Heterogeneous Protooncogene Amplification Correlates with Tumor Progression and Presence of Metastases in Gastric Cancer Patients[1]

Guglielmina N. Ranzani,[2] Natalia S. Pellegata, Carlo Previderè, Ariele Saragoni, Antonio Vio, Marco Maltoni, and Dino Amadori

Department of Genetics and Microbiology, University of Pavia, Via S. Epifanio 14, 27100 Pavia [G. N. R., N. S. P., C. P.], and Department of Oncology, Morgagni-Pierantoni Hospital, 47100 Forlì [A. S., A. V., M. M., D. A.], Italy

ABSTRACT

In order to evaluate the relevance of protooncogene alterations in gastric cancer and to specifically relate these alterations to types and stages of the neoplasia, we studied oncogenes of possible interest in gastric tumors with different clinical parameters. Fifty DNAs from primary gastric adenocarcinoma were analyzed, by the Southern blotting technique, for the presence of amplification or rearrangements of seven different protooncogenes: c-myc, c-erbB2, c-Ki-ras, c-Ha-ras, c-N-ras, hst, and c-mos. All the tumors analyzed were histologically classified and staged. Amplification of the following genes was found: c-myc (2 of 50), hst (3 of 50), c-erbB2 (3 of 50), and c-Ki-ras (5 of 50). The simultaneous amplification of hst (3 cases), c-myc (1 of 3), or c-Ki-ras (2 of 3) was observed. Analysis of DNAs from atrophic and metaplastic gastric mucosa (which can be regarded as preneoplastic lesions) of the 10 patients showing gene amplification demonstrated that this was limited to neoplastic cells. Considering protooncogene amplification in general (i.e., involving different genes and occurring to different degrees) and clinical parameters of tumors, we found a statistically significant association between amplification and both tumor progression and presence of metastases. Therefore, at least for the genes analyzed, amplification is a relatively infrequent phenomenon and represents a late event in the temporal development of gastric cancer.

INTRODUCTION

Protooncogenes are cellular genes with a fundamental role in cell growth, development, or differentiation. They can be activated (oncogenes) by a variety of molecular mechanisms resulting in qualitative and/or quantitative changes of their products. These mechanisms include point mutations, rearrangements, and amplification (for a review, see Ref. 1).

Numerous findings suggest that oncogenes are involved in human cancer both in the initial conversion of normal to malignant cells and in the progression of less aggressive cell types to more aggressive ones (1). A specific mutation of the Ki-ras gene has been found in human colon cancer, not only in neoplastic cells but also in premalignant lesions (2). This indicates that the mutation precedes the development of the malignancy. On the other hand, amplification of N-myc gene in neuroblastoma (3) and of erbB2 in breast cancer (4) has been correlated with a more advanced stage of the neoplasia and a poorer prognosis.

Although stomach cancer is a common malignancy in man, little is known about the oncogenes which may be associated with the origin or development of the tumor. Findings on amplification and single base mutations of several oncogenes have already been reported (5–13). However, in most cases the available data derive from sporadic observations, making it difficult to evaluate the relevance of these alterations. More-

over, no analyses have been made to specifically relate different gene alterations to types and stages of the neoplasia.

We therefore carried out a systematic study on 50 cases of gastric adenocarcinoma characterized by different clinical parameters. DNAs from neoplastic and control tissue samples were hybridized by the Southern blotting technique with probes homologous to seven different protooncogenes: c-myc, c-erbB2, c-Ki-ras, c-Ha-ras, c-N-ras, hst, and c-mos. These genes were chosen since they are possibly involved in stomach cancer, as suggested by previous data, or they are frequently activated in other types of solid tumors.

We found amplification of c-myc, c-erbB2, c-Ki-ras, and hst oncogenes. Our data indicate that amplification is a relatively infrequent phenomenon in gastric cancer, is limited to tumors at advanced stages, and is associated with the presence of metastases.

MATERIALS AND METHODS

Patients. We examined 50 untreated patients affected by gastric adenocarcinoma who were all from the Province of Forlì, a small area of north-central Italy, characterized by an incidence of stomach cancer among the highest in the world (14).

Tissue Samples. Primary tumors were obtained during surgery. Control tissues, both atrophic and metaplastic mucosa, were available in most cases. Tumors were histologically classified and staged according to both the Laurén (15) and the World Health Organization (16) classifications. Tissue fragments were stored in liquid nitrogen until utilized as a source of DNA.

