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The *TPR–MET* oncogenic rearrangement is present and expressed in human gastric carcinoma and precursor lesions

(polymerase chain reaction/transcript amplification/solution hybridization/*RAS* gene mutation/restriction fragment length polymorphism)

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ABSTRACT **The *TPR–MET* oncogenic rearrangement was originally observed in an *in vitro* transformed human osteosarcoma cell line. Recently, we detected the expression of this rearrangement at very low levels in several cell lines derived from human tumors of nonhematopoietic origin using a highly sensitive method based on polymerase chain reaction amplification of the transcript. We report here the results of analysis of *TPR–MET* expression in cell lines derived from human gastric tumors and 22 biopsy samples of human gastric mucosa showing cancer or precursor lesions. The rearranged RNA was expressed in all four cell lines as well as in biopsy samples from 12 of the 22 patients. Overexpression of *TPR–MET* RNA in superficial gastritis lesions with hyperplasia of glandular neck cells suggests the possible involvement of this oncogene at an early stage of gastric tumorigenesis. Analysis of gastric biopsy samples for *RAS* gene mutations showed base substitutions occurring in the codon 12 region of Ki- and Ha-*RAS* genes in four cases, including two precursor lesions.**

The *TPR–MET* oncogenic rearrangement was first observed in a human osteosarcoma cell line (HOS) transformed *in vitro* by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (1, 2). The rearrangement involved the fusion of a *TPR* (translocated promoter region) locus present on chromosome 1 to the 5' region of *MET* gene sequences located on chromosome 7 (3, 4). The oncogenic *TPR–MET* RNA is a 5.0-kilobase (kb) hybrid transcript encoding a 65-kDa fusion protein (3, 4). To permit detection of the rearranged RNA in tumor tissues, we developed a sensitive analytical procedure in which cDNA generated from the fused transcript was used as a template for PCR amplification of the breakpoint region of the *TPR–MET* gene. Using this method, we found *TPR–MET* RNA to be expressed at low levels by cell lines derived from several human tumors of nonhematopoietic origin (5). Amplification and overexpression of the c-*MET* gene was recently observed in a human gastric tumor cell line, GTL-16 (6), suggesting a possible role of the *MET* oncogene in gastric tumorigenesis.

The human model of gastric carcinogenesis (7) shares certain features with colorectal cancer, with respect to the progress of its development. Epidemiological and laboratory data have shown the disease to progress through at least six stages, in which morphological features and biochemical markers of differentiation define the progression to malignancy: superficial gastritis (SG); chronic atrophic gastritis (CAG); intestinal metaplasia of the small intestinal type followed by the colonic type (IM-CT); dysplasia (DYS); and carcinoma (Ca). The progression from normal to neoplastic cells appears to take place over a period of many years, under the combined influence of modulating forces of different types. These include carcinogens (e.g., *N*-nitroso compounds), irritants (especially salt), bacterial infection (*Helicobacter pylori*), protective agents (micronutrients and nonnutrient factors), and genetic susceptibility. The multitude of phenotypic changes seen in the precancerous phases of the disease and their apparently gradual occurrence suggest a complex interplay of modulating factors and genetic changes in cells of the gastric epithelium. Genetic alterations in gastric carcinogenesis have not been extensively studied, in contrast to the molecular genetics of colorectal cancer, a disease that also develops through a series of morphologically characteristic stages, which have been the subject of intense investigation. Current evidence supports a model of colorectal cancer in which events required for tumor development often include the mutational activation of an oncogene (the Ki-*RAS* gene), DNA hypomethylation, and loss of the tumor suppressor genes p53 and DCC (8).

As noted above, the *TPR–MET* rearrangement was originally identified in cells transformed by MNNG, a potent mutagen and carcinogen that induces gastric tumors in experimental animals (9). On the basis of evidence suggesting involvement of environmental carcinogens as risk factors for human gastric cancer, it was of interest to determine whether genetic alterations take place during gastric cancer development that could plausibly be related to carcinogen exposure and also relate to those found in colorectal cancer. To study the possible involvement of the *TPR–MET* oncogenic rearrangement in human gastric tumorigenesis, we analyzed four cell lines derived from human gastric tumors as well as biopsy samples of human gastric mucosa containing histopathologic lesions characteristic of various stages in the development of gastric cancer. We found *TPR–MET* RNA to be expressed frequently in gastric Ca as well as preneoplastic tissues. Concurrent analysis of these tissues for *RAS* gene mutations also demonstrated base substitution mutations in the exon 1 region of Ki-*RAS* and Ha-*RAS* genes in some cases.

