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Advances in Brief

The *Insulin-like Growth Factor II Receptor* Gene Is Mutated in Genetically Unstable Cancers of the Endometrium, Stomach, and Colorectum[1]

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Abstract

Disruption of the DNA mismatch repair system, characterized by microsatellite instability (MI), plays an important role in the course of human carcinogenesis. Repetitive sequences constitute targets for mutation in MI+ cells, and frequent mutations have indeed been reported in such regions within the *transforming growth factor β receptor II* (*RII*) gene in genetically unstable colorectal and gastric cancers. However, other genes that are targets for mutations in MI+ cells during the course of carcinogenesis have proven elusive. Because the *insulin-like growth factor II receptor* (*IGFIIR*) gene contains several repetitive sequences within its coding region, we examined mutations of this gene in MI+ cancers occurring at various primary sites. We found frameshift mutations in the poly(G)$_8$ tract of *IGFIIR* in eight tumors, all of which were MI+: 4 of 26 (15%) MI+ endometrial cancers, 3 of 12 (25%) MI+ gastric cancers, and 1 of 18 (6%) MI+ colorectal cancers. In contrast, no mutation was found in 51 pancreatic cancers, 7 of which (14%) were MI+. These results implicate abnormal IGFIIR-mediated growth control in carcinogenesis involving the endometrium, stomach, and colorectum but not the pancreas.

Introduction

IGFIIR[3] is a multifunctional protein. It plays a role in lysosomal enzyme trafficking, endocytosis, and activation of the TGFβ, which is known to be a potent growth inhibitor (1, 2). This receptor also inhibits cell proliferation mediated by the IGFII ligand, itself a potent growth stimulant, by internalizing and degrading this protein (3). The human *IGFIIR* gene has been mapped to chromosome bands 6q26–q27 (4). Frequent allelic deletions that include this region have been observed in numerous tumors, including those of the breast, ovary, liver, stomach, colorectum, and pancreas (5–14). Moreover, mutations of this gene were reported in cancers of the breast and liver (13, 15). These findings suggest that *IGFIIR* functions as a tumor suppressor gene in several human organs. MI, or instability in simple repeated sequences, has been associated with hereditary nonpolyposis colorectal cancer, as well as several sporadic forms of human cancer (16–19). This behavior is thought to be caused by disruption of the mismatch repair system, leading to increased rates of mutation within genes, some of which presumably are cancer related or even cancer causing. However, the target gene(s) for mutations in MI+ cells remain difficult to find. Recently, frequent somatic mutations in poly(A)$_{10}$ tract in the coding region of the *RII* gene were reported in MI+ colorectal and gastric cancers (20–23). However, mutation within this gene was infrequent in MI+ endometrial and pancreatic cancers (22, 23). *IGFIIR*, a candidate tumor suppressor gene, harbors a repetitive sequence within its coding region, and somatic mutations in this region were recently reported in gastric and colorectal tumors with MI+ (24). In this connection, it is of interest to examine whether somatic mutations of *IGFIIR* play important roles in other MI+ human cancers. Herein, we report mutational analyses of the *IGFIIR* gene in primary human tumors of the stomach, colorectum, pancreas, endometrium, breast, and ovary.

Materials and Methods

Materials and DNA Extraction. A total of 405 paired tumors and corresponding normal tissues from Japanese patients at Tohoku University Hospital and its related hospitals (Sendai, Japan) and from Cancer Institute Hospital (Tokyo, Japan) were analyzed. These samples are listed in Table 1. DNAs were extracted according to methods described previously (23). Histopathological diagnoses were classified according to the WHO criteria for endometrial and colorectal cancers (25, 26), and that for gastric cancer was classified according to the criteria of Lauren (27) and the WHO (28). Clinical stages were determined by the criteria of the International Federation of Gynecology and Obstetrics (29) for endometrial cancers and Dukes' criteria for colorectal cancers. For gastric cancers, clinical stages were determined by the Japanese Research Society for Gastric Cancer study (30). In brief, the stages were as follows. Stage I: (*a*) tumors of up to the subserosal layer without nodal involvement; or (*b*) tumors of up to the submucosal layer, the nodal involvement of which is limited to group 1 lymph nodes. Stage II: (*a*) tumors with suspected invasion to the serosal layer without nodal involvement; (*b*) tumors of up to the subserosal layer, the nodal involvement of which is limited to group 1 lymph nodes; or (*c*) tumors of up to the submucosal layer, the nodal involvement of which is limited to group 2 lymph nodes. Stage III: (*a*) tumors with invasion limited to one surrounding organ, the nodal involvement of which is limited to group 1 lymph nodes; (*b*) tumors with suspected invasion to the serosal layer, the nodal involvement of which is limited to group 2 lymph nodes; or (*c*) tumors of up to the subserosal layer, the nodal involvement of which is limited to group 3 lymph nodes. Stage IV, others.

