Amplification of *c-erb*B-2 Proto-oncogene in Cancer Foci, Adjacent Normal, Metastatic and Normal Tissues of Human Primary Gastric Adenocarcinomas

Genetic damages are frequently found in both tumor and normal cells at carcinogen exposed areas in the patients with upper aerodigestive tract cancer. These phenomena are explained by the multistage process and/or field cancerization theories. The *c-erb*B-2 proto-oncogene has been amplified in many human tumors including breast, stomach, kidney and lung cancers. To study the possible evidence of multistage process and/or field cancerization in the development of gastric adenocarcinoma, the amplification statuses of *c-erb*B-2 proto-oncogene using the Southern hybridization technique were evaluated at the 45 gastric adenocarcinoma specimen sets consisting of tumor tissue, adjacent normal tissue (within 2 cm of the primary tumor), metastatic tissue and normal stomach tissue (at least 5 cm away from primary tumor). As a result, *c-erb*B-2 proto-oncogene at 2 specimen sets (4.4%) was amplified 2- to 4-fold to normal control status. In these 2 cases, *c-erb*B-2 proto-oncogene at histologically normal tissue adjacent to tumor tissue was amplified. And, the metastatic tissue of 1 case also exhibited *c-erb*B-2 proto-oncogene amplification of which the degree was less than that of tumor tissue. From these results, we were able to suspect that *c-erb*B-2 proto-oncogene amplification in the normal tissue adjacent to tumor tissue could be a biomarker of premalignant changes in a small proportion of gastric adenocarcinoma patients. And, this result might suggest the possibile role of multistage process and/or field cancerization in the development of gastric adenocarcinoma.

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Key Words : *Gastric adenocarcinoma, Field cancerization, c-erbB-2 proto-oncogene*

Jun Suk Kim, M.D., Chul Won Choi, M.D., Byung Soo Kim, M.D., Sang Won Shin, M.D., Yeul Hong Kim, M.D., Yong Jae Mok*, M.D., Jong Suk Kim*, M.D. and Bum Hwan Koo*, M.D.

Department of Internal Medicine and General Surgery*, School of Medicine, Korea University, Seoul, Korea

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Address for correspondence
Jun Suk Kim, M.D., Department of Internal Medicine, Guro Hospital, Korea University, School of Medicine, #80, Guro-dong, Guro-gu, Seoul 152-050, Korea
Tel : (02) 818-6651, Fax : (02) 862-4453

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INTRODUCTION

Carcinogenesis in the upper aerodigestive tract is supported by the multistage process and/or field cancerization theories (1). There are many reports supporting these theories such as histologic findings (1, 2), chromosomal abnormalities (3~6), and proto-oncogene changes (3~8) in the normal epithelium not affected by the tumors, resulting in the increased incidence of second primary tumors among the patients whose first primary tumors were successfully treated (9~11). Although the incidence of gastric adenocarcinoma has been decreased significantly in Western countries (12), this disease still represents the leading cause of death from all malignant diseases in Korea. To understand the pathophysiology of gastric adenocarcinoma and its prognostic factors, the studies (13~26) for chromosome and proto-oncogenes such as *c-erb*B-2, *c-myc*, *c-yes*-1, *c-ras*-Ki, *Int*-2/*hst*-1, and *c-met* had been done. Especially, *c-erb*B-2 proto-oncogene expression has been reported in carcinomas of epithelial gland origin such as breast carcinoma (13~15), renal cell carcinoma (16), colon carcinoma (17), salivary gland carcinoma (18), and stomach carcinoma (16~21). This proto-oncogene encodes a 185 kDa glycoprotein mapped to band q21 of chromosome 17 (12, 22). There has been a little research for the role of multistage process and/or field cancerization in the gastric adenocarcinoma development using a proto-oncogene amplification method. The amplification status of *c-erb*B-2 proto-oncogene, which is not detected in normal adult tissues, has been researched in many studies (16~26) to assess the role at the carcinogenesis of stomach. So, we selected *c-erb*B-2 proto-oncogene to evaluate the role of multistage process and/or field cancerization in gastric adenocarcinoma development.

MATERIALS AND METHODS

Materials

Forty-five sample sets of primary gastric adenocarcinoma, collected from Nov.1992 to Aug.1993 at Guro Hospital, were used in this study. One sample set consisted of the tumor tissue, adjacent normal tissue (within 2 cm of the primary tumor), metastatic tissue and normal stomach tissue (at least 5 cm away from primary tumor). Each sample set was obtained simultaneously in the operation room and examined by a pathologist to verify its adequacy for the study.

