COMMENTARY

Pre-cancerous lesions for colorectal cancers in rodents: a new concept

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It is widely believed that colorectal carcinogenesis is a representative multi-step tumorigenesis with events of genetic alterations. Aberrant crypt foci (ACF) recognized on the surface of cancer-predisposed colons of rodents have been regarded as early-appearing pre-neoplastic lesions. However, it is not clear if such lesions are truly pre-cancerous lesions for colorectal cancers in rodents. Recently, β-catenin-accumulated crypts (BCAC) were identified in colonic mucosa at the early stages of colon carcinogenesis. Accumulating evidence indicates that they are independent small dysplastic lesions of ACF. Here we discuss the importance of BCAC as pre-cancerous lesions in colon carcinogenesis.

Aberrant crypt foci in the colon

Many colorectal cancers are thought to develop through a series of histological tumorigenesis. Such an advent of epithelial change in the colon has been proved to accompany genetic alteration (1), and colon carcinogenesis is now regarded as a multi-step event with genetic alterations. In humans, adenomatous polyposis coli (APC), β-catenin (CTNNB1), Ki-ras (KRAS1) oncogene and p53 (TP53) genes are thought to play important roles at different stages of colorectal carcinogenesis (2–4). Aberrant crypt foci (ACF) are early-appearing lesions recognized in the colonic surface of rats treated with colon-specific carcinogens like azoxymethane (AOM) (5). ACF were first described by Bird (6) and defined as crypts that: (i) have altered luminal openings; (ii) exhibit thickened epithelia; and (iii) are larger than adjacent normal crypts (Figure 1A and B). Numerous studies including molecular analysis have focused on the significance of ACF in early events in colon carcinogenesis (5). It is known that the number of crypts of ACF increases with time after the carcinogen treatment, that they have an increased proliferative activity and some ACF reveal histological dysplasia (5). Therefore, ACF are now regarded as putative pre-neoplastic lesions for colon cancers and are used to evaluate potential chemopreventive agents against colon carcinogenesis (7). Nevertheless, there is evidence that documents the lack of correlation between tumor development and expression of ACF (8,9). The significance of ACF during colon carcinogenesis remains inconclusive so far.

β-Catenin signaling pathway plays a critical role in colon carcinogenesis

During the last decade, it has become increasingly apparent that the β-catenin signaling pathway is closely associated with the development of colon cancer. The β-catenin protein and its Drosophila homolog armadillo have been established as critical downstream factors in the Wnt signaling pathway in vertebrates and the conserved Drosophila Wingless pathway (10). Glycogen synthase kinase 3, when complexed with the APC tumor suppressor protein and the axin or conductin proteins, phosphorylates specific serine and/or threonine residues in the N-terminus of β-catenin (11,12). Phosphorylation of these N-terminal sequences of β-catenin promotes its interaction with F-box proteins and subsequent ubiquitination and rapid degradation by the proteasome (13,14). When the β-catenin protein escapes from degradation and accumulates in the nucleus, it binds to T-cell factor (TCF) or lymphoid-enhancer factor (Lef) proteins and stimulates transcription of TCF/Lef-target genes (15). Recently, the target genes of the β-catenin signaling pathway were determined as growth-promoting genes, such as c-myc and cyclin D1, suggesting that the pathway is potentially oncogenic (16,17). As might be predicted from such models of β-catenin function in the Wnt pathway, bi-allelic inactivation of the APC gene or mutations in the β-catenin gene in colon cancers give rise to stabilization of β-catenin, its translocation to the nucleus and constitutive activation of TCF/Lef transcription (4). These alterations finally lead to activation of the oncogenic β-catenin signaling pathway. It is now apparent that the majority of colon cancers in both humans and rodents have alterations in the Apc gene or β-catenin gene, which lead to β-catenin accumulation (18,19). Furthermore, it has recently been indicated that expression of nuclear β-catenin is correlated with the size of colon neoplasms (20).

