RAPID COMMUNICATION Jpn. J. Cancer Res. 81, 707–710, August 1990

# Isolation of an Amplified DNA Sequence in Stomach Cancer

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**By use of the in-gel DNA renaturation method, the presence of amplified DNA sequences was demonstrated in KATO-III, a cell line established from a signet ring cell carcinoma of the stomach. A DNA fragment from one of these amplified regions in KATO-III cells was cloned and designated $SAM_{0.2}$; the locus containing the $SAM_{0.2}$ fragment was referred to as SAM. The SAM locus was shown to be amplified not only in KATO-III cells, but also in three of 24 surgical specimens of stomach cancers and in two of 13 xenografts of human stomach cancers, all of these specimens being poorly differentiated adenocarcinoma or mucinous adenocarcinoma of the stomach. The SAM locus was not amplified in 14 cell lines of cancers of other organs or in 42 surgical specimens of lung cancers.**

Key words: Stomach cancer — Gene amplification — In-gel DNA renaturation method

Increased content of DNA per cell and aneuploidy are often associated with more malignant types of cancers.[1,2] However, amplification of oncogenes has been found only in a limited number of human cancers.[3,4] Amplification of genes other than known oncogenes is expected to occur more commonly in cancers. Previously, we used in-gel DNA denaturation and renaturation following gel electrophoresis to demonstrate the presence of amplified DNA sequences in cell lines established from stomach cancers.[5] Here we report the successful isolation of a DNA fragment from one of these amplified genomic regions in a gastric cancer cell line. The isolated fragment was termed $SAM_{0.2}$, and the locus containing $SAM_{0.2}$ was designated SAM. The SAM locus was found to be amplified not only in this cell line, but also in two of 13 xenografts of human stomach cancers and in three of 24 surgical specimens of human stomach cancers; two of these three specimens were stomach cancers metastasized to lymph nodes. SAM was not amplified either in 14 cell lines derived from cancers of other organs or in 42 surgical specimens of lung cancers.

In-gel DNA renaturation was performed as previously described.[5-13] Fig. 1 shows the result of a DNA renaturation analysis of the gastric cancer cell line, KATO-III. KATO-III was established from pleural effusion of metastatic gastric cancer cells.[14] The original tumor was a signet ring cell carcinoma. The KATO-III cells were hypertriploid with a modal chromosomal number of 79 and have many marker chromosomes and a homogeneously staining region (HSR) on the long arm of chromosome 11 at band q23.[6] As shown in Fig. 1, many DNA fragments with sizes ranging from 1 kbp to 20 kbp were amplified. The total size of these fragments was estimated to be about 200 kbp. The degree of amplification was more than 100-fold based on the intensity of the bands. No amplification of c-Ha-*ras*, c-Ki-*ras*, N-*ras*, c-*myc*, N-*myc*, L-*myc*, c-*myb*, c-*abl*, c-*erb*B-1, c-*erb*B-2, *hst*-1, *hst*-2, *mdr* or *ets* was detected in KATO-III cells by Southern blot hybridization analysis (data not shown).

To characterize the nature of the gene amplification in KATO-III cells, a DNA fragment in one of the amplified regions of the genome was cloned as follows; 150 $\mu$g of *Hin*dIII-digested DNA of KATO-III cells was processed basically according to the in-gel DNA renaturation method except for the step of S1 nuclease digestion. The portion of the gel corresponding to 3.0 kbp arbitrarily chosen and excised, and DNA was eluted electrophoretically. Since the ends of the eluted DNA fragments might have been damaged in the process of in-gel renaturation, the DNA was further digested with *Mbo*I to offer fresh cohesive ends for ligation with the *Bam*HI-digested pUC8 vector. *E. coli* JM101 was transformed by the recombinant plasmids, and 155 colonies were identified to carry DNA inserts by their inactivation of the $\beta$-galactosidase gene of the pUC8 vector. Five clones were randomly picked up, and the inserts were analyzed for amplification in the KATO-III cells by Southern blot analysis. One clone, $pSAM_{0.2}$, was found to carry a 0.2-kbp insert termed $SAM_{0.2}$, and the corresponding genomic locus, SAM, was amplified about 30-fold in KATO-III cells. The other four clones did not contain fragments amplified in KATO-III (data not shown). The