Methods

Frozen tissues were ground in liquid nitrogen and digested with 100 μg/ml proteinase K (Promega, USA) in a lysis buffer containig 10 mM Tris-HCl (pH 8.0), 0.1 M EDTA (pH 8.0) and 0.5% sodium dodecyl sulfate (SDS). Then, DNA was purified by phenol-chloroform extraction and precipitated with ethanol. 10 micrograms of DNA were digested with *Eco*R I (Promega, USA) and electrophoresed through 0.8% agarose gel and transferred to a positively-charged nylon membrane (Boehringer Mannheim, Germany). The hybridization probe was a 1.6 kb human *c-erb*B-2 internal *Eco*RI fragment probe, and was labelled with [α.^{32}P] dCTP, 3,000 Ci/mMol using random DNA labeling kit (Boehringer Mannheim, Germany). Washing was carried out under stringent conditions and autoradiographed against Hyperfilm™ (Amersham, UK) at -70℃ for 72 hours. The blots were stripped and then rehybridized with human β-actin cDNA (Clontech, USA) to control for loading differences between samples. Signal intensity on autoradiographs were quantitated using an Ultroscan XL densitometer (Pharmacia LKB).

RESULTS

The cases of forty-five patients with primary gastric adenocarcinoma were classified according to histologic type and TNM stage. Well-differentiated, moderately-differentiated, poorly-differentiated, and signet-ring type were diagnosed in 4, 17, 15, and 9 patients, respectively.

