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AMPLIFICATION OF THE *hst*-1 GENE IN HUMAN ESOPHAGEAL CARCINOMAS

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The *hst*-1 gene, previously designated as the *hst* gene, and seven other oncogenes were examined for possible structural changes in esophageal, gastric and colorectal carcinomas by Southern blot hybridization. The *hst*-1 gene was amplified in eight (42.1%) of the nineteen esophageal squamous cell carcinomas and in all four metastatic tumors of lymph nodes. The degree of amplification ranged from two to eight times. Coamplification of the *hst*-1 and c-*erbB*-1 gene was found in one case of esophageal carcinoma. However, no amplification of the *hst*-1 gene was detected in gastric and colorectal carcinomas.

Key words: *hst*-1 — Amplification — Esophageal carcinoma

In Japan esophageal carcinoma is less frequent than gastric carcinoma and colorectal carcinoma, and the prognosis of esophageal carcinoma is poorer than that of gastric or colorectal carcinoma. Cytophotometric DNA analysis of esophageal carcinoma shows that most of the advanced esophageal carcinomas have a high ploidy pattern and show a high grade of biological malignancy.[1] However, no information is available on alteration of the oncogenes in esophageal carcinomas except for elevated level of expression and amplification of c-*erbB*-1 proto-oncogene.[2]

The *hst*-1 gene, previously designated the *hst* gene, was first isolated from a gastric carcinoma using NIH3T3 transfection assay.[3] We applied Southern blot analysis using *hst*-1 and seven other oncogenes as probes not only to esophageal carcinomas but also to gastric and colorectal carcinomas.

A total of 76 gastrointestinal carcinomas comprising 19 esophageal carcinomas, 37 gastric adenocarcinomas and 20 colorectal adenocarcinomas were employed. DNAs were extracted from these tumor tissues, normal adjacent mucosas and four metastatic tumors of lymph nodes. A small piece of each tissue was frozen in liquid nitrogen as soon as the tumor tissues were removed, and the diagnosis was confirmed microscopically by cryostat sectioning. Total cellular DNAs were prepared using the phenol-chloroform method after treatment with sodium dodecyl sulfate (SDS) and proteinase K. DNAs were digested with a restriction enzyme under the conditions suggested by the manufacturers.[4] The completely digested DNAs (10 μg) were subjected to electrophoresis on 0.8% agarose gel. After the electrophoresis, DNAs in the agarose gel were transferred to nitrocellulose filters according to the method of Southern.[5] The *hst*-1 specific probe was probe c, a 0.79 kbp *Eco*RI-*Eco*RI fragment of the *hst*-1 gene.[3]

Hybridization was performed at 42° for 16 to 24 hr in 50% formamide, 7×SSC (1×SSC is 0.15*M* NaCl, 0.015*M* sodium citrate), 5× Denhardt's solution, 100 μg/ml denatured salmon testis DNA, 0.1% SDS and 10% dextran sulfate. The probe was labeled with [α-^{32}P]dCTP by the multiprime DNA labeling system (Amersham RPN. 1601Y) to a specific activity of more than 2×10^8 cpm/μg of DNA. After hybridization, the filters were washed for 1 hr at 65° in 0.1×SSC, 0.1% SDS and exposed overnight at −70° to Kodak XRP-5 film with an intensifying screen.

Table I shows the clinical and pathological diagnoses of 19 esophageal carcinomas examined. Age, sex, degree of *hst*-1 amplifica-

Table I. Summary of Patients Examined in This Study

Case No.	Age	Sex	Degree of *hst*-1 amplification	Location of the lesion[a),b)]	Clinical stage[a)]	Histologic type[a),c)]	Depth of invasion[a),d)]
1	62	male	×2, ×4(L)	Im	IV	poorly	a_3
2	68	male	×4, ×4(L)	Im	III	moderately	a_2
3	74	male	—[f)]	Iu	II	moderately	a_2
4	79	female	—	Im	III	moderately	a_2
5	62	female	—	Ce	IV	well	a_3
6	60	male	—	Im	I	poorly	mp
7	55	male	—	Im	IV	poorly	a_3
8	58	mele	×4(L_1), ×8(L_2)	Im	IV	well	a_3
9[e)]	64	female	×4	Ce	IV	well	a_3
10	61	female	×6	Im	IV	well	a_2
11	74	female	×4	Ea	III	well	a_2
12	61	female	—	Ei	III	well	a_2
13	46	female	—	Ce	II	well	a_3
14	54	female	—	Ce	IV	well	a_3
15	65	male	—	Im	IV	poorly	a_0
16	61	male	×4	Im	III	moderately	a_1
17	67	male	×6	Iu	II	moderately	a_1
18	72	male	—	Ei	III	poorly	a_1
19	72	male	×4	Im	IV	moderately	a_1

