Activation-induced cytidine deaminase promotes oncogenesis of ultraviolet light-independent squamous cell carcinoma of the skin

Taichiro Nonaka[1,2,3], Munehiro Uemura[1], Hiroshi Hiai[3] , & Kazuo Kinoshita[1]

[1]Shiga Medical Center Research Institute, Moriyama, Japan; [2]Department of Microbiology and Immunology, University of San Francisco, USA; [3]Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Most squamous cell carcinoma (SCC) of the skin develops after ultraviolet (UV) light-induced DNA damage and repair errors. However, significant portion of SCC appears to occur independently from UV light because SCC often occur in light-protected areas such as chronic ulcers and burn scars underneath clothing, and oropharyngeal mucosa. Such UV-independent SCC is speculated to have a causal link with chronic inflammation. However, how chronic inflammation leads to cancer is unclear. We propose here that activation-induced cytidine deaminase (AID) is a mechanistic link between chronic inflammation and SCC. AID is an enzyme essential for DNA cleavage involved in immunoglobulin class switch and somatic hypermutation. We found that tumor frequencies were elevated by transgenic expression of AID from keratin14 promoter, and decreased by genetic deletion of AID in a mouse skin cancer model using 7,12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoylphorbol 13-acetate (TPA). Significant numbers of mutations were observed in the *Trp53* gene of SCC developed spontaneously in AID transgenic mice. Human primary cultured keratinocytes and SCC cell line expresses AID after stimulation with LPS, TPA, poly(I:C), or inflammatory cytokines such as TGF-β and TNF-α. Overexpression of AID in human keratinocyte cell line caused point mutations in *TP53* gene. These results suggest that AID is a DNA damaging factor involved in inflammation-associated SCC.