MATERIALS AND METHODS

Osteosarcoma cell lines HOS 6374 (passage 62) and MNNG-HOS were grown as described (5). Gastric cell lines MKN1, MKN28, MKN45, and SCH were grown in RPMI 1640 medium containing 10% fetal bovine serum (10). The MKN1 line was established from an adenosquamous Ca; the MKN28 line was from a well-differentiated, tubular adenocarcinoma; the MKN45 line was from a poorly differentiated adenocarcinoma; and the SCH line was from a choriocarcinoma (10). The Calu-1 cell line was obtained from the American Type

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Abbreviations: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; DEPC, diethylpyrocarbonate; HLO, *Helicobacter*; SG, superficial gastritis; DAG, diffuse antral gastritis; CAG, chronic atrophic gastritis; IM-CT, intestinal metaplasia, colonic type; IM-SIT, intestinal metaplasia, small intestinal type; DYS, dysplasia; Ca, carcinoma(s).

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Culture Collection and grown according to instructions provided.

RNA was prepared from 2×10^5 cells and analyzed by the transcript-PCR amplification method involving generation of a 205-base-pair (bp) cDNA fragment carrying the *TPR–MET* breakpoint region, using primers (T_2 and M_2) as described (5). Internal probes (T_a and M_b) were end-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase enzyme and purified using an Elutip-d (Schleicher & Schuell) column. The labeled primers were ethanol precipitated, resuspended in 30 μl of sterile water, and used for solution hybridization analysis (5).

TPR–MET expression was determined in biopsy samples of human gastric mucosa containing cancer or precursor lesions, based on histopathologic examination. Frozen tissue (about 1 mg) was homogenized in 50 μl of 0.15% diethylpyrocarbonate (DEPC)-treated deionized water using a tissue microhomogenizer and boiled for 5 min to release RNA. After pelleting the cell debris (5 min at 12,000 $\times$ *g* in a Microfuge at 4°C), 5 μl of the supernatant was used for transcript amplification as described earlier. Genomic DNA was isolated from frozen samples by an SDS/proteinase K digestion method (11) and from paraffin-embedded tissues as described by Shibata *et al.* (12).

Oligonucleotides were purchased from Research Genetics (Huntsville, AL). The sequences of primers used for analysis of exon 1 region, codon 12 (position 1 or 2), of Ki-*RAS* and Ha-*RAS* gene by a PCR-mismatched primer restriction fragment length polymorphism method were as follows: Ki-*RAS*, KHE5′ (30-mer), 5′-GGAATTCATAAACTTGTGGTAGTTGGACCT-3′; K3′ (13) (20-mer), 5′-TCAAAGAATGGTCCTGGACC-3′; and β (20-mer), 5′-ATCCACAAAGTGATTCTGAA-3′. The primers KHE5′ and K3′ amplified a 161-bp DNA fragment. The wild-type allele carried two *Bst*NI recognition sites, giving rise to three fragments (114 bp, 29 bp, and 18 bp) on digestion with *Bst*NI endonuclease, whereas mutations in position 1 or 2 of codon 12 abolished one *Bst*NI site, giving rise on digestion to only two DNA fragments (143 bp and 18 bp). Incorporation of a *Bst*NI site at the 3′ region of the amplified product aided in monitoring complete digestion.

About 10–15 μl of PCR aliquot was digested overnight by *Bst*NI enzyme under the conditions specified by the manufacturer and the products were separated on an 8% polyacrylamide gel. The mutant DNA fragment was recovered from the gel and directly sequenced using a ^{32}P-end-labeled internal primer, β, using Sequenase enzyme as described above. In cases in which an adequate amount of DNA was not available for direct sequencing, the 143-bp DNA was further reamplified using primers, KHE5′ and β, to produce an 87-bp fragment and used for sequence analysis.

