**ABSTRACT**

Since the first detection of aberrant crypt foci (ACF) in carcinogen-treated mice, there have been numerous studies focusing on these microscopically visible lesions both in rodents and in humans. ACF have been generally accepted as precancerous lesions in regard to histopathological characteristics, biochemical and immunohistochemical alterations, and genetic and epigenetic alterations. ACF show variable histological features, ranging from hyperplasia to dysplasia. ACF in human colon are more frequently located in the distal parts than in the proximal parts, which is in accordance with those in colorectal cancer (CRC). The immunohistochemical expressions of carcinoembryonic antigen (CEA), β-catenin, placental cadherin (P-cadherin), epithelial cadherin (E-cadherin), inducible nitric oxide synthase (iNOS), cyclooxygenase (COX-2), and P16^INK4a are found to be altered. Genetic mutations of K-ras, APC and p53, and the epigenetic alterations of CpG island methylation of ACF have also been demonstrated. Genomic instabilities due to the defect of mismatch repair (MMR) system are detectable in ACF. Two hypotheses have been proposed. One is the "dysplasia ACF-adenoma-carcinoma sequence", the other is "heteroeplastic ACF-adenoma-carcinoma sequence". The malignant potential of ACF, especially dysplastic ACF, makes it necessary to reveal the nature of these lesions, and to prevent CRC from the earliest possible stage. The technique of magnifying chromosome makes it possible to detect "in vivo" ACF, which is beneficial to colon cancer research, identifying high-risk populations for CRC, and developing preventive procedures.

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**INTRODUCTION**

Genetic changes in the malignant transformation process of colorectal mucosa include deletions, rearrangements, and mutations leading to either inactivation or activation of specific target genes[1-3]. A number of biomarkers associated with genetic changes have been identified for early detection of CRC. Two major classes of genes, oncogenes and suppressor genes, are involved in addition to mismatch repair (MMR) genes[4-6], which are associated with genomic instability. The major advances in understanding CRC include identification of the involvement of APC, p53, K-ras and MMR genes, as well as epigenetic alterations, such as CpG island methylation, in the formation and progression of the disease. Identification of ACF as an early preinvasive lesion and its relationship to the development of cancer have aroused an increasing interest in recent years.

Since the first detection of ACF in carcinogen-treated mice by Bird in 1987 and the hypothesis of ACF as the earliest precursors of CRC, there have been numerous studies focusing on these microscopically visible lesions both in rodents and in humans. The following table (Table 1) shows some highlights of studies on human ACF.