1. Establishment of novel skin cancer mouse model

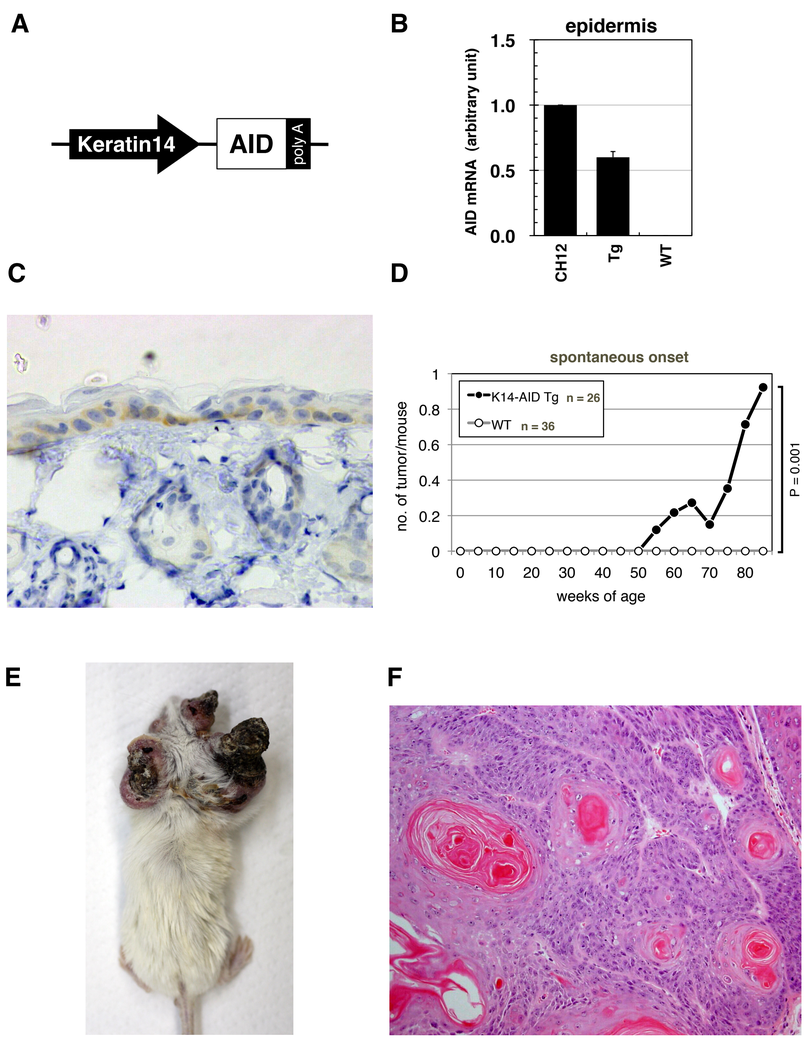

Figure 1. A. Generation of AID transgenic mice driven by keratin14 promoter in FVB/N background. Black arrow indicates human keratin14 promoter, and rectangle indicates mouse AID transgene. Polyadenylation signal (poly A) is attached after AID sequence. B. mRNA expression of AID in mouse epidermis were compared with murine B lymphoma cell line CH12 without cytokine stimulation by qRT-PCR. C. Immunohistochemistry of the skin of AID transgenic mouse by anti-AID antibody. D. AID transgenic mice developed skin tumor spontaneously. E. squamous cell carcinoma (SCC) developed in AID transgenic mouse. F. Microscopic image of SCC. H&E stain. Abbreviations: Tg, transgenic mice; WT, wild type mice; K14-AID Tg, keratin14 promoter-driven AID transgenic mice.

2. AID contributes to skin tumorigenesis as a role of both an initiator and promoter

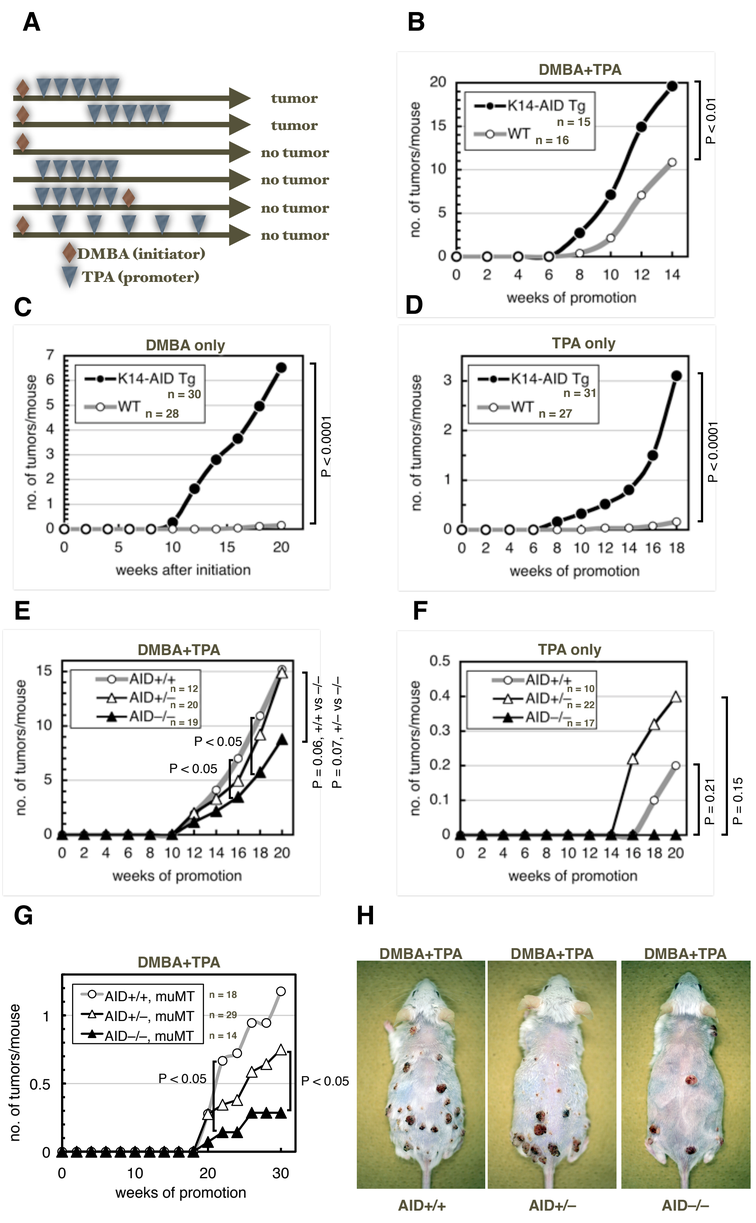

Figure 2. A. Scheme of chemical-induced skin tumorigenesis using DMBA (initiator) and TPA (promoter). Repetitive topical administrations of TPA after a single topical administration of DMBA to the shaved dorsal skin of the mice cause skin papilloma and SCC. B. Tumor frequency after DMBA+TPA administration in K14-AID Tg and WT mice. C. Tumor frequency measured as in B except only TPA was administered. D. Tumor frequency measured as in B except only DMBA was administered. E. Tumor frequency after DMBA+TPA administration in WT and AID-deficient mice. F. Tumor frequency measured as in E except only DMBA was administered. G. Tumor frequency in DMBA+TPA-treated AID-deficient mice with muMT background. As muMT mice lack mature B lymphocytes and antibody, absence of antibody diversification (CSR and SHM) is not the cause of reduced number of papilloma. H. Skin tumors developed in WT and AID-deficient mice. Abbreviations: AID+/+, wild-type mice; AID+/–, heterozygously AID-deficient mice; AID–/–, homozygously AID-deficient mice.

