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Mini Review

Molecular mechanism of colitis-associated colorectal carcinogenesis

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The development of colorectal cancer caused by chronic inflammatory bowel disease (IBD) is the representative example of inflammation-associated carcinogenesis. The mechanism underlying the development of colorectal cancers through chronic inflammation, however, is not known. Activation-induced cytidine deaminase (AID) was originally identified as an inducer of somatic hypermutation in the immunoglobulin gene. We recently demonstrated that the mutagenic activity of AID expression links colonic inflammation to the development of colitis associated colorectal cancers. Immunohistochemistry revealed enhanced expression of endogenous AID protein not only in the inflamed colonic mucosa of ulcerative colitis patients, but also in tumor lesions of colitis-associated colorectal cancers. Pro-inflammatory cytokine TNF-α and/or T helper cell-2-driven cytokines IL-4 and IL-13 induced aberrant expression of AID in human colonic epithelial cells. *In vivo*, aberrant AID expression in the inflamed colon is associated with the accumulation of somatic mutations in tumor suppressor *Trp53* gene, and AID deficiency resulted in a reduced incidence of colitis-associated colon cancers. These findings suggested that pro-inflammatory cytokine-mediated aberrant expression of AID in colonic epithelial cells plays a role as a genotoxic factor that enhances genetic instability during chronic colonic inflammation, leading to colitis-associated colorectal cancer development.

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Nonstandard abbreviations used:
AID, activation-induced cytidine deaminase; TNF, tumor necrosis factor; NF-κB, nuclear factor κB; IKK, IκB kinase; IL, interleukin

Key words AID, inflammatory bowel disease, colitis associated colorectal cancer, cytokine, TP53

Introduction

A causal relationship between inflammation and cancer development is proposed in a variety of chronic inflammatory diseases. In particular, many cancers of gastrointestinal organs, some of which are caused by infectious agents, are known to arise in a background of chronic inflammation. Inflammatory bowel disease (IBD) is an important etiologic risk factor for the development of colorectal cancer[1] and the incidence of colorectal cancer is significantly higher in patients with IBD than in the general population. For example, the relative risk of colorectal cancer in patients with ulcerative colitis (UC) is 20 times higher than that in the general population[2], and the cumulative risk of colitis-associated colorectal cancer (CAC) increases according to the number of years after the disease onset. The cumulative probability of cancer in patients with UC regardless of the disease extent is 2% at 10 years, 8% at 20 years, and 18% at 30 years[3]. Patients with extensive colitis, colitis lasting 8 years or more, more severe inflammation, and early-age onset colitis have the greatest risk of developing cancer[4]. Recently, several studies showed that not only patients with UC but also those with Crohn's disease (CD) are at risk of developing colon cancer[5]. There is an 18-fold increase in the risk of developing colorectal cancer in patients with CD compared with the general population[6]. The absolute cumulative risk frequency for developing colorectal cancer is 8% at 22 years from the onset of symptoms in patients with CD. Surveillance colonoscopy is frequently used to detect early cancer lesions in patients with IBD. Surveillance programs are not sufficiently effective to prevent cancers, however, because its diagnosis is difficult. To improve the detection of dysplastic lesions, the mechanisms by which chronic inflammation of the mucosa increases the risk of colorectal cancer development should be examined.

Nucleotide-editing enzymes that can induce mutations in DNA

Genetic changes, such as nucleotide alterations and chromosomal translocation occurred in oncogenes and tumor-suppressor genes, have an important role in cancer development. In this aspect, CAC is different from sporadic colorectal cancer that originates from colorectal adenomatous polyps in the molecular pathogenesis of cancer development[7]. Adenomatous polyps are the major precursor of sporadic colorectal cancer and inactivation of the *APC* gene is known to be the initial event in many sporadic colorectal cancer, followed by changes in the *K-ras*, *DCC*, and *TP53* genes[8]. In contrast, mutations in the *TP53* gene appear to be an early event and already present in the colonic mucosa of patients with UC before CAC onset[9, 10]. The molecular mechanisms underlying the development of the *TP3* mutations in chronic inflamed mucosa that lead to carcinogenesis, however, are not known.

