Numerical Aberrations of Chromosome 8 in Gastric Cancer Detected by Fluorescence In Situ Hybridization

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Abstract. Background: Limited data are available on the genetic events underlying gastric cancer. Studying a few cases by conventional cytogenetic techniques, we previously reported that chromosome 8 might be frequently involved. The aim of our study was to evaluate the numerical aberrations of chromosome 8 in gastric cancer using fluorescence in situ hybridization (FISH). Materials and Methods: FISH, with an a-satellite DNA probe specific for chromosome 8, was applied to 37 primary gastric tumors directly processed for cytogenetic study. Results: Numerical aberrations of chromosome 8 were observed in 23 out of 37 tumors (62.16%). Trisomy was detected in 16 cases (43.24%), tetrasomy in 4 cases (10.81%) and monosomy in 3 cases (8.10%). No correlation was found between polysomy 8 and the histopathologic characteristics of the tumors. Conclusion: An increase in the number of chromosome 8 may frequently occur in gastric cancer. Advanced and more aggressive gastric tumors did not harbor polysomy 8. Further studies at molecular and clinical level must be carried out to identify the gene alterations reflected by polysomy 8 and possibly to facilitate the detection of specific tumors subtypes.

Gastric cancer is of major importance world-wide. Statistical data on mortality rates identified that gastric carcinoma represents the second most common cause of cancer-related death in the world (