Review Article

Inflammation-mediated genomic instability: roles of activation-induced cytidine deaminase in carcinogenesis

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Chronic inflammation is a strong risk factor for the development of cancer. Many previous studies have demonstrated that a transcriptional factor, nuclear factor (NF)-κB, plays an important role in the association between inflammation and cancer development, particularly tumor promotion and tumor progression. Although it is well recognized that cancer develops via stepwise accumulation of genetic aberrations, the mechanisms underlying the generation of these genetic alterations in normal epithelial cells under inflammatory conditions are not known. We recently demonstrated that pathogenic bacterial or viral factors and the subsequent inflammatory reactions lead to the aberrant expression of a DNA mutator enzyme, activation-induced cytidine deaminase (AID), in various epithelial cells via NF-κB activation, which causes the accumulation of genetic alterations in tumor-related genes. AID activation is widely observed in gastrointestinal tissues with cancer-associated inflammation, such as chronic viral hepatitis, *Helicobacter pylori*-related gastritis, Barrett's esophagus and inflammatory bowel disease. Furthermore, a deficiency of endogenous AID expression reduces both accumulation of somatic mutations in tumor-related genes and tumor incidence in a mouse model of inflammation-associated cancer development. These findings strongly suggest that AID plays an integral role in inflammation-associated carcinogenesis and is therefore a potential target molecule for the prevention and treatment of cancers. (*Cancer Sci* 2012; 103: 1201–1206)

Cancer cells have various genetic alterations, including somatic mutations, chromosomal rearrangements, copy number alterations and epigenetic changes.[1] Recent genome technology innovations, such as ultra-deep sequencing and comprehensive genome hybridization analyses, have allowed investigators to better understand the landscape of genetic alterations in cancer tissues.[2] Using these technologies, comprehensive genome analyses of various cancer tissues have clarified that cancer cells have numerous nucleotide alterations, including "passenger mutations, which might not be involved in cancer development, and 'driver mutations", which directly contribute to carcinogenesis.[3] In contrast, chronic inflammation plays important roles in the development of various human cancers, and tumor cells are considered to be generated from stepwise accumulation of genetic alterations in various genes during the process of inflammation-associated carcinogenesis. In some diseases, including hereditary non-polyposis colorectal cancer, genetic abnormalities in the DNA repair system result in accumulation of genetic alterations in various genes, leading to colorectal carcinogenesis.[4,5] However, in most sporadic cancers the molecular mechanisms for acquiring genetic alterations under inflammatory conditions are unknown.

Nuclear Factor (NF)-κB is a Key Molecule in Inflammation-Associated Carcinogenesis

Many epidemiological studies have clearly demonstrated that chronic inflammation predisposes to tumor formation in various organs, including hepatocellular carcinoma (HCC) caused by hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, gastric cancer caused by *Helicobacter pylori* infection, colorectal cancer caused by inflammatory bowel diseases (IBD), bile duct cancer caused by primary sclerosing cholangitis and esophageal cancer caused by Barrett's esophagus.[6–11] Accordingly, over 25% of human cancer cases are thought to be associated with chronic inflammation.[12]

Chronic inflammation is characterized by a continued active inflammatory response and tissue destruction, followed by irreversible tissue remodeling. Various cytokines, chemokines and transcription factors contribute to pathogenesis of chronic inflammation, and are also involved in the carcinogenesis process.[13] One of the most well known molecules is the transcription factor NF-κB, which is heavily involved in the processes of both tumor promotion and tumor progression and is considered a key regulator of cancer-associated inflammation. NF-κB is activated by various proinflammatory cytokines and microbial products, and regulates various cytokines and chemokines related to the determination of cell fate by binding their gene promoter.[14] Although NF-κB is physiologically associated with immune and inflammatory cell function, activated NF-κB proteins are detected in epithelial cells under many chronic inflammatory conditions related to carcinogenesis, such as *H. pylori*-related chronic gastritis, HCV-associated chronic liver disease and IBD.[15–18] NF-κB activation in inflamed epithelial cells, premalignant cells and malignant cells is involved not only in regulating cell survival, proliferation and growth, but also in the epithelial-to-mesenchymal transition.[19] In addition to these crucial processes that could contribute to tumorigenesis, a novel molecule, activation-induced cytidine deaminase (AID), a nucleotide-editing enzyme that is directly involved in DNA instability, has been recently identified to be regulated by NF-κB[20] (Fig. 1).