Analyses of MI. MI was determined using five or more of the microsatellite markers. In each case, one of the primers was ^{32}P end labeled, and paired DNAs of normal and cancerous tissues were amplified by PCR followed by electrophoresis in 6% polyacrylamide/8 M urea gels as described previously (23). Nucleotide sequences of the primers and detailed conditions for PCR amplifications of microsatellite analyses are available from the authors upon request. Tumors in which altered sized bands were observed at two or more (or 40% or more) of the microsatellite loci were defined as MI+.

Mutational Analyses of *IGFIIR* and *RII*. Mutations of *IGFIIR* and *RII* at repetitive sequences within their coding regions were identified using a PCR-based assay (23) that was essentially identical to that used for the MI analyses. Five regions within *IGFIIR*, as well as the poly(A)$_{10}$ tract in *RII*, were

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[3] The abbreviations used are: IGFIIR, insulin-like growth factor II receptor; IGFII, insulin-like growth factor II; TGFβ, transforming growth factor β; RII, TGFβ receptor type II; MI, microsatellite instability.

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Table 1 *Summary of mutations detected in IGFIIR and RII*

Tumor Histologic diagnosis and clinical stage[a]	No. of tumors	MI+ cases	Mutation of *IGFIIR*	Mutation of *RII* (No. of MI− cases[b])
Endometrial cancer	100	26	4	0 (0)
G1	48	11	3	0 (0)
G2	29	8	1	0 (0)
G3	20	6	0	0 (0)
Clear	2	1	0	0 (0)
Serous	1	0	0	0 (0)
Stage I	65	19	3	0 (0)
Stage II	16	3	1	0 (0)
Stage III	18	4	0	0 (0)
Stage IV	1	0	0	0 (0)
Gastric cancer	81	12	3	9 (3)
Intestinal	35	5	2	5 (0)
Diffuse	36	3	0	2 (2)
Other	10	4	1	2 (1)
Stage I	26	3	1	4 (1)
Stage II	13	3	0	0 (0)
Stage III	31	4	1	3 (1)
Stage IV	11	2	1	2 (1)
Colorectal cancer[c]	114	18	1	12 (1)
Well	40	4	0	3 (0)
Moderately	41	0	0	1 (1)
Poorly	29	13	1	8 (0)
Mucinous	4	1	0	0 (0)
Dukes' A	49	6	0	5 (1)
Dukes' B	10	2	0	1 (0)
Dukes' C	43	7	1	4 (0)
Dukes' D	12	3	0	2 (0)
Ovarian cancer	39	0	0	0 (0)
Pancreatic cancer	51	7	0	0 (0)
Breast cancer	20	0	0	0 (0)

[a] G1, endometrioid adenocarcinoma grade 1; G2, endometrioid adenocarcinoma grade 2; G3, endometrioid adenocarcinoma grade 3; clear, clear cell adenocarcinoma; serous, serous adenocarcinoma; intestinal, intestinal type of gastric cancer; diffuse, diffuse type of gastric cancer; well, well differentiated adenocarcinoma; moderately, moderately differentiated adenocarcinoma; poorly, poorly differentiated adenocarcinoma; mucinous, mucinous adenocarcinoma.

[b] No. of MI− cases with mutated *RII* gene are shown in parentheses.

