Carcinogenesis by activation-induced cytidine deaminase

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Activation-induced cytidine deaminase (AID), the only enzyme that is known to be able to induce mutations in the human genome, is required for somatic hypermutation and class-switch recombination in B lymphocytes. Recently, we demonstrated that AID is implicated in the pathogenesis of human cancers including hepatitis C virus (HCV)-induced human hepatocellular carcinoma (HCC). In the present study, we established a new AID-transgenic mouse model (TNAP-AID) in which AID is expressed in cells producing tissue-nonspecific alkaline phosphatase (TNAP), which is a marker of primordial germ cells and immature stem cells, including ES cells. High expression of TNAP was found in the liver of the embryos and adults of TNAP-AID mice. HCC developed in 27% of these mice at the age of approximately 90 weeks. The HCC that developed in TNAP-AID mice expressed α-fetoprotein and had deleterious mutations in the tumor suppressor gene *Trp53*, some of which corresponded to those found in human cancer. Therefore, TNAP-AID is a mouse model that spontaneously develops HCC, sharing genetic and phenotypic features with human HCC. In a mouse model of chemical-induced skin cancer, AID was shown to play dual roles of both initiation and promotion of tumor. Notably, there was a significant difference in the tumor frequency between AID-deficient and wild-type mice, indicating involvement of endogenous AID expression in this skin cancer model. These observations suggest causal role of AID in epithelial tumor. Since AID is an inducible enzyme by inflammatory stimuli, it might be a link between cancer and chronic inflammation caused by infection or physical stimuli.

1. TNAP-AID: A mouse model of liver cancer triggered by AID

Previous AID transgenic mice (JEM 197:1173) develops lethal T-cell lymphoma, which kills mice before reaching 1 year of age. To prevent the early death and observe late appearing cancer in other organs, conditional AID transgenic mouse (**AID cTg**) was generated, in which AID is overexpressed in cells expressing Cre recombinase. In this experiment, AID cTg was crossed with TNAP-Cre mouse, which expresses Cre under the promoter of *Alpl* gene, encoding tissue nonspecific alkaline phosphatase (TNAP), a marker of primordial germ cells and ES cells. TNAP is also expressed in fetal and adult liver. The resulting double transgenic mouse was designated as **TNAP-AID**. Despite our initial expectation for germ cell tumors, TNAP-AID mice frequently developed liver tumors.

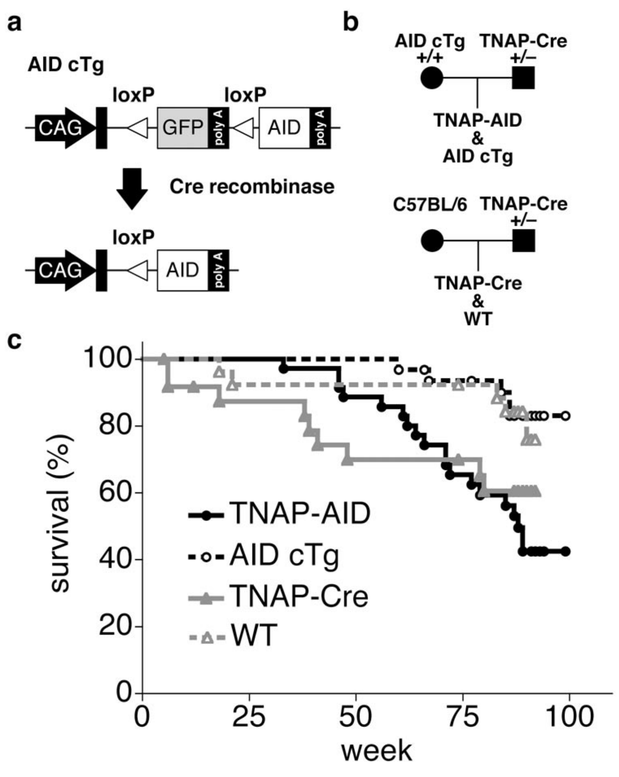


Figure 1. Generation of TNAP-AID mice and their controls.
(a) Structure of transgene used to generate AID conditional transgenic mice (AID cTg) and the structure after Cre-mediated recombination. Arrows indicate the CAG promoter and rectangles indicate exons. Grey and open boxes represent the coding sequences of GFP and AID, respectively. A polyadenylation signal (poly A) is attached after each coding sequence. Triangles indicate loxP sites. (b) Mating scheme. (c) Kaplan–Meier survival curves for the four genotype groups. There were no statistical significance between the curve for TNAP-AID and that for TNAP-Cre, making it unable to conclude death-promoting effect of AID expression.