Similarly, presence of a codon 13 aspartic acid mutation was determined by *Hph* I analysis. The wild-type allele did not carry any *Hph* I recognition site, whereas substitution by an adenosine residue at position 2 of codon 13 created a new *Hph* I site, giving rise to two fragments (118 bp and 43 bp, respectively) upon *Hph* I digestion.

For Ha-*RAS*, HA12E5′ (24-mer), 5′-GGAATTCATGACGGAATATAAGCT-3′; HA12B3′ (24-mer), 5′-GGATCCACAAAATGGTTCCGGATC-3′; and H_β (20-mer), 5′-GGATCCATCAGCTGGATGGT-3′, the primers HA12E5′ and HA12B3′ amplified a 99-bp DNA fragment. The wild-type allele carried two *Msp* I recognition sites, giving rise to three DNA fragments (40 bp, 41 bp, and 18 bp) upon *Msp* I digestion. Mutations at position 1 or 2 of codon 12 or position 2 or 3 of codon 11 abolished one *Msp* I site, thus producing only two DNA fragments (81 bp and 18 bp) on digestion. The mutant bands were sequenced either directly or following reamplification with primer HA12E5′ and an internal primer, H_β, using end-labeled H_β as sequencing primer. The restriction enzymes *Bst*NI and *Hph* I were purchased from New England Nuclear Biolabs and *Msp* I enzyme was obtained from IBI.

PCR amplifications were carried out on genomic DNA using *Thermus aquaticus* DNA polymerase (Taq polymerase) and a thermal DNA cycler (Perkin–Elmer/Cetus). The reaction mixture contained 200 μM, 1.0 μM of each primer in 50 μl of PCR buffer (50 mM Tris·HCl/50 mM KCl/2.5 mM $MgCl_2$/100 μg of bovine serum albumin per ml, pH 8.4). Forty reaction cycles at 94°C, 55°C, and 72°C for 1 min, 1 min, and 2 min, respectively, were performed, at the end of which the mixtures were incubated at 72°C for an additional 7 min and then stored at 4°C. Aliquots (10–15 μl) were digested overnight by the appropriate restriction enzymes at 60°C for *Bst*NI and 37°C for *Hph* I and *Msp* I enzymes. The products were separated on native polyacrylamide gels and visualized after ethidium bromide staining under UV light. For amplification of DNA isolated from paraffin blocks, concentration of the template required for maximal amplification was determined for each sample.

RESULTS

TPR–MET gene expression was first examined in four cell lines derived from human gastric tumors of different types (10). As shown in Fig. 1, *TPR–MET* RNA was expressed at different levels by cells of all four lines, suggesting possible involvement of the *TPR–MET* oncogenic rearrangement in gastric tumorigenesis. On the basis of these findings, we sought to determine whether the rearrangement was also present in gastric tissues containing histologic evidence of pathologic changes associated with the development of gastric cancer, and, if so, whether it was related to the simultaneous presence of other risk factors for the disease. For this purpose, we analyzed gastric biopsy samples from 22 patients residing in the New Orleans area for *TPR–MET* gene expression, mutations in Ki-*RAS* and Ha-*RAS* genes, and evidence of *Helicobacter* (HLO) infection. Histological analysis confirmed that these samples contained lesions representing various stages of neoplastic progression. Tissue from one subject showed no abnormalities and was included as a control.

Data produced by these analyses are summarized in Table 1. *TPR–MET* RNA was detected in many samples representing all stages of gastric carcinogenesis, from early SG through end-stage Ca. Although the highest level of expression was found in one sample with SG, there was no evident relationship between the amount of *TPR–MET* RNA present and progression of the disease. Evidence of HLO infection was found in the majority of samples analyzed (15/22), representing all precancerous stages. Severity of infection appeared to be somewhat greater in biopsies exhibiting advancing gastritis [diffuse antral gastritis (DAG) and CAG] than in other precancerous stages; however, the severity of infection appeared to be diminished in the more advanced stages of disease (IM-CT, DYS, and Ca). The two Ca analyzed,

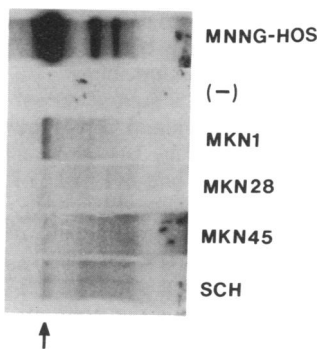

FIG. 1. Autoradiogram of a 7% polyacrylamide gel showing expression of *TPR–MET* RNA in the gastric cell lines tested. The 205-bp *TPR–MET* cDNA is indicated by the arrow. Each lane represents RNA from 5×10^4 cells.

