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Co-amplification of c-*myc* and c-*erbB*-2 Oncogenes in a Poorly Differentiated Human Gastric Cancer

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c-*erbB*-2 oncogene has been reported to be frequently amplified in differentiated, tubular type of gastric cancer. Here we report a human gastric cancer which bore co-amplified c-*myc* and c-*erbB*-2 oncogenes: a portion of the amplified c-*erbB*-2 oncogene was found to be rearranged. Furthermore, c-*myc* and c-*erbB*-2 oncogenes were over-expressed in the tumor cells. In contrast to the previous reports, this gastric adenocarcinoma was classified as a poorly differentiated type, and was highly tumorigenic in nude mice. These results might suggest that activated c-*myc* and c-*erbB*-2 oncogenes co-operate and influence the malignant state of some gastric carcinomas.

Key words: Co-amplification — c-*myc* — c-*erbB*-2 — Gastric cancer

Carcinogenesis is considered to be a multi-step process of alteration of genes which are involved in growth control of cells. As positively regulating factors, a variety of proto-oncogenes have been shown to be activated in tumor cells via quantitative and qualitative mechanisms.[1–7] Gene amplification is one of the most common alterations of proto-oncogenes in malignant cells, and in some tumors structurally and functionally altered proto-oncogenes were found to be amplified.[8,9] In gastric cancer, c-*myc*,[10–12] c-*erbB*-2,[13] *sam*[14] and c-*erbA*[13] genes have been reported to be frequently amplified and over-expressed. Among these genes, amplification of c-*erbB*-2, which encodes a cell surface receptor similar to EGF receptor,[15] has been found in differentiated and tubular type of gastric adenocarcinoma, suggesting a relationship between the type of activated oncogene and the histological features.[13] Since activation of multiple proto-oncogenes in a single tumor has been reported in several cases[9,16–20] such as Lu65[9] and HL60,[16] we have screened co-amplification of cellular genes in gastric cancer.

About twenty human gastric adenocarcinoma cell DNAs (including 10 cases of poorly differentiated type and 7 cases of tubular type) were hybridized with c-*erbB*-2 0.44 kb genomic DNA fragment[21] as a probe. As shown in Fig. 1A, we found that one case, 4-1ST (Shiraishi strain), of poorly differentiated adenocarcinoma carried amplification of c-*erbB*-2 gene: digestion of the DNA with restriction enzyme *Hin*dIII showed two amplified fragments, genomic type of 13 kb long and an extra band of 5.0 kb long. The degrees of amplification of these fragments were 10- to 15-fold and 2- to 3-fold, respectively.

4-1ST tumor is one of the three cases of transplantable gastric cancer which were previously shown to have amplification of c-*myc* gene.[11] Using the same nitrocellulose sheet as in Fig. 1A, we confirmed the c-*myc* amplification in 4-1ST by Southern blot analysis (Fig. 1B). The degree of c-*myc* amplification was about 30-fold. It is of interest to note that all cases of gastric cancer bearing c-*erbB*-2 gene amplification reported so far were a type of well-differentiated, tubular adenocarcinoma, and did not carry c-*myc* gene amplification.[13] Thus, 4-1ST tumor seems to be the first case of poorly differentiated gastric cancer associated with co-amplification of c-*erbB*-2 and c-*myc* genes.

With respect to the extra band of amplified c-*erbB*-2 gene in 4-1ST (Fig. 1A), a restriction fragment length polymorphism of the allelic c-*erbB*-2 gene in the tumor cells seems unlikely, since no other tumor cell line or normal tissue of humans examined so far showed this 5.0 kb *Hin*dIII fragment (data not shown). We have not yet determined precisely the domain(s) of c-*erbB*-2 gene included in the rearranged 5.0 kb *Hin*dIII fragment. However, *Xba*I-*Kpn*I 0.44 kb probe, which corresponds to the middle portion of the tyrosine kinase domain in the c-*erbB*-2 gene, and 0.65 kb cDNA probe, covering the amino-terminal half of its kinase domain, clearly detected this extra band (Fig. 1A and C). These results suggest that at least a half of the kinase domain in the c-*erbB*-2 gene is present in this rearranged *Hin*dIII fragment.