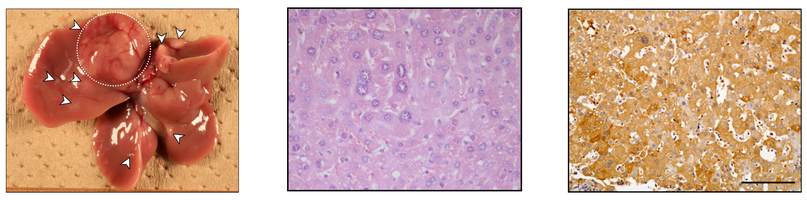


Figure 2. HCC developed in TNAP-AID mice
(left) The liver tumor are often but not always multiple Each nodule is indicated by arrowheads or a dotted circle. (middle) HE staining image reveals highly differentiated HCC. (right) Immunohistochemistry for α-fetoprotein in liver tumor. The scale bar is 500 μm.

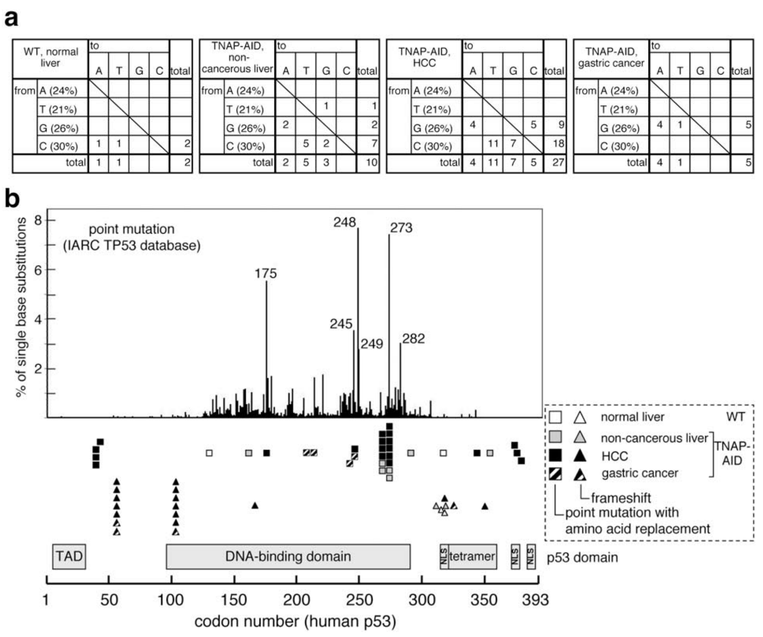


Figure 3. *Trp53* gene mutation profiles in liver and HCC of TNAP-AID mice.
(a) Base substitution patterns seen in liver of WT mice (left), non-cancerous liver of TNAP-AID mice (centre) and HCC of TNAP-AID mice (right) extracted from the same data sets as those used for the mutation frequency analysis in Table 2. Percentages in parentheses are compositions of the indicated bases in the sequenced region. (b) Top: Distribution of *TP53* somatic mutations in human cancer, reproduced from the IARC TP53 Database (version R12), November 2007 (http://www-p53.iarc.fr/) (Petitjean et al., 2007). Middle: Distribution of mouse *Trp53* mutations found in WT and TNAP-AID mouse liver. Codon positions are converted into human equivalents. Squares and triangles indicate point mutations with amino acid replacements and frameshifts, respectively. Open, grey, filled and hatched symbols indicate normal liver of WT mice, non-cancerous liver, HCC and gastric cancer of TNAP-AID mice, respectively. Bottom: p53 domain structure with transactivation (TAD), DNA-binding and tetramerization domains and nuclear localization signal (NLS).

Table 1 Frequencies of tumours observed in TNAP-AID and control mice

Genotype	*Mean age at killing (weeks)*	*HCC*	*Lymphoma*	*Lung cancer*	*Stomach cancer*
TNAP-AID (15)	88.5	26.7% (4)	40.0% (6)	6.7% (1)	6.7% (1)
AID-cTg (24)	89.9	0.0% (0)	29.2% (7)	0.0% (0)	0.0% (0)
TNAP-Cre (14)	88.4	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)
WT (23)	89.1	0.0% (0)	17.4% (4)	0.0% (0)	0.0% (0)

Abbreviations: HCC, hepatocellular carcinoma; WT, wild type.
Frequencies were calculated from the numbers of mice with macroscopic tumours of the indicated organs at approximately 90 weeks of age. Numbers in parentheses are the number of individuals killed for tumour inspection (genotype column) and those with macroscopic tumours (right four columns).