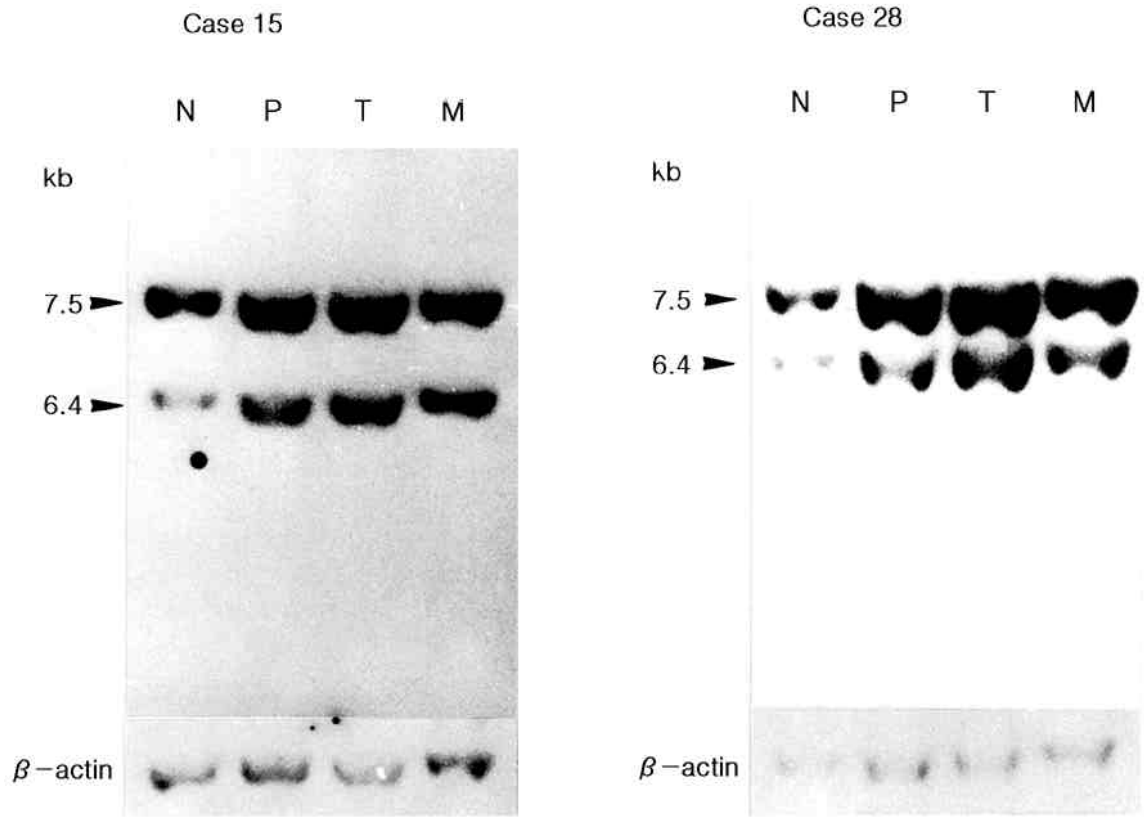

Fig. 1. Southern blot analysis of *c-erb*B-2 and beta actin genes in gastric adenocarcinomas (N : DNA from normal gastric tissue, P : DNA from histologically normal tissue adjacent to tumor, T : DNA from tumor focus, M : DNA from metastatic tissue). The *c-erb*B-2 proto-oncogene is amplified 2-fold in case 15 and 2- to 4-fold in case 28.

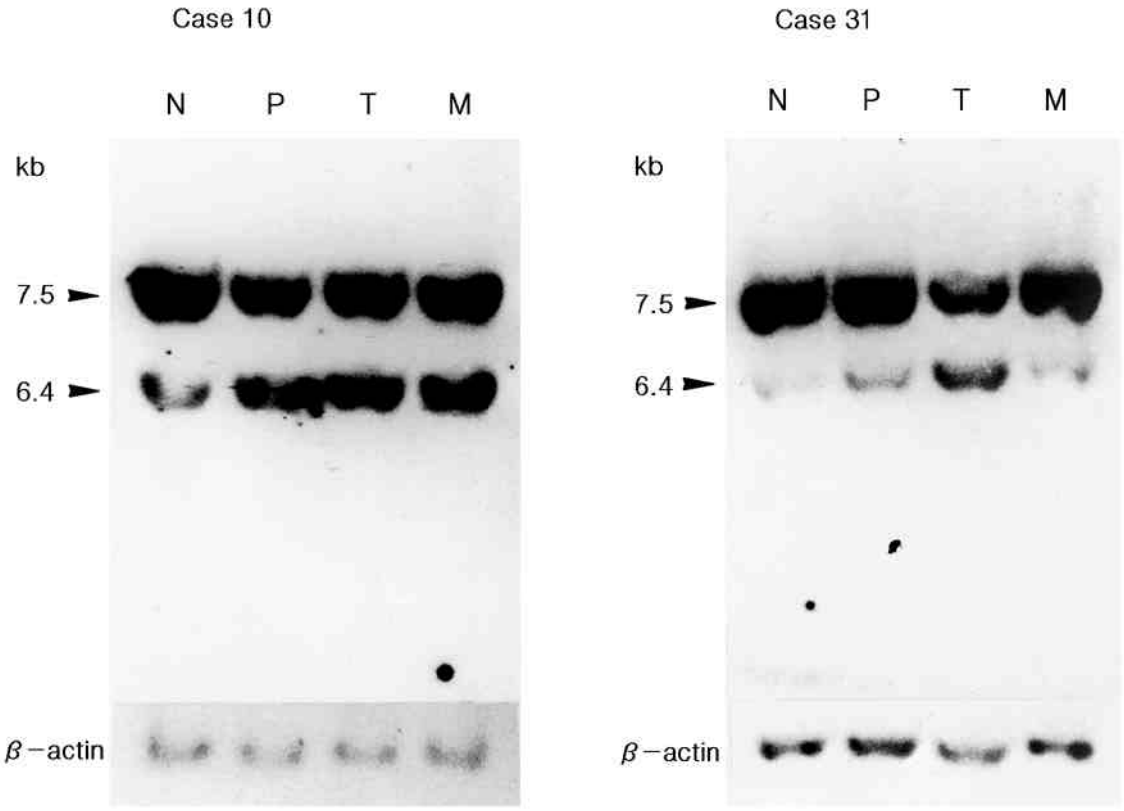

Fig. 2. Southern blot analysis of *c-erb*B-2 and beta actin genes in gastric adenocarcinomas using the same conditions described in Fig. 1. The *c-erb*B-2 proto-oncogene is not amplified in these cases.

Thirty-one patients were classified as TNM stage III or IV and the others were stage I or II.

