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Point Mutation of c-Ki-*ras* Oncogene in Gastric Adenoma and Adenocarcinoma with Tubular Differentiation

Toshimasa Kihana,[1] Hitoshi Tsuda,[1] Teruyuki Hirota,[1] Yukio Shimosato,[1] Hiromi Sakamoto,[2] Masaaki Terada[2] and Setsuo Hirohashi[1,3]

[1]Pathology and [2]Genetics Divisions, National Cancer Center Research Institute and Hospital, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104

The presence of point mutation at codons 12, 13 and 61 of the c-Ki-*ras* oncogene was investigated in 7 cases of gastric adenoma and 35 cases of gastric adenocarcinoma using DNA samples from formalin-fixed and paraffin-embedded tissues. Oligonucleotides encompassing the three codons were amplified by using the polymerase chain reaction (PCR), and then examined for point mutation by the selective oligonucleotide hybridization technique. Point mutation was detected in three of the 7 adenomas (43%) and three of the 35 carcinomas (9%). All the gastric adenomas showed the histology of tubular adenoma, being very similar to that of colonic adenoma. The 35 cases of gastric adenocarcinoma were classified into 17 cases of differentiated type and 18 cases of undifferentiated type including signet-ring cell carcinoma. The point mutation of c-Ki-*ras* oncogene was detected only in the differentiated type (3/17, 18%), and there was no case with point mutation in the undifferentiated type. These results suggest that the genetic mechanism of carcinogenesis differs between the differentiated type and the undifferentiated type of gastric adenocarcinoma, and also that c-Ki-*ras* activation is possibly involved in a relatively early step of the "adenoma-carcinoma sequence," which leads to the development of a portion of differentiated adenocarcinomas in the stomach.

Key words: Human gastric cancer — c-Ki-*ras* oncogene — Histogenesis of gastric cancer

The *ras* oncogene family consists of three members, namely c-Ha-*ras*, c-Ki-*ras* and N-*ras*. A single base substitution at specific codons within these genes is reported to be responsible for their transforming activity, and the activated forms of these oncogenes have been detected in various human cancers by means of the NIH/3T3 transfection assay. Among the three *ras* genes, point-mutational activation of c-Ki-*ras* oncogene has been frequently detected in several kinds of human cancers; in 95% of pancreatic cancer,[1] in more than 40% of colorectal cancer,[2-4] and in 26% of adenocarcinoma of the lung.[5] The point-mutational activation of the c-Ki-*ras* oncogene has been detected not only in cancers but also in benign tumors, e.g. in more than half of all colorectal adenomas larger than 1 cm.[4] Since colorectal adenoma is important as a precursor of colorectal carcinoma, activation of the c-Ki-*ras* oncogene is considered to play an important role in carcinogenesis of the colon and rectum.

Gastric cancer is still the most frequent cancer not only in Japan but also in the world, and approximately 50,000 persons die of gastric cancer every year in Japan. Gastric cancer can be grouped into two major forms histologically, i.e. adenocarcinomas of differentiated type and of undifferentiated type. In addition to carcinomas, increasing numbers of adenomas are detected as a result of the progress made in endoscopic diagnosis. Among these gastric tumors, adenocarcinomas of the differentiated type and adenomas closely resemble the corresponding colorectal tumors, in which point-mutational activation of c-Ki-*ras* oncogene is frequently detected.

With regard to gastric cancer, however, only three papers have reported a total of four cases of gastric cancer carrying the activated form of the *ras* oncogene.[6-8] Although Sakamoto *et al.* previously failed to detect the activated forms of *ras* genes in 26 cases of gastric cancer using the NIH/3T3 transfection assay,[9] this type of assay may not be efficient for detecting c-Ki-*ras* activation, since the c-Ki-*ras* oncogene has long introns in its own structure. Therefore, we have examined the incidence of the activated c-Ki-*ras* oncogene in gastric tumors using the polymerase chain reaction (PCR)[4] and specific oligonucleotide hybridization technique, which is more sensitive than the NIH/3T3 transfection assay. Using these techniques, we were able to demonstrate clearly the presence of point-mutational activation of the c-Ki-*ras* oncogene in gastric tumors which resemble colorectal tumors.