2. Two roles of AID: Initiation and promotion in chemical-induced skin cancer model

In a classical skin cancer model, two classes of chemicals are required for tumor formation: initiator and promoter. A DNA alkylating agent DMBA is an initiator causing DNA mutations. A phorbol ester TPA is a promoter, which drives proliferation of keratinocytes by activating protein kinase C. As AID is a mutagen, we speculated that AID can substitute DMBA but not TPA.

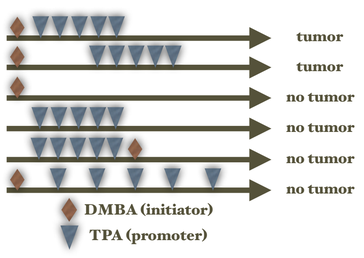


Figure 4. Relationship between administration protocols of chemicals and tumor occurrence.
Single dose of DMBA must precede multiple doses of TPA (every 3-4 days) for skin tumors (papilloma) to occur.

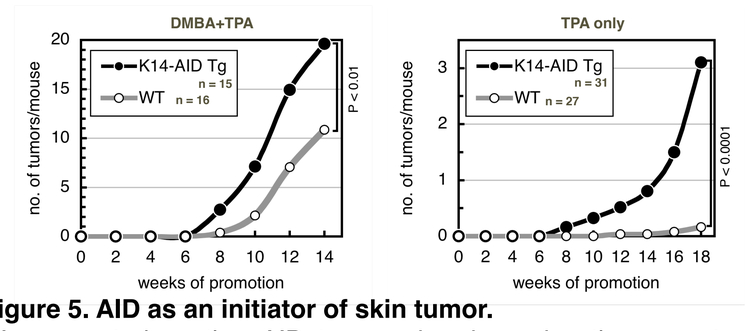


Figure 5. AID as an initiator of skin tumor.
We generated another AID transgenic mice using the promoter of keratin 14 gene, which is highly expressed in the basal layer of the epidermis (K14-AID Tg) in the genetic background of FVB/N, which is more susceptible for skin tumor than C57BL/6 strain. Back skin of K14-AID Tg mice and control wild-type mice (WT) were treated either with DMBA and TPA (DMBA+TPA) or with TPA only (TPA only). K14-AID Tg mice developed papilloma without DMBA. This indicates that AID can partially substitute the role of DMBA in skin tumor formation. *n* in legends indicates number of mice used.

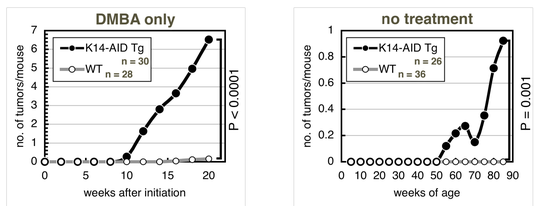


Figure 6. AID as a promoter of skin tumor.
When K14-AID mice and WT mice were treated only once with DMBA, K14-AID mice developed papilloma (left, DMBA only), suggesting role of AID as a promoter. Supporting this notion, K14-AID mice developed papilloma even without chemicals (middle, no treatment). Induction of AID in primary culture of human keratinocytes by TPA as well as LPS and TGFβ (qRT-PCR, right) suggests that promotion by TPA may be mediated partly through expression of AID.

