Advances in Brief

Occurrence of Microsatellite Instability in Gastric Carcinoma Is Associated with Enhanced Expression of erbB-2 Oncoprotein1

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Abstract

To investigate the molecular mechanism of gastric carcinogenesis, we examined simultaneously the frequency of microsatellite instability and the immunoreactivities to ras, erbB-2, and p53 in 42 gastric adenocarcinoma tissues. Microsatellite instability, measured by DNA replication error, was detected in 33.3% (14/42) of patients with gastric carcinoma while positive immunostaining was demonstrated in 31% (1/32) for ras, 46.5% (17/42) for erbB-2, and 28.6% (12/42) for p53. There was no statistical difference between the intestinal type and the diffuse type of carcinoma with respect to microsatellite instability, ras, or erbB-2 expression. The expression of p53 occurred more frequently in the intestinal type of carcinoma (41.7%, 10/24) than in the diffuse type of carcinoma (11.1%, 2/18; P < 0.01). There was no association between microsatellite instability and ras or p53 expression, while enhanced expression of erbB-2 occurred more frequently in carcinomas with microsatellite instability (64.3%, 9/14) than in those without microsatellite instability (28.6%, 8/28; P < 0.05). Such a strong association between microsatellite instability and erbB-2 oncogene may be responsible for the increase of other oncogenic mutations and tumor progression in gastric carcinogenesis.

Introduction

Recent studies on the molecular genetics have a great impact on the understanding of carcinogenesis of colorectal cancer. A model of multistep carcinogenesis has been proposed involving activation of oncogenes and inactivation of tumor suppressor genes (1). The presence of the "mutator" phenotype due to alterations in the "stability" gene has been considered critical for promoting multistep carcinogenesis (2). Recently, DNA RER3 resulting in microsatellite instability has been demonstrated in hereditary nonpolyposis colorectal cancer and certain sporadic cancers (3–5). The existence of DNA RER, a marker for the mutator phenotype of cancers, may be responsible for the secondary mutations throughout the genome (2). However, preliminary studies in colorectal carcinomas failed to find an association between microsatellite instability and chromosomal losses of tumor suppressor genes such as the APC gene at 5q and the p53 gene at 17p (4).

GC is one of the most common cancers in the world (6). Marked heterogeneity and functional difference exists in GC. It has been demonstrated that the prevalence of expression of several oncogenes and tumor suppressor genes may vary according to histological types of GC, and affect the biological behavior of GC (7, 8). Among them, the mutation of the p53 gene has been found to correlate well with histological subtypes, while the presence of ras mutation usually implicates distant metastasis (7, 8). In addition, erbB-2 overexpression generally indicates a more aggressive phenotype and a poor prognosis in GC (9). Recently, microsatellite instability has also been shown to occur in GC and play a role in tumor progression (10–13). However, because studies are scanty it remains unclear whether there is any relationship between RER and expression of different oncogenes or tumor suppressor genes in GC (7–13). To obtain a better understanding of the molecular mechanism of gastric carcinogenesis, we examined the frequency of DNA microsatellite instability and the immunoreactivity to ras, erbB-2, and p53 in the same GC tissues. Our results revealed that GC tumors with the RER (+) phenotype tend to have enhanced expression of erbB-2, a marker indicative of tumor progression in GC (9).

Materials and Methods

GC Tissues. A total of 42 gastric specimens resected due to GC were studied. They are obtained from 24 males and 18 females with a mean age of 63.0 years. All GC tissues were examined histologically by the same pathologist, and subdivided into 6 early GC and 36 advanced GC according to the Lauren's classification (15). Both tumorous tissues and nontumorous neighboring tissues were individually dissected off each resected GC specimen, immediately frozen in the optimal cooling temperature embedding compound (Miles Scientific), and then stored at −80°C until later use.