Table 1. *TPR–MET* expression, HLO status, and *RAS* mutations in gastric biopsies

Histologic diagnosis	*TPR–MET* RNA*	HLO†	*RAS* mutation‡ Ki-	Ha-
Normal				
1	–	–	–	–
SG				
1	+++	–		
2	+	–		
3	–	++	–	–
4	–	–	–	–
DAG				
1	+	++	–	–
2	+	+++	–	–
3§			+	
CAG				
1	–	+++	–	–
2	–	+++	–	–
3	+	++		
4	+	+++		
5	+	+++		
6	+	++		
7	+	+		
CAG + IM-SIT				
1	–	–		
2	+	+	–	+
3	–	+++	–	–
CAG + IM-CT				
1	+	+	–	–
2	–	+	–	–
Severe DYS				
1	–	++	–	–
Ca				
1	++	–	++	
2	–	–	–	–
3§			–	+
4§			–	–
5§			–	–

*Expression level of *TPR–MET* RNA by transcript amplification method.
†HLO status as determined by Warthin–Starry staining.
‡*RAS* mutation determined by PCR restriction fragment length polymorphism method. Ki, Kirsten; Ha, Harvey.
§Paraffin-embedded tissue.

including the one sample expressing *TPR–MET* RNA, were negative for HLO infection.

As shown in Fig. 2 *A* and *B*, variable levels of expression of *TPR–MET* RNA were observed in some biopsy samples representing each stage of progression from SG to Ca. Interestingly, the highest level of expression was observed in one sample at the earliest SG stage (Fig. 2*A*; case 1). The *TPR–MET* rearrangement was expressed in two of four biopsy samples with SG lesions. Comparatively low levels of expression of the rearranged RNA were observed in later stages, with the exception of one sample containing gastric Ca (Fig. 2*B*; case 1). The SG tissue with a high level of *TPR–MET* RNA showed only mild inflammation, but it also showed hyperplasia of the glandular necks, a sign of recent injury to the gastric mucosa followed by gland repair by means of hyperproliferation of neck cells (Fig. 3*A*). Neither case of SG showing *TPR–MET* expression had evidence of infection with HLO.

One of two Ca studied expressed relatively high levels of *TPR–MET* RNA (see Fig. 2*B* and Table 1). Histologically, this was a well-differentiated adenocarcinoma of the intestinal or "expansive" type (see Fig. 3*B*). This is the predominating type of gastric Ca found in high-risk populations and