Several molecules that possess nucleotide editing activity were recently identified. These molecules are called nucleotide editing enzymes and include the apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) family[11]. The APOBEC family molecules are thought to have an important role in maintaining homeostasis and the immunologic response by inducing somatic mutations in targeted DNA or RNA sequences. Among the APOBEC family molecules, only activation-induced cytidine deaminase (AID) induces genetic changes in human DNA sequences[12]. AID is expressed only in activated B cells under physiologic conditions and contributes to two unique molecular mechanisms for antigen-driven immunoglobulin (Ig) gene diversification[13, 14]. These mechanisms include somatic hypermutation (SHM) and class switch recombination (CSR). SHMs are point mutations introduced into the variable (V) region of Ig gene at a high frequency, leading to the production of a variety of high-affinity antibodies. For this purpose, AID deaminates cytidine (C) on target DNA to produce a thymidine (T), leading to the generation of changes in human genome DNA sequences. The activity of AID as a genome mutator raises the question of whether AID induces inappropriate mutations in non-Ig genes. We recently demonstrated that the expression of AID links inflammation to the development of human gastrointestinal and hepatobiliary cancers[14-20].

Under physiologic conditions, AID expression is restricted to activated B cells, but we observed aberrant expression of AID in liver tissues exposed to chronic inflammation[16]. Immunohistochemistry and real time-polymerase chain reaction (PCR) showed that AID expression is not normally detected in hepatocytes of noninflamed liver, but endogenous AID expression is significantly elevated in liver tissues with chronic hepatitis and liver cirrhosis. Moreover, aberrant AID expression is induced in response to pro-inflammatory cytokine stimulation or hepatitis C virus infection via the nuclear factor (NF)-κB signaling pathway in human hepatocytes[17]. Similarly, aberrant expression of AID

is triggered by *Helicobacter pylori* infection or pro-inflammatory cytokine stimulation in human gastric epithelial cells[15, 21]. We also found that endogenous AID expression is also induced after treatment with pro-inflammatory cytokines in both human biliary cells and esophageal epithelium[22]. These findings indicate that aberrant AID expression could be commonly induced in response to inflammatory stimulation in gastrointestinal epithelial cells.

Pro-inflammatory cytokines are involved in the regulation of AID expression in human colonic epithelial cells

To study the expression of AID protein in human colonic tissues, we performed immunohistochemistry in human colonic tissues from UC lesions, colitis-associated neoplasms, and nontumorous regions of patients with sporadic colon cancers. In normal colonic mucosa, no immunostaining for AID was observed. In contrast, in UC tissues, immunoreactivity for AID was detected in colonic epithelial cells as well as in infiltrating lymphocytes around the inflamed colonic mucosa[23]. In the colitis-associated neoplasms, AID protein expression was also observed in neoplastic cells in the tumor lesions. These findings indicated that AID protein is expressed in the colonic epithelial cells that are chronically inflamed and in tumor cells of CAC.

To determine whether pro-inflammatory cytokines regulate AID transcription in human colonic epithelial cells, we analyzed the expression level of endogenous AID by quantitative reverse transcription-PCR and immunoblotting in cultured human colon cancer cells. First, we focused on tumor necrosis factor (TNF)-α, which is constitutively activated in the colonic epithelial cells of patients with UC. Only a small amount of endogenous AID expression was detected in quiescent human colonic epithelial cells, but AID expression was markedly elevated after TNF-α treatment. The transcription factor NF-κB is activated by TNF-α signaling[24]. We therefore examined whether AID expression is regulated by the NF-κB signaling pathway in human colon cancer cells. Expression of the positive NF-κB regulators IKKα, IKKβ, and NF-κB itself resulted in an increased expression of endogenous AID protein. A negative regulator of NF-κB, the super-repressor form of IκBα, reduced TNF-α mediated AID expression. We also revealed that AID expression is regulated by IL-4 and IL-13 in a STAT6-dependent manner in human colonic epithelial cells. These findings support the idea that AID expression in colonic epithelial cells is regulated through the NF-κB-dependent and STAT6-dependent signaling pathway.

AID expression in colonic epithelial cells results in an accumulation of TP53 mutations

The findings that AID expression is induced in colonic cells with chronic inflammation prompted us to examine whether aberrant AID expression could lead to the generation of somatic mutations in tumor-related genes in human colonic epithelial cells. We established a cultured human colonic cell line with constitutive AID expression using a retroviral system, and investigated the mutation frequencies in the *TP53*, *APC*, and *K-ras* genes of colonic epithelial cells in which AID was overexpressed for 8 weeks. No change or only a single nucleotide alteration was detected in all genes from cells transfected with control vectors. In contrast, however, several nucleotide alterations appeared in the *TP53* gene after AID activation[23]. Mutation frequencies in the *TP53* gene indicated that AID expression increased the mutations in a time-dependent manner. In contrast to the *TP53* gene, no nucleotide alterations were detected in the *APC* and *K-ras* genes, even after 8 weeks of AID activation. These findings indicate that aberrant AID expression in human colonic epithelial cells preferentially targets the *TP53* gene and longer AID activation might induce more mutations. Why the *TP53* gene is more sensitive to AID in human colonic epithelial cells remains unknown. Further studies are required to identify the specific target genes of AID in human colonic epithelial cells. Those genes may become new markers for predicting the development of CAC lesions.