**Table 1 Highlights of human ACF in recent years (from NCBI)**

| Major fields                  | Magazines and authors | Year |
|------------------------------|-----------------------|------|
| Histopathology               | Kristt D, et al. Hum Pathol. | 1999 |
|                             | Nascimbeni R, et al. Am J Surg Pathol. | 1999 |
|                             | Shipitz B, et al. Hum Pathol. | 1998 |
|                             | Siu IM, et al. Am J Pathol. | 1997 |
|                             | Di Gregorio C, et al. Histopathology. | 2007 |
|                             | Roncucci L, et al. Cancer Epidemiol Biomarkers Prev. | 1991 |
|                             | Roncucci L, et al. Hum Pathol. | 1991 |
| Gene mutations               | Yuan P, et al. World J Gastroenterol. | 2001 |
|                             | Takayama T, et al. Gastroenterology. | 2001 |
|                             | Otori K, et al. Cancer. | 1998 |
|                             | Bjerknes M, et al. Am J Pathol. | 1997 |
|                             | Losi L, et al. J Pathol. | 1996 |
|                             | Zaidi NH, et al. Carcinogenesis. | 1995 |
|                             | Smith AJ, et al. Cancer Res. | 1994 |
| Epigenetic/ phenotype alterations of genes | Chan AO, et al. Am J Pathol. | 2002 |
|                             | Sakurazawa N, et al. Cancer Res. | 2000 |
| Microsatellite instability   | Pedroni M, et al. Cancer Res. | 2001 |
|                             | Heinen CD, et al. Cancer Res. | 1996 |
|                             | Augenlicht L, et al. Oncogene. | 1996 |
| Cell dynamics and proliferation | Roncucci L, et al. Cell Prolif. | 2000 |
|                             | Kristt D, et al. Pathol Oncol Res. | 1999 |
|                             | Otori K, et al. Cancer Res. | 1995 |
|                             | Roncucci L, et al. Cancer Res. | 1993 |
| Oncoproteins                | Dong M, et al. Carcinogenesis. | 2003 |
|                             | Hao XP, et al. Cancer Res. | 2001 |
|                             | Shipitz B, et al. Anticancer Res. | 1999 |
|                             | Pretlow TP, et al. Gastroenterology. | 1994 |
| Dietary agents              | Nascimbeni R, et al. Cancer Epidemiol Biomarkers Prev. | 2002 |
|                             | Johnson IT, et al. Food Chem Toxicol. | 2002 |
|                             | Alabaster O, et al. Mutat Res. | 1996 |
| Magnifying/ chromosomic coloreoscopy | Hurlstone DP, et al. Br J Surg. | 2002 |
|                             | Takayama T, et al. N Engl J Med. | 1998 |
|                             | Yokota T, et al. Gastrointest Endosc. | 1997 |
| Chemoprevention             | Dolora P, et al. Cancer Detect Prev. | 1997 |
|                             | Murillo G, et al. Int J Cancer. | 2003 |
|                             | Osaka E, et al. Gastroenterology. | 2003 |
|                             | Kassie F, et al. Carcinogenesis. | 2003 |
|                             | Osaka E, et al. Life Sci. | 2002 |
|                             | Mori H, et al. Biofactors. | 2000 |
| Signal transduction pathways | Boon EM, et al. Cancer Res. | 2002 |

a: ileal microadenoma; b: in colorectal cell lines.
Identification of ACF both in carcinogen-treated rodents and in human colon makes the study of CRC at precancerous stages possible. The growth, morphological and molecular features of ACF support the contention that ACF are putative preneoplastic lesions. The traditional “adenoma-carcinoma” sequence of colorectal carcinogenesis has been extended to “ACF-adenoma-carcinoma” sequence. A better understanding of the underlying cellular and molecular events affected by cancer preventive or promoting agents will provide more insights into colorectal carcinogenesis and lead to the development of different cancer preventive strategies for high-risk individuals and the general population.

DEFINITION OF ACF
ACF was first reported by Bird in 1987[1] in the colons of carcinogen-treated C57BL/6J or CF1 female mice, and the assumption of ACF as potential preneoplastic lesions in murine colon was put forward one year later, coming up with the methodological approach to detect ACF[9]. Under microscope, aberrant crypts appeared larger and had a thicker epithelial lining compared to normal crypts, and usually gathered into a focus, consisting of aberrant crypts from one to hundreds[8,9]. These aberrant crypts sometimes were slightly elevated from the surrounding normal mucosa and often had oval or slit-like lumens[9,11-13]. It has been described as single or clusters of abnormally large crypts of the colon mucosal surface after stained with methylene blue. On colonoscopy, ACF were defined as being deeper with methylene blue staining than normal surrounding mucosa, and as a cluster of two or more crypts with dilated or slit-like openings rising above the surrounding mucosa[14,15].

ACF with a single crypt met the following criteria by McLellan[16]. The size of the crypt was at least twice that of the normal surrounding ones, the luminal opening was more elliptical than circular, and the epithelial lining was thicker than that of the normal surrounding crypts. ACF consisting of more than one crypt were defined as crypts to form a distinct focus. Individual crypts within the focus had a thicker epithelial lining and an elliptical luminal opening, and the total area occupied by the crypts of ACF was greater than that occupied by an equivalent number of surrounding morphologically normal crypts.