Samples (about 10 ml) of peripheral blood from patients were taken in the presence of an anticoagulant and buffy coat cells were stored at −20°C until utilized for DNA extraction.

DNA Extraction and Southern Blotting. Frozen tissue fragments were converted to fine powder with a Brown Mikro-dismembrator (17). The powder was resuspended in the extraction buffer (18). High molecular weight DNA was isolated by proteinase K digestion and phenol/chloroform extraction (18).

Frozen buffy coat cells were used as a source of DNA, as previously reported (19).

DNA (10 μg) was digested to completion with the EcoRI restriction enzyme, fractionated on 0.8% agarose gel, and transferred to Zetabind filters (CUNO) as described (19). Filters were hybridized, washed, and exposed as reported previously (19). Suggestions from the membrane supplier were followed to remove probes and rehybridize filters.

Probes. The various probes, inserts from recombinant plasmids, were ^{32}P-labeled at high specific activity ($>1 \times 10^8$ cpm/μg) by nick-translation (20).

The following oncogene-specific DNA fragments were used as probes: 1.4-kilobase ClaI-EcoRI fragment corresponding to the III exon of the c-myc gene, from the pMC413RC clone (21); 1-kilobase HincII v-Ki-ras fragment, from the pHiHi3 clone (22); 6.4-kilobase BamHI fragment corresponding to the c-Ha-ras1 locus from the pT24-C3 clone (23); 1.5-kilobase EcoRI N-ras-specific fragment, from the p52c+ clone (24); 1.1-kilobase EcoRI hst (KS) fragment, from the KS3 clone (25); 2.7-kilobase EcoRI mos-specific insert, from the pHM2A clone (26); 4.6-kilobase HindIII-SalI fragment corresponding to a full-length complementary DNA of the rat neu gene, from the pSV2neuNT clone (27).

RESULTS

We studied a total of 50 tumors from patients with gastric adenocarcinoma. Table 1 shows a clinical classification of the tumors examined.

Genomic DNAs extracted from neoplastic tissue samples were digested with EcoRI and hybridized by the Southern blotting technique to seven different probes, homologous to the following cellular protooncogenes: c-myc, c-erbB2, c-Ki-ras, c-Ha-ras, c-N-ras, hst, and c-mos.

In all cases, probes detected the genomic DNA fragments expected on the basis of restriction maps previously reported (5, 22, 25, 27–30). In 2 cases only was an extra fragment observed in addition to the EcoRI fragment commonly detected by the c-myc probe (5). Its presence in DNA from peripheral blood leukocytes of the same patients indicated that it was due to a genomic alteration rather than to a somatic rearrangement in neoplastic cells. (Molecular characterization of the c-myc variants will be given elsewhere.)

In 13 cases, with probes homologous to c-erbB2, c-myc, c-Ki-ras, and hst genes, we found an increased intensity of hybridization signals (Fig. 1). Rehybridization of all filters with at least three different protooncogene probes allowed us to exclude the possibility that this was due to a difference in the amount of DNA bound to filters (Fig. 2).

An increased hybridization signal could result from a true gene amplification or from the presence in neoplastic cells of extra copies of the chromosome carrying the gene under analysis. We ruled out this last possibility by rehybridizing relevant samples with a second probe lying on the same chromosome. In particular, c-mos was used as a control probe to rehybridize filters with samples showing c-myc amplification since both genes map to chromosome 8. c-Ha-ras was used as a control for hst (both genes lying on chromosome 11), int1 for c-Ki-ras (both on chromosome 12) and c10-HOX 2.1, for c-erbB2 (both on chromosome 17) (see Fig. 2 for an example).

Dilution experiments (Fig. 3) and densitometric scanning allowed us to estimate the degree of gene amplification. This was low (3- to 5-fold) in all samples except two, in which the hybridization signal obtained with the c-erbB2 and c-Ki-ras probes was increased at least 20-fold. The simultaneous amplification of hst (3 cases) and c-myc (1 of 3 cases) or c-Ki-ras (2 of 3 cases) was observed. Amplification was found to occur in tumors of different histological types at progression stages III or IV. Six of 10 DNAs showing amplification were from the 12 patients with metastases. All these observations and a classification of the tumors which were characterized by gene amplification are summarized in Table 2.