[c] Two of the 114 colorectal cancers developed in one HNPCC patient.

analyzed. Two regions of the former were reported previously (24). A repetitive sequence in the histone gene (a polymorphic eight- or seven-(G) tract in the 3′ noncoding region) was also analyzed as a non-cancer-related gene control. Primers used for mutational analyses are listed in Table 2. Mutant bands were cut from the gel, and their DNAs were purified and ligated into pKRX, a T-tailed PCR product cloning vector (31). Each ligated DNA was subjected to determination of DNA sequence as follows. Reamplification with (*a*) the sense primer and the BS-A primer (upstream from the T3 promoter in pBluescript) and (*b*) the antisense primer and BS-B primer (upstream from the T7 promoter in pBluescript), followed by sequencing with (*a*) the T3 primer and (*b*) the T7 primer, respectively, to determine the sequence on both strands. Sequencing analyses were performed using the Thermo Sequenase fluorescent-labeled primer cycle sequencing kit with 7-deaza-GTP (Amersham Corp., Little Chalfont, United Kingdom) and an SQ-5500 DNA sequencer (Hitachi Electronics Engineering Co., Ltd., Tokyo, Japan) according to the suppliers' recommendations.

Results

We first examined *IGFIIR* for mutations at repetitive sequences within its coding region in 405 human tumors of several different organs as summarized in Table 1. We analyzed five regions of this gene using primer pairs listed in Table 2, but we observed mutations only in the poly$(G)_8$ region in four endometrial cancers, three gastric cancers, and one colorectal cancer: all of them were MI+. These results are shown in Fig. 1*A*. DNAs of endometrial cancer cases E510, E517, E87, and E90 showed 1- or 2-bp insertions, whereas gastric cancer case G139, G84, and G47 and colorectal cancer case C31D showed 1-bp deletions at the G_8 microsatellite within *IGFIIR*. It is interesting that all four endometrial cancers with mutations in *IGFIIR* had insertions, whereas all other cancers with *IGFIIR* mutation had deletions. Moreover, case E90 contained a two-hit mutation: intensity of a band corresponding to the wild-type sequence was very faint, presumably contributed by contaminating normal cells, and two unique bands, both of greater length than the wild-type band, were clearly visible in this case. In case E87, two greater bands were also visible. However, the band corresponding to the wild type was also visible in this case. Because this tumor had massive contamination of normal stromal cells (data not shown), we think that E87 also had a two-hit mutation.

To confirm that these alterations occurred in the repetitive poly$(G)_8$ region of each PCR product showing mutation, nucleotide sequences of these tumors along with DNAs from their corresponding normal tissues were analyzed. Examples are shown in Fig. 1*B*. DNA from the endometrial cancer E90 gained one or two guanine residues in the G_8 region of *IGFIIR*. (Note that nucleotide sequences of the antisense strands are shown in Fig. 1*B*.) Similarly, in endometrial cancer E510 and gastric cancer G139, a 1-bp insertion and a 1-bp deletion, respectively, in the poly$(G)_8$ tract were clearly observed. Identical findings were obtained in all other tumors containing mutant bands (data not shown).

We also analyzed mutations of the *RII* gene in its poly$(A)_{10}$ microsatellite region and found 21 tumors with frame-shift mutations in *RII*. These results are also summarized in Table 1. Some of these results were already reported in our previous study (23). Two of the gastric cancer cases (G47 and G84) and one colon cancer case (C31D) contained mutations in both *IGFIIR* and *RII*.

The G_8/G_7 region within the histone gene was analyzed as a non-cancer-related gene control. This polymorphic region was first examined using DNA samples from 25 normal Japanese volunteers; we found an allele frequency of 32% for the G_8 allele and 68% for the G_7 allele. All tumors containing mutations either in *IGFIIR* or in *RII* were analyzed at this site, but no somatic mutations were observed.

To further characterize the significance of *IGFIIR* mutation, we determined whether there was a correlation between mutation and clinical features. However, we did not find any correlation between *IGFIIR* mutation and age, stage, or histological type.

Discussion

Recently, MI has been found to occur in a significant proportion of human tumors (16–19, 32). Genetically unstable tumor cells tend to have mutations in numerous repetitive sequences throughout the genome (33). Thus, repetitive sequences within genes could be targets for genetic alterations. The *RII* gene was the first reported target gene;