With the Southern hybridization technique, *c-erb*B-2 proto-oncogene at 2 specimen sets (4.4%) was amplified 2- to 4-fold to normal control status (Fig. 1) and the remaining cases showed no amplification (Fig. 2). In the two cases exhibiting amplification of *c-erb*B-2 proto-oncogene, all the histologically normal tissues adjacent to the tumors also exhibited amplification. These cases were poorly-differentiated (stage IIIb) and moderately-differentiated (stage IIIa) adenocarcinomas, respectively. And in case #28, metastatic tissue exhibited *c-erb*B-2 proto-oncogene amplification of which the degree was less than that of tumor tissue. In summary, we could reveal the amplification of *c-erb*B-2 proto-oncogene at adjacent normal tissue as well as at tumor tissue.

DISCUSSION

In our study, the amplification status of *c-erb*B-2 proto-oncogene, known as to be involved in the development of stomach carcinoma, was measured to assess the role of multistage process and/or field cancerization in the development of gastric adenocarcinoma. If gastric adenocarcinoma was developed through a multistage process and/or field cancerization, we could expect that genetic alterations occur in normal stomach tissues as well as in cancer tissues exposed to carcinogens. To test the multistage process and/or field cancerization hypothesis for carcinogenesis of the stomach, Kim and Lee (24) analyzed alterations in chromosomes in normal epithelial glands, intestinal metaplasia and early gastric cancer. This study showed alterations in chromosomal numbers in normal epithelial glands and intestinal metaplasia as well as early gastric cancer suggesting that carcinogenesis in the stomach could be explained by concepts of multistage process and/or field cancerization.

According to the study of *c-erb*B-2 proto-oncogene amplification in stomach carcinoma, Yokota et al.(16) reported that the *c-erb*B-2 proto-oncogene was amplified specifically in adenocarcinomas and the frequency was 22% (2/9). At the studies of Tal et al.(17), Sasaki et al.(18), Houldsworth et al.(19), and Ranzani et al.(20), the frequency of *c-erb*B-2 proto-oncogene amplification in gastric adenocarcinomas was between 6% and 10.7%. Park et al.(21) reported that four cases among the fifty-one cases of primary gastric adenocarcinomas exhibited

amplification of the *c-erb*B-2 proto-oncogene ranging from 2- to 8-fold to normal control status, and postulated that the variations of the reported *c-erb*B-2 proto-oncogene amplification frequencies in stomach adenocarcinoma might be due to the relative differences of tubular adenocarcinoma numbers included in the analysis. This result was consistent with the previous report of Yokota et al.(16) linking *c-erb*B-2 proto-oncogene amplification to the histologic type of stomach adenocarcinoma. However, Tsujino et al.(25) found that *c-erb*B-2 proto-oncogene was also amplified at metastatic signet ring cell type stomach adenocarcinomas. Therefore, it could not be suspected that the specific histologic type of stomach cancer is more frequently associated with the increased incidence of *c-erb*B-2 proto-oncogene expression. In our study, the *c-erb*B-2 proto-oncogene amplifications were found at moderately-differentiated and poorly-differentiated types and the incidence was 4.4% (2/45), which was less than that of previous reports (16~21). Tsujino et al.(25) tried to determine whether alterations in *c-erb*B-2 proto-oncogene might be associated with tumor progression and metastasis and found that the incidence of *c-erb*B-2 proto-oncogene amplification in metastatic gastric carcinomas was significantly higher than that in non-metastatic gastric carcinomas. From this finding, they assumed that the multi-alterations in *c-erb*B-2 proto-oncogenes might occur during progression of human gastric carcinomas, suggesting the role of multistage process and/or field cancerization. Mizutani et al.(26) also found that *c-erb*B-2 proto-oncogene was more frequently amplified in the advanced or metastatic gastric adenocarcinomas compaired to early stage of gastric adenocarcinomas and this finding might be associated with multistage process and/or field cancerization. In our study, the incidence of *c-erb*B-2 proto-oncogene amplification at the cases associated with metastatic lesions was 6.5% (2/31) which were alike with the incidence of previous reports (16~21), and the entire gastric adenocarcinoma cases without metastasis were not associated with *c-erb*B-2 proto-oncogene amplification.

From this study, we could suspect that *c-erb*B-2 proto-oncogene amplification in the normal tissue adjacent to tumor tissue could be a biomarker of premalignant changes in a small proportion of gastric adenocarcinoma patients. But, the sample size was relatively small and the incidence of *c-erb*B-2 proto-oncogene amplification was low in our study. So, we could not be able to conclude that multistage process and/or field cancerization had a important role in the development of gastric adenocarcinoma. Therefore, further studies will be required to confirm the role of multistage process and/or field cancerization in the development of gastric adenocarcinoma.

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