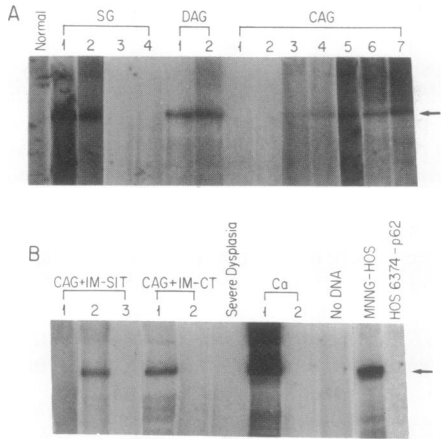

FIG. 2. Expression of *TPR–MET* RNA in human gastric biopsy tissues and cell lines. (*A* and *B*) Autoradiograms of 7% native polyacrylamide gels showing a 205-bp DNA fragment spanning the *TPR–MET* breakpoint region of cDNA. (*A*) RNAs from 2×10^4 cells of the MNNG-HOS cell line and 5×10^4 cells of the HOS 6374 (passage 62) cell line were used per lane as positive and negative controls, respectively. The analysis was carried out in two separate experiments. SG (case 1) represents an overnight exposure of the autoradiograph (from the first experiment), whereas SG (case 2), CAG (cases 3, 6, and 7), CAG plus IM-SIT (intestinal metaplasia, small intestinal type; case 1), normal, MNNG-HOS, and HOS 6374 (passage 62) lanes represent a longer exposure time (3 days using Kodak x-ray film with a single intensifier screen). A negative control lane with no template RNA was also included (see *B*). Ca (case 1) is a short exposure (2 days with intensifier screen) of the autoradiograph from the second experiment, whereas all of the remaining lanes represent a prolonged exposure time (5 days) of the autoradiogram.

is generally preceded by a complex series of precancerous lesions. The other Ca, which was negative for *TPR–MET* rearrangement, was of the signet-ring diffuse or "infiltrative" type. This pathological type of gastric Ca is usually found in low-risk populations and is not preceded by chronic gastritis or metaplasia (see Fig. 3*C*). Among samples from 12 patients with CAG, 7 expressed *TPR–MET* RNA (see Fig. 2 *A* and *B* and Table 1). Tissues from two patients with DAG, a lesion that has not been implicated in the gastric precancerous process, were found to be positive for *TPR–MET* rearrange-

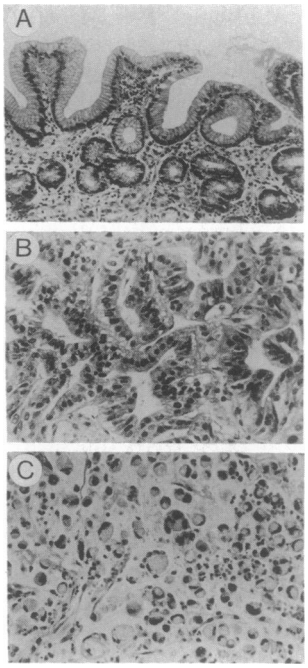

FIG. 3. Analysis of gastric tissues. (*A*) Histopathological diagnosis of a biopsy sample of mild SG (case 1) with mild hyperplasia of neck glands. (×20.) (*B*) Histopathological diagnosis of a well-differentiated adenocarcinoma, "intestinal type" (case 1). (×110.) (*C*) Histopathological diagnosis of a signet ring ("diffuse") type adenocarcinoma (case 2). (×90.)

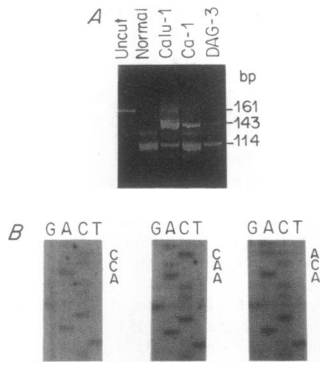

FIG. 4. *RAS* codon 12 polymorphism. (*A*) Electrophoretic analysis of *Bst*NI-digested DNA, following PCR amplification, on a 10% PAGE gel. Mutations in position 1 or 2 of Ki-*RAS* codon 12 region could be detected as a larger band. (*B*) Sequence analysis of the wild-type and mutant bands shown in *A*. The lanes from left to right represent DNA from the following biopsy samples: normal, Ca (case 1), and DAG (case 3), respectively. The codon 12 DNA sequence of the antisense strand is indicated on the righthand side of each lane.

ment (Fig. 2*A* and Table 1). In both cases, evidence of severe HLO infection was present.

Table 1 also summarizes the results of analysis of *RAS* gene mutations in samples for which adequate amounts of tissue were available for DNA isolation. Fig. 4*A* shows the result of *Bst*NI digestion of the PCR-amplified product for Ki-*RAS* codon 12 mutation. Complete digestion by the enzyme was ensured by including a *Bst*NI site at the 3′ end of the product. The undigested DNA and the wild-type and mutant alleles migrated differently in the gel. Fig. 4*B* shows the sequence of the DNA from two cases showing Ki-*RAS* gene codon 12 mutations. Ki-*RAS* codon 13 and Ha-*RAS* codon 12 and 61 sequences were also analyzed for mutations by the PCR restriction fragment length polymorphism method. No codon 61 mutation in the Ha-*RAS* gene or codon 13 mutation in the Ki-*RAS* gene was observed for any of the samples analyzed (data not shown). As shown in Table 1, two biopsy samples showed Ha-*RAS* codon 12 mutations. Sequence analysis revealed that the DNA from both samples carried mutations at codon 11 and codon 12 (GCT and GGA, respectively). Both of these mutations were cryptic, encoding the same amino acids as wild-type DNA.