MATERIALS AND METHODS

Tumor specimens We analyzed 42 gastric tumor specimens, comprising 7 adenomas and 35 carcinomas, which

[3] To whom reprint requests should be addressed.

[4] Abbreviations: PCR, polymerase chain reaction; EDTA, ethylenediaminetetraacetic acid.

had been obtained from 41 patients by surgical resection or biopsy at the National Cancer Center Hospital, Tokyo. The size of the adenomas, ranging from 4.5 mm to 14 mm, was larger than 10 mm in 5 cases and less than 10 mm in the other 2 cases. The specimens were fixed in 10% formalin and embedded in paraffin. Histological diagnoses of the tumors were made according to the histological classification of gastric cancer defined by the Japanese Research Society for Gastric Cancer.[10]

Synthetic oligonucleotides for probes and primers All of the oligonucleotides used in this study, as either probes or primers, were the same as those described by Verlaan-de Vries *et al.*,[11] and were synthesized on an Applied Biosystems 380A DNA Synthesizer (Applied Biosystems Japan, Tokyo) by the β-cyanoethyl phosphoramidite method. The sequences of the synthesized oligonucleotides are shown in Table I.

DNA samples and PCR DNA samples isolated from cultured cell lines, c-Lu-65 and KP-2, which are known to carry a point mutation within codon 12 of the c-Ki-*ras* oncogene,[12, 13] were used as positive controls. By histological examination of hematoxylin-eosin-stained sections, we selected an area where viable tumor cells were plentiful (>60%) and accompanied with a few necrotic and inflammatory cells (Fig. 1). In 25 out of the 42 gastric tumors, DNA was isolated from the paraffin-embedded tissue block using the method described previously.[14, 15] In the other 17 cases of gastric tumor, one 5-μm-thick section cut from formalin-fixed, paraffin-embedded tissue was placed in a 500-μl microtube. The surface area of the tissue ranged between 4 and 25 mm^2. These sections were deparaffinized with xylene, cleared with ethanol and completely dried before PCR without extracting the DNA.

Using PCR, we amplified a sequence spanning 108 base pairs across codons 12 and 13 of the c-Ki-*ras* oncogene and a sequence spanning 128 base pairs across codon 61 of the c-Ki-*ras* oncogene.[11, 16] For each PCR, 0.7–3.2 μg

Table I. Synthetic Oligonucleotide Primers and Probes Used to Analyze the Point Mutation of the c-Ki-*ras* Gene

		Sequence						
Primers								
Ki-12	sense	G	ACT	GAA	TAT	AAA	CTT	GTG	G
	anti-sense	C	TAT	TGT	TGG	ATC	ATA	TTC	G
Ki-61	sense	T	TCC	TAC	AGG	AAG	CAA	GTA	G
	anti-sense	C	ACA	AAG	AAA	GCC	CTC	CCC	A
Probes								
Ki-12	wild type	CC	TAC	GCC	ACC	AGC	TCC	AAC	
	mutant type							
	a	—	—	—	ACA	—	—	—	
	b	—	—	—	ACT	—	—	—	
	c	—	—	—	ACG	—	—	—	
	d	—	—	—	AAC	—	—	—	
	e	—	—	—	ATC	—	—	—	
	f	—	—	—	AGC	—	—	—	
Ki-13	mutant type							
	a	—	—	GCA	—	—	—	—	
	b	—	—	GCT	—	—	—	—	
	c	—	—	GCG	—	—	—	—	
	d	—	—	GAC	—	—	—	—	
	e	—	—	GTC	—	—	—	—	
	f	—	—	GGC	—	—	—	—	
Ki-61	wild type	TA	CTC	CTC	TTG	ACC	TGC	TGT	
	mutant type							
	a	—	—	—	TTC	—	—	—	
	b	—	—	—	TTT	—	—	—	
	c	—	—	—	TCG	—	—	—	
	d	—	—	—	TAG	—	—	—	
	e	—	—	—	TGG	—	—	—	
	f	—	—	—	ATG	—	—	—	
	g	—	—	—	GTG	—	—	—	

