Chronic lung injury by constitutive expression of AID leads to focal alveolar regeneration and cancer

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Introduction

Activation-induced cytidine deaminase, AID, is an enzyme required for somatic hypermutation and class switch recombination in antibody genes. AID causes some cancer-related mutations and generates widespread DNA double-strand breaks. Uncontrolled expression of AID is cytotoxic. Constitutive AID expression in mice invariably causes lung lesions morphologically similar to human atypical adenomatous hyperplasia (AAH), which can be a precursor of bronchioloalveolar carcinoma and about 10 percent of these mice develop visible lung tumors.

A recent study reported that p63 and cytokeratin 5 expressing cells in the bronchiolar epithelium were involved in alveolar regeneration of damaged lung using the mouse model for H1N1 influenza viral infection.

In the present study, we examined these mouse AAH-like lesions (MALLs) and discussed the relationship between lung regeneration following chronic lung injury and lung cancer.

Results

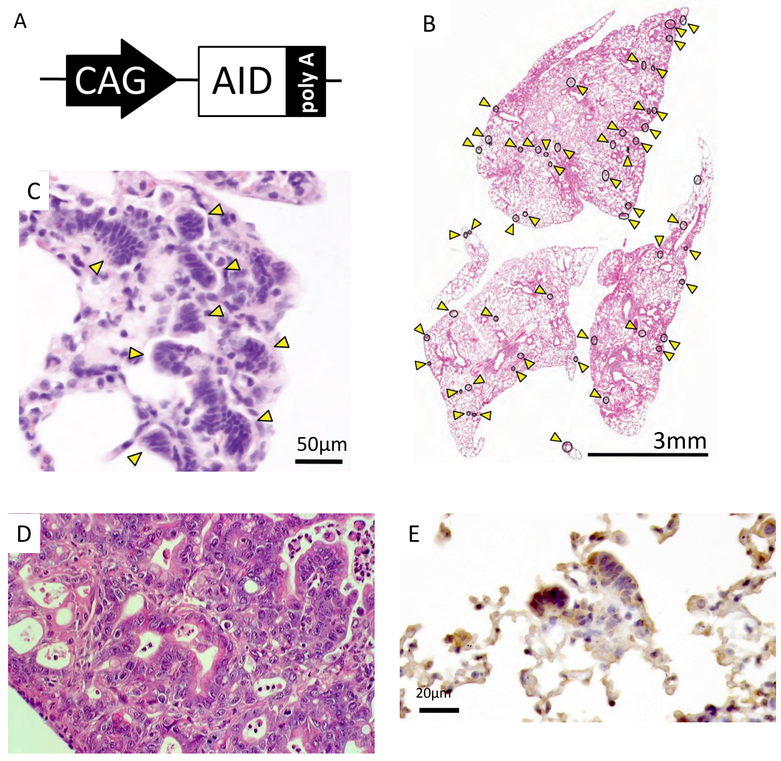

Figure 1. AID transgenic mouse.
(A) Structure of transgene used to generate AID transgenic mice. Arrow indicates the CAG promoter. Open box represents the coding sequences of AID. Polyadenylation signal (poly A) is attached after the coding sequence. (B) HE staining of the lung of AID transgenic mouse. Arrow heads and open circles indicate the locations of MALLs. (C) Higher power view of a representative MALLs in the lung of AID transgenic mouse. Arrow heads indicate MALLs. (D) Histopathological analysis shows lung adenocarcinoma in AID transgenic mouse. (E) Immunohistochemitry indicates AID expression in MALLs.

	p53		KRAS		EGFR	
MALL	3.6%	(4/110)	0.0%	(0/110)	0.0%	(0/110)
AAH	0.0%	Yamasaki, 2000	33.0%	Sakamoto, 2007	25.0%	Yoo, 2010
	9.0%	Slebos, 1998	26.7%	Yoshida, 2005	3.0%	Sakamoto, 2007
			15.0%	Cooper, 1997	35.0%	Yoshida, 2005
			39.0%	Westra, 1996		

Table 1. Lung cancer related-gene mutation frequencies in MALLs in comparison with published data for human AAH.

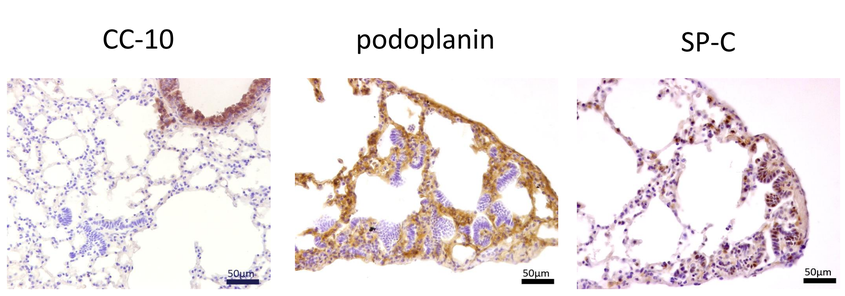

Figure 2. Immunohistochemistry for airway epithelial cell markers.
CC-10 (clara cell marker) and podoplanin (alveolar type I cell marker) were negative, but SP-C (alveolar type II cell marker) was partially positive in MALL cells.