DISCUSSION

Activation of cellular protooncogenes can take place through several mechanisms, including mutations or other chromosomal structural changes such as translocations, overexpression of normal or mutated protooncogenes, or amplification of protooncogene sequences. Although currently available information concerning the possible involvement of oncogene activation in human gastric carcinogenesis is limited and fragmentary, examples of each of the above mechanisms have been reported. Evidence of *RAS* gene mutations has been sought in several investigations, with contradictory results. In one instance, 5/18 gastric tumors analyzed contained mutations in the 12th codon of Ha-*RAS* (14). In contrast, other investigators failed to find evidence of *RAS* mutations in analyses of 21 (15), 7 (16), or 27 (17) gastric tumors, respectively. However, individual gastric tumors and cancer cell lines containing mutated Ha-*RAS*, Ki-*RAS*, or N-*RAS* alleles have been described (14, 18–21). The reasons for these discrepancies are not known but may in part be attributable to use of analytical methods with differing limits of detection and analysis of tumors at different stages of development and from several population groups, therefore possibly reflecting different etiologic agents.

In addition to mutational activation, overexpression of the normal *RAS* gene product (p21) has been shown to cause cell transformation. Several previous investigations have dealt with the possible role of enhanced oncogene expression in gastric carcinogenesis, but, as in the case of *RAS* mutations, the evidence currently available on this point is both fragmentary and limited in amount. Overexpression of *RAS* oncogene p21 protein in advanced gastric tumors has been reported by several investigators (16, 22, 23). Enhanced p21 expression was found in nearly all advanced adenocarcinomas, in which it was also associated with invasiveness, metastasis, and poor prognosis. Recently, overexpression was also found in early Ca as well as in the dysplastic and/or metaplastic mucosal alterations accompanying intestinal type of gastric cancer (24). Overexpression of the c-*MYC* gene product (p62) in advanced, but not early, gastric adenocarcinomas has also been reported (25).

Gene transfer experiments have resulted in the identification of a transforming gene, *HST*, in human stomach cancers (26, 27). This sequence was shown to be closely related to the v-*RAF* oncogene of the murine transforming retrovirus 3611-MSV. The transforming sequences were found at a frequency of 3/58 tumors in the initial study, but no further information is available concerning this gene or its role in gastric carcinogenesis.

Limited information is available regarding the precise role of the various known modulating factors and genetic changes involved in the progression of gastric carcinogenesis. The most common precursor of gastritis is the multifocal atrophic gastritis (MAG) complex, in which gland loss (atrophy) is followed by the expression of abnormal phenotypes resembling either small intestinal epithelium (small intestinal metaplasia or IM-SIT) or colonic epithelium (or IM-CT). In this setting, dysplastic changes take place that may eventually lead to invasive Ca. In high gastric cancer risk areas, gastric peptic ulcer is more frequent than duodenal ulcer. Duodenal ulcer is usually accompanied by DAG, which is not considered as a cancer precursor and in which gastric atrophy (gland loss) is not prominent. Recently, *H. pylori* has been recognized as a cause of chronic gastritis: it appears to play a dominant role in DAG and an adjuvant role in MAG (28). HLO infection was severe in the two cases of DAG studied (Table 1), which could be interpreted as lending some support to the hypothesis that *H. pylori* may play a role in the gastric precancerous process (28, 29). These tissues also expressed *TPR–MET* RNA. However, the small number of samples analyzed does not establish a direct correlation between *TPR–MET* expression and *H. pylori* infection. In six patients, HLO was present in the absence of *TPR–MET* rearrangement, whereas *TPR–MET* RNA was expressed in the absence of infection in three cases studied, including the SG (case 1) and Ca (case 1) tissues showing elevated expression of *TPR–MET* RNA.