AID deficiency reduced both an accumulation of *TP53* mutations and incidence of CAC development

To clarify whether AID is a crucial mediator of the genetic alterations required for inflammation-mediated carcinogenesis, we recently investigated the impact of AID deficiency in the pathogenesis of colitis-associated colon cancer using IL-10$^{-/-}$ mice, a representative model of human IBD. We first confirmed that persistent inflammation in the cecum of IL-10$^{-/-}$ mice is closely associated with the enhanced production of various inflammatory cytokines such as such as TNF-α, IL-1β, and IL-6, leading to the

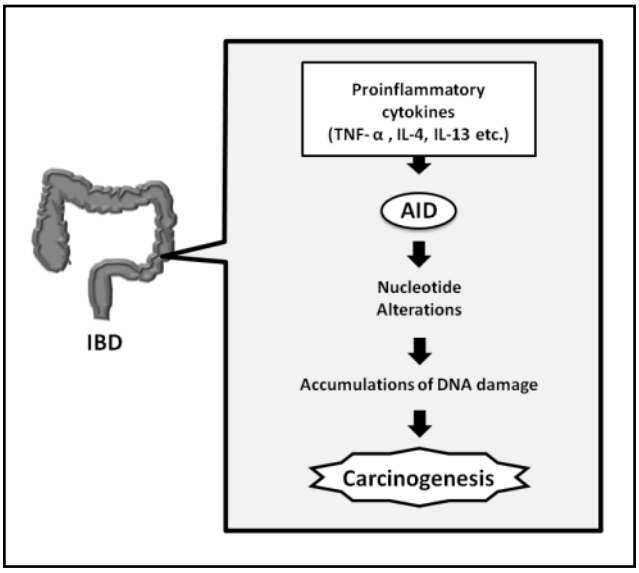

Figure AID links inflammation to cancer development in colitis-associated colorectal carcinogenesis

Proinflammatory cytokines induce aberrant AID expression in human colonic epithelial cells. Constitutive AID activation in these epithelial cells results in the accumulation of DNA damage.

induction of aberrant AID expression in inflamed colonic mucosa. Consistent with the previous report, we found that nucleotide alterations had accumulated in the *Trp53* gene in the inflamed cecal mucosa of the IL-10$^{-/-}$AID$^{+/+}$ mice. In contrast, the mutation frequency of the *Trp53* gene in the inflamed epithelial cells of IL-10$^{-/-}$AID$^{-/-}$ mice was significantly lower than that of IL-10$^{-/-}$AID$^{+/+}$ mice, suggesting that the accumulation of genetic changes in the *Trp53* gene of the inflamed colonic mucosa was due to AID activity. Notably, invasive adenocarcinomas were detected in 6 of 22 IL-10$^{-/-}$AID$^{+/+}$ mice and all the tumors characteristically developed from the dysplastic mucosa in the cecum. In contrast, IL-10$^{-/-}$AID$^{-/-}$ mouse developed no tumors in the inflamed colonic mucosa except only one non-invasive tumor in the distal colon. These findings clearly indicated that aberrant AID expression in the inflamed colon is associated with the accumulation of somatic mutations in tumor suppressor *Trp53* gene, and AID deficiency resulted in a reduced incidence of colitis-associated colon cancers.

Conclusion

Until recently, the precise molecular mechanism underlying cancer development due to the chronic inflammation has been unclear. The findings of the present study showed that pro-inflammatory cytokines, which play important roles in the pathophysiology of IBD, result in the aberrant expression of AID in human colonic epithelial cells, and lead to the generation of somatic mutations in the *TP53* gene (Figure). This is new evidence that might link chronic inflammation of the colonic mucosa to the accumulation of *TP53* mutations, leading to the development of colorectal cancer.

Conflicts of interest

No conflicts of interest exist.