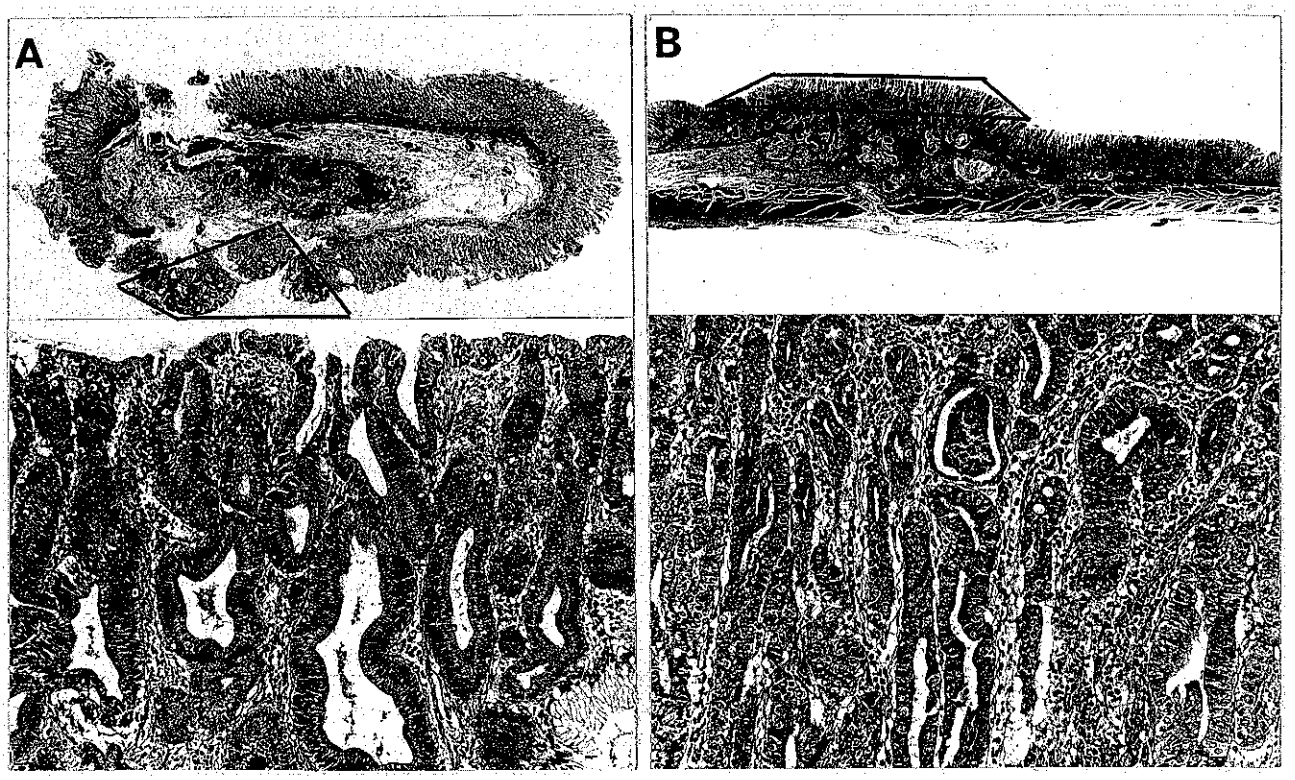


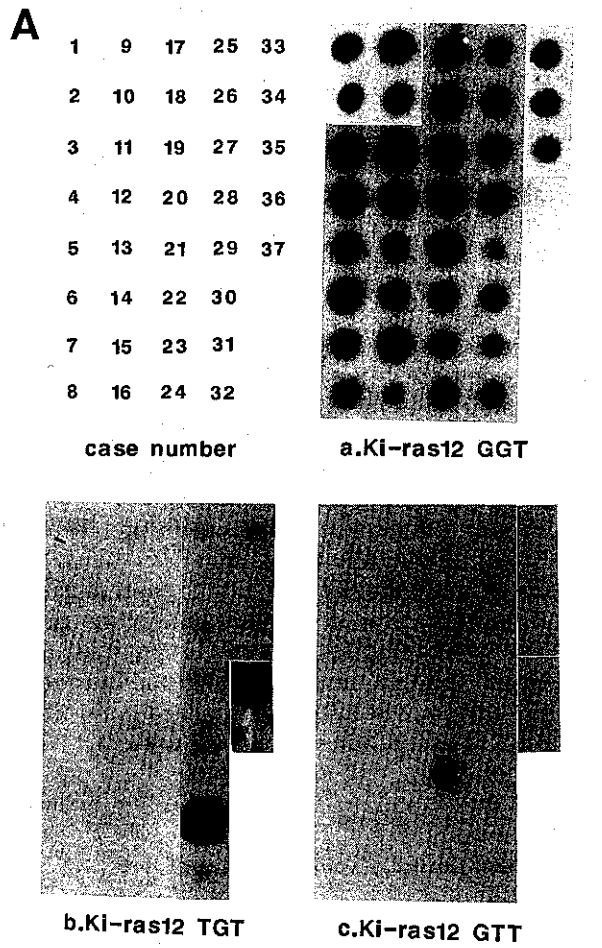
Fig. 1. A. Photomicrographs of a tubular adenoma of the stomach (case 5). Upper: Low-magnification view (×15). One 5-μm-thick section of the area of adenoma enclosed by the line was cut and used for PCR. Lower: Moderately magnified view (×100). Tubules are lined by columnar tumor cells in which uniform nuclei are located at the basal side. Hematoxylin and eosin stain. B. Photomicrographs of well differentiated tubular adenocarcinoma of the stomach (case 31). Upper: Low-magnification view (×15). The area where neoplastic cells are predominant is shown by the line. One 5-μm-thick section of the area was cut and used for PCR. Lower: Moderately magnified view (×100). B. Tubular structures are slightly irregular and lined by tumor cells with atypical nuclei. Hematoxylin and eosin stain.

of DNA or one 5-μm-thick deparaffinized section, 20 pmol each of two primers, 2.5 units of Taq polymerase (Perkin-Elmer Cetus, Norwalk, CA) and 10 μl of Taq buffer (500 m*M* potassium chloride, 100 m*M* Tris-hydrochloride pH 8.3, 15 m*M* magnesium chloride, 0.1% (w/v) gelatin) were mixed and the final volume was adjusted to 100 μl. Thirty-five cycles each consisting of denaturation for 1 min, annealing for 45 s, and extension for 2 min were performed, employing an automated heat-block (DNA thermal cycler, Perkin-Elmer Cetus). The PCR products were electrophoresed in 3% NuSieve GTG agarose gel (FMC, Rocklard, ME) using a Mupid gel electrophoresis apparatus (Advance, Tokyo) for 1–1.5 h at 50 V, and the gel was stained with ethidium bromide in order to confirm the successful amplification of the expected sequence.