The expression of the *TPR–MET* gene at the early stage of SG together with high expression in one of two cases examined suggest a possible functional role of this oncogene during the initial stages of gastric carcinogenesis. One of the early morphological changes observed in the pathogenesis is inflammation followed by atrophy or gland loss involving cell loss and regenerative hyperplasia. The *TPR–MET* gene may therefore be expressed in the early stages of gastric tumorigenesis as a consequence of the inflammatory response. Several factors, including irritants and micronutrient deficiency, may also play roles at this stage of the disease. The *TPR–MET* RNA expression continued through the later stages of the tumorigenesis process, including CAG, IM, and Ca. It is possible that endogenous mutagens such as *N*-nitroso compounds may cause cellular damage in the stomach epithelium contributing to the gradual progression of atrophic cells to cancer cells. Nitric oxide required for the synthesis

of these nitroso compounds is often produced in the gastric cavity of patients as well as by macrophages participating in the inflammatory process (7). Our finding that the *TPR–MET* RNA is expressed in a majority of tissues analyzed shows that the *TPR–MET* oncogenic rearrangement is frequently associated with human gastric carcinogenesis and may constitute one genetic alteration associated with the progression of gastric epithelial cells to malignancy.

The *MET* protooncogene is a member of the protein tyrosine kinase growth factor receptor gene family. Activation of *MET* oncogene was shown to involve a chromosome translocation event, giving rise to the rearranged *TPR–MET* gene (3, 4). Other investigators have reported a similar rearrangement mechanism leading to activation of *BCR–ABL* in the Philadelphia chromosome translocation in chronic myeloid leukemia (30–33) and the *TRK* gene (34). Similar to *MET, TRK* protooncogene also codes for a growth factor receptor associated with protein tyrosine kinase activity. Another oncogene, *PTC*, associated with papillary thyroid Ca and showing rearrangement of *H4* and the *RET* protooncogene sequences, also is a member of the same family (35–37). Information about these protooncogenes is limited to the knowledge of their nucleotide sequences and biochemical properties of their products. No information is currently available regarding the biological function of *MET* and other similar gene products, mainly due to the unknown nature of their putative growth factor ligands. The *TPR* domain involved in the activation of *MET* gene was also shown to activate the *RAF* gene in rat by a similar rearrangement event (38). In both cases, the breakpoint occurred within the intron region and did not interfere with the reading frame of the downstream oncogene domains of *MET* and *RAF*. Involvement of a single 5′ *TPR* domain in the activation of *MET* and *RAF* genes is suggestive of a putative role of the *TPR* gene in activation events. Recently, pulsed-field gel electrophoresis analysis of the chemically transformed human cell line MNNG-HOS showed that the *TPR–MET* rearrangement may have occurred as a result of an insertion of at least 500 kbp of a portion of chromosome 1 carrying the *TPR* domain into the *MET* locus (39). As noted by King *et al.* (38), *TPR* DNA may have a high propensity to translocate into various genes and thereby disrupt their normal regulation. Some of the common features of growth control genes are that they often contain a heptad leucine repeat motif or leucine zipper domain required for protein dimerization and that their products are phosphorylated and involved in signal transduction events. It is interesting to note that the *TPR* domain involved in the activation of *MET* and *RAF* genes shares structural homology with the DNA binding leucine zipper motif of the protooncogene products of *FOS* and *JUN*, the cyclic AMP-response element binding protein, and the intermediate filament protein, vimentin (40).

Two of 18 gastric samples analyzed showed base substitutions in the codon 12 region of the Ki-*RAS* gene and both of these mutations corresponded to activating mutations often associated with human neoplastic tissue. Two other samples showed cryptic mutations in the codon 12 region of the Ha-*RAS* gene. Whether such mutations are associated with changes in *RAS* expression needs to be investigated, inasmuch as mutational activation and overexpression of *RAS* gene products have been reported to occur in gastric tumors. Although we found that *RAS* gene mutations were present in some gastric tumors and precursor lesions, the frequency of these changes was substantially lower than that reported to be involved in colorectal carcinogenesis.

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