a) According to the classification of the Japanese Society for Esophageal Diseases.
b) Ce, cervical esophagus; Iu, upper intra-thoracic esophagus; Im, middle intra-thoracic esophagus; Ei, lower intra-thoracic esophagus; Ea, abdominal esophagus.
c) well, well differentiated squamous cell carcinoma; moderately, moderately differentiated squamous cell carcinoma; poorly, poorly differentiated squamous cell carcinoma.
d) mp, invasion to muscularis propria; a_0, no invasion to the adventitia; a_1, invasion reaching the adventitia; a_2, definite invasion to the adventitia; a_3, invasion into the neighboring structures.
e) Esophageal carcinoma with amplified c-*erbB*-1 gene.
f) Not amplified.

tion, location of the lesion, clinical stage, histologic type and depth of the invasion are summarized in the table.

Figure 1 shows the results of Southern blot hybridization with probe c and c-*myc*. The *hst*-1 gene was amplified in the primary tumor of Cases 1, 2, 9, 10, 11, 16, 17 and 19, and in the metastatic tumor of Cases 1 and 2 and two metastatic tumors in Case 8. The probe was removed, and the same filters were reused for Southern blot analysis using c-*myc* as the probe. A 0.4 kbp *Pst*I-*Pst*I fragment of pHSR *myc* including exon II was used as the c-*myc* probe. Each lane shows equal intensity of the c-*myc* bands indicating each lane contains the same amount of DNAs. No amplification of the *hst*-1 gene was detected in 37 gastric adenocarcinomas and 20 colorectal adenocarcinomas (data not shown).

Figure 2 shows the sequential dilution analysis of *hst*-1 gene amplification. Considering that normal cells such as fibroblasts, endothelial cells, lymphocytes or neutrophils contaminated the tumor tissues, the degree of amplification of the *hst*-1 gene was more than 4 fold in Case 16. The degree in other cases ranged from 2 to 8 times, judging from the photographic densitometrical analysis.

Figure 3 shows the result of Southern blot analysis (Fig. 3A) and sequential dilution analysis (Fig. 3B) using the c-*erbB*-1 gene as the probe. A 2.4 kbp *Cla*I-*Cla*I fragment of pE7 was used as c-*erbB*-1/EGF receptor gene.[6] Only in Case 9 was the c-*erbB*-1 gene also amplified. The degree of the c-*erbB*-1 gene amplification was 4 times, the same as that of *hst*-1 amplification.

Amplification of the c-*erbB*-1 gene has been reported in only one out of five surgically resected esophageal carcinomas,[2] but alteration of another oncogene has not been shown so far. We demonstrated that the *hst*-1 gene was amplified in 8 (42.1%) out of 19 esophageal carcinomas and all the 4 metastatic

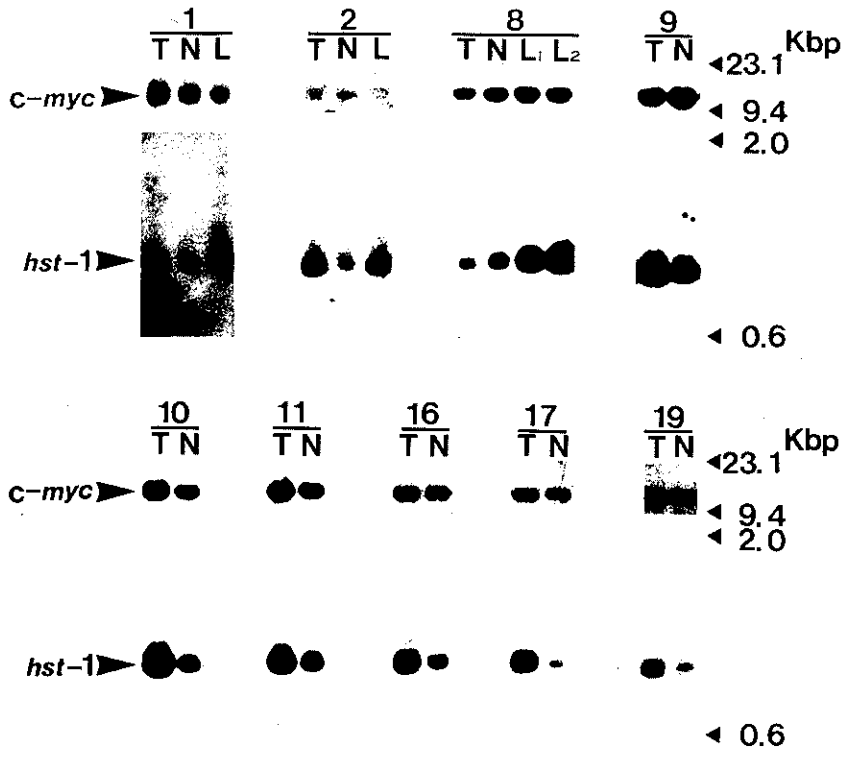

Fig. 1. Amplification of the *hst*-1 gene in esophageal carcinomas. Each lane contains 10 μg of *Eco*RI-digested DNA, Southern blot analysis was performed using *hst*-1 and c-*myc* as probes. The size of the band of genomic *hst*-1 was 0.8 kbp and that of c-*myc* was 12.5 kbp. Each number is the case number. T, The DNA from esophageal carcinomas; N, the DNA from adjacent normal mucosas; L, the DNA from metastatic carcinoma of lymph nodes.