β-Catenin-accumulated crypts and ACF in cancer-predisposed colon

In recent investigations for pre-malignant lesions of colon cancer, it was found that the β-catenin gene mutation and accumulation of the protein are involved in small dysplastic crypts on histological sections of cancer-predisposed colon in rats, and the dysplastic crypts with excessive β-catenin were designated as β-catenin-accumulated crypts (BCAC) (Figure 1C and E) (21,22). Principally, BCAC have histological dysplasia with disruption of cellular morphology. It is also known that the grade of BCAC dysplasia increases with time after the carcinogen treatment. Some of the BCAC are recognized as adenomatous crypts with extensive branchings (22). Interestingly, in the whole mount colons, BCAC do not have a typical ACF-like appearance, which was originally characterized by Bird (6,22). The majority of BCAC is not recognized in the mucosal surface, and is identified only in the histological sections of en face preparations. In the en face

Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; APC, adenomatous polyposis coli; BCAC, β-catenin-accumulated crypts.
sections, typical ACF do not represent excessive β-catenin protein (Figure 1D), which is associated with the majority of colon cancers and suggested to play a critical role in colon carcinogenesis (21). These results suggest that BCAC are independent early-appearing lesions of ACF. It is noteworthy that BCAC have a higher grade of histological dysplasia and proliferative activity than ACF (Figure 1B and C) (22). Thus, it is reasonable to conclude that BCAC are more likely to progress into malignant transformation than ACF.

Fig. 1. (A) Mucosal topography of rat colon treated with AOM. Arrows indicate ACF that have large crypts, altered luminal openings and thickened epithelium. (B and C) Histological sections of ACF (B) and BCAC (C). In upper sections, it is clear that crypts in ACF are larger than adjacent normal crypts, whereas crypts in BCAC are not. (B) (Inset) Mucosal topography of the corresponding ACF. (D and E) β-Catenin immunohistochemistry of ACF (D) and BCAC (E). Accumulation of β-catenin is apparent in BCAC. Crypts in ACF show only membranous staining of β-catenin, which is the same staining in surrounding normal crypts. (F) β-Catenin immunohistochemistry of microadenomatous crypts in Apc min/+ mouse. Microadenomatous crypts have the increased expression levels of β-catenin protein, indicating that they are the identical lesions with BCAC. (G) Mucosal topography of the colon of Apc min/+ mouse. Note that there are no ACF identified in the mucosal surface.
Paneth cell differentiation of BCAC and colon tumors

It is also interesting to note that Paneth cells are frequently associated with BCAC (22). In fact, mature Paneth cells are sometimes present in colonic tumors, whereas they rarely occur in normal colonic epithelium. Consistent with this, lysozyme expression, which is considered to be a marker for Paneth cell lineage (23), is prominent in both BCAC and colon cancers (unpublished data). Despite extensive examinations, the biological role of Paneth cells has not yet been defined clearly. However, Paneth cells are known to provide a number of cell growth-related factors, such as tumor necrosis factor-α, guanylin, epidermal growth factor and matrilysin (24–27). Of them, matrilysin has been shown to play a role in the early stage of intestinal carcinogenesis (28), suggesting that Paneth cell metaplasia could promote intestinal carcinogenesis. Indeed, we confirmed that BCAC have an increased expression of matrilysin at both mRNA and protein levels (unpublished data). These findings indicate a differentiating potential of BCAC towards the Paneth cell lineage and the results suggest that BCAC have the similarity to colon cancers in terms of their biological characteristics. It may be possible that Paneth cell differentiation could be a molecular target for early detection of colon cancers.

Genetic alterations in BCAC and ACF

It was shown that the most common mutation of the β-catenin gene seen in BCAC is the same and representative type detected in colon tumors (21,29). Such genetic evidence seems to support the notion that BCAC is the pre-malignant lesion and implies that these mutations play gatekeeper roles in the development of colon tumors. The results that BCAC have β-catenin gene mutations more frequently than typical ACF may therefore indicate the potential of BCAC to progress into malignant lesions. It is also interesting that a portion of ACF harbors the mutation in the β-catenin gene and lack accumulation of β-catenin (21,29). Such data suggest that although the β-catenin mutation itself is insufficient for the detectable accumulation of β-catenin, a part of ACF with β-catenin mutations may possess neoplastic potential leading to colon cancers. Thus, it is conceivable that other genetic and/or epigenetic alterations relating to the translocation of β-catenin into the cytoplasm and/or nuclei are present in BCAC, but not in most ACF.