Microsatellite Analysis. Cryosections of 5–7 μm were prepared for each tumorous tissue, and sections containing predominantly neoplastic cells were used to prepare genomic DNA by a conventional procedure (1). DNA from nontumorous parts of the stomach was also extracted from the same patient to serve as a control. Analysis of microsatellite instability was performed by PCR using seven primers, i.e., D2S114, D2S123, D3S1260, D5S395, D10S193, D10S197, and D17S785, obtained from Research Genetics. PCR was performed in a 25-μl reaction volume containing 5 μM each primer, 0.125 mm dATP, 1.25 mm each of dGTP, dCTP, and dTFP, 3 @Ci [α-35S]dATP, 25 ng DNA, and 0.75 units Taq DNA polymerase (Boehringer Mannheim, Mannheim, Germany). The reaction condition consisted of 30 s at 94°C, 75 s at 55°C, and 15 s at 72°C for 27 cycles followed by a final extension for 5 min at 72°C. The PCR products were diluted in a ratio of 3:2 by the loading buffer, heated at 95°C for 5 min, and loaded (5 μl) onto 7% polyacrylamide sequencing gels. After electrophoresis, gels were dried at 80°C and exposed to X-ray film from 24 to 72 h. The band pattern was compared between tumorous and nontumorous tissues for each patient.

Immunohistochemical Stainings of ras, erbB-2, and p53. To demonstrate the expression of ras, erbB-2, and p53 proteins, frozen sections were stained immunohistochemically with each respective antibody purchased from Oncogene Science, Inc. using a standard avidin-biotin-peroxidase complex detection system. Briefly, cryosections mounted on slides were first treated with 3% hydrogen peroxide to block endogenous peroxidase. They were then nonspecific protein binding, primary mouse antibodies overnight at 4°C, biotinylated goat anti-mouse secondary antibody for 30 min, peroxidase-conjugated streptavidin for 10 min, and finally diaminobenzidine tetrachloride/ 

H2O2 chromogen substrate for 10 min. Slides were then counterstained with Mayer’s hematoxylin for p53 and with methyl green for ras or erbB-2. Negative control sections were processed in the same manner by replacing the

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3 The abbreviations used are: RER, replication error; GC, gastric carcinoma.
primary antibody with buffered saline. Positive control sections were from breast adenocarcinoma known to express high levels of ras, erbB-2, and p53. Immunostainings were performed in all 42 patients for p53 and erbB-2, but in 32 patients for ras due to limited tissues.

Results

Among the 42 patients examined, 14 (33.3%) manifested microsatellite instability at two or more chromosomal loci. The RER(+) tumors showed patterns of electrophoretic shifts or expansion of the microsatellite repeats (Fig. 1a), and all patterns were reproducible using repeated assays. Instability was found in 1 (16.7%) of 6 patients with early GC and in 13 (36.1%) of 36 patients with advanced GC (P > 0.05). There was no difference in the frequency of RER(+) between the intestinal type (29.2%, 7/24) and the diffuse (38.9%, 7/18) type of GC (P > 0.05).

Table 1 Frequency of positive immunohistochemical stainings of erbB-2, p53, and ras in gastric carcinoma with and without microsatellite instability

<table>
<thead>
<tr>
<th>Microsatellite instability</th>
<th>RER(+)</th>
<th>RER(-)</th>
<th>Statistics</th>
</tr>
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<tbody>
<tr>
<td>erbB-2</td>
<td>9/14(64.3%)</td>
<td>8/28(28.6%)</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>p53</td>
<td>5/14(35.7%)</td>
<td>7/28(25.9%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>ras</td>
<td>1/12(8.3%)</td>
<td>0/20(0%)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Typical examples of positive immunohistochemical staining of p53 and erbB-2 are shown in Fig. 1, b and c. The positive rate of immunohistochemical stainings in ras, erbB-2, and p53 was 3.1% (1/32), 40.5% (17/42), and 28.6% (12/42), respectively. GC of the intestinal type had a statistically higher positive rate of p53 (41.7% (11/27), versus 33.3% (6/18), respectively; P > 0.05). The association between RER(+) and positive immunohistochemical stainings of erbB-2, p53, and ras is shown in Table 1. Nine (64.3%) of 14 cancers exhibiting microsatellite instability also exhibited overproduction of the erbB-2 oncogene. In contrast, only 8 (28.6%) of 28 cancers without microsatellite instability exhibited erbB-2 overexpression (P < 0.05 by Fisher’s exact test). However, the frequency of positive immunostaining to ras or p53 was not statistically different between RER(+) and RER(-) tumors.