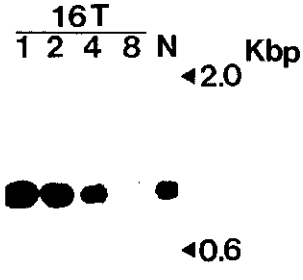

Fig. 2. The degree of amplification of the *hst*-1 gene. *Eco*RI-digested DNAs were diluted 2-, 4-, and 8-fold sequentially in Case 16.

tumors of lymph nodes. Moreover, in one case coamplification of the *hst*-1 and the c-*erbB*-1 gene was found. No correlation between the *hst*-1 gene amplification and clinical stage, histologic type, or depth of the invasion was observed. We also performed Southern blot analysis using Ha-*ras*, Ki-*ras*, c-*myc*, v-*sis*, c-*erbB*-2 and v-*erbA* as probes, but amplification of these oncogenes could not be detected in these esophageal carcinomas.

The *hst*-1 gene was first isolated and identified as a transforming gene, by using NIH3T3 transfection assay, from a gastric carcinoma, a metastatic tumor of the lymph node and a noncancerous mucosa of gastric carcinoma.[3] The *hst*-1 gene was subsequently isolated from other human gastric carcinomas,[7] a colon carcinoma,[7] hepatomas[8,9] and Kaposi sarcomas.[10] The sequence analysis of the *hst*-1 cDNA has revealed the presence of two open reading frames, one of which is responsible for the transforming ac-

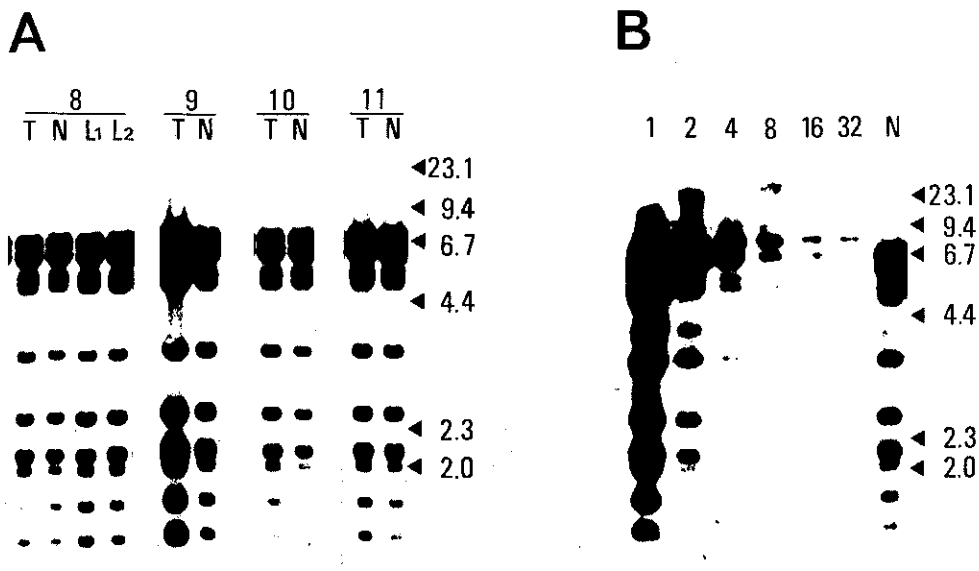

Fig. 3. Amplification of the c-*erbB*-1/EGF receptor gene and the degree of amplification in Case 9 (B).

tivity.[11] The *hst*-1 gene encodes a protein related to fibroblast growth factor and *int*-2 protein.[12, 13] The *hst*-1 gene from a patient with acute leukemia[14] and that from a normal person (unpublished data) have shown transforming activity. This is the first report which has unequivocally shown the relation between the *hst*-1 gene amplification and carcinoma. It is of interest to note that the *hst*-1 gene was amplified in over 40% of the esophageal carcinomas. Whether amplification of the *hst*-1 gene participates in carcinogenesis and metastasis of esophageal carcinomas or whether it occurs in other squamous cell carcinomas will be elucidated in the future.

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