AID Triggers Both Somatic Mutations and DNA Double-Strand Breaks

Several molecules that possess the capacity to induce mutations in target DNA or RNA have been identified. Most of these molecules are members of the apolipoprotein B

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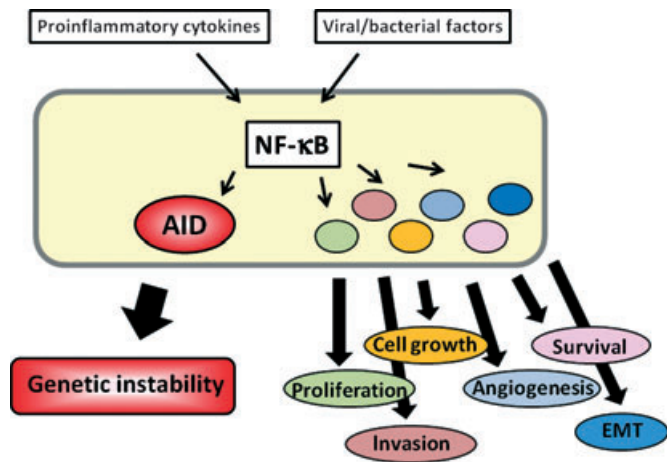E-mail: maru@kuhp.kyoto-u.ac.jp

Fig. 1. Link between inflammation and carcinogenesis. Nuclear factor (NF)-κB is activated by various proinflammatory cytokines and microbial products and regulates various cytokines and chemokines related to the determination of cell fate. NF-κB activation in epithelial cells and malignant cells is involved in generating genetic instability via aberrant activation-induced cytidine deaminase (AID) activation, as well as cell growth, survival, proliferation, angiogenesis and epithelial-to-mesenchymal transition (EMT).

mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) family.[21] Among the APOBEC family members, AID is the only molecule that can induce genetic alterations in human DNA sequences.[22] AID is a key molecule for generating immune diversity and is essential for inducing both somatic hypermutation, which occurs in variable regions of the immunoglobulin (Ig) genes, and class-switch recombination, which occurs in switch regions of the Ig genes (Fig. 2A).[23,24] It is considered that AID acts on single-strand DNA during the transcriptional stage, resulting in the conversion of cytosine (C) to uracil (U). The generated U:G mismatches can usually be repaired to C:G by the high-fidelity repair system. However, if the U:G mismatch is not repaired before the onset of DNA replication and is replicated over, it give rise to C:G to T:A transitions.[25] In contrast, nicks in the near sites of both strand sequences of switch regions are generated by the repair process of AID-induced U:G mismatches, resulting in DNA double-strand breaks that are required for class-switch recombination[26] (Fig. 3).

Constitutive AID Expression Contributes to Tumorigenesis

Under physiological conditions, AID has a strong preference for targeting the Ig genes in activated B cells, but it also mutates a number of non-Ig genes, including Bcl6, Pax5, mirR142, Pim1 and c-myc.[27,28] Strikingly, approximately 25% of all genes expressed in germinal center B cells are mutated by AID activity.[29] Additionally, a number of studies demonstrated that high AID expression is frequently observed in various types of human B-cell lymphomas and leukemias, and AID expression is associated with various gene mutations and chromosomal translocations.[30–34] These findings call attention to the concept that while the nucleotide-editing function of AID is physiologically essential for immune diversity in activated B cells, AID could be involved in the malignant transformation of lymphocytes via the accumulation of genetic alterations in tumor-related genes. This hypothesis was confirmed by generating a transgenic (Tg) mouse model with constitutive and ubiquitous AID expression.[35] All AID-Tg mice developed T cell lymphomas via the accumulation of mutations in non-Ig genes, such as T cell receptor and c-myc. Nota-

bly, the AID-Tg mice not only developed malignant lymphomas but also various epithelial tumors, including liver, lung and gastric cancers.[36] Thus, the cancer phenotypes of AID-Tg mice further suggest that epithelial cells with constitutive AID expression are transformed into malignant cells via the accumulation of genetic alterations in tumor-related genes (Fig. 2B).