Oligonucleotide hybridization Five microliters of the final PCR product from each sample mixed with 95 μl of 0.25 *M* sodium hydroxide/0.8 m*M* EDTA/8 m*M* Tris-hydrochloride, pH 7.4, was dotted onto a GeneScreen Plus nylon filter (New England Nuclear, MA) using a Bio-Dot apparatus (Bio-Rad Japan, Tokyo).

The filters were prehybridized for about 2 h at 55°C in 3 *M* tetramethylammonium chloride/50 m*M* Tris-hydrochloride, pH 8.2/2 m*M* EDTA/0.1% sodium dodecyl sulfate/5×Denhardt's solution (1×Denhardt's solution consists of 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin)/denatured salmon testis DNA (10 μg/ml). The filters were then hybridized to a 20-mer synthetic oligonucleotide probe which had been end-labeled with [γ-^{32}P]ATP, for approximately 16 h at 55°C in buffer with the same constituents as those used for prehybridization. The filters were washed twice in 6×SSC (1×SSC is 0.15 *M* sodium chloride/0.015 *M* sodium citrate) for 15 min at room temperature, and washed additionally twice in 6×SSC for 30 min at 4°C. After the filters had been rinsed in 3 *M* tetramethylammonium chloride buffer (3 *M* tetra-

methylammonium chloride/50 m*M* Tris-hydrochloride (pH 9.0)/2 m*M* EDTA/0.3% sodium dodecyl sulfate), they were washed in this solution for 30–60 min at 57–60°C.[11, 17] Finally the filters were autoradiographed at −70°C for 30 min to 6 h, on Kodak XAR-5 film using intensifying screens. When the removal of the one-base mismatched probe from the nylon filter was insufficient, as determined by referring to the positive and negative controls on the film, the filter was washed again for another 30 min at a temperature 1 or 2°C higher to obtain a highly specific autoradiogram.