Table 2 *Primers used in the present study*

Primer		Nucleotide sequence	Location
IGFIIR $(AT)_3$	Sense	5′-ATTCGGATGGAGACCTCACC-3′	1361–1380
	Antisense	5′-GTTTATGACGCTCATCCGCT-3′	1440–1421
IGFIIR $(TG)_4$	Sense	5′-GGTGCCTATAAAGTTGAGAC-3′	2089–2108
	Antisense	5′-TTTTTGCCACCTGGCAGGCT-3′	2197–2178
IGFIIR $(AC)_4$	Sense	5′-TCCAACTGAACTACAGAGGC-3′	2271–2290
	Antisense	5′-TGATATTCAGGGAAGCCCAC-3′	2375–2356
IGFIIR G_8	Sense	5′-GCAGGTCTCCTGACTCAGAA-3′	4030–4049
	Antisense	5′-GAAGAAGATGGCTGTGGAGC-3′	4140–4121
IGFIIR $(CT)_5$	Sense	5′-GAAACACAAAACCTACGACC-3′	6141–6160
	Antisense	5′-GGTGGACTGGGAAGGCAC-3′	intron 40
RII A_{10}	Sense	5′-ATGCTGCTTCTCCAAAGTGC-3′	679–698
	Antisense	5′-GTCATTGCACTCATCAGAGC-3′	770–751
Histone G_8/G_7	Sense	5′-GTGCAAGGACAGCAACAACC-3′	1135–1154
	Antisense	5′-ACCTTCATGGCAAACAAGCC-3′	1265–1246
BS-A[a]	Antisense	5′-GTATGTTGTGTGGAATTGTGAG-3′	867–846
BS-B[a]	Sense	5′-GATTAAGTTGGGTAACGCCAG-3′	561–581

[a] These primers are located in pBluescript II SK(+).

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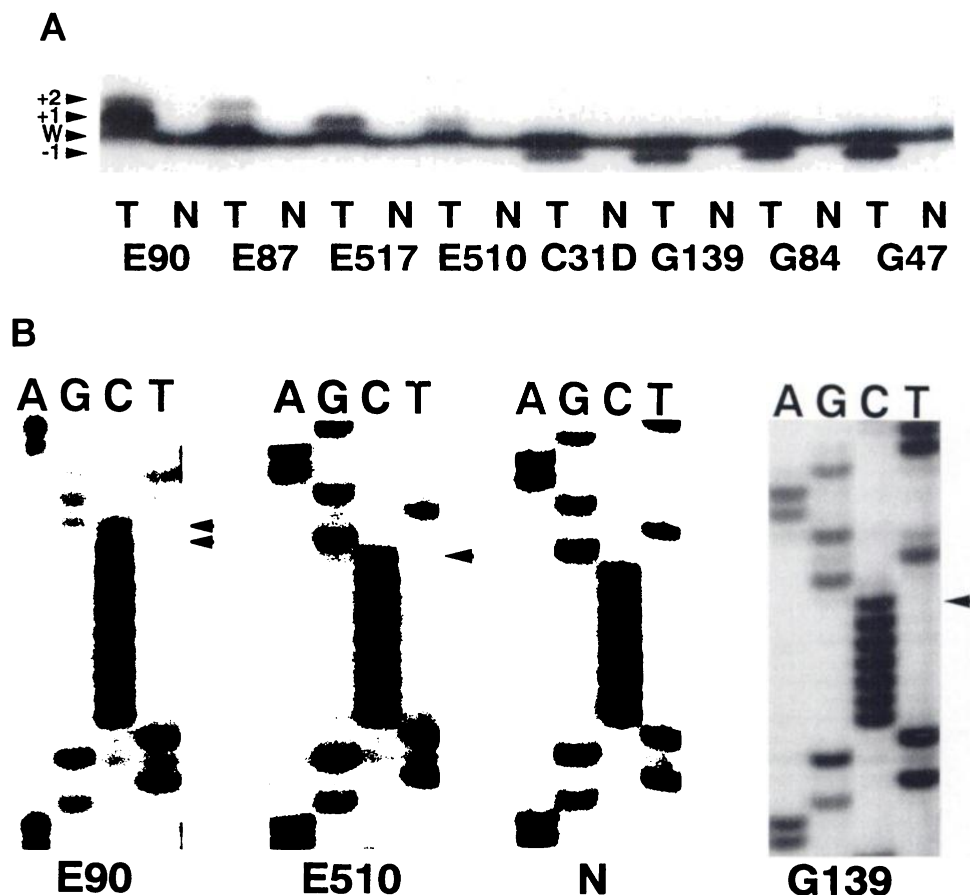

