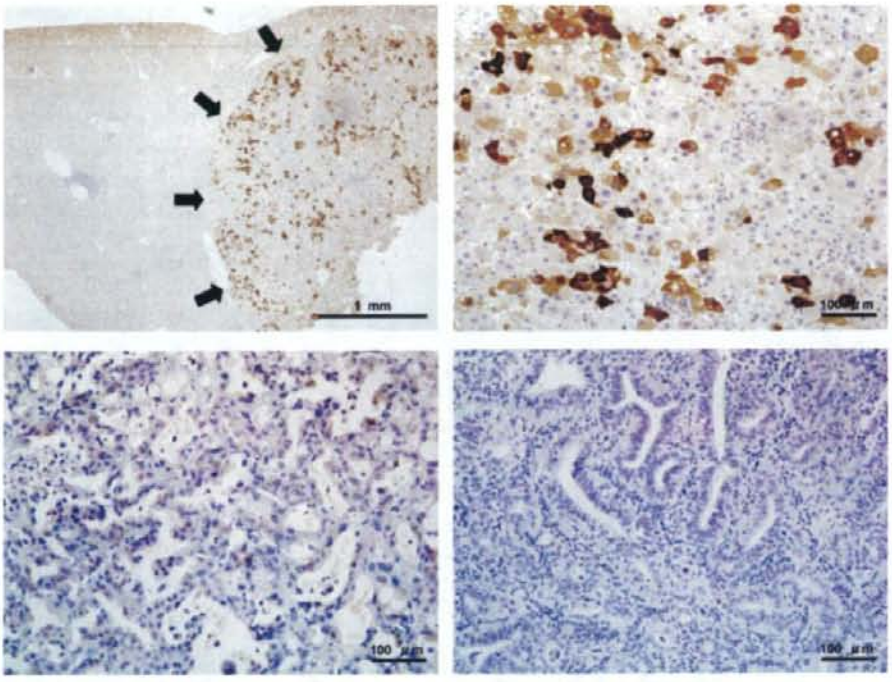

FIGURE 3 – Immunostaining for alpha-fetoprotein (Afp) in epithelial tumors from AID Tg mice. Top left, liver section immunostained for Afp in a 53-week-old AID Tg mouse (magnification ×40). The HCC (arrowheads) is clearly distinct from nontumor tissues. Top right, higher magnification (magnification ×200) of the same HCC. Afp-immunostained tumor cells are scattered in this view. Bottom left, lung cancer section immunostained for Afp (magnification ×200). Lung cancer cells are not immunostained for Afp. Bottom right, stomach cancer section immunostained for Afp (magnification ×200). Stomach cancer cells, like lung cancer cells, are not immunostained for Afp.

gene is that increased levels of the *c-myc* gene transcription in lung tissue might cause the accumulation of somatic hypermutations induced by *AID* activity, as AID-induced hypermutation depends on the transcription level of the target gene.[38–41] It must be noted that cancer cells with the specific *c-myc* or *K-ras* gene mutations might survive selectively during tumorigenesis in AID Tg mice, although all the nucleotide changes detected in the current analyses were sporadic, not clonal, in the tumor tissues. Thus, further analysis would be required to determine whether AID-mediated genotoxic effects lead to clonal mutations in certain tumor-related genes and how AID-induced mutagenesis contributes to changes in the expression levels of the target genes.

Interestingly, the *Afp* transcript was specifically upregulated in HCC, but not in the stomach and lung cancers that developed in AID Tg mice. Afp is a glycoprotein synthesized by the fetal liver and is the best known and widely used tumor marker for human HCC. The significance of the specific aberrant upregulation of Afp in the cancers that developed in the liver of AID Tg mice cannot be overemphasized, as it suggests that the molecular process of hepatocarcinogenesis in AID Tg mice might be very similar to that in the development of human HCC.[28] It remains unclear why *Afp* overexpression was specifically observed in HCC tissues. It might be tempting to assume that the regulatory pathway for *Afp* gene expression is a specific target of AID-mediated genotoxic effects in hepatocytes during hepatocarcinogenesis. Another possibility is that Afp-expressing cells survived selectively as the tumor cells during the process of liver cancer development, irrespective of AID-induced mutagenesis.

In conclusion, the present study clearly demonstrates that the genetic changes induced by the genotoxic activity of AID show organ-specific profiles and are quite different in liver, stomach and lung cancers. As *AID* expression is induced by proinflammatory cytokine stimulation in various human epithelial tissues, our findings suggest that the target preference of AID-induced mutagenesis might contribute to the diversity of tissue-specific oncogenic pathways in the various epithelial organs.

Further analyses would be required to determine whether organ specific mutations observed in the AID Tg mice were the result of the preferential target selection of the AID activity or the characteristics of the tumor cells selectively surviving in each organ during the process of tumorigenesis. Moreover, a recent study has demonstrated that AID is required for the translocation between the *c-myc* and *IgH* loci by a mechanism common to class-switch recombination in immunoglobulin genes.[11] This suggests that aberrant AID expression contributes to the occurrence of chromosomal abnormalities in epithelial organs. Therefore, determining the role of AID in the generation of chromosomal abnormalities in epithelial tissues will be a future challenge.

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