RESULTS

The normal c-Ki-*ras* oncogene was detected by hybridization with wild-type probes in all of the 42 tumor tissues, and the reported mutated sequences were clearly detected in the positive controls (Fig. 2).

Point mutation of the c-Ki-*ras* oncogene was detected in six out of 42 gastric tumors (14%) (Table II). Histologically, three of these were well or moderately differentiated tubular adenocarcinoma and the other three were tubular adenoma. Five of the 6 tumors carried the mutation at codon 12, and the other carried the mutation at codon 13. There was no tumor with a point mutation at codon 61 of the c-Ki-*ras* oncogene among the cases examined. The alterations of the DNA sequence and the amino acid changes in these cases are shown in Table II. Four of the 6 cases with the activated c-Ki-*ras* gene showed a base replacement from guanine to thymine.

The incidence of point mutation of c-Ki-*ras* oncogene in relation to tumor histological type is shown in Table

Fig. 2. Dot-blot hybridization analysis of point mutation of the c-Ki-*ras* gene in gastric tumors. A. Point mutation in adenocarcinoma of the stomach. A DNA sequence encompassing codons 12 and 13 was amplified by PCR, applied to nylon filters and hybridized with the probes using 35 samples from 35 patients with adenocarcinoma of the stomach (samples 1–35). Two samples from positive controls were also applied, i.e., DNA from c-Lu-65, carrying a point mutation from GGT to TGT at codon 12 of the c-Ki-*ras* gene (sample 36) and DNA from KP2, carrying a point-mutation from GGT to CGT at codon 12 of the c-Ki-*ras* gene (sample 37).[11, 12] a. Autoradiogram after hybridization to wild-type probe detecting GGT at codon 12. All gastric tumor samples are shown to contain a normal allele at codons 12 and 13. b and c Autoradiogram after hybridization with mutant probes; b, probe for TGT; c, probe for GTT at codon 12. In b, case 31 and a positive control c-Lu-65 show a positive reaction, and in c, case 22 shows a positive reaction. B. Point mutation in tubular adenoma of the stomach. DNA samples from seven cases (samples 1–7) and two positive controls, i.e., c-Lu-65 (sample 8) and KP-2 (sample 9), were hybridized with probes of wild type and mutant types. Autoradiogram after hybridization with the probes of; a, wild type; b, mutant type for TGT at codon 12; c, mutant type for GTT at codon 12; d, mutant type for TGC at codon 13 in the c-Ki-*ras* gene. Case 7 and c-Lu-65 are shown to have a point mutation of the first type, case 5 to have a point-mutation of the second type and case 1 to have a point-mutation of the third type.

III. The incidence was highest in tubular adenomas (3/7, 43%), followed by differentiated-type adenocarcinoma (3/17, 18%), whereas point mutation was not detected in undifferentiated-type adenocarcinoma (0/18). The sizes of the adenomas with point mutation were 1.4 cm, 0.9 cm and 4.5 mm, and there was no obvious association between tumor size and point mutation of the c-Ki-*ras* oncogene among the 7 adenomas examined.

Table II. Cases of Gastric Adenocarcinoma and Adenoma with Point Mutation of the c-Ki-ras Oncogene

Tumor No.[a)]	Patient age/sex	Codon	Mutation	Histological type[b)]
C22	74/M	12	GGT-GTT (Gly-Val)	M/D tubular adenocarcinoma
C31	56/F	12	GGT-TGT (Gly-Cys)	W/D tubular adenocarcinoma
C33	63/M	12	GGT-GAT (Gly-Asp)	W/D tubular adenocarcinoma
A1	80/F	13	GGC-TGC (Gly-Cys)	tubular adenoma
A5	79/M	12	GGT-GTT (Gly-Val)	tubular adenoma
A7	65/M	12	GGT-TGT (Gly-Cys)	tubular adenoma

a) C, carcinoma; A, adenoma.
b) M/D, moderately differentiated; W/D, well differentiated.