In humans, ACF were described and partially characterized for the first time in 1991[9,11], and in 1994, Pretlow first detected altered enzymatic activity, crypt dynamics and proliferation. ACF in human colon closely resembled aberrant crypt seen in rodents treated with carcinogens[9]. Some lines of evidences supported the view that ACF or at least some of them, might be precursors of CRC. In particular, ACF in human colon were more often located in the distal parts than in the proximal parts[17], which was verified in animal model[18]. Aberrant crypts had a hyperproliferative epithelial[19-21]. The size of ACF could increase with time, and it was evident that the nuclear atypia noted in some ACF was similar to those seen in the crypts of adenocarcinomas in rat colons[19]. In ACF, the immunohistochemical expressions of carcinoembryonic antigen[22] and β-catenin[22-24] were increased. K-ras, APC and PTEN mutations, have been shown to be important genetic alterations in the development of CRC[25,26,27], have been demonstrated in ACF[22,25]. Identification of dysplasia and monoclonoality in a putative precursor lesion would strongly link this lesion to neoplastic progression. ACF have been confirmed to arise from independent initiation events[25,30,34] and when examined histologically, ACF showed variable features, ranging from mild hyperplasia to dysplasia[12,22,35,36].

HISTOPATHOLOGICAL CHARACTERISTICS OF ACF
The final histological criteria for ACF are generally accepted as “nondysplasia”, “dysplasia” and “mixed type”[10,12,16,17,35-40].

ACF without dysplasia
ACF with normal mucosa: lacking significant modifications of the epithelium lining the crypts, enlarged crypts (at least 1.5 times larger than normal) with only slightly enlarged and elongated nuclei, but no crowding or stratification, and no mucin depletion. Crypt cells with positive staining of PCNA and Ki-67 remain at the lower part of the crypts.

ACF with hyperplasia: analogous to the manifestations of hyperplastic polyps of colon. The crypts are larger or longer than normal crypts sometimes showing apical branching. The luminal opening is serrated and slightly elevated from the surrounding normal mucosa, but without dysplasia. Goblet cells are mixed with absorbing cells, with partial mucin depletion. Nuclei are enlarged or sometimes crowded without stratification. Cells with positive staining of PCNA and Ki-67 remain at the lower and middle parts of the crypts.

ACF with dysplasia (microadenoma)
Both crypts and cells have different degree abnormalities, with enlarged, elongated and sometimes stratified and depolarized nuclei. The number of goblet cells is decreased obviously and mucin is depleted. The major site of positive staining cells of PCNA and Ki-67 is extended to the upper part of the crypts. Serrated adenomatous ACF[41] could also be found to have similar histopathological manifestations of serrated adenomas[42].

Dysplastic ACF were common in FAP patients, and also occurred in sporadic patients at a low frequency[12,31,35]. Sporadic ACF had the characteristics similar to those of dysplastic ACF in FAP patients with less frequent APC mutations[39,43] and more frequent methylation[44].

Investigations showed the possibility of ACF transition from one pathological type to another. ACF have also been found to contain carcinoma in situ, or severe dysplasia in focal areas of human colon[36,40,41], and invasive carcinoma in the rat model[11], showing evidences of ACF as precancerous lesions[10,21,30,38-40,45].

ACF with mixed type of hyperplasia and dysplasia
ACF with mixed hyperplastic and adenomatous components histologically showed the combination of various proportions of pure adenomatous pattern with dysplasia and pure hyperplastic pattern without dysplasia[41].

The WHO classifications of ACF are simplified as hyperplastic ACF and dysplastic ACF.