Fig. 1. *A*, screening for mutations of the poly(G)$_8$ (eight deoxyguanine repeat) region of *IGFIIR*. Insertions of 1 or 2 bp in endometrial cancers (E90, E87, E517, and E510) and deletions of 1 bp in descending colon (C31D) and gastric (G139, G84, and G47) cancers are shown. *T* and *N*, DNAs from tumors and corresponding normal tissues, respectively. *W*, *−1*, *+1*, and *+2*, wild type, 1-bp deletion, and 1- and 2-bp insertions, respectively. *B*, nucleotide sequencing analysis at the poly(G)$_8$ tract of the *IGFIIR* gene in E90 and E510 (endometrial cancers) and G139 (gastric cancer). Nucleotide sequences of the antisense strands are shown. *Arrows*, deletion of 1 bp and insertions of 1 or 2 bp at the poly(G)$_8$ tracts, which were represented by poly(C)$_8$ tracts in the antisense strand. *N*, nucleotide sequence of the normal DNA.

frequent mutations of MI+ colorectal and gastric cancers were reported (20–23). However, additional genes that constitute targets for mutations in MI+ cells have been hard to identify. Recently, frequent allelic loss of the long arm of chromosome 6, where the *IGFIIR* gene resides, was reported in cancers of the breast, liver, ovary, stomach, colorectum, and pancreas (5–14), and somatic mutations of *IGFIIR* in cancers of the breast and liver were reported (13, 15). Moreover, frequent frameshift mutations of this gene in genetically unstable tumors of the colorectum and stomach were also observed (24). In the present study, we observed somatic frameshift mutations in the poly(G)$_8$ microsatellite of *IGFIIR* in MI+ endometrial [4 of 26 (15%)], gastric [3 of 12 (25%)], and colorectal [1 of 18 (6%)] cancers; this type of alteration should result in the production of truncated IGFIIR protein. It was suggested that: (*a*) mutations of the *IGFIIR* gene play an important role in the genesis of endometrial, gastric, and colorectal cancers with MI+; and (*b*) the *IGFIIR* gene is one of the targets for allelic loss in the course of gastric and colorectal carcinogenesis.

All mutations of *IGFIIR* detected in endometrial cancers were insertions, whereas all mutations detected in other cancers were deletions. This finding may reflect differences between mutagens in the endometrium and digestive tract, or it may be due simply to chance (because the number of tumors analyzed was not large). Alternatively, the difference may represent tissue-specific sensitivities to different types of mutation.

Only one case of colorectal cancer, which developed in a hereditary nonpolyposis colorectal cancer patient, harbored mutated *IGFIIR*. Although Souza *et al.* (24) reported somatic frameshift mutations in the *IGFIIR* gene in 9% of MI+ sporadic colorectal cancers, we did not find any mutations of this gene in our sporadic colorectal cancers. This difference may be accounted for by differences in mutagens, possibly in food, between Japanese and American populations, or it may be due simply to chance (because the number of tumors analyzed was not large).

Somatic frameshift mutations in either *IGFIIR* or *RII* were observed in 7 of 12 (58%) MI+ gastric cancers and 11 of 18 (61%) MI+ colorectal cancers. It was suggested that the tumorigenic pathway comprising *RII*, the receptor for TGFβ, and *IGFIIR*, a possible activator of TGFβ, is very important in these tumors.

Four of 26 (15%) MI+ endometrial cancers had mutations in *IGFIIR*. We and others reported that mutation of *RII* is rare in MI+ endometrial cancers (22, 23). Thus, there may be differences in mutational susceptibility or in the carcinogenicity of *RII* or *IGFIIR* mutations occurring in cells of the endometrium *versus* cells of the colorectum and stomach.

Although the incidence of MI is high in pancreatic cancers, we did not observe any mutation in *IGFIIR* in 51 pancreatic cancers, 7 of which (14%) were MI+. There are several possible explanations for this finding, notably (*a*) *IGFIIR* and *RII* do not play important roles in pancreatic carcinogenesis; or (*b*) mutations in other regions of these genes are crucial in pancreatic carcinogenesis. Because repetitive sequences as analyzed in this study are frequent targets for mutation in genetically unstable cells, we suspect that the former possibility is more likely. If this is the case, then mutations in a gene(s) other than *IGFIIR* and *RII* may play important roles in tumorigenesis in the pancreas. Further studies are necessary to identify other possible targets of MI in this tumor type.