Additional analysis of DNA from atrophic and metaplastic mucosa of patients showing gene amplification demonstrated, without exception, that the amplification was only detectable in neoplastic cells (Fig. 3).

DISCUSSION

Using the Southern blotting technique we hybridized 50 DNAs from gastric adenocarcinoma with seven different probes homologous to c-myc, c-erbB2, c-Ki-ras, c-Ha-ras, c-N-ras, hst, and c-mos protooncogenes. The hybridization patterns allow us to exclude the occurrence of gross rearrangements (such as deletion/insertion, translocation) involving the genes analyzed. The two unexpected patterns found with the c-myc probe can be explained by a genomic alteration of the c-myc locus.

Ten of 50 DNAs showed gene amplification involving different protooncogenes, namely: c-myc (4%), c-erbB2 (6%), c-Ki-ras (10%), and hst (6%). Our finding that these genes can be amplified in gastric cancer is in agreement with previous observations (5–7, 31). The amplification frequencies we estimate for c-myc, c-Ki-ras, and hst genes are comparable to those obtained by other authors (31–33) surveying a more limited number of cases. During a recent study of Japanese patients, Yokota et al. (30) observed that the amplification of the c-erbB2 gene is frequent (5 of 13) in tubular adenocarcinoma and absent (0 of 30) in other histological types of stomach cancer. Our studies show an occurrence of only 3 of 41 cases in tubular

Table 1
Classification of tumors

	Classification	No. of tumors
Histological type		
Lauren's classification (15)	Intestinal	38[a]
	Diffuse	3
	Mixed	9
World Health Organization classification (16)	Signet ring	2
	Mucinous	5
	Tubular	41
	Papillar	2
Dimension	T_1-T_2	8
	T_3	22
	T_4	20
Lymphonodal status	N_0	14
	N_1	18
	N_2	14
	N_3	4
Stage	I-II	15
	III	8
	IV	27
Metastases	M_1	12
	M_0	38

[a] Including 19 poorly differentiated tumors.

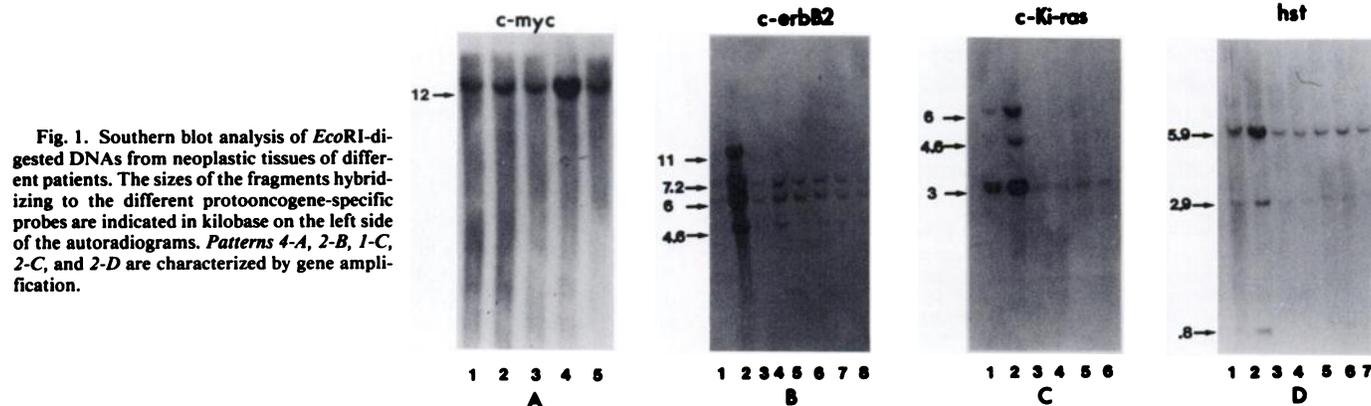Fig. 1. Southern blot analysis of EcoRI-digested DNAs from neoplastic tissues of different patients. The sizes of the fragments hybridizing to the different protooncogene-specific probes are indicated in kilobase on the left side of the autoradiograms. Patterns 4-A, 2-B, 1-C, 2-C, and 2-D are characterized by gene amplification.