Role of AID During Inflammation-Associated Human Carcinogenesis

Under physiological conditions, expression of the AID gene *Aicda* is strictly regulated by many factors, because AID has a dangerous ability to induce genomic instability.[37] Four regions with transcription factor-binding motifs have been identified in and around the *Aicda* locus.[38] Among these regions, the transcription enhancer activity of region 4, the area approximately 8 kb upstream of the transcription start site, has a primary role in the induction of AID by environmental stimuli.[39] The major transcription factors that mediate the signals initiated by CD40L, interleukin (IL)-4 and transforming growth factor-β stimuli are identified as NF-κB, STAT6 and Smad3/4, respectively. Furthermore, NF-κB-binding sites are also found immediately upstream of the transcription start site in region 1.[40,41] The NF-κB site in region 4 is considered to have a main role against CD40 stimulation in activated B cells, while the site in region 1 is responsible for the cell's response to viral infection and tumor necrosis factor (TNF)-α (Fig. 4).[39] These findings indicate that NF-κB plays a key role in the regulation of AID expression and suggest that activation of NF-κB in epithelial cells under many inflammatory conditions might induce AID, leading to genetic instability.

Liver carcinogenesis related to chronic HCV infection. Epidemiological studies have demonstrated that most HCC arise in the setting of chronic liver disease with features of chronic hepatitis or liver cirrhosis. HCV infection is one of the leading causes of chronic liver damage and induces the development of HCC at an annual rate of 0.5–1.0% in chronic hepatitis and 5–8% in liver cirrhosis.[6] Recent studies demonstrated that a number of genetic alterations, including the tumor-suppressor *TP53* gene, are present not only in human HCC but also in the surrounding hepatitis or liver cirrhosis.[42] Strikingly, aberrant AID expression is observed in both HCC cells and the surrounding non-cancerous hepatocytes, while no AID expression is observed in normal liver tissues.[42] Previous studies have revealed that high TNF-α expression levels are found in the liver of patients with chronic viral hepatitis, which activates the NF-κB classical pathway, and the HCV core protein has the potential to induce NF-κB activation in human hepatocytes.[43,44] Consistent with the regulation of AID by NF-κB in activated B cells, AID expression is induced in response to TNF-α stimulation in cultured human hepatocytes through the NF-κB pathway.[45] Likewise, AID is expressed in cultured human hepatocytes expressing HCV core protein via the NF-κB pathway. Furthermore, aberrant expression of AID in hepatocytes results in the accumulation of genetic alterations of various genes, including the *TP53* gene.[45] These findings provide the concept that both the response to chronic inflammation and HCV infection itself induce the aberrant expression of AID in hepatocytes, contributing to the genomic instability required for hepatocarcinogenesis.