ACF EVALUATION
The density of ACF is the number of ACF per square centimeter of mucosal surface, which was higher in diseases at a high risk of malignancy such as familial adenomatous polyposis (FAP) and in CRC, and was lower in patients with benign diseases of the colon such as diverticulosis[11,31]. ACF density is also significantly and progressively higher from proximal colon toward distal, being the highest in sigmoid and rectum, which was in accordance with the location of CRC[19,37]. It has been found that the density of FAP ACF was significantly higher than that of sporadic CRC and benign large bowel diseases[9]. In a comparative research of sporadic CRC, Gardner’s syndrome, and benign diseases such as diverticulosis, ACF density in Gardner’s syndrome was more common and ACF occupied a larger area of mucosa as compared to sporadic CRC[31]. All these findings showed that hereditary diseases prone to colon cancer had a higher density and crypt multiplicity of ACF than CRC, which shed lights on the differences between hereditary ACF and sporadic ACF, and helped to reveal the neoplastic nature of ACF.
The mechanism by which ACF increase in size seems to be a process of crypt division, which begins at the base of the crypt and proceeds upwards until two crypts are generated. Thus, the number of crypts per ACF, also termed “crypt multiplicity”, would be an important parameter for evaluating ACF progression. It has been demonstrated that crypt multiplicity was significantly lower from proximal toward distal colon, which was opposite to that of ACF density, and was significantly larger when it was associated with carcinoma or adenoma than with nonneoplastic diseases[49]. Also no gradient in ACF density and crypt multiplicity was observed according to the distance from the tumor[11,17,39].

Increased mitotic activity, which has been proposed as a biomarker of colon cancer at early stages, was observed in a majority of ACF. Most of the crypts showed a mitotic pattern similar to that of normal adjacent crypts[19,40]. In some of the dysplastic foci, mitotic activity was seen to distribute throughout the crypts, as reported in adenomas. The above findings may be consistent with the assumption that ACF were preneoplastic lesions[52].

Protein kinase C (PKC) is a family of twelve distinct serine/threonine kinases that participate in signaling pathways involved in cell proliferation, differentiation, and apoptosis. Alterations in PKC isozyme levels also played a role in colon carcinogenesis[44,47]. Elevated expression of PKC βII was associated with neoplastic transformation both in rat and in human colonic epithelia[48,49]. It has been demonstrated that overexpression of protein kinase C βII (PKC βII) made transgenic mice more susceptible to carcinogen-induced colonic hyperproliferation and ACF formation[50], and the level of PKC βII protein expression was strikingly increased in ACF compared with that in normal colonic epithelium[51].

The study of ACF and their relationships to growth factors, such as TGF-α, TGF-β, EGFR, TGFβRII, phosphorylated cellular tyrosine (P-tyr) revealed a strong correlation between altered expression of TGFs in all ACF that have been examined and the degree of dysplasia and crypt multiplicity[52]. TGF-α was undetectable in ACF[52,53], which had a high incidence of apoptosis (AI). The result was similar to that both in adenomas and in carcinomas[53]. Apoptosis provides a protective mechanism against neoplasia by moving genetically damaged stem cells from the epithelium before they can undergo clonal expansion. Manifestations are indicative of a high level of apoptosis in human ACF and carcinogen-treated animal ACF, in which apoptosis was said to eliminate cells damaged by carcinogen administration[54].

DIFFERENCES OF FAP AND SPORADIC ACF

Most FAP ACF are histopathologically, phenotypically and genetically different from sporadic ACF. Apart from the differences in ACF density between FAP ACF and sporadic ACF, there are significant differences in regard to dysplasia. Most FAP ACF were dysplastic, whereas sporadic ACF had the histopathological features of hyperplastic polyps with little or no dysplasia[38]. The degree of dysplasia in FAP ACF was severer than that of sporadic ACF[59]. Most ACF from FAP patients have phenotypic characteristics of adenomas, which are vital to carcinogenesis, and lack ras gene mutations, while sporadic ACF and a subset of FAP ACF closely resemble hyperplastic polyps, which are benign, but usually have ras gene mutations. There are also evidences identified in the colon of Min/+ mice after azoxymethane (AOM) treatment. Germline mutations in murine APC homologous to human APC, caused multiple intestinal neoplasia in mice (Min/+ mice)[55]. ACFsp resemble dysplastic ACF, which were described previously as potential precursors of adenomas in rodent and human colon carcinogenesis[35,36,39,52], and more severe in FAP ACF than in sporadic ACF. ACFsp, followed a continuous development from a single crypt to adenoma with fast crypt multiplication and altered expression of β-catenin, while classical ACF homologous to hyperplastic human ACF showed slow-growing crypts with normal β-catenin expression, and were probably not related to tumorigenesis[60]. In carcinogen-treated rodents and patients with sporadic CRC, only a very small fraction of ACF progressed to tumor[31]. This was consistent with the observation that a large fraction of ACF was hyperplastic whereas only a small fraction of ACF showed dysplasia, a hallmark of malignant potential[38,35]. In contrast, most ACF in FAP patients were dysplastic, resembling that of adenomas[31]. It has been proposed that only dysplastic ACF progressed to adenomas and carcinomas[51]. In non-FAP cases, K-ras mutations were detected in 82 % (89/106) of nondysplastic ACF and 63 % (17/27) of dysplastic ACF. FAP patients showed K-ras mutations in only 13 % (1/8) of dysplastic ACF, which was the predominant form of ACF found in FAP. In FAP patients, somatic APC mutations were found in 100 % of dysplastic ACF, as they were in adenomas[61]. A previous study showed an association between CpG island methylation in cancer and K-ras mutations[52]. It was found that CpG island methylation was present in more ACF from sporadic cancer than in FAP ACF, implying that FAP ACF usually lacked methylation or K-ras mutations and were frequently dysplastic, while sporadic ACF usually had methylation and/or K-ras mutations and lacked dysplasia[57].

