Mitotic Kinase Expression and Colorectal Cancer Progression

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Loss of chromosomal integrity as well as genomic stability is considered to act as a driving force during the processes of tumorigenesis and tumor progression *(1–3)*. Recently, two kinase genes involved in mitosis, the genes for aurora and IPL1-like midbody-associated protein kinase-1 *(4,5)* [AIM-1, registered in UniGene and also known as aurora1 *(6)* and ARK2 *(7)*] and for serine/threonine kinase-6 [STK6, also known as BTAK/STK15 *(8,9)*, Aik *(10)*, aurora2 *(6)*, and ARK1 *(7)*], which are related to Ipl1 in *Saccharomyces cerevisiae* and aurora in *Drosophila*, have been found to be expressed at high levels in cancer cells *(5,6,9)*. These genes encode serine/threonine protein kinases whose functional roles during chromosomal segregation processes in mitosis have been examined *(4–10)*. In transfected human cells *in vitro*, the overexpression of either AIM-1 or STK6 causes chromosomal abnormalities, which are presumably attributed to a defect in the mitotic processes *(5,9)*. Thus, these genes may be involved in the loss of chromosomal integrity during human cancer development via mitotic subversion.

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See "Notes" following "References."

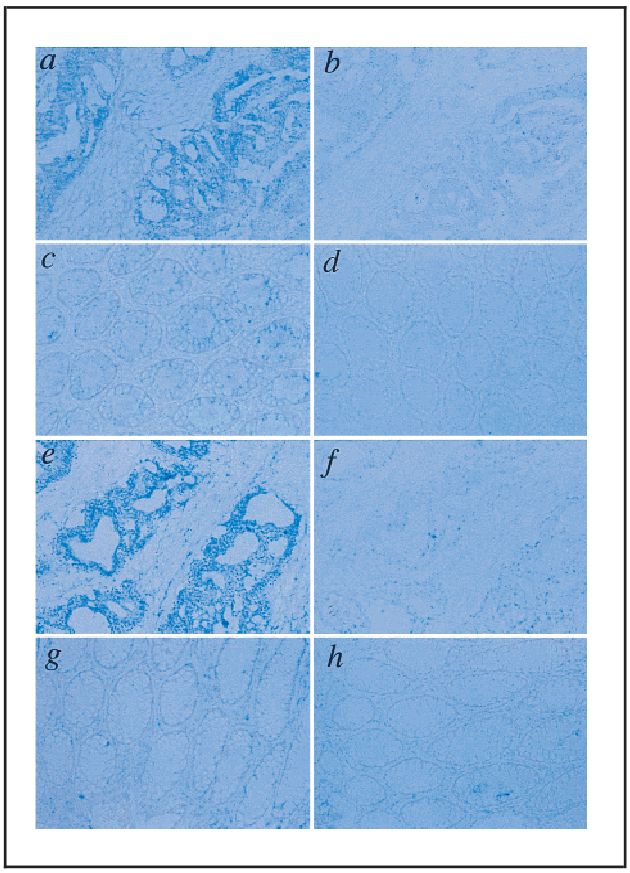

Fig. 1. *In situ* messenger RNA expression of aurora- and IPL1-like midbody-associated protein kinase-1 (AIM-1) (***a***–***d***) and serine/threonine kinase-6 (STK6) (***e***–***h***) in human cancerous (***a*** and ***e***) and normal (***c*** and ***g***) colon tissue by use of the appropriate antisense probes. Higher positive staining with AIM-1 (***a***) and STK6 (***e***) probes is seen in colon carcinoma than in normal colon; no substantially elevated positive signal is observed in the stroma. In normal colon mucosa, some crypt cells show weakly positive signals (***c*** and ***g***). Control hybridization, with the use of sense-orientation probes, shows background staining (***b, d, f,*** and ***h***).