Meanwhile, K-ras mutations are recognized in >70% of total ACF in rats treated with AOM (29). Such a fact is consistent with reports in which the majority of human nondysplastic ACF have K-ras mutations (30,31), indicating that K-ras mutations are closely associated with the formation of typical ACF. It is important that mouse strains carrying oncogenic alleles of K-ras develop ACF in the colon (32), suggesting again that K-ras activation may be implicated in the formation of ACF.

Early dysplastic lesions are not always pre-malignant lesions

The advent of epithelial dysplasia has been indicated to reflect the frequency of genetic alteration in the process of colon carcinogenesis as these in other organs, and numerous reports have suggested that colonic dysplasia can be a hallmark of malignant potential (1). Furthermore, this evidence seems to lie at the base of the concept of multi-step carcinogenesis in the colon. Although frequent mutations in the β-catenin gene are confirmed in both BCAC and cancers in the colons of rats (19,29), it must be noted that mutational spectrum of early-appearing lesions is different from that of colon cancers. It is already clear that all mutations detected in colon cancers converge at codons encoding functionally important residues that may directly mediate β-catenin degradation (14,33), whereas mutations in the early dysplastic lesions were scattered in the same exon of the gene. These findings suggest that, although all BCAC express histological dysplasia (22), the only lesions with the specific type of the mutations will preferentially progress into colon cancers. If the early dysplastic lesions possessed β-catenin mutations without the specific site, they will not be regarded as genuine pre-malignant lesions. It may be true that the grade of epithelial dysplasia represents not only the frequency of genetic alterations but also the degree of mutational activation of the oncogenic pathway. The concept might provide a novel insight into the multi-step carcinogenesis theory, especially in phenotypic terms.

Microadenomatous crypts in the colon of ApcMin/+ mice

Mutant mouse lineage being predisposed to multiple intestinal neoplasms (Min) is regarded as one of the models for colorectal tumorigenesis (34). Originally, this lineage was established from an ethylnitrosourea-treated C57BL/6J (B6) male mouse, and its phenotype is a fully penetrant autosomal dominant trait. The dominant mutation is known to be located in Apc, the mouse homolog of the human APC gene, resulting in truncation of the gene product at amino acid 850 (35). It is suggested that, although homozygous ApcMin/Min mice die as embryos, ApcMin/+ mice develop multiple intestinal neoplasias in the intestinal tracts within a few weeks after birth. However, it is well demonstrated that the distributing pattern of intestinal tumors in ApcMin/+ mice is different from that in human cases. Importantly, most adenomatous polyps in humans arise in the colon, whereas the highest frequency of tumors in ApcMin/+ mice is seen in the small intestine (36). Very recently, our group found that there are a large number of microadenomatous lesions in the colonic mucosa of the ApcMin/+ mice (37). Such microadenomatous crypts in the colon were found to have lost the remaining allele of Apc, indicating that loss of Apc function has already occurred in such crypts. Accordingly, it seems to be reasonable to apply Knudson’s ‘two-hit’ theory (38) to the formation of microadenomatous lesions in the colon of ApcMin/+ mice.

It is also interesting that almost all intramucosal adenomatous lesions in the colon were <300 μm in their greatest dimension (37). As ApcMin/+ mice used in the experiment were older than 20 weeks of age, such microadenomatous crypts are suggested to be self-limiting lesions and not grow into colonic tumors. The results strongly suggest that inactivation of the Apc is not sufficient for development of colonic tumors. The previous report demonstrating that only small numbers of tumors occur in conditional knockout mice of the Apc, also supports this notion (39).