Gastric carcinogenesis related to *H. pylori* infection. *Helicobacter pylori* causes chronic gastric inflammation and is defined as a class one carcinogen for human gastric cancer.[46] Patients with a previous history of *H. pylori*-related gastric cancer are at a higher risk of subsequent development of gastric cancers.[47] Although eradication of *H. pylori* reduces the development of metachronous gastric cancer, *H. pylori*-infected
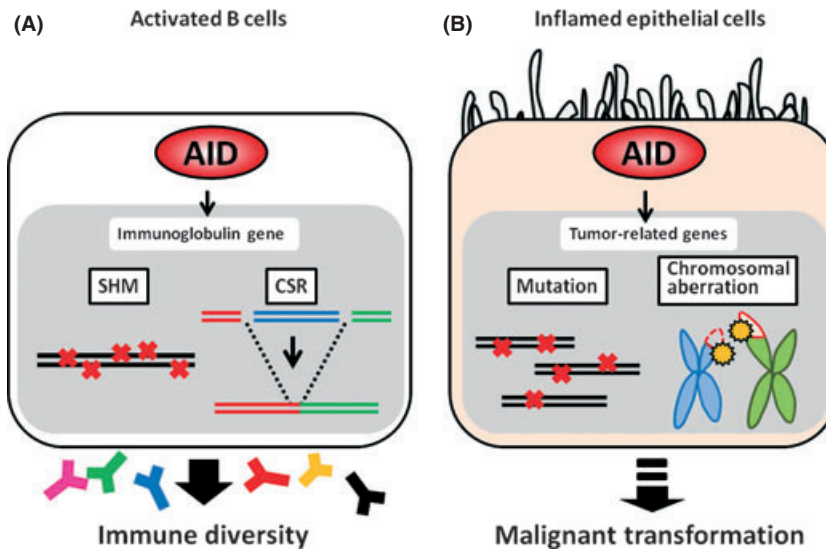
Fig. 2. Activation-induced cytidine deaminase (AID) function under physiological and inflammatory conditions. (A) Under physiological conditions, AID is expressed only in activated B cells and is a key molecule for generating immune diversity by inducing somatic hypermutation (SHM) and class-switch recombination (CSR) in immunoglobulin genes. (B) Under inflammatory conditions, AID is aberrantly expressed in epithelial cells. AID can induce somatic mutations and chromosomal aberrations in tumor-related genes, contributing to malignant transformation.
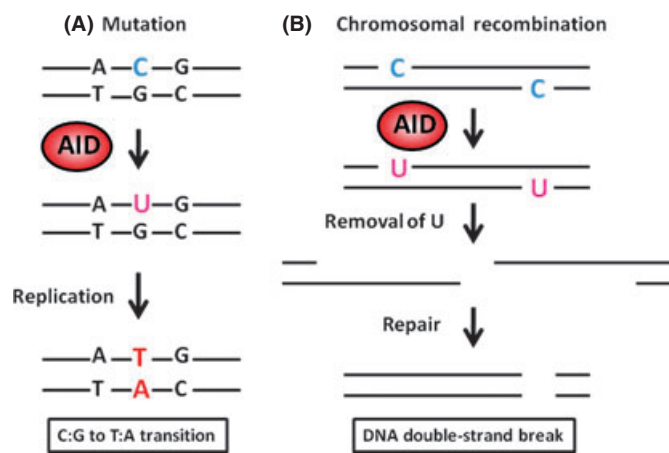

Fig. 3. Molecular mechanisms of the induction of mutation and chromosomal recombination by activation-induced cytidine deaminase (AID). (A) AID acts on single-strand DNA during the transcriptional stage. AID binds to the target DNA and deaminates deoxycytidine (dC) to deoxyuracil (U), creating a U–G mismatch. The general DNA replication without repair pathways can convert U to T and G to A, generating C:G to T:A mutations. (B) DNA double-strand breaks are generated through the repair process of AID-induced U:G mismatches through the production of abasic sites. Class-switch recombination (CSR) occurs by recombination of double-strand breaks.

mucosa might already have a number of genetic alterations, leading to multicentric cancer development.[48,49] Interestingly, AID protein is aberrantly expressed in a substantial proportion of *H. pylori*-associated human gastric epithelium and gastric cancer tissues, although no AID expression is observed in normal gastric mucosa or in gastric tissues after *H. pylori* eradication.[50]

Helicobacter pylori can be subclassified into "*cag*" pathogenicity island (*cag*PAI)-positive and *cag*PAI-negative strains based on the presence or absence of *cag*PAI, a 40-kb genome fragment containing 31 genes.[51] The *cag*PAI-positive isolates are more virulent strains that produce severe pathological infection in humans and are deeply associated with an

increased risk for gastric cancer.[52] Previous studies showed that infection with *cag*PAI-positive *H. pylori* is associated with increased expression of NF-κB in gastric epithelial cells both *in vitro* and *in vivo*.[53,54] Intriguingly, infection with *cag*PAI-positive *H. pylori* ectopically induces a high expression of AID in human gastric epithelial cell lines, while cag*PAI*-negative *H. pylori* has no effect on AID expression. Similar to cag-*PAI*-positive *H. pylori* infection, TNF-α stimulation increases the expression of endogenous AID protein in gastric epithelial cells through the NF-κB pathway. Furthermore, aberrant AID expression in gastric epithelial cells causes a number of somatic mutations in tumor-related genes, including the *TP53* gene, and knockdown of endogenous AID significantly reduces the number of *TP53* mutations observed in *H. pylori*-infected cells.[50]