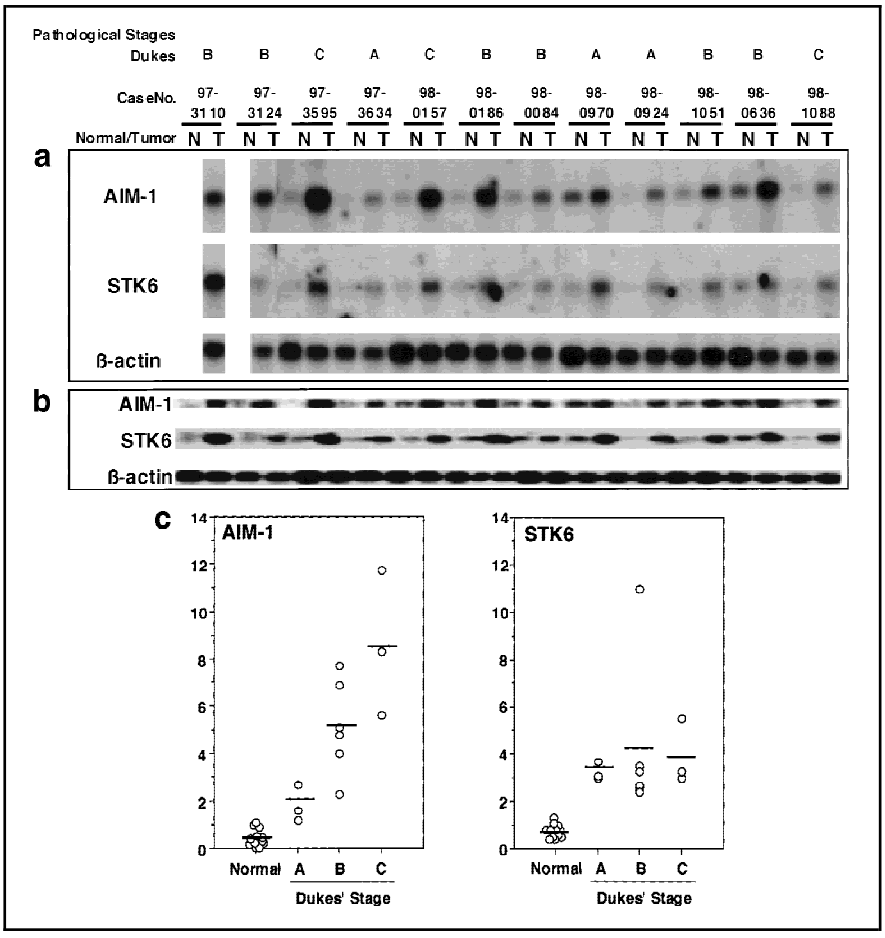

Fig. 2. Expression levels of AIM-1 and STK6 in primary human colorectal cancers of various pathologic stages. (**a**) AIM-1 and STK6 messenger RNA (mRNA) expression was estimated by northern blot analysis, and (**b**) AIM-1 and STK6 protein expression was estimated by western blot analysis with the use of anti-AIM-1 antibody *(4)* and anti-IAK1 antibody (Transduction Laboratory, Lexington, KY). (**c**) AIM-1 (**left panel**) and STK6 (**right panel**) mRNA expression levels were measured by quantitative reverse transcription–polymerase chain reaction analysis *(11)*. The ratio of the values between the targeted genes and the control gene, glyceraldehyde-3-phosphate dehydrogenase, was plotted against Dukes' stage. The relative expression levels of AIM-1 and STK6 in cells from tumors classified as Dukes' A, B, and C were statistically significantly higher than those in normal colorectal tissue ($P<.01$ by two-tailed Mann–Whitney *U* test). The AIM-1 mRNA expression levels in Dukes' A, B, and C samples were statistically significantly different between each group of tumor samples, i.e., Dukes' C > B > A ($P<.04$ by two-tailed Mann–Whitney *U* test). There were no statistically significant differences in the expression levels of STK6 among samples from Dukes' A, B, and C tumors. The **vertical axis** indicates the relative expression levels. **Bars** indicates mean values.

Herein, we report the expression levels of these genes in human colorectal cancers of different pathologic grades. We found that both genes were highly expressed. In addition, we observed that the expression levels of AIM-1 showed a tendency to group in higher grades of malignancy defined by pathologic observation.

Fresh tumor tissues and their normal counterparts were obtained from surgically resected specimens from 12 patients with primary colorectal cancer. Highly elevated, positive signals were observed by *in situ* hybridization to AIM-1 and STK6 genes in cancerous colon as compared with normal colon tissue (Fig. 1). These differences cannot account for the different cell cycle progression profiles between cancerous and normal cells because highly elevated positive signals were clearly observed during mitosis in tumors and in cultured colon cancer cells but not in normal mitotic colon cells. Moreover, such elevated positive signals were not detected in inflammatory cells, smooth muscle cells, endothelial cells in the blood vessels, or other actively growing cells such as in the testis and thymus (data not shown). Northern and western blot analyses confirmed the higher expression levels of both genes in the tumors (Fig. 2, a and b). The quantitation of the levels of AIM-1 and STK6 expression revealed that both genes were overexpressed in tumors, and only the AIM-1 expression levels were increased as a function of Dukes' stage, which suggests that AIM-1 overexpression is closely implicated in tumor progression (Fig. 2, c).

The STK6 gene is amplified on chromosome 20q13 *(8,9)*, but AIM-1 (on chromosome 17p13) is not amplified and is overexpressed by a transcriptional mechanism *(5)*. These two structurally related mitotic kinases are periodically expressed in G_2/M phases during the normal cell cycle progression *(4–6)*, but the regulatory mechanisms for each gene's expression may be different. In the endomitotic differentiation of megakaryocytic lineage cells, the AIM-1 transcript was diminished, but the STK6 transcript was transiently increased *(11)*. In addition, AIM-1 expression and its kinase activity were reduced in a TP53-dependent manner when cells were irradiated with X-irradiation (Tatsuka M, Katayama H: unpublished data). Thus, AIM-1 expression levels are considered to be multifunctionally regulated by "caretaker" and "gatekeeper" genes for multistep carcinogenesis. Higher expression levels of AIM-1 in pathologically more aggressive cancers indicate that AIM-1 expression may serve as a marker for prognostic evaluation of malignant tumors.

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NOTES

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