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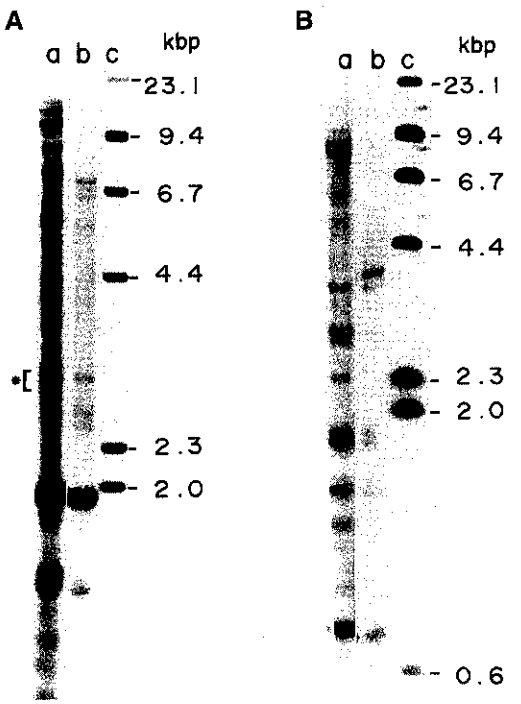


Fig. 1. Analysis of amplified DNAs in a gastric cancer cell line, KATO-III, by the in-gel DNA renaturation method.[5-13] DNAs digested with *Hin*dIII (A) or *Eco*RI (B) were subjected to in-gel DNA renaturation analysis. Lane a, KATO-III; lane b, spleen; lane c, labeled *Hin*dIII digests of $\lambda$ phage DNA used as size markers. The asterisk on the left side of the KATO-III lane shows the portion of the 1.5 cm-wide agarose strip from which amplified DNA fragments were extracted.

$SAM_{0.2}$ fragment hybridized to 3.0-kbp *Hin*dIII and 4.4-kbp *Eco*RI fragments of normal human DNA on Southern blot analysis (Figs. 2A, 2B). The degree of amplification of the SAM locus in KATO-III cells was around 30-fold, less than the 100-fold amplification of the major 3.0-kbp *Hin*dIII fragment we observed in the in-gel DNA renaturation assays (Fig. 1); The $SAM_{0.2}$ fragment may not be in the 3.0-kbp *Hin*dIII fragment. It is also possible that the degree of amplification of DNA fragments detected by in-gel DNA renaturation analysis was overestimated because of fortuitous comigration with other amplified 3.0 kbp *Hin*dIII DNA fragments.

DNAs from 24 surgical specimens of stomach cancers, including three well or moderately differentiated tubular adenocarcinomas, 18 poorly differentiated adenocarcinomas, one mucinous adenocarcinoma, one signet ring cell carcinoma and one undifferentiated adenocarcinoma were analyzed by Southern blot hybridization with the $SAM_{0.2}$ fragment. In three of 24 surgical specimens of human stomach cancers, the SAM locus was amplified 30- to 50-fold: one was a mucinous adenocarcinoma and the other two were poorly differentiated adenocarcinomas (Fig. 2A, 2B, 2C). SAM amplification was not found in 42 surgical specimens of lung cancers. In one case of stomach cancer with metastasis, there was an amplification of the SAM locus only in the metastatic tumor but not in the primary tumor (Fig. 2C). This result suggested that this case had developed heterogeneous populations of cells in the tumor, and only the cancer cells with the amplified SAM locus metastasized to the lymph node. Furthermore, in two of 13 human stomach cancers transplanted into nude mice, the SAM locus was amplified 30- to 50-fold. These were xenografts NSC4 and NSC10 (Fig. 2D). NSC4 and NSC10 xenografts were established from a poorly differentiated adenocarcinoma and a mucinous adenocarcinoma, respectively.[15] In *Hin*dIII-digested DNAs from these two xenografts of stomach cancers, novel bands of 10.0 kbp and 5.6 kbp were detected by the $SAM_{0.2}$ probe, suggesting that the amplification of the SAM locus was accompanied with rearrangement in these tumors. None of the other 11 stomach cancers maintained as transplantable tumors in nude mice[15] contained amplified sequences detected by $SAM_{0.2}$; these were St4, NSC3, NSC8, SCK41, SCK16, SCK33, H-111, NSC7, SCK29, St15 and SC7-JCK xenografts. St4, NSC3, NSC8 and SCK41 xenografts were established from poorly differentiated adenocarcinomas, while SCK16 xenograft was from a papillary adenocarcinoma. SCK33, H-111, NSC7, SCK29, St15 and SC7-JCK xenografts were derived from well or moderately differentiated tubular adenocarcinomas.