7812

Table 2
Cases characterized by gene amplification

| | Adenocarcinoma classification | | | | | | | |
Patient	World Health Organization type[a]	Lauren's type[a]	Dimension	Lymphonodal status	Stage	Metastases	Amplified oncogene	Degree of amplification
B. C.[b]	M	M	T₃	N₁	III	M₀	c-*myc*	3–4
M. R.	T	I[e]	T₃	N₂	IV	M₁	c-*myc*	3–4
V. M.	T	I	T₄	N₁	IV	M₀	c-*erb*B2	>20
R. A.	T	I[e]	T₃	N₂	IV	M₁	c-*erb*B2	5
C. E.	T	I	T₄	N₃	IV	M₁	c-*erb*B2	3
C. E.[c]	M	I	T₄	N₁	IV	M₁	c-Ki-*ras*	2–3
V. E.	S	D	T₃	N₃	IV	M₁	c-Ki-*ras*	2–3
L. R.[d]	T	I	T₄	N₀	IV	M₀	c-Ki-*ras*	>20
R. R.	T	I[e]	T₄	N₁	IV	M₀	c-Ki-*ras*	2–3
N. A.	T	I[e]	T₃	N₂	IV	M₁	c-Ki-*ras*	2–3
C. E.[c]							*hst*	5
L. R.[d]							*hst*	2–3
B. C.[b]							*hst*	3

[a] M, mixed or mucinous; T, tubular; S, signet ring; I, intestinal; D, diffuse.
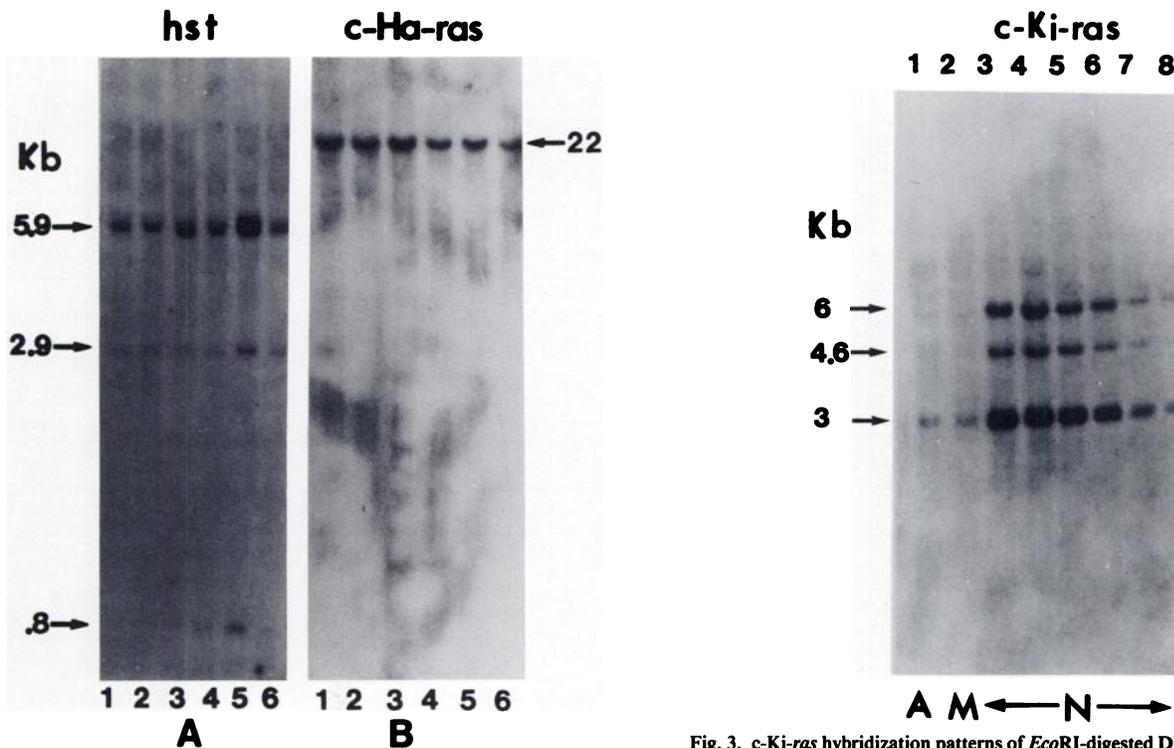[b,c,d] Same patients in this table.
[e] Poorly differentiated.