In contrast, it is reasonable to assume that AID is capable of inducing chromosomal aberrations in epithelial cells because of its ability to trigger DNA double-strand breaks. Consistent with this hypothesis, AID expression in gastric epithelial cells causes chromosomal aberrations, mainly submicroscopic deletions, at various chromosomal loci.[55] Among these deleted loci, the recurrently deleted chromosomal regions harbored the tumor-suppressor genes cyclin-dependent kinase inhibitor (*CDKN*)2A/*CDKN*2B. Oral infection of wild-type mice with *H. pylori* reduces the copy number of the *Cdkn2b-Cdkn2a* locus, whereas no such changes are observed in the gastric mucosa of *H. pylori*-infected AID-deficient mice.[55] Taken together, these findings suggest that both *H. pylori* infection and the resulting inflammatory responses induce aberrant AID expression in gastric epithelial cells via NF-κB activation, leading to accumulation of genetic alterations, including somatic mutations and submicroscopic deletions in tumor-related genes during *H. pylori*-related gastric carcinogenesis.

Colitis-associated carcinogenesis. Inflammatory bowel diseases is an important etiological risk for the development of colorectal cancer.[56] It is well known that the risk of colitis-associated colorectal cancer increases according to the number of years after disease onset.[57] The cumulative risk of developing colorectal cancer in patients with ulcerative colitis (UC) is estimated to be 1.6% at 10 years, 8.3% at 20 years and 18.4% at 30 years from disease onset.[9] Recent studies have shown that patients with Crohn's disease are also at high risk of developing colon cancer.[58] Importantly, colitis-associated
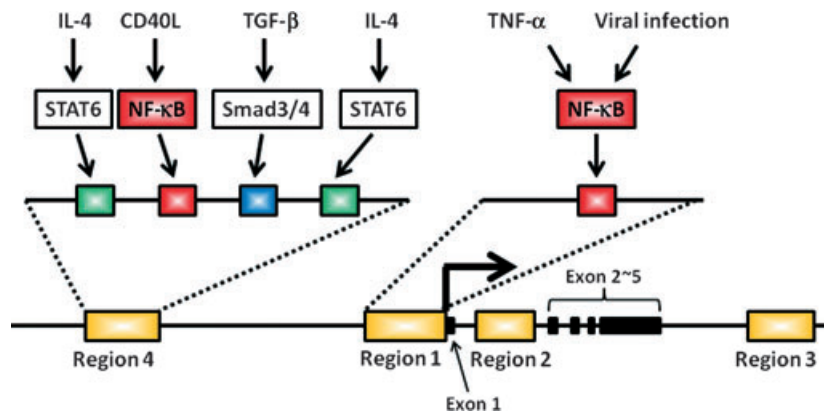
Fig. 4. Transcriptional regulation of activation-induced cytidine deaminase (AID) expression induced by environmental stimuli. The transcriptional regulatory elements for AID are localized to four regions. Nuclear factor (NF)-κB-binding sites are present in region 4 and region 1. The site in region 4 has a main role against CD40 stimulation in activated B cells, while the site in region 1 might be responsible for the cell's response to viral infection and tumor necrosis factor (TNF)-α.

colorectal cancer has several distinct characteristics compared with sporadic colorectal cancers. In most sporadic colorectal cancers, adenomatous polyps first develop through inactivation of the *APC* gene, and the subsequent acquisition of other gene abnormalities, such as *KRAS*, *TP53* and *DCC* genes, contribute to cancer development.[59] In contrast, mutations in the *TP53* gene are already present in dysplastic lesions with a background of UC, suggesting that colonic inflammation induces the accumulation of mutations in the *TP53* gene.[60,61] Interestingly, AID protein is expressed in colonic epithelial cells of patients with IBD, while AID is not detected in normal colonic mucosa.[62]