It was found that the SAM locus was not amplified in eight gastric cancer cell lines[14-17] other than KATO-III cells: OKAJIMA, SCH, MKN1, MKN7, MKN28, MKN45, MKN74 and TMK-1 cells. OKAJIMA, MKN45 and TMK-1 cells were derived from poorly differentiated adenocarcinomas, while MKN7, MKN28 and MKN74 cells were derived from well differentiated adenocarcinomas. SCH cells were established from a choriocarcinoma of the stomach and MKN1 from an adenosquamous carcinoma. The amplification was also not detected in 11 cancer cell lines of other cancers[2-5]: two neuroblastoma cell lines, NB1 and C26 cells, one retinoblastoma cell line, Y79 cells, two small cell lung cancer cell lines, H69 and Lu135, two colon cancer cell lines, COLO205 and COLO320, one promyelocytic leukemia cell line, HL60, one chronic myelocytic leukemia cell line, K562, one epidermoid cancer cell line, A431 and one cervical cancer cell line, HeLa.

Some of the cell lines tested were found to have amplification of various oncogenes,[2-5, 15] including K-*ras* in MKN1 cells (unpublished data), N-*myc* in NB1, C26, Y79 and H69 cells, c-*myc* in St4, NSC-3, Lu135, COLO320 and HL60 cells, L-*myc* in Lu135 cells, c-*myb*