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References

1. Dahms, N. M., Lobel, P., and Kornfeld, S. Mannose 6-phosphate receptors and lysosomal enzyme targeting. J. Biol. Chem., *264:* 12115–12118, 1989.
2. Dennis, P. A., and Rifkin, D. B. Cellular activation of latent transforming growth factor β requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. Proc. Natl. Acad. Sci. USA, *88:* 580–584, 1991.
3. Kornfeld, S. Structure and function of the mannose 6-phosphate/insulin-like growth factor II receptors. Annu. Rev. Biochem., *61:* 307–330, 1992.
4. Laureys, G., Barton, D. E., Ullrich, A., and Francke, U. Chromosomal mapping of the gene for the type II insulin-like growth factor receptor/cation-independent mannose 6-phosphate receptor in man and mouse. Genomics, *3:* 224–229, 1988.
5. Dutrillaux, B., Gerbault-Seureau, M., Zafrani, B. Characterization of chromosomal anomalies in human breast cancer. Cancer Genet. Cytogenet., *49:* 203–217, 1990.
6. Lee, J. H., Kavanagh, J. J., Wildrick, D. M., Wharton, J. T., Blick, M. Frequent loss of heterozygosity on chromosomes 6q, 11, and 17 in human ovarian carcinomas. Cancer Res., *50:* 2724–2728, 1990.
7. Devilee, P., van Vliet, M., van Sloun, P., Dijkshoorn, N. K., Hermans, J., Pearson, P. L., Cornelisse, C. J. Allelotype of human breast carcinoma: a second major site for loss of heterozygosity is on chromosome 6q. Oncogene, *6:* 1705–1711, 1991.
8. Saito, S., Saito, H., Koi, S., Sagae, S., Kudo, R., Saito, J., Noda, K., Nakamura, Y. Fine-scale deletion mapping of the distal long arm of chromosome 6 in 70 human ovarian cancers. Cancer Res., *52:* 5815–5817, 1992.
9. De Souza, A. T., Hankins, G. R., Washington, M. K., Fine, R. L., Orton, T. C., and Jirtle, R. L. Frequent loss of heterozygosity on 6q at the mannose 6-phosphate/insulin-like growth factor II receptor locus in human hepatocellular tumors. Oncogene, *10:* 1725–1729, 1995.
10. Hahn, S. A., Seymour, A. B., Hoque, A. T., Schutte, M., da Costa, L. T., Redston, M. S., Caldas, C., Weinstein, C. L., Fischer, A., Yeo, C. J., Hruban, R. H., Kern, S. E. Allelotype of pancreatic adenocarcinoma using xenograft enrichment. Cancer Res., *55:* 4670–4675, 1995.
11. Queimado, L., Seruca, R., Costa, P. A., and Castedo, S. Identification of two distinct regions of deletion at 6q in gastric carcinoma. Genes Chromosomes Cancer, *14:* 28–34, 1995.
12. Honchel, R., McDonnell, S., Schaid, D. J., and Thibodeau, S. N. Tumor necrosis factor-α allelic frequency and chromosome 6 allelic imbalance in patients with colorectal cancer. Cancer Res., *56:* 145–149, 1996.
13. Hankins, G. R., De Souza, A. T., Bentley, R. C., Patel, M. R., Marks, J. R., Iglehart, J. D., and Jirtle, R. L. M6P/IGF2 receptor: a candidate breast tumor suppressor gene. Oncogene, *12:* 2003–2009, 1996.
14. Kimura, M., Abe, T., Sunamura, M., Matsuno, S., and Horii, A. Detailed deletion mapping on chromosome arm 12q in human pancreatic adenocarcinoma: identification of a 1-cM region of common allelic loss. Genes Chromosomes Cancer, *17:* 88–93, 1996.
15. De Souza, A. T., Hankins, G. R., Washington, M. K., Orton, T. C., Jirtle, R. L. *M6P/IGF2R* gene is mutated in human hepatocellular carcinomas with loss of heterozygosity. Nat. Genet., *11:* 447–449, 1995.
16. Aaltonen, L. A., Peltomäki, P., Leach, F. S., Sistonen, P., Rylkkanen, L., Mecklin, J. P., Järvinen, H., Powell, S. M., Jen, J., Hamilton, S. R., Petersen, G. M., Kinzler, K. W., Vogelstein, B., and de la Chapelle, A. Clues to the pathogenesis of familial colorectal cancer. Science (Washington DC), *260:* 812–816, 1993.
17. Thibodeau, S. N., Bren, G., Schaid, D. Microsatellite instability in cancer of the proximal colon. Science (Washington DC), *260:* 816–819, 1993.
18. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D., Perucho, M. Ubiquitous somatic mutation in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature (Lond.), *363:* 558–561, 1993.
19. Han, H-J., Yanagisawa, A., Kato, Y., Park, J-G., Nakamura, Y. Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. Cancer Res., *53:* 5087–5089, 1993.
20. Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L., Lutterbaugh, J., Fan, R. S., Zborowska, E., Kinzler, K. W., Vogelstein, B., Brattain, M., Willson, J. K. V. Inactivation of the Type II TGF-β receptor in colon cancer cells with microsatellite instability. Science (Washington DC), *268:* 1336–1338, 1995.
21. Parsons, R., Myeroff, L. L., Liu, B., Willson, J. K. V., Markowitz, S. D., Kinzler, K., W., and Vogelstein, B. Microsatellite instability and mutations of the transforming growth factor β type II receptor gene in colorectal cancer. Cancer Res., *55:* 5548–5550, 1995.
22. Meyeroff, L. L., Parsons. R., Kim, S-L., Hedrick, L., Cho, K. R., Orth, K., Mathis, M., Kinzler, K. W., Lutterbaugh, J., Park, K., Bang, Y-J., Lee, H. Y., Park, J-G., Lynch, H. T., Roberts, A. B., Vogelstein, B., and Markowitz, S. D. A transforming growth factor β receptor type II gene mutation common in colon and gastric but rare in endometrial cancers with microsatellite instability. Cancer Res., *55:* 5545–5547, 1995.
23. Abe, T., Ouyang, H., Migita, T., Kato, Y., Kimura, M., Shiiba, K., Sunamura, M., Matsuno, S., and Horii, A. The somatic mutation frequency of the transforming growth factor β receptor type II gene varies widely among different cancers with microsatellite instability. Eur. J. Surg. Oncol., *22:* 474–477, 1996.
24. Souza, R. F., Appel, R., Yin, J., Wang, S., Smolinski, K. N., Abraham, J. M., Zou, T-T., Shi, Y-Q., Lei, J., Cottrel, J., Cymes, K., Biden, K., Simms, L., Leggett, B., Lynch, P. M., Frazier, M., Powell, S. M., Harpaz, N., Sugimura, H., Young, J., and Meltzer, S. J. Microsatellite instability in the insulin-like growth factor II receptor gene in gastrointestinal tumours. Nat. Genet., *14:* 255–257, 1996.
25. WHO. Histological Typing of Female Genital Tract Tumors, Ed. 2, pp. 13–18. Springer-Verlag, Heidelberg, Germany, 1994.
26. WHO. Histological Typing of Intestinal Tumors, Ed. 2, pp. 29–33. Springer-Verlag, Heidelberg, Germany, 1989.
27. Lauren, P. The two histological main types of gastric carcinoma, diffuse and so called intestinal type carcinoma. An attempt at a histo-clinical classification. Acta Pathol. Microbiol. Scand., *64:* 31–49, 1965.
28. WHO. Histological Typing of Oesophageal and Gastric Tumors, Ed. 2, pp. 20–26. Springer-Verlag, Heidelberg, Germany, 1990.
29. International Federation of Gynecology and Obstetrics. FIGO Stages: 1988 Revision. Gynecol. Oncol., *35:* 125–127, 1989.
30. Japanese Research Society for Gastric Cancer. The general rules for the gastric cancer study in surgery and pathology. Jpn. J. Surg., *11:* 127–139, 1981.
31. Schutte, B. C., Ranade, K., Pruessner, J., and Dracopoli, N. Optimized conditions for cloning PCR products into an *Xcm*I T-vector. Biotechniques, *22:* 40–44, 1997.
32. Aaltonen, L. A., Peltomäki, P., Mecklin, J-P., Järvinen, H., Jass, J. R., Green, J. S., Lynch, H. T., Watson, P., Tallqvist, G., Luhola, M., Sistonen, P., Hamilton, S. R., Kinzler, K. W., Vogelstein, B., and de la Chapelle, A. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. Cancer Res., *54:* 1645–1648, 1994.
33. Strand, M., Prolla, T. A., Liskay, R. M., and Petes, T. D. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature (Lond.), *365:* 274–276, 1993.

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