Accumulation of β-catenin in microadenomatous crypts and absence of ACF in ApcMin/+ mice

The importance of BCAC in early stages of colon carcinogenesis is highlighted by the recent findings derived from mouse
models for colon carcinogenesis. We have identified the presence of BCAC in cancer-predisposed colons of mice treated with AOM (unpublished results). Additionally, in Apc<sup>Min/+</sup> mice, microadenomatous crypts involving Apc LOH possess prominent accumulation of β-catenin protein, indicating that the microadenomatous crypts are the identical lesions with BCAC in rats (Figure 1F). Furthermore, it should be noted that no typical ACF are recognized in the mucosal surface of the colon of Apc<sup>Min/+</sup> mice (Figure 1G) (40), indicating again that BCAC are independent lesions of ACF in rodents. Such results imply that the activation of β-catenin signaling pathway is involved in the formation of BCAC, whereas the K-ras activation is associated with the formation of ACF. Interestingly, the contribution of BCAC and ACF to colon carcinogenesis could be appreciated by examining the phenotype of Apc<sup>Min/+</sup> mice and mice carrying oncogenic alleles of K-ras (32). It is important to note that Apc<sup>Min/+</sup> mice develop numerous intestinal tumors as well as BCAC, whereas activated K-ras mice develop only ACF but no tumors in their colon.

ACF and BCAC as biomarkers for colon carcinogenesis

ACF have been utilized as biomarkers to evaluate a number of agents for their potential chemopreventive properties. However, some compounds like 2-(carboxyphenyl) retinamide or genistein with a potency to prevent occurrence of ACF were or genistain with a potency to prevent occurrence of ACF were found to enhance development of colon cancers (8,9). Very prominently accumulation of ACF in rats (Figure 1F). Furthermore, it should be noted that BCAC and ACF as biomarkers for colon carcinogenesis involved in the formation of BCAC, whereas the K-ras activation is associated with the formation of ACF. Interestingly, the contribution of BCAC and ACF to colon carcinogenesis could be appreciated by examining the phenotype of Apc<sup>Min/+</sup> mice and mice carrying oncogenic alleles of K-ras (32). It is important to note that Apc<sup>Min/+</sup> mice develop numerous intestinal tumors as well as BCAC, whereas activated K-ras mice develop only ACF but no tumors in their colon.

Pre-malignant colon lesions in humans

Similar lesions as rodent ACF have been detected in the mucosal surface of human colon (42). Pretlow and Bird (43) have emphasized the role of ACF in the early stages of human colorectal carcinogenesis, and ACF are now also considered to be pre-neoplastic lesions for human colorectal cancers. Especially, they proposed the concept of ‘dysplastic ACF’ and insisted that such lesions are truly pre-malignant lesions. Indeed, in contrast to the findings in rodents, some human ACF have apparent accumulation of β-catenin (44,45).

In the human colon, orifices of the colonic crypts have been referred to as ‘pits’, and the specific arrangement of the openings of the colonic glands is called the ‘pit pattern’. Kudo et al. (46,47) classified the pit patterns into five types: type I, round pit detected in the normal mucosa; type II, asteroid; type III, tubular or round pit, which is larger than a normal pit (type I); type III, tubular or round pit, which is smaller than a normal pit (type I); type IV, dendritic or gyrus-like pit; and type V, irregular or amorphous pit. Applying their classification, typical ACF seem to correspond to type II and type III pit patterns. In the previous studies, types III and V pit patterns, which seemed to be different lesions from ACF, had stronger dysplasia than type II and type III pit patterns (typical ACF pattern) (46,47). It is noteworthy that some lesions with type III and type V pit patterns are smaller than ACF (48). The evidence strongly suggests that, as the rodent cases, there will be small dysplastic lesions without typical ACF appearance and such lesions could be direct precursors for colon cancers in humans. Further investigations—including those for intramusosal lesions, which cannot be identified in mucosal surface of colons—are necessary to understand genuine pre-malignant lesions in humans.

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