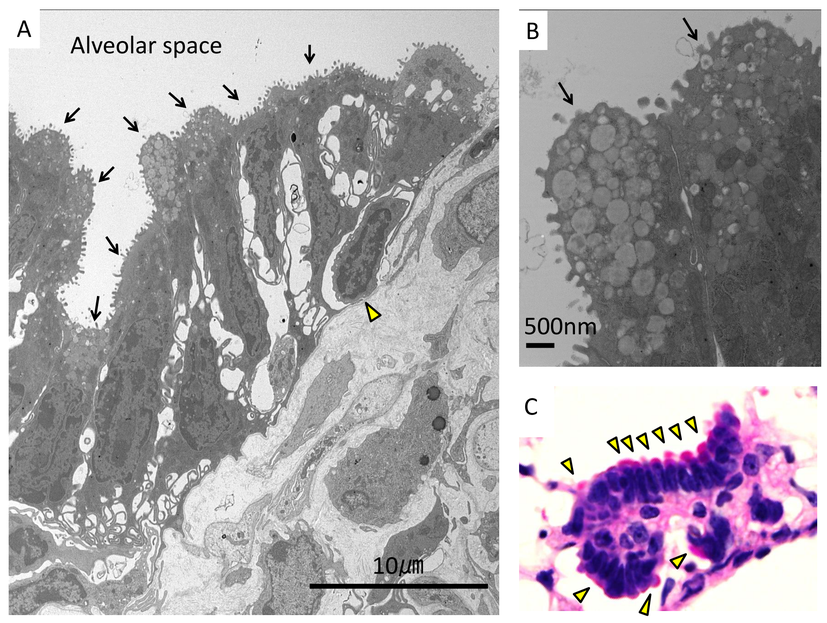

Figure 3. Characteristics of MALL cells as immature mucous cells.
Electron micrographs of MALL. (A) overview of MALL cells. (B) apical region of MALL cells. Arrows indicate immature secretory vesicles in the cytoplasm. Arrow head indicates a basal cell residing at the base of MALL cells. (C) PAS-positive materials in the apex of MALL cells suggest the presence of mucin (arrow heads).

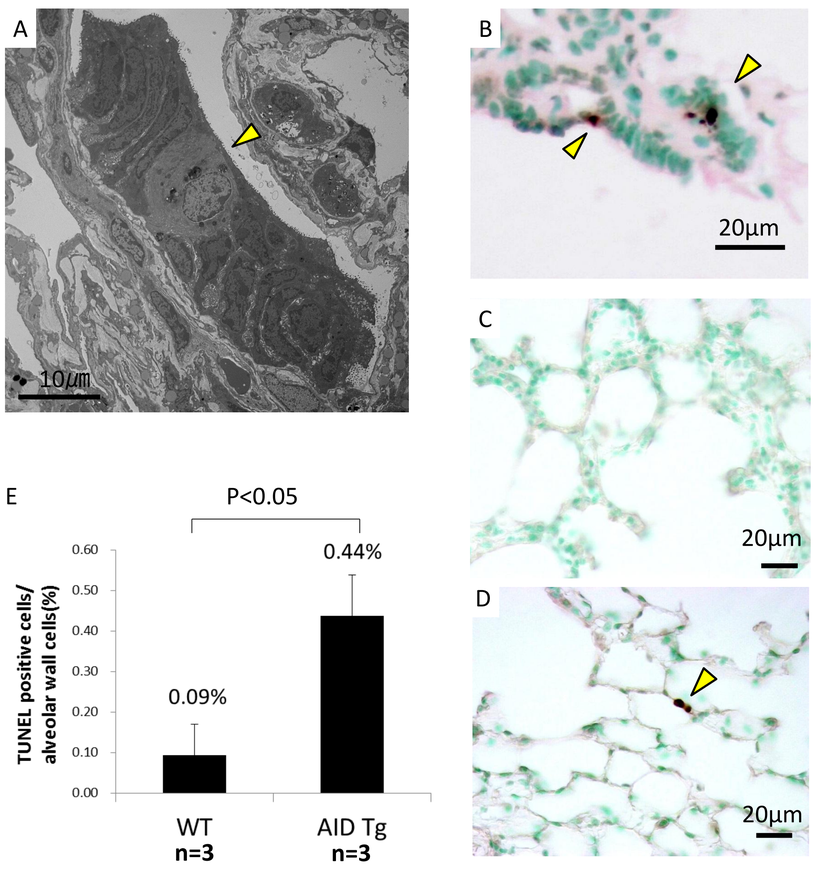

Figure 4. Cell death in the lung of AID transgenic mice.
(A) Electron micrograph shows a phagocytic epithelial cell within MALL (Arrow head). This finding suggests the clearance of apoptotic cells. (B-D) TUNEL staining for MALL. (B), wild type mouse (C), and AID transgenic mouse (D). Arrow heads indicate TUNEL positive cells in MALL (B) and in the alveolar wall (D). (E) Frequency of TUNEL positive cells among alveolar wall cells in AID transgenic mice was compared to that in wild type mice. The frequencies were calculated by counting TUNEL positive and negative alveolar wall cells. Abbreviations: WT, wild type mice: AID-Tg, AID transgenic mice.