Discussion

Microsatellite instability due to RER(+) is a novel genetic mechanism of tumorigenesis (3–5). It has been found in sporadic GC regardless of histological types and may play a role in tumor progression (10–13). The occurrence of RER(+) (33.3%) shown in this report was within the range reported in GC from 22.7 to 62.5% (10–13). Also consistent with other reports (12, 13), we did not find any significant difference in the frequency of RER(+) between the intestinal type and the diffuse type of GC.

Recent studies have indicated sequential changes in oncogenes and tumor suppressor genes in human colorectal carcinoma (1). It is, thus, also important to examine these genetic alterations in the same tumorous tissue so as to understand the multistep nature of carcinogenesis. In GC, abnormalities of ras, erbB-2, and p53 have been reported to affect such biological behaviors of the tumor as histopathological type, depth of invasion, lymph node metastasis, and survival (7, 16–18). Mutations of the p53 gene correlate well with histological subtypes, while the presence of ras mutation usually implicates distant metastases and a poor outcome (7). Because there is a good correlation between the genetic change and its oncoprotein production, immunohistochemical analysis is clinically useful and convenient to screen the expression of ras, erbB-2, and mutant p53 (9, 17, 18). However, this method does present some limitations. For example, nonsense, frame shift, and silent mutations in the p53 gene will not result in overexpression of the p53 protein, which is to be detected by immunohistochemical staining (18). In the present study, enhanced expression of p53 was noted both in early and advanced GC and was more frequently found in the intestinal type of carcinoma. These results were in agreement with others showing the molecular heterogeneity of these two distinct histological types (18). As for the ras expression, there has been marked disparity among previous reports presumably due to variations in the antibodies used, methods of tissue preservation, and extents of epitope presentation (17). Although a very low rate of ras expression was noted in the current study, which
is contradictory to some earlier studies, a recent report also confirmed infrequent ras mutations in GC using direct DNA sequencing (19). The erbB-2/neu oncogene encodes a transmembranous protein of 185 KDa with tyrosine kinase activity, and its sequence is homologous to that of the epidermal growth factor receptor (20). Various immunohistochemical studies have demonstrated erbB-2 overexpression ranging from 10 to 55% of patients with GC (9, 20). Despite the existence of conflicting data concerning erbB-2 oncoprotein expression in different histological types, there is a consensus that erbB-2 overexpression tends to have a more aggressive phenotype and a poor prognosis in GC (9). In this study, we noted a high positive rate of erbB-2 (40.5%) in GC, a finding consistent with previous reports. The follow-up period of the current study was not long enough to determine whether the strong association of RER with erbB-2 expression did not reach a statistically significant level. The interaction between RER and other genetic alterations remains unclear (10–13). Chong et al. (12) have demonstrated that chromosomal losses in 5q and 17p occur more frequently in GC with microsatellite instability. Nevertheless, Mironov et al. (11) and Strickler et al. (13) suggest that RER(+) and p53 mutations are two independent pathways in gastric carcinogenesis. In the present study, we noted that the RER(+) phenotype was not associated with enhanced expression of p53 or ras, a finding supporting those of others (11, 13). However, we noted that the RER(+) phenotype was associated with a significantly higher rate of erbB-2 immunoreactivity, a marker indicative of tumor aggressiveness and poor prognosis (9). The follow-up period of the current study was not long enough to determine whether the strong association of RER with erbB-2 expression can be used as another marker to predict tumor progression. Such strong association also implies that microsatellite instability may increase the probability of other oncogenic mutations (2). Since the biological significance of microsatellite instability in GC remains unclear, further studies to include more GC cases and to have a longer term of patient follow-ups will be necessary to verify our speculations.

References

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