p53 were examined. We found that CI, as indicated by the expression of p53 and APC, were both absent. The tumor showed the loss of MLH1 expression, but MSH2 expression was normal. Our patient also had MI at BAT26 and D2S123, and the tumor was classified as MSI-H. In conclusion, MSI may contribute to the histogenesis of fistula cancer, although our patient was not diagnosed as having hereditary nonpolyposis colorectal cancer (HNPCC) according to the Bethesda criteria[9,10].

The diagnosis of anal carcinoma associated with anal fistula is not always easy to establish. Repeated, deep and multiple biopsies are required to make the correct diagnosis[21]. Moreover, all tissues removed by fistulectomy should be routinely examined with microscopy, particularly for long-standing fistulae because of the rarity of these tumors[22]. Furthermore, the treatment of these tumors is still controversial. Some surgeons suggest abdominoperineal resection, whereas others prefer wide local excision of the carcinoma. Complementary radiotherapy allows the sterilization of a large colloid and ischiorectal neoplastic hollows. Chemotherapy together with neoadjuvant radiotherapy has been proposed, and some surgeons have also described the use of the preoperative chemoembolization of large tumors to decrease the extent of perineal tissue resection[23].

The prognosis of anal carcinoma is quite poor despite aggressive surgical therapy[24,25]. The 5-year survival rate is less than 20% in most series, reflecting the advanced stage of the disease at the time of presentation. By using different modalities of treatment, a better prognosis is expected in the future.

Here, we have reported on a patient with long-standing perianal fistulae giving rise to cancer who was treated with abdominoperineal resection. In addition to the presence of HPV infection being determined, CI and MSI were examined. Our results suggest that MI may contribute to the histogenesis of fistula cancer.

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(Received, April 9, 2007)
(Accepted, May 14, 2007)