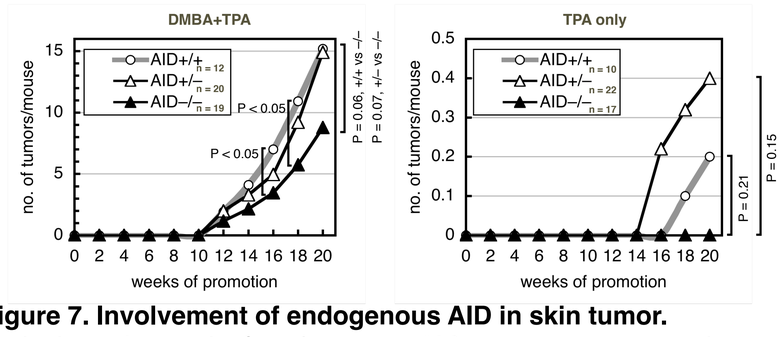
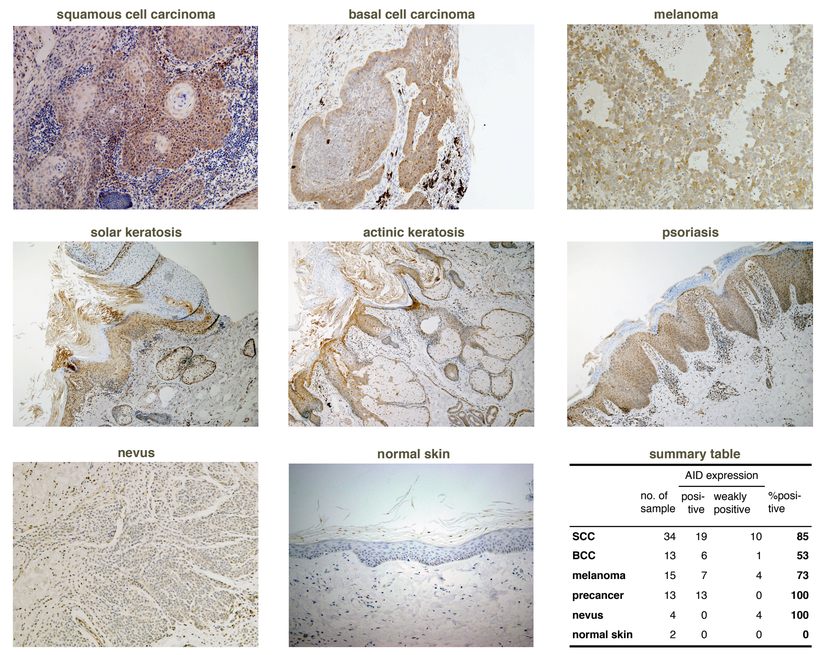


Figure 7. Involvement of endogenous AID in skin tumor.
To test involvement of endogenous AID expression in skin tumor, AID-knockout mice (–/–) were treated with DMBA and TPA (left). Compared to the control (+/+, +/–), tumor incidence was slightly decreased. When mice were treated with TPA alone (right), both controls developed skin tumor, while knockout mice did not. Although low incidence of tumors in TPA-only protocol precluded statistical significance, these data hint the possibility that endogenous AID plays a role in skin tumor formation.



	no. of sample	AID expression: positive	weakly positive	%positive
SCC	34	19	10	**85**
BCC	13	6	1	**53**
melanoma	15	7	4	**73**
precancer	13	13	0	**100**
nevus	4	0	4	**100**
normal skin	2	0	0	**0**

Figure 8. Expression of AID in human skin cancer.
Expression of AID in skin cancer specimens from patients was examined by immunohistochemistry. As shown in the table (right), majority of skin tumors of various types (squamous cell carcinoma, basal cell carcinoma, and melanoma) and precancerous legions (including solar keratosis, actinic keratosis, psoriasis and nevus) expressed AID. SCC is an abbreviation for squamous cell carcinoma and BCC for basal cell carcinoma. These data suggest that AID may be involved in the pathogenesis of skin cancer in human.

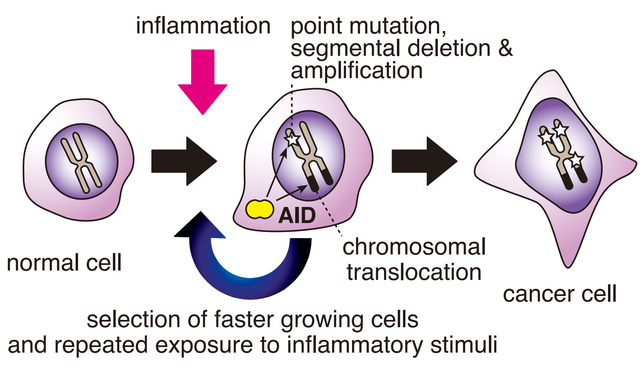


Figure 9. Model of AID causing cancer.
Stimulation by inflammation due to infection and chemical stress, or other stimuli may induce normal lymphoid and epithelial cells (left) to express AID, which induces point mutations and chromosomal translocations (middle). When inflammation is persistent, continuous AID expression may drive successive rounds of genetic changes and selection of proliferating cells, resulting in the evolution of cancerous cells (right).

CONCLUDING REMARKS

Mutational targets of AID are not confined to antibody genes. It has been reported that AID causes alteration of non-immunoglobulin genes in B cells as well as in epithelial cells. Recently, we reported AID-induced deletion of tumor suppressor *CDKN2A* gene in human gastric cancer cell (Matsumoto et al. *Gastroenterology* online, 2010). Combining data from animal experiments and clinical human samples, it is becoming more plausible that AID is a missing link between inflammation and mutation in inflammation-associated cancers by infection and physical stimuli. Therefore, inhibition of AID might be a new anti-cancer strategy that stops evolvability of cancers.