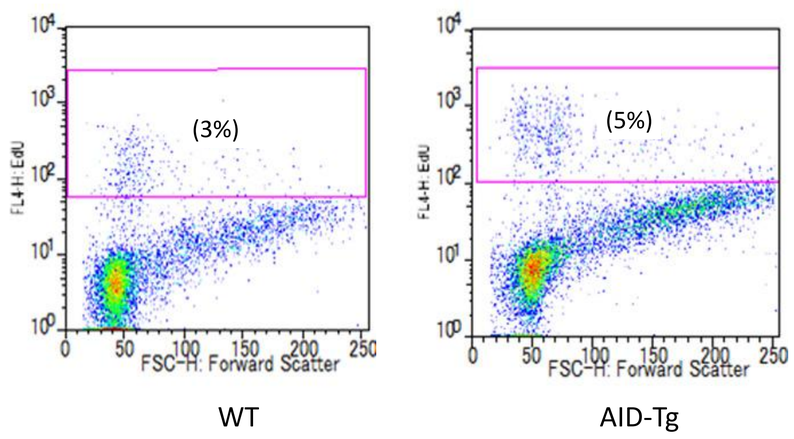

Figure 5. Flow cytometric analysis of cell proliferation by Edu labeling in the lung of AID transgenic mouse and wild type mouse.
The number in the parentheses indicates the percentage of EdU positive cells among the whole lung cells. Abbreviation: EdU, 5-ethynil-2'-deoxyuridine.

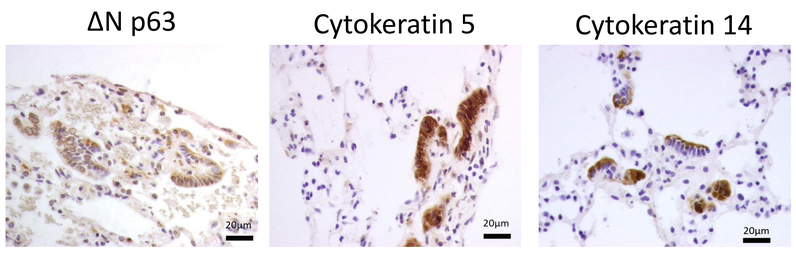

Figure 6. Immunohistochemistry for lung alveolar regeneration markers.
Delta N p63 (left), cytokeratin 5 (middle), and cytokeratin14 (right) were positive in MALLs respectively.

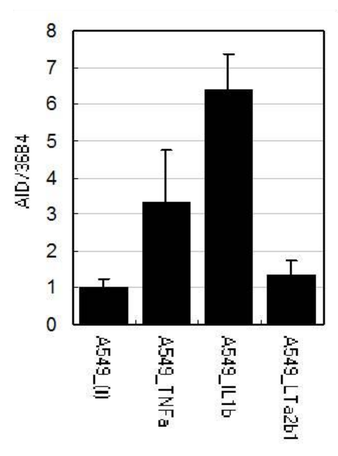

Figure 7. AID expression induced in human lung cancer cell in response to TNFα and IL-1β stimulation.
AID transcripts were measured with TNFα and IL-1β stimulation for 24 hours by quantitative real-time RT–PCR and the expression levels of AID were normalized to 36B4 as an endogenous control. Abbreviations: TNFα, tumor necrosis factor α : IL-1β, interleukin-1β.

	MALL	AAH
cell	columnar	cuboidal
nuclei	elongated	round
SP-C	+	+
lamellar body	-	+
mucous granule	+	-

Table 2. Difference between MALL and AAH.

CONCLUDING REMARKS

Based on these observations, we speculate that occasional alveolar epithelial cell death in the lung of AID transgenic mice are caused by AID-induced genotoxic stress. MALL is a regenerating tissue compensating for the cellular loss. Considering that 10 percent of AID transgenic mice develop visible lung tumors, the AID expression in such regenerating tissue should predispose cells to malignant transformation by its mutagenic activity.

We analyzed the expression of endogenous AID transcripts in human lung adenocarcinoma cell lines by quantitative reverse transcription–polymerase chain reaction. The expression of AID was induced after the treatment of the cells with TNFα and IL-1β for 24 hours (Figure 7). Recently, AID expression in human lung adenocarcinoma has been reported. Combining our data and the report, it is plausible that AID is related to lung carcinogenesis. AID transgenic mice could be a mouse model that may provide the link between lung regeneration after injury and the development of lung cancer.