DISCUSSION

Except for a very few cases, all gastric carcinomas are adenocarcinoma, and can be divided into two major types, differentiated type and undifferentiated type. The differentiated type is composed of well or moderately differentiated tubular adenocarcinoma and papillary adenocarcinoma, and the undifferentiated type includes signet-ring cell carcinoma in addition to poorly differentiated adenocarcinoma without special features.[10] Adenocarcinomas of the differentiated type differ from those of the undifferentiated type not only in histology but also in biochemical features and histogenetic background. Colorectal carcinomas, on the other hand, show a histology of well or moderately differentiated adenocarcinoma in most cases, and some differentiated adenocarcinomas of the stomach closely resemble colorectal carcinoma histologically (Fig. 1B). In addition, most differentiated adenocarcinomas of the stomach are accompanied with intestinal metaplasia of the surrounding gastric mucosa. Intestinal metaplasia is the replacement of gastric mucosa by a mucosa resembling that of the intestine, and occurs very commonly in elderly Japanese. It is considered that this underlying condition is important for the development of differentiated adenocarcinoma. Tubular adenomas are occasionally detected in the stomach of elderly Japanese. The number of detected cases of gastric adenoma has been rising in parallel with the increasing refinement of endoscopic techniques. Histologically, gastric adenomas closely resemble colorectal adenomas (Fig. 1A) and they are usually associated with diffuse intestinal metaplasia of the surrounding mucosa.

Table III. Histological Classification of 42 Gastric Tumors and the Incidence of Point Mutation of the c-Ki-*ras* Oncogene

Histological type	Number of cases examined	Number of cases with c-Ki-*ras* point mutation	Incidence (%)
Differentiated type	17	3	18
W/D[a)] tubular adenocarcinoma	4	2	50
M/D[b)] tubular adenocarcinoma	10	1	10
papillary adenocarcinoma	3	0	0
Undifferentiated type	18	0	0
P/D[c)] adenocarcinoma	12	0	0
signet-ring cell carcinoma	5	0	0
mucinous adenocarcinoma	1	0	0
Tubular adenoma	7	3	43
Total	42	6	14

a) Well differentiated.
b) Moderately differentiated.
c) Poorly differentiated.

The activated form of c-Ki-*ras* oncogene due to point mutation was clearly detected in some cases of differentiated gastric adenocarcinoma, whereas it was not detected in the undifferentiated type. Point mutation of the c-Ki-*ras* oncogene was also detected in gastric tubular adenoma, and the incidence, up to 43% (3/7), was rather high. It is also known that differentiated adenocarcinoma often develops within gastric adenoma when the latter lesion is larger than 2.0 cm. Thus, the existence of an "adenoma-carcinoma sequence" in the stomach is indicated from genetic features as well as from clinical and histological observations.[18] Our present study disclosed the possible involvement of c-Ki-*ras* gene activation at a relatively early step of the adenoma-carcinoma sequence, leading to the development of a portion of differentiated adenocarcinomas in the stomach. This putative adenoma-carcinoma sequence in gastric tumors seems to be similar in several respects to that in colorectal tumors with regard to point mutation of the *ras* oncogene. First, according to Vogelstein *et al.*,[4] the incidence of *ras* gene mutation is higher in colorectal adenomas larger than 1 cm (58%) than in colorectal cancers (47%). Second, the substitution of guanine by thymine at codon 12 of c-Ki-*ras* oncogene was predominant among point mutations in both gastric and colorectal tumors. It is strongly suggested that multiple genetic abnormalities are required for the development of overt gastric cancer, as is the case in colorectal cancer.[4] Allelic loss of chromosomes and inactivation of tumor suppressor genes may also contribute to the development of gastric cancer.[19, 20] The details remain to be elucidated.