BIOCHEMICAL AND IMMUNOHISTOCHEMICAL ALTERATIONS OF ACF

What we have discussed above is concerned about the determination of ACF under a microscope, and the histopathological characteristics of ACF. Yamada reported another kind of crypts with normal appearance harboring altered β-catenin expression, which was regarded as lesions different from ACF, and might occur earlier than ACF in rat model[39,40]. Previous studies explored phenotype alterations of ACF by means of biochemical and immunohistochemical methods. Pretlow reported enzyme-altered foci with normal morphology in colon and decreased expression of hexosaminidase in these lesions for the first time[53]. By using serial glycolmethacrylate-embedded sections of grossly normal colons from F344 rats treated with colon carcinogen, Pretlow’s group was able to detect multiple lesions with altered enzyme activities in the distal colon and rectum of those rats, and found that histochemically decreased demonstrable hexosaminidase activity could be observed in more than 95 % of ACF in rats[22,29,61], and was also able to demonstrate two groups of lesions with decreased hexosaminidase activity: one with aberrant morphology resembling ACF, the other with normal appearance[55]. A decrease in hexosaminidase activity was also seen in colon tumors developed in those animals[13]. Hexosaminidase provided a marker of colon epithelial cells throughout the carcinogenesis progression[61]. Human ACF clearly resembled those seen in animals in morphology and histological appearance[11,12,22,36,61], but hexosaminidase activity was not a useful marker of human ACF because human colonic neoplasia was accompanied by a number of phenotypic changes that frequently included increased expressions of a variety of tumor-associated glycoproteins[64]. Carcinoembryonic antigen (CEA), a member of the immunoglobulin superfamily, was first isolated from human colon cancers, and seems to function as an intracellular adhesion molecule. The immunohistochemical expression of CEA was altered in as many as 93 % (39/42) of ACF in 15 patients, and was related to the size of the foci, but not to the presence or degree of dysplasia in Pretlow’s study[62]. The immunohistochemical localization of CEA in the
cytoplasm and on the basolateral membranes in ACF was similar to that of carcinomas. The finding that CEA appeared to be a marker of human ACF by means of immunohistochemistry should facilitate the identification and characterization of these lesions in human.