Fig. 2. Autoradiograms obtained by hybridizing the same filter with *hst* (*A*) and c-Ha-*ras* (*B*) probes. *Lane 5* is characterized by *hst* gene amplification. This control experiment allows us to exclude both differences in the amount of DNA bound to the filter and the presence in sample 5 of extra copies of chromosome 11, on which *hst* and c-Ha-*ras* genes are located.

Fig. 3. c-Ki-*ras* hybridization patterns of *Eco*RI-digested DNAs from atrophic (*A*), metaplastic (*M*), and neoplastic (*N*) gastric mucosa of the same patient. The c-Ki-*ras* gene is only amplified in DNA from cancer tissue (*compare lanes 1, 2, and 4,* where 10 μg of DNA are loaded). Decreasing amounts of DNA (12.5–1 μg) from cancer tissue are loaded in *lanes 3–8* in order to evaluate the c-Ki-*ras* amplification degree (>20-fold).

adenocarcinoma. Variations in genetic and environmental background might account for the different frequencies found for the c-*erb*B2 gene amplification between Italian (see "Materials and Methods") and Japanese (30) patients.

During our survey, we found *hst* amplified simultaneously with c-*myc* or c-Ki-*ras*. Cases of coamplification involving *hst* in gastric cancer have been so far reported for the *int*2 gene which maps to the same chromosomal band (11q13) (31). Our observation suggests that a cooperative expression of amplified *hst* and c-*myc* or c-Ki-*ras* might be involved in the genesis and/ or progression of gastric cancer.

Our results also show that gene amplification is limited, in all cases, to transformed gastric mucosa. It is, in fact, completely undetectable in atrophic and metaplastic tissue areas, which can be regarded as preneoplastic lesions. The observed gene amplification should therefore represent a late event in the temporal development of gastric cancer. Obviously, we cannot exclude the presence of a small number of cells, in preneoplastic areas, harboring amplified oncogenes and having some proliferative advantage. These cells would be undetected due to the dilution of their DNA during extraction from the whole tissue sample.

We checked for correlations between protooncogene ampli-

7813

fication and the clinical parameters reported in Table 1 (*i.e.*, tumor type, dimension, stage, lymphonodal status, and metastases). We considered one gene at a time and found no statistically significant correlation, probably because of the low number of cases showing amplification. However, for all genes, amplification was mostly observed in patients with advanced and metastatic tumors. Due to this precise trend, data were pooled to check for correlations between amplification in general (*i.e.*, involving different protooncogenes and occurring to different degrees) and clinical parameters. In this way, although involving oncogenes coding for different functions, amplification was regarded as a general phenomenon deeply affecting cell growth regulation. We found a significant χ^2 value for the association between tumor progression and gene amplification which turned out to be typical of stage IV tumors (9 of 27 *versus* 1 of 23; $P < 0.025$, 1 d.f.). Moreover, 6 of 10 DNAs showing amplification were from the 12 patients with metastases. The χ^2 for the association between amplification and presence of metastases was highly significant (6 of 12 *versus* 4 of 38; $P = 0.003$, 1 d.f.).

In conclusion, protooncogene amplification in gastric adenocarcinoma is a relatively infrequent phenomenon and generally occurs at a low degree. Amplification is absent in tissue areas showing preneoplastic characteristics, being limited to advanced or metastatic tumors. If our findings are considered as a whole, amplification seems to represent a late event in the temporal development of gastric cancer and a marker of progression in this type of tumor. We cannot, however, completely exclude the possibility that amplification occurs early in the neoplastic process and results in a more aggressive phenotype. In this case it should represent a negative prognostic marker.

Two groups of patients with advanced tumors showing and not showing amplification are being followed up clinically on a long-term basis. If amplification actually represents a negative prognostic marker, patients with advanced tumors characterized by amplification will have a poorer prognosis. Otherwise, the idea that amplification represents a late event in the development of gastric cancer could be definitively confirmed.