3. Gene mutation profile of squamous cell carcinoma of the skin developed in AID transgenic mice

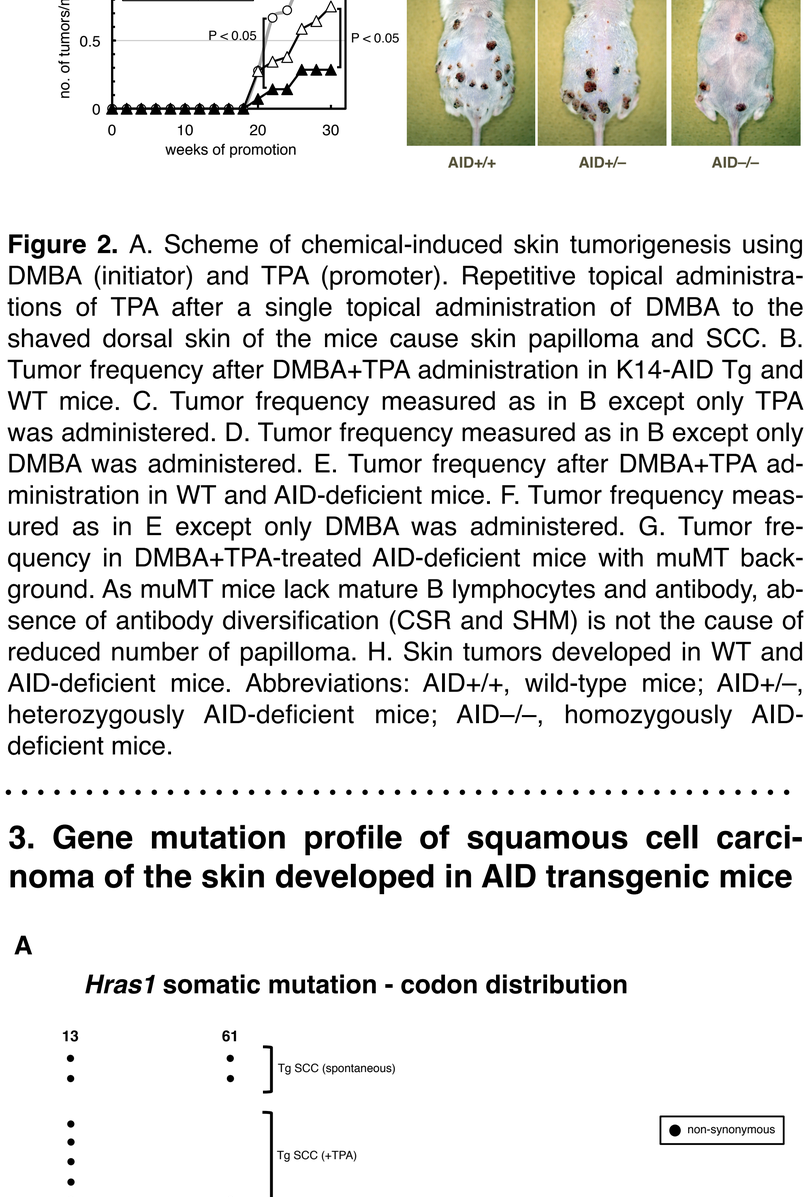

Table. Mutations in *Hras1* gene in K14–AID Tg and WT tissue

Genotype	tissue	Mutated/total samples	Mutated/total bases	Mutation frequency x 10^{-4}	
K14–AID Tg	SCC	3/25	4/14250	2.81	
K14–AID Tg (+TPA)	SCC	6/21	6/11970	5.01	P = 0.19
K14–AID Tg	epidermis	0/6	0/3420	0	
WT	epidermis	0/2	0/1140	0	