The other two members of the cadherin family of cell adhesion molecules were placental cadherin (P-cadherin) and epithelial cadherin (E-cadherin). It has been shown that striking membranous and/or cytoplasmic P-cadherin up-regulation and its co-expression with E-cadherin usually represented a pre-invasive dysplastic transformation. P-cadherin was expressed from ACF to hyperplastic and adenomatous polyps, and was prior to and independent of disturbance in E-cadherin and β-catenin expression in ACF. P-cadherin was aberrantly expressed at the earliest stage of aberrant crypt formation, before the disturbance in E-cadherin and β-catenin. β-catenin, which was originally discovered as a cadherin-binding protein, has been proved to function as a transcriptional activator. Inactivation of β-catenin with the product of adenomatous polyposis coli (APC) gene highlighted a role of catenins in epithelial tumorigenesis. Target genes of the β-catenin-Tcf pathway were determined to be growth-promoting genes, such as c-myc and cyclin D1, suggesting β-catenin-Tcf pathway was oncogenic. Excessive β-catenin protein has been shown in colon cancers of rats and humans, and the aberrant expression of β-catenin in ACF was also seen. It was reported that in most ACF, namely ACF with hyperplasia, β-catenin was localized at the cell membrane like normal colon epithelium, and in ACF with dysplasia, reduced membranous expression of β-catenin was associated with increased nuclear and cytoplasmic expression. The membranous expression of β-catenin was reduced, and cytoplasmic and nuclear expression increased in ACF according to their degrees of dysplasia. Likewise, membranous expression of β-catenin was reduced, and the nuclear expression increased from ACF to adenoma and carcinoma, strongly suggesting that ACF and their aberrant expression of β-catenin played an important role in colorectal carcinogenesis, and the immunohistochemical staining of ACF for β-catenin could evaluate the malignant potential of ACF.

In carcinogen-treated animal carcinomas, it has been reported frequent mutation and altered cellular localization of β-catenin, and inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) were also found to be expressed in these carcinomas. In carcinogen-treated animal colon, an altered cellular localization of β-catenin in all dysplastic ACF, adenomas and adenocarcinomas was shown, and iNOS expression was also observed in all but one of the lesions in which β-catenin alterations were observed. Neither iNOS expression nor β-catenin alterations were observed in any hyperplastic ACF. Nitric oxide (NO) was known to cause DNA damage and nitrosylation of proteins, and increased production in tumor cells would be expected to facilitate accumulation of sequential mutations. Since altered localization of β-catenin was apparent in all lesions expressing iNOS, it might be possible that β-catenin alteration was related to induction of iNOS expression. The positive expression of iNOS in dysplasia, but not hyperplastic ACF suggested that iNOS, like β-catenin, could be important at the early stages of tumor formation, and the cause of dysplastic changes. There was also an overexpression of COX-2 detected in ACF, adenomas and carcinomas, which has been demonstrated to render tumor cells resistant to apoptosis and to enhance neovascularization, thus conferring a survival advantage. The overexpression of COX-2 may contribute to ACF growth and sequential tumor growth.

p16 expression was seldom seen in epithelial cells at the base of normal colonic crypts. But at all the stages of tumor progression, including ACF, a higher fraction of epithelial cells was seen to express p16INK4a. The staining of p16INK4a correlated inversely with that of Ki67, cyclin A, and the retinoblastoma protein in ACF, adenomas and carcinomas, suggesting that cell cycle progression was inhibited. Thus, p16INK4a appeared to constrain the proliferation of subsets of cells throughout intestinal tumorigenesis, however, the exact mechanisms remain unclear.

GENETIC AND EPIGENETIC ALTERATIONS OF ACF

Further development of ACF described by Gregorio had two pathways. One was headed for dysplastic ACF, which were to progress to adenomas, and the other was headed for hyperplastic polyps, which had little malignant potentials. The investigation of ACF by using 25 different genetic markers, such as microsatellite instabilities and mutations of APC, H-ras, k-ras, p53, DCC, and DNA repair genes hMLH1, hMSH2, showed no difference between hyperplastic ACF and normal mucosa, which was in accordance with the latter hypothesis. Dysplastic ACF have been further identified to be precancerous lesions of CRC. However, an alternative pathway to the general adenoma-carcinoma sequence was also proposed as a hyperplastic polypl/serrated adenoma-carcinoma one. Nucci’s study showed genetic differences between hyperplastic ACF and hyperplastic polyps in that the former had more frequent K-ras mutations. Therefore hyperplastic ACF should be named as “heteroplasia ACF” (“hetero” meaning “other”, and “plasia” meaning “form”) to avoid potential misleading as to their relationships. Though the role of heteroplastic ACF in colorectal carcinogenesis remains controversial because of the lack of dysplasia in spite of the high frequency of K-ras mutations, there have been still lines of evidences supporting heteroplastic ACF as a precursor to a subset of CRC. They were clonal and had genetic alterations of K-ras mutations, chromosome 1p loss, and CpG island methylation, hence the heteroplastic ACF-adenoma-carcinoma sequence, in which K-ras mutations preceded APC mutations in rodents and humans.