REFERENCES

1. Garrett, C. T. Oncogenes. Clin. Chim. Acta, *156:* 1–40, 1986.
2. Bos, J. L., Fearon, E. R., Hamilton, S. R., Verlaan-de Vries, M., van Boom, J. H., van der Eb, A. J., and Vogelstein, B. Prevalence of *ras* gene mutations in human colorectal cancers. Nature (Lond.), *327:* 293–297, 1987.
3. Brodeur, G. M., Seeger, R. C., Schwab, M., Varmus, H. E., and Bishop, J. M. Amplification of N-*myc* in untreated human neuroblastoma correlates with advanced disease stage. Science (Washington DC), *224:* 1121–1125, 1984.
4. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. Science (Washington DC), *235:* 177–182, 1987.
5. Shibuya, M., Yokota, J., and Ueyama, Y. Amplification and expression of a cellular oncogene (c-*myc*) in human gastric adenocarcinoma cells. Mol. Cell. Biol., *5:* 414–418, 1985.
6. Yokota, J., Yamamoto, T., Toyoshima, K., Terada, M., Sugimura, T., Battifora, H., and Cline, M. J. Amplification of c-*erb*B-2 oncogene in human adenocarcinomas *in vivo*. Lancet, *1:* 765–766, 1986.
7. Bos, J. L., Verlaan-de Vries, M., Marshall, C. J., Veeneman, G. H., van Boom, J. H., and van der Eb, A. J. A human gastric carcinoma contains a single mutated and an amplified normal allele of the Ki-*ras* oncogene. Nucleic Acids Res., *14:* 1209–1217, 1986.
8. Deng, G., Lu, Y., Chen, S., Miao, J., Lu, G., Li, H., Cai, H., Xv, X., E, Z., and Liu, P. Activated c-Ha-*ras* oncogene with a guanine to thymine transversion at the twelfth codon in a human stomach cancer cell line. Cancer Res., *47:* 3195–3198, 1987.
9. O'Hara, B. M., Oskarsson, M., Tainsky, M. A., and Blair, D. G. Mechanism of activation of human *ras* genes cloned from a gastric adenocarcinoma and
10. a pancreatic carcinoma cell line. Cancer Res., *46:* 4695–4700, 1986.
10. Sakamoto, H., Mori, M., Taira, M., Yoshida, T., Matsukawa, S., Shimizu, K., Sekiguchi, M., Terada, M., and Sugimura, T. Transforming gene from human stomach cancers and a noncancerous portion of stomach mucosa. Proc. Natl. Acad. Sci. USA, *83:* 3997–4001, 1986.
11. Shimizu, K., Nakatsu, Y., Sekiguchi, M., Hokamura, K., Tanaka, K., Terada, M., and Sugimura, T. Molecular cloning of an activated human oncogene, homologous to v-*raf*, from primary stomach cancer. Proc. Natl. Acad. Sci. USA, *82:* 5641–5645, 1985.
12. Jiang, W., Kahn, S. M., Guillem, J. G., Lu, S. H., and Weinstein, B. I. Rapid detection of *ras* oncogenes in human tumors: applications to colon, esophageal, and gastric cancer. Oncogene, *4:* 923–928, 1989.
13. Gutman, M., Ravia, Y., Assaf, D., Yamamoto, T., Rozin, R., and Shiloh, Y. Amplification of c-*myc* and c-*erb*B2 proto-oncogenes in human solid tumors: frequency and clinical significance. Int. J. Cancer, *44:* 802–805, 1989.
14. Amadori, D., Falcini, F., Biserni, R., and Bonaguri, C. Il carcinoma dello stomaco. *In:* D. Amadori (ed.), I tumori maligni in Romagna, pp. 37–68. Rimini, Italy, 1985.
15. Laurén, P. The two histological main types of gastric adenocarcinoma: diffuse and so-called intestinal-type carcinoma. Acta Pathol. Microbiol. Scand., *64:* 31–49, 1965.
16. Oota, K. Histological Typing of Gastric and Esophageal Tumours. Geneva, Switzerland: World Health Organization, 1977.
17. Krieg, P., Amtmann, E., and Sauer, G. The simultaneous extraction of high molecular weight DNA and RNA from solid tumors. Anal. Biochem., *134:* 288–294, 1983.
18. Wigler, M., Sweet, R., Sim, G. K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S., and Axel, R. Transformation of mammalian cells with genes from procaryotes and eucaryotes. Cell, *16:* 777–785, 1979.
19. Ranzani, G. N., Salerno-Mele, P., Maltoni, M., Talarico, D., Della Valle, G., and Amadori, D. Study of c-Ha-*ras*1 locus polymorphism in an Italian population with high incidence of gastric cancer. Mol. Biol. Med., *5:* 145–153, 1988.