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REFERENCES

1) Almoguera, C., Shibata, D., Forrester, K., Martin, J., Arnheim, N. and Perucho, M. Most human carcinomas of the exocrine pancreas contain mutant c-K-*ras* genes. *Cell*, **53**, 549–554 (1988).
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*, **327**, 293–297 (1987).
3) Forrester, K., Almoguera, C., Han, K., Grizzle, W. E. and Perucho, M. Detection of high incidence of K-*ras* oncogenes during human colon tumorigenesis. *Nature*, **327**, 298–303 (1987).
4) Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M. and Bos, J. L. Genetic alterations during colorectal tumor development. *N. Engl. J. Med.*, **319**, 525–532 (1988).
5) Rodenhuis, S., Slebos, R. J. C., Boot, A. J. M., Evers, S. G., Mooi, W. J., Wagenaar, S. S., van Bodegom, P. C. and Bos, J. L. Incidence and possible clinical significance of K-*ras* oncogene activation in adenocarcinoma of the human lung. *Cancer Res.*, **48**, 5738–5741 (1988).
6) 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).
7) 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 a pancreatic carcinoma cell line. *Cancer Res.*, **46**, 4695–4700 (1986).
8) Nagata, Y., Abe, M., Kobayashi, K., Yoshida, K., Ishibashi, T., Naoe, T., Nakayama, E. and Shiku, H. Glycine to aspartic acid mutations at codon 13 of the c-Ki-*ras* gene in human gastrointestinal cancers. *Cancer Res.*, **50**, 480–482 (1990).
9) 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).
10) Japanese Research Society Committee on Histological Classification of Gastric Cancer. The general rules for the gastric cancer study in surgery and pathology. II. Histological classification of gastric cancer. *Jpn. J. Surg.*, **11**, 140–145 (1981).
11) Verlaan-de Vries, M., Bogaard, M. E., van den Elst, H., van Boom, J. H., van der Eb, A. J. and Bos, J. L. A dot-blot screening procedure for mutated *ras* oncogenes using synthetic oligodeoxynucleotides. *Gene*, **50**, 313–320 (1986).
12) Taya, Y., Hosogai, K., Hirohashi, S., Shimosato, Y., Tsuchiya, R., Tsuchida, N., Fushimi, M., Sekiya, T. and Nishimura, S. A novel combination of K-*ras* and *myc* amplification accompanied by point mutational activation of K-*ras* in a human lung cancer. *EMBO J.*, **3**, 2943–2946 (1984).
13) Yamada, H., Sakamoto, H., Taira, M., Nishimura, S.,

Shimosato, Y., Terada, M. and Sugimura, T. Amplifications of both c-Ki-*ras* with a point mutation and c-*myc* in a primary pancreatic cancer and its metastatic tumors in lymph nodes. *Jpn. J. Cancer Res.*, 77, 370–375 (1986).

14) Goelz, S. E., Hamilton, S. R. and Vogelstein, B. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. *Biochem. Biophys. Res. Commun.*, **130,** 118–126 (1985).

15) Tsuda, H., Shimosato, Y., Upton, M. P., Yokota, J., Terada, M., Ohira, M., Sugimura, T. and Hirohashi, S. Retrospective study on amplification of N-*myc* and c-*myc* genes in pediatric solid tumors and its association with prognosis and tumor differentiation. *Lab. Invest.*, **59,** 321–327 (1988).

16) Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. and Erlich, H. A. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science,* **239,** 487–491 (1988).

17) Wood, W. I., Gitschier, J., Lasky, L. A. and Lawn, R. M. Base composition-independent hybridization in tetramethylammonium chloride: a method for oligonucleotide screening of highly complex gene libraries. *Proc. Natl. Acad. Sci. USA,* **82,** 1585–1588 (1985).

18) Hirota, T., Okada, T., Itabashi, M. and Kitaoka, H. Histogenesis of human gastric cancer with special reference to the significance of adenoma as a precancerous lesion. *In* "Precursors of Gastric Cancer," ed. S. C. Ming, pp. 233–252 (1984). Praeger Co., New York.

19) Wada, M., Yokota, J., Mizoguchi, H., Sugimura, T. and Terada, M. Infrequent loss of chromosomal heterozygosity in human stomach cancer. *Cancer Res.*, **48,** 2988–2992 (1988).

20) Motomura, K., Nishisho, I., Takai, S., Tateishi, H., Okazaki, M., Yamamoto, M., Miki, T., Honjo, T. and Mori, T. Loss of alleles at loci on chromosome 13 in human primary gastric cancers. *Genomics,* **2,** 180–184 (1988).