References

1) Podolsky DK: Inflammatory bowel disease. N Engl J Med. 2002; 347: 417-429.
2) Mellemkjaer L, Olsen JH, Frisch M, Johansen C, Gridley G, McLaughlin JK: Cancer in patients with ulcerative colitis. Int J Cancer. 1995; 60: 330-333.
3) Eaden JA, Abrams KR, Mayberry JF: The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001; 48: 526-535.
4) Lennard-Jones JE, Morson BC, Ritchie JK, Williams CB: Cancer surveillance in ulcerative colitis. Experience over 15 years. Lancet. 1983; 2: 149-152.
5) Jess T, Gamborg M, Matzen P, Munkholm P, Sorensen TI: Increased risk of intestinal cancer in Crohn's disease: a meta-analysis of population-based cohort studies. Am J Gastroenterol. 2005; 100: 2724-2729.
6) Gillen CD, Walmsley RS, Prior P, Andrews HA, Allan RN: Ulcerative colitis and Crohn's disease: a comparison of the colorectal cancer risk in extensive colitis. Gut. 1994; 35: 1590-1592.
7) Ullman TA, Itzkowitz SH: Intestinal inflammation and cancer. Gastroenterology. 2011; 140: 1807-1816.
8) Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. Cell. 1990; 61: 759-767.
9) Yin J, Harpaz N, Tong Y, Huang Y, Laurin J, Greenwald BD, Hontanosas M, Newkirk C, Meltzer SJ: p53 point mutations in dysplastic and cancerous ulcerative colitis lesions. Gastroenterology. 1993; 104: 1633-1639.
10) Kern SE, Redston M, Seymour AB, Caldas C, Powell SM, Kornacki S, Kinzler KW: Molecular genetic profiles of colitis-associated neoplasms. Gastroenterology. 1994; 107: 420-428.
11) Wedekind JE, Dance GS, Sowden MP, Smith HC: Messenger RNA editing in mammals: new members of the APOBEC family seeking roles in the family busi-

ness. Trends Genet. 2003; 19: 207-216.
12) Honjo T, Kinoshita K, Muramatsu M: Molecular mechanism of class switch recombination: linkage with somatic hypermutation. Annu Rev Immunol. 2002; 20: 165-196.
13) Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, Catalan N, Forveille M, Dufourcq-Labelouse R, Gennery A, Tezcan I, Ersoy F, Kayserili H, Ugazio AG, Brousse N, Muramatsu M, Notarangelo LD, Kinoshita K, Honjo T, Fischer A, Durandy A: Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). Cell. 2000; 102: 565-575.
14) Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T: Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell. 2000; 102: 553-563.
15) Matsumoto Y, Marusawa H, Kinoshita K, Endo Y, Kou T, Morisawa T, Azuma T, Okazaki IM, Honjo T, Chiba T: Helicobacter pylori infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. Nat Med. 2007; 13: 470-476.
16) Kou T, Marusawa H, Kinoshita K, Endo Y, Okazaki IM, Ueda Y, Kodama Y, Haga H, Ikai I, Chiba T: Expression of activation-induced cytidine deaminase in human hepatocytes during hepatocarcinogenesis. Int J Cancer. 2007; 120: 469-476.
17) Endo Y, Marusawa H, Kinoshita K, Morisawa T, Sakurai T, Okazaki IM, Watashi K, Shimotohno K, Honjo T, Chiba T: Expression of activation-induced cytidine deaminase in human hepatocytes via NF-kappaB signaling. Oncogene. 2007; 26: 5587-5595.
18) Kinoshita K, Nonaka T: The dark side of activation-induced cytidine deaminase: relationship with leukemia and beyond. Int J Hematol. 2006; 83: 201-207.
19) Okazaki IM, Hiai H, Kakazu N, Yamada S, Muramatsu M, Kinoshita K, Honjo T: Constitutive expression of AID leads to tumorigenesis. J Exp Med. 2003; 197: 1173-1181.
20) Morisawa T, Marusawa H, Ueda Y, Iwai A, Okazaki IM, Honjo T, Chiba T: Organ-specific profiles of genetic changes in cancers caused by activation-induced cytidine deaminase expression. Int J Cancer. 2008; 123: 2735-2740.
21) Chiba T, Marusawa H, Seno H, Watanabe N: Mechanism for gastric cancer development by Helicobacter pylori infection. J Gastroenterol Hepatol. 2008; 23: 1175-1181.
22) Komori J, Marusawa H, Machimoto T, Endo Y, Kinoshita K, Kou T, Haga H, Ikai I, Uemoto S, Chiba T: Activation-induced cytidine deaminase links bile duct inflammation to human cholangiocarcinoma. Hepatology. 2008; 47: 888-896.
23) Endo Y, Marusawa H, Kou T, Nakase H, Fujii S, Fujimori T, Kinoshita K, Honjo T, Chiba T: Activation-induced cytidine deaminase links between inflammation and the development of colitis-associated colorectal cancers. Gastroenterology. 2008; 135: 889-898, 898 e1-e3.
24) Ben-Neriah Y, Karin M: Inflammation meets cancer, with NF-kappaB as the matchmaker. Nat Immunol. 2011; 12: 715-723.