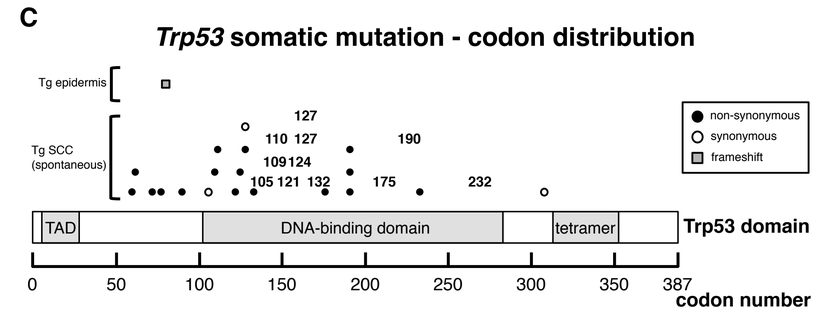

Table. Mutations in *Trp53* gene in K14–AID Tg and WT tissue

Genotype	tissue	Mutated/total samples	Mutated/total bases	Mutation frequency x 10^{-4}	
K14–AID Tg	SCC	6/17	19/13023	14.58	P = 0.02
K14–AID Tg	epidermis	1/6	1/5162	1.93	
WT	epidermis	0/2	0/1729	0	

Figure 3. Mutations seen in SCC of K14-AID Tg. A. Distribution of somatic mutation in *Hras1* found in Tg SCC developed spontaneously, and Tg SCC induced by TPA only protocol. Filled circles indicate non-synonymous mutation. The number above circles is substituted codon number. Sites of G-box-motifs (G1-G5), P-loop, switch regions are indicated. B. Mutation frequency of transcribed *Hras1* gene of Tg and WT tissues. C. Distribution of somatic mutation in *Trp53* found in Tg SCC developed spontaneously. Filled circles, open circles, gray square indicate non-synonymous, synonymous, frameshift mutation, respectively. The number above circles is substituted codon number. Sites of transactivation (TAD), DNA-binding domain, tetramerization domain are indicated. D. Mutation frequency of transcribed *Trp53* gene of Tg and WT tissues.

4. Expression of AID in human skin keratinocytes

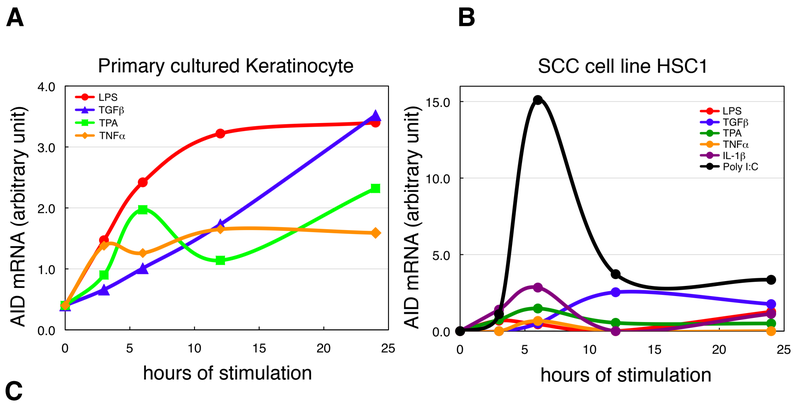

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Table. Mutations in *TP53* gene in human keratinocyte cell line HaCaT retrovirally transfected with AID

Cell	Retrovirus	Mutated/total clones	Mutated/total bases	Mutation frequency x 10^{-4}	
HaCaT	human AID–Ugi	7/24	7/28368	2.47	P < 0.05
	mock–Ugi	1/24	1/28179	0.35	

Figure 4. A. Stimulation-dependent AID expression in human primary cultured keratinocyte. B. in human skin SCC cell line HSC1. C. Mutation frequency of transcribed *TP53* gene of AID-transfected human keratinocyte HaCaT. HaCaT cells were infected with AID retrovirus, and co-transfected with Ugi (uracil-DNA glycosylase inhibitor) to increase mutation by AID. Transfectants were cultured under antibiotics selection for 100 days. Abbreviations: LPS, lipopolysaccharide; TGFβ, transforming growth factor β; TPA, 12-O-tetradecanoylphorbol 13-acetate; TNFα, tumor necrosis factor α; IL-1β, Interleukin-1β; Poly I:C, polyinosinic-polycytidylic acid.

CONCLUDING REMARKS

It has been reported that AID causes alteration of non-immunoglobulin genes in gastric cancer and liver cancer. Our transgenic mouse model demonstrated that ectopic overexpression of AID in epidermis triggered mutation in *Hras1* and *Trp53* genes and lead to skin tumorigenesis. In addition, we revealed that AID overexpression induces skin tumors in AID alone, and in concert with chemical tumor initiator or promoter. Combining data from animal experiments and human-derived cells, it became more plausible that AID is a missing link between inflammation and gene mutation in inflammation-associated cancers by infection and physical stimuli. Therefore, inhibition of AID might be a new anti-cancer strategy.

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