Stopena identified K-ras point mutation in carcinogen-induced colonic aberrant crypts in Sprague-Dawley rats in 1992 for the first time, and numerous studies have proved K-ras mutation as one of the major events in ACF formation since then. The mutation rate of K-ras in ACF was similar to that of small adenomas, and was found to be even significantly higher than that identified in CRC. K-ras 12 codon mutation was most frequently observed, and in Losi’s study, different mutational types of GTT mutation, occurred in ACF of the same patient. There were also different mutational types between carcinomas and ACF, the former had predominant GTT mutation, while the latter had GAT mutations almost as frequently as GTT in codon 12 of K-ras. Therefore each ACF might originate independently from different clones.

Identification of monoclonality in a putative precursor lesion would strongly link this lesion to neoplastic progression. The monoclonality of various degrees of dysplastic ACF was determined by studying the differential methylation of a highly polymorphic site in the first exon of the androgen receptor gene to determine the pattern of X chromosome inactivation. Identification of monoclonality in a putative precursor lesion would strongly link this lesion to neoplastic progression. The monoclonality of various degrees of dysplastic ACF was determined by studying the differential methylation of a highly polymorphic site in the first exon of the androgen receptor gene to determine the pattern of X chromosome inactivation. Controversial results were produced also by using the same method of clonality analysis based on X chromosome inactivation of the polymorphically X-linked human androgen receptor (HUMARA) gene, in addition to K-ras mutation detection. It was observed that a significant fraction of individual aberrant crypts that made up an ACF to be polyclonal, although by K-ras mutation genotyping, all ACF appeared to be monoclonal.

Other oncogenes as COX and c-myc were also found to
have an increased mRNA or protein expression in carcinogen-induced rats[18,88]. The immunoreactivity of oncoproteins of c-fos, ras, bcl-2 and p53 was evaluated in ACF, and abnormal expression and coexpression of oncoproteins could be identified in colorectal tumorigenesis at the earliest stages[89].

APC gene is considered to be “gatekeeper”, maintaining the stability of colon epithelium. In carcinogen-treated rats, a decreased mRNA expression of APC was observed[19], APC mutations could be detected in human ACF, but the mutation rates of APC in ACF were lower or undetectable compared to those in adenomas and carcinomas[26,41,90], which were the same as in animal models[83], suggesting that APC mutation was unlikely to initiate ACF. If a ras gene mutation occurred first, ACF would be nondysplasia, and if an APC mutation was the first to occur, it would result in a dysplastic ACF, whose progression was driven by subsequent K-ras mutations[19]. It has been proposed that in sporadic colorectal carcinogenesis, assuming the biological potential of ACF as a precursor of adenomas, there was a route where K-ras mutations mainly occurred during the formation of ACF. Some ACF then became adenomas in which APC mutations occurred. In FAP, however, somatic mutations of APC predominantly occurred during ACF formation, followed by K-ras mutations[19]. Apart from oncogene and tumor suppressor gene alterations in ACF, there is some other kind of genes concerning DNA repair. Inactivation of the mismatch repair (MMR) system can result in instability of the whole genome and an increased rate of spontaneous mutations of other vital genes to carcinogenesis. In addition to the cause of genomic instability, DNA mismatch repair proteins have several other functions that are highly relevant to cancer progression. Some MMR components have been found to be involved in cell-cycle regulation, and p53-dependent apoptotic response to a variety of DNA damages[91-97]. Germline mutations of any of them, especially hMsh2 and hMLH1, gave rise to hereditary nonpolyposis of colorectal cancer (HNPPC)[98,99]. There was also a link between hMsh6 mutations and a high incidence of endometrial cancer[99], and prevalence of colon cancer with TGFβRII gene and Tcf-4 gene[100,101], hMsh2 deficiency resulted in the development of many ACF in mice colons, as well as reduced survival of the mice[102].