Colonic mucosal inflammation is usually mediated by either an excessive T helper cell (Th) 1 T-cell response associated with increased interferon-γ and IL-12 secretion, or an excessive Th2 T-cell response related to increased IL-4, IL-5 and IL-13 secretion.[63] Although the concentration of the Th2 cell-driven cytokine IL-4 varies in UC colon tissue, UC is considered to have a Th2 profile.[64] In addition to the finding that the proinflammatory cytokine TNF-α induces AID expression through the NF-κB pathway, Th2 cytokines IL-4 and IL-13 enhance the aberrant expression of AID via STAT6 activation in cultured colonic epithelial cells.[62] Moreover, constitutive AID expression in colonic epithelial cells generates a number of mutations of the *TP53* gene.

Recent studies have revealed that IL-10 has a pivotal role in mediating the signals that control inflammation in the human gut.[65] Moreover, a mouse model with target disruption of the IL-10 gene spontaneously develops intestinal inflammation followed by colon cancer, and thus this mouse model has been used to represent human IBD.[66,67] We found that AID expression is observed in the inflamed cecal mucosa of IL-10-deficient mice, and mice deficient in both IL-10 and AID do not develop colon cancer in the cecum, whereas IL-10-deficient mice develop spontaneous colon cancers.[68] Furthermore, somatic mutation rates of *Trp53* in the cecal mucosa of mice deficient in both IL-10 and AID are significantly lower than those in the mucosa of mice deficient in IL-10 only, regardless of the same inflammation conditions. Therefore, it is conceivable that aberrant AID expression in the inflamed colonic mucosa plays an integral role in the development of colitis-associated cancers via accumulation of genetic abberations.

Barrett's esophageal carcinogenesis related to bile acid exposure. Barrett's esophagus is a well known high-risk factor for the development of esophageal adenocarcinoma.[11] Barrett's esophagus is a metaplastic change from the normal stratified squamous epithelium of the lower esophagus to a columnar-lined epithelium with intestinal-type differentiation due to chronic duodenogastro-esophageal reflux and resultant inflammation.[69] Several studies have demonstrated that NF-κB is activated by bile acid components, resulting in the upregulation of various genes involved in the development of metaplasia of Barrett's esophagus and cancer formation.[70] Interestingly, AID is aberrantly expressed in the columnar cell-lined Barrett's esophagus and Barrett's adenocarcinoma, whereas weak or no expression of AID is observed in normal squamous epithelial cells of the esophagus.[71] *In vitro* experiments using human non-neoplastic esophageal squamous-derived cells showed that exposure to deoxycholic acid induces endogenous AID upregulation via NF-κB activation. Moreover, constitutive AID expression in esophageal squamous cells causes the accumulation of mutations in *TP53*.[71] Taken together, these findings suggest that bile acid reflux-mediated aberrant AID expression in Barrett's columnar-lined epithelium enhances the susceptibility to genetic alterations, contributing to the development of Barrett's adenocarcinoma.

Conclusion

Recent epidemiological studies have revealed that removing the causes of chronic inflammation, such as eradication of HCV or *H. pylori*, reduced cancer development, suggesting that regulation of inflammation contributes to suppressing cancer development.[48] To date, we have demonstrated that aberrant AID expression in various epithelial cells induces the accumulation of genetic alterations, and a deficiency of endogenous AID reduces the accumulation of somatic mutations in various genes in inflamed tissues, resulting in reduced incidence of inflammation-associated cancer development.[68] These findings might provide a novel strategy for cancer prevention by targeting AID-related pathways. Recently, several studies have shown that deamination activity of AID promote active DNA demethylation via the base excision repair pathway, suggesting that AID is involved in the epigenetic regulation of various genes.[72,73] Thus, it is hoped that further elucidation of the link between AID and the DNA methylation system will lead to a better understanding of the mechanisms of inflammation-associated carcinogenesis.

Disclosure Statement

The authors declare no potential conflict of interest.

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