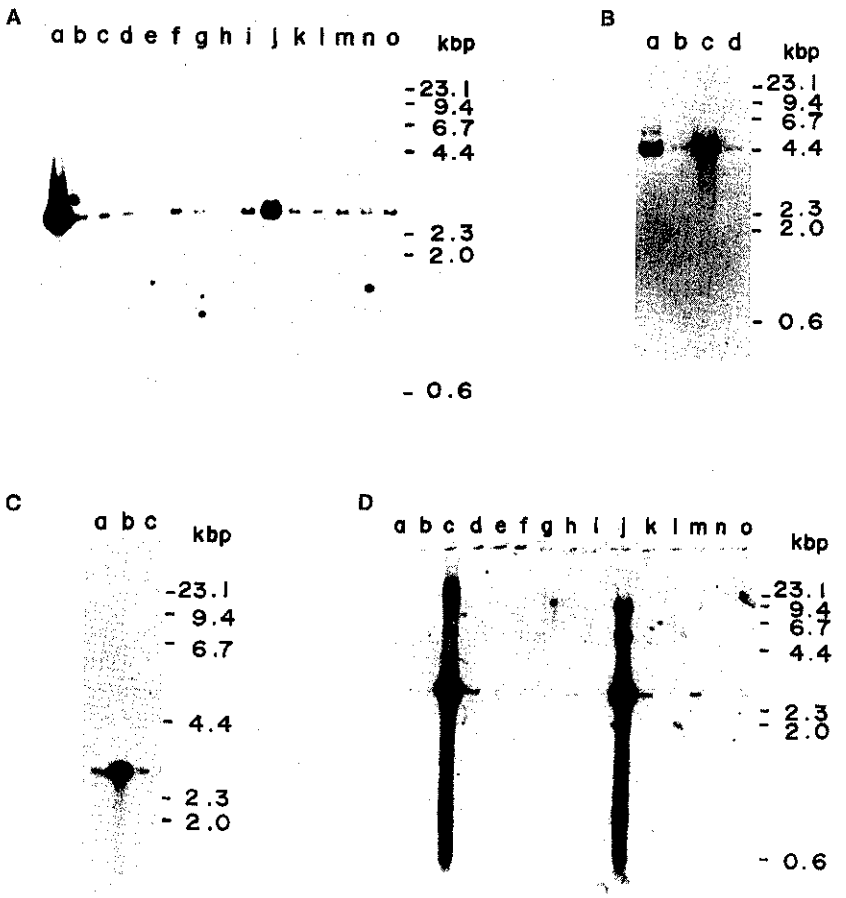


Fig. 2. Southern blot analysis by the $SAM_{0.2}$ probe. DNAs were digested with *Hin*dIII (A, C, D) or *Eco*RI (B). Hybridization was performed at 42°C in a buffer containing 50% formamide, and the filters were washed with 0.1×SSC/0.1% SDS at 65°C as described previously.[19,20] A. Amplification of the SAM locus in DNA samples from gastric cancer cell lines and surgical specimens. Lane a, KATO-III; lane b, OKAJIMA; lane c, SCH; lane d, MKN1; lane e, MKN7; lane f, MKN28; lane g, MKN45; lane h, MKN74; lane i, TMK-1; lane j, 81; lane k, 311; lane l, 351; lane m, 372; lane n, 633; lane o, 653. KATO-III, OKAJIMA, SCH, MKN1, MKN7, MKN28, MKN45, MKN74 and TMK-1 are gastric cancer cell lines. Samples 81, 311, 351 and 372 were lymph node metastases of stomach cancers. Samples 633 and 653 were spleen tissues. B and C. Amplification of the SAM locus in DNA samples from surgical specimens of primary gastric cancers and metastatic lymph nodes. B. Lane a, 81; lane b, 82; lane c, 101; lane d, 102; C. lane a, 561; lane b, 562; lane c, 563. Samples 81 and 562 were gastric cancers metastasized to lymph nodes, while samples 101 and 561 were primary gastric cancers. Samples 82, 102 and 563 were non-cancerous portions of gastric mucosae adjacent to the cancer lesion. Samples 81, 82; 101, 102; and 561, 562, 563 were obtained from the same patients. The histological diagnosis of sample 81 was mucinous adenocarcinoma, while samples 101 and 561 were poorly differentiated adenocarcinomas. D. Amplification of the SAM locus in DNAs from xenografts of human gastric cancers. Lane a, St4; lane b, NSC3; lane c, NSC4; lane d, NSC8; lane e, SCK16; lane f, SCK29; lane g, SCK41; lane h, SCK33; lane i, NSC7; lane j, NSC10; lane k, H-111; lane l, SC7-JCK; lane m, St15; lane n, nude mouse liver; lane o, 633.

in COLO205 cells, c-*abl* in K562 cells, c-*erb*B-1 in A431 cells and c-*erb*B-2 in MKN7 cells. The results indicated that the SAM locus is not linked to any of these oncogenes.

The stomach cancers found to contain amplified SAM locus were poorly differentiated adenocarcinoma, signet ring cell carcinoma and mucinous adenocarcinoma. We could not detect amplification of SAM in well or mod-

erately differentiated stomach cancers. Isolation and characterization of the regions containing the $SAM_{0.2}$ fragment will certainly provide important information on the molecular mechanisms underlying the development of poorly differentiated types of stomach cancers *in vivo*.

In-gel DNA renaturation assay was first developed by Roninson *et al.* to detect amplified DNA sequences in multidrug-resistant cancer cells.[7-10] Based on the results of analyses of amplified sequences with this method, the *mdr* gene was subsequently isolated.[10, 11] Using the same approach, *gli* was identified as a gene that is amplified in a malignant glioma.[12, 18] The in-gel DNA renaturation method followed by isolation and characterization of the amplified sequences will be useful for detection of amplified genes in various types of cancers.

We thank Drs. Y. Shimosato and T. Hirota for histological diagnosis of the surgical specimens, and Dr. M. Sekiguchi for providing the KATO-III cell line. We are grateful to the following scientists for providing cell lines or xenografts used in the present studies: Drs. H. Watanabe, T. Suzuki, S. Takao and K. Nakatani. This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control, Japan.

*(Received March 15, 1990/Accepted April 20, 1990)*

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