20. Maniatis, T., Fritsch, E. F., and Sambrook, J. Molecular Cloning: A Laboratory Manual, pp. 109–112. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1982.
21. Dalla Favera, R., Wong-Staal, F., and Gallo, R. C. Onc gene amplification in promyelocytic leukaemia cell line HL-60 and primary leukaemic cells of the same patient. Nature (Lond.), *299:* 61–63, 1982.
22. Ellis, R. W., DeFeo, D., Shih, T. Y., Gonda, M. A., Young, H. A., Tsuchida, N., Lowy, D. R., and Scolnick E. M. The p21 *src* genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. Nature (Lond.), *292:* 506–511, 1981.
23. Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S., and Barbacid, M. T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes. Nature (Lond.), *298:* 343–347, 1982.
24. Murray, M. J., Cunningham, J. M., Parada, L. F., Dautry, F., Lebowitz, P., and Weinberg, R. A. The HL-60 transforming sequence: a *ras* oncogene coexisting with altered *myc* genes in hematopoietic tumors. Cell, *33:* 749–757, 1983.
25. Delli Bovi, P., Curatola, A. M., Kern, F. G., Greco, A., Ittmann, M., and Basilico, C. An oncogene isolated by transfection of Kaposi's sarcoma DNA encodes a growth factor that is a member of the FGF family. Cell, *50:* 729–737, 1987.
26. Watson, R., Oskarsson, M., and Vande Woude, G. F. Human DNA sequence homologous to the transforming gene (*mos*) of Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. USA, *79:* 4078–4082, 1982.
27. Bagmann, C. I., Hung, M. C., and Weinberg, R. A. Multiple independent activations of the *neu* oncogene by a point mutation altering the transmembrane domain of p185. Cell, *45:* 649–657, 1986.
28. Cang, E. H., Gonda, M. A., Ellis, R. W., Scolnick, E. M., and Lowy, D. R. Human genome contains four genes homologous to transforming genes of Harvey and Kirsten murine sarcoma viruses. Proc. Natl. Acad. Sci. USA, *79:* 4848–4852, 1982.
29. Koda, T., Sasaki, A., Matsushima, S. and Kakinuma, M. A transforming gene, *hst*, found in NIH 3T3 cells transformed with DNA from three stomach cancers and a colon cancer. Jpn. J. Cancer Res. (Gann.), *78:* 325–328, 1987.
30. Yokota, J., Yamamoto, T., Miyajima, N., Toyoshima, K., Nomura, N., Sakamoto, H., Yoshida, T., Terada, M., and Sugimura, T. Genetic alterations of the c-*erb*B2 oncogene occur frequently in tubular adenocarcinoma of the stomach and are often accompanied by amplification of the v-*erb*A homologue. Oncogene, *2:* 283–287, 1988.
31. Yoshida, M. C., Wada M., Satoh, H., Yoshida, T., Sakamoto, H., Miyagawa, K., Yokota, J., Koda, T., Kakinuma, M., Sugimura, T., and Terada, M. Human *HST*1 (HSTF1) gene maps to chromosome band 11q13 and coamplifies with the *INT*2 gene in human cancer. Proc. Natl. Acad. Sci. USA, *85:* 4861–4864, 1988.
32. Koda, T., Matsushima, S., Sasaki, A., Danjo, Y., and Kakinuma, M. c-*myc* gene amplification in primary stomach cancer. Jpn. J. Cancer Res. (Gann.), *76:* 551–554, 1985.
33. Fujita, K., Ohuchi, N., Yao, T., Okumura, M., Fukushima, Y., Kanakura, Y., Kitamura, Y., and Fujita, J. Frequent overexpression, but not activation by point mutation, of *ras* genes in primary human gastric cancers. Gastroenterology, *93:* 1339–1345, 1987.

AA**C**R American Association
for Cancer Research

Heterogeneous Protooncogene Amplification Correlates with Tumor Progression and Presence of Metastases in Gastric Cancer Patients

Guglielmina N. Ranzani, Natalia S. Pellegata, Carlo Previderè, et al.

Updated version	Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/50/24/7811

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/50/24/7811. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.