Epigenetic alterations such as CpG island methylation, and genetic phenotype alterations such as microsatellite instability (MSI) as a result of MMR defect, also play important roles as in ACF. Inactivation of genes might occur not by mutation or loss, but through silencing mediated by CpG island methylation of the gene’s promoter region. hMLH1 and MGMT are examples of DNA repair genes that were silenced by methylation. It had been demonstrated[103-105] that hMLH1 gene promoter had aberrant methylation in sporadic CRC. A novel pathway characterized by methylation of multiple CpG islands in colorectal carcinomas and adenomas, including genes known to be vital in tumorigenesis, such as p16 tumor suppressor gene and hMLH1 mismatch repair gene, was proposed as a CpG island methylator phenotype (CIMP)[97,106]. Ahuja questioned whether hMLH1 methylation preceded or was a consequence of the MSI phenotype[107]. The answer was presented by Chan that methylation of hMLH1 mismatch repair gene in ACF was not associated with the development of MSI, suggesting that hMLH1 methylation preceded MSI[104]. The frequency of CpG island methylation in ACF was related to the type of patients, as was concluded in Chan’s research, that methylation was detected in 53 % of ACF from sporadic colorectal carcinomas but only in 11 % of ACF from FAP patients[104], and methylation was more frequent in dysplastic sporadic ACF than in dysplastic FAP ACF. The epigenetic differences of CpG island methylation in ACF were similar to those in colorectal carcinomas.

Extensive genomic instabilities due to the defect of MMR system could be detected as MSI both in animal model and in human ACF[108,109]. MSI could occur either in dysplastic or hyperplastic (or “heteroplastic” as mentioned before) ACF from HNPCC patients, suggesting no association of MSI with histological features, being present even in small ACF. MSI in sporadic ACF also seemed to be independent of ACF size[110]. Heinen et al.[43] detected MSI status in ACF, and found two ACFs from the same patient with different MSI status. One showed MSI-positive, while the other was MSI-negative, indicating independent initiation of individual ACF, as has been verified by sequential studies with different methods[26,30,34,81]. The role of ACF as precursors of some CRCs was verified by the presence of MSI in ACF from HNPCC patients, in whom carcinoma with high levels of MSI was the hallmark, and in ACF from sporadic CRC as well[104,108].

SUMMARY
The malignant potential of ACF, especially dysplastic ACF, makes it necessary to reveal the nature of these lesions. Many researches nowadays are focused on the mechanisms of ACF initiation, their genetic and environmental backgrounds, methods of early detection, dietary and chemoprevention. In spite of the increasing evidences of ACF as precancerous lesions of colorectal carcinomas, a lot remain to be clarified so as to reduce the incidence of CRC at the earliest stages.

First reported by Yokota in 1997[40], the application of endoscopy, either magnifying or dye/chromaticoscopy, made it possible to detect “in vivo” ACF. It is of great advantage over the detection of surgical specimens from with CRC. It may offer much benefit to colon cancer researches and identify high-risk populations for colorectal tumors. Recent studies have proved the validity of chromoendoscopy, or dye colonoscopy, which allows easy detection of mucosal lesions in the colon and facilitates visualization of the margins of flat lesions[111,112].

In summary, ACF, especially dysplastic ACF, are widely accepted as precursors of colon cancer morphologically, histologically, biologically, and genetically. They have an aberrant appearance of crypts, and display both hyperplasia and dysplasia. They are supposed to have alterations of enzymology and cell dynamics. They also harbor gene mutations that are vital to tumor formation and progression. By studying these lesions both grossly and genetically, it may be possible to learn more about the causes of colon carcinogenesis. By testing new compounds through ACF assay, it is possible to discover not only potentially new chemopreventive drugs, but also the mechanisms behind them. In addition, by developing available methods for “in vivo” ACF examination, it may be possible to evaluate high-risk individuals at the earliest possible stages, as well as the preventive procedures.

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