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ORIGINAL ARTICLE

# Comparison of chromosomal aberrations between primary tumors and their synchronous lymph-node metastases in intestinal-type gastric carcinoma ☆

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## Abstract

Lymph-node metastasis is a main factor causing poor prognosis of patients with gastric cancer (GC). In order to determine the genes involved in lymph-node metastasis, we compared primary tumors with their synchronous lymph-node metastases for DNA sequence copy number aberrations (DSCNAs) in 20 patients diagnosed as having intestinal-type GC using comparative genomic hybridization (CGH). The results showed that some DSCNAs (gains at 8q, 13q, 5p, 7 and X, and losses at 1p, 17p, 19, 21q and 22q) were frequently found in both primary tumors and their metastases. However, metastases often contained DSCNAs that were not found in corresponding primary tumors, and gain at 20q12–13 and losses at 21qcen-21, 4q and 14q22-ter were significantly more frequently observed in metastatic lesions than in their primary tumors (10:2, 9:0, 6:0, and 7:0 between metastases and corresponding primary tumors, respectively). Our data indicate that gain at 20q12–13 and losses at 21qcen-21, 4q, and 14q22-ter are involved in lymph-node metastases, and that these chromosomal regions may contain the genes related to lymph-node metastases in intestinal-type GC.


***Keywords:*** Comparative genomic hybridization; Gastric carcinoma; Lymph-node metastasis; DNA sequence copy number aberrations

## Introduction

Gastric carcinoma (GC) is the second most common tumor. Regarding mortality, it is the third most common malignant tumor in China. Metastasis is one of the main factors causing poor prognosis of patients with GC. It is clear that metastasis is generally regarded as a multistep process in which many factors are involved, including the chromosome genes of the tumor cell itself, environment of the host, etc. Although influenced by numerous factors,

*Abbreviations:* CGH, comparative genomic hybridization; DSCNAs, DNA sequence copy number aberrations; GC, gastric carcinoma; LOH, loss of heterozygosity.

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the most important affected factor is the genomic alteration of tumor cells. In general, the number of genetic aberrations parallels the clinical progression of tumors [30,28].

A widely accepted concept is that accumulation of genetic aberrations is necessary for carcinogenesis, and that the multistep progression of genetic alterations reflects clinical tumor progression in numerous tumors [14]. According to many detailed reports, several genomic aberrations are required for the initiation and progression of GC. These aberrations include expression or amplification of various genes, for example, genes for p27 [21], smad4 [35]-met [5]-erbB2 [34], E-cadherin [32], $\beta$-catenin [32], VEGF [12], and FHIT [7], and mutations in the gene for K-ras [6] have been associated with gastric carcinogenesis. Some studies have reported loss of heterozygosity (LOH) in some chromosomal regions [20]. Previous studies have revealed that two histological subtypes of GC, intestinal-type (IT-GC) and diffuse-type (DT-GC), have distinct epidemiological, clinical, and prognostic characteristics [16,18], and that these two subtypes might result from different genetic routes taken by normal gastric cells during oncogenesis. Different molecular mechanisms are possibly involved in their development and progression [29,22]. However, genetic aberration profiles associated with lymph-node metastasis are still unclear in IT-GC. Therefore, we compared primary tumors with their synchronous lymph-node metastases for genetic aberrations in 20 patients with lymph-node metastatic IT-GC using comparative genomic hybridization (CGH). We concluded that chromosomal regions, including 20q12–13, 21qcen-21, 4q, and 14q22-ter, may contain the genes related to lymph-node metastases in IT-GC.

## Materials and methods

### Tumor samples

Tumorous gastric tissues (primary lesions and lymph-node metastases) of 20 cases were collected from 80 patients having undergone curative surgery for advanced GC between January 1990 and December 1995. All these 20 chosen cases were diagnosed as IT-GC by two pathologists according to the Lauren classification [17]. Microscopic photographs of representative IT-GC are shown in Fig. 1. All these 20 cases were found to have lymph-node metastasis by examining at least 10 lymph nodes in regions close to the stomach. There was no distant organ metastasis in all 20 cases. Age of the 20 patients (12 men and 8 women) ranged between 47 and 72 years (mean age 61 years), and clinicopathologic findings such as age, gender, tumor size, lymphatic invasion, blood vessel invasion, and lymph-node metastasis were reviewed according to the *Japanese Classification of Gastric Carcinoma* (2nd English edition) [4]. The clinicopathological data are summarized in Table 1. None of the patients had received chemotherapy or radiotherapy before surgery.

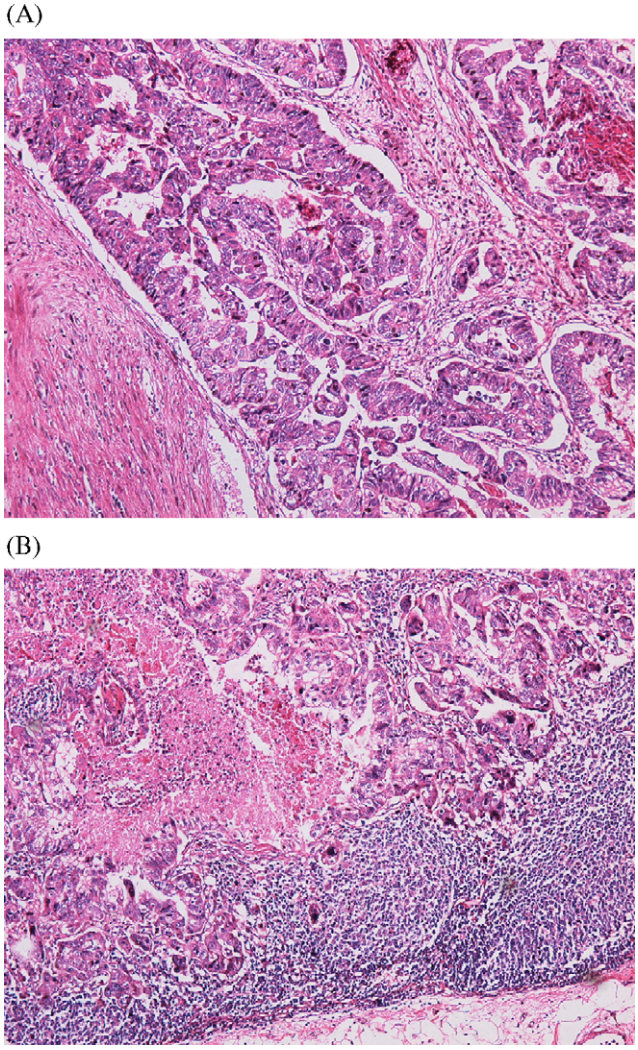


**Fig. 1.** Microscopic photograph of representative intestinal-type gastric adenocarcinoma. Hematoxylin and eosin (H–E stain), original magnification $\times 200$. (A) Primary lesion and (B) lymph-node metastasis of case 6.

After surgical resection, tissues were frozen and stored at $-80\,^{\circ}C$ until used.

### Microdissection and DNA extraction

A previously described microdissection technique was used to reduce contamination of normal tissue [30,28]. Test DNA was extracted from tumor tissues (primary lesion or lymph-node metastatic lesion), while normal DNA for reference DNA was extracted from peripheral blood lymphocytes of a cytogenetically normal male. High-molecular-weight DNA was isolated using a DNA