Although the size of rearranged c-*erbB*-2 fragment (about 5 kb) is close to those of plasmid vectors such as

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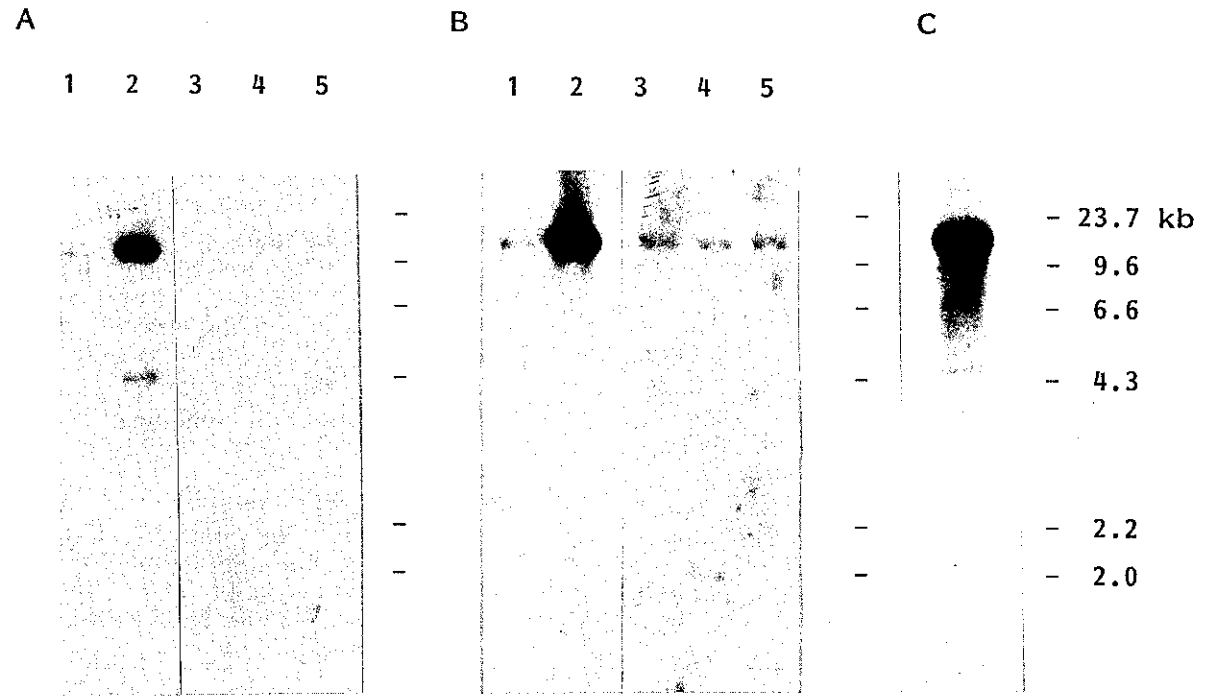
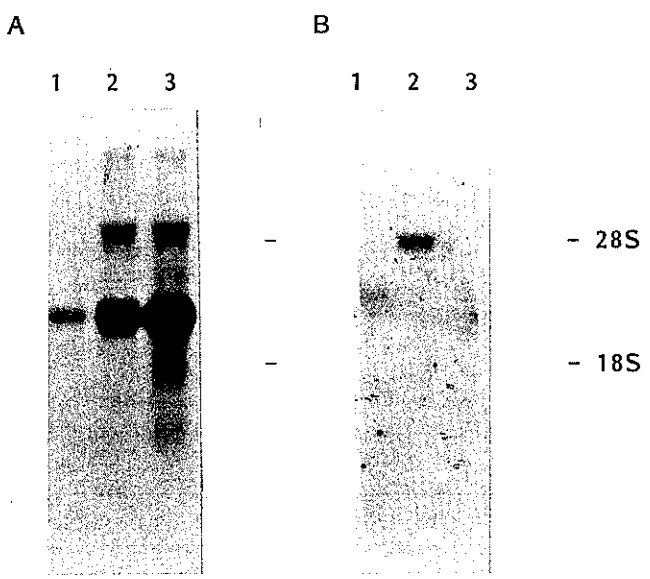


Fig. 1. Amplification of c-*erbB*-2 and c-*myc* genes in a human gastric cancer 4-1ST. A, About 10 μg of cellular DNA was digested with restriction enzyme *Hin*dIII, transferred to a nitrocellulose filter and hybridized with ^{32}P-labeled *Xba*I-*Kpn*I 0.44 kb fragment of c-*erbB*-2 DNA. The size of the genomic DNA of c-*erbB*-2 gene detected with this probe was about 13 kb. The gastric cancers analyzed were SC-4-JCK (lane 1), 4-1ST (lane 2), SC-9-JCK (lane 3), NS-8 (lane 4) and OSS (lane 5). B, The same nitrocellulose sheet as in A was rehybridized with ^{32}P-labeled *Cla*I-*Eco*RI 1.5 kb human c-*myc* DNA as a probe after removal of the first c-*erbB*-2 probe. The size of the genomic c-*myc* DNA fragment detectable with this probe was about 11 kb. C, About 10 μg of 4-1ST DNA was digested with *Hin*dIII and hybridized with *Acc*I-*Acc*I 0.65 kb of c-*erbB*-2 cDNA. This fragment covers the amino-terminal half of the tyrosine kinase domain.



pBR322, contamination of plasmid sequences into tumor DNA samples seems unlikely, because only two c-*erbB*-2 probes out of 10 kinds of *onc* probes examined (including c-*myc* gene) detected this extra band in 4-1ST DNA.

To examine the level of mRNA in 4-1ST cells, poly-(A)-containing RNA was electrophoresed in a formaldehyde-agarose gel, transferred to a nitrocellulose sheet, and hybridized with c-*myc* or c-*erbB*-2 probe. As shown in Fig. 2A, c-*myc* gene was strongly expressed in 4-1ST cells and the level of c-*myc* mRNA was similar to that in HL60 cells, which carry 20- to 30-fold amplification of

Fig. 2. High expression of c-*erbB*-2 and c-*myc* genes in gastric cancer 4-1ST. A, About 2 μg of poly(A)-containing RNA was separated on an agarose gel, transferred to a nitrocellulose sheet and hybridized with *Cla*I-*Eco*RI c-*myc* probe. The RNAs are from a transplantable gastric cancer SC-4-JCK (lane 1), 4-1ST (lane 2) and a promyelocytic leukemia cell line HL60 (lane 3). B, After removal of the c-*myc* probe, the same nitrocellulose sheet as A was rehybridized with ^{32}P-labeled c-*erbB*-2 cDNA.

c-*myc* DNA.[5,6] When the same sheet was hybridized with c-*erbB*-2 cDNA probe, 4-1ST cells showed a significant amount of c-*erbB*-2 mRNA compared with those in HL60 and another cell line of gastric cancer: in the latter cases this mRNA was almost undetectable (Fig. 2B). The size of c-*erbB*-2 mRNA in 4-1ST cells was about 4.8 kb and appeared to be identical to the normal c-*erbB*-2 mRNA described previously[21]: no abnormal transcripts were detected by Northern blot analysis. These results may indicate that the major portion of the c-*erbB*-2 mRNA in 4-1ST cells is derived from non-rearranged, genomic type c-*erbB*-2 DNA. Therefore, the biological significance of the rearranged c-*erbB*-2 DNA shown in Fig. 1 is not clear at this moment. A similar rearrangement of c-*erbB*-2 gene, but without association of gene amplification, was observed in a gastric cancer by Yokota *et al.*[13]

Since 4-1ST gastric cancer is a transplantable tumor cell line in athymic nude mice and the original cancer tissue was not stored, we cannot completely rule out the possibility that co-amplification of c-*myc* and c-*erbB*-2 took place during or after establishment of this cell line. Although such an oncogene amplification in *in vitro* culture is thought to be very rare, further screening experiments of primary gastric cancer are necessary to clarify the biological significance of *myc* and *erbB*-2 co-amplification.

In addition to the morphological characteristics of poorly differentiated type, 4-1ST cells cause tumors within 3 weeks after inoculation of small pieces of tumor into nude mice, which is rather faster than has been found with most cases of well-differentiated, tubular adenocarcinomas of the stomach.

Multi-step activation of proto-oncogenes appears to be very important for carcinogenesis in naturally occurring tumors. In view of the co-amplification of c-*myc* and c-*erbB*-2 genes shown here, 4-1ST may represent an interesting model system of co-operation of two proto-oncogenes for progression of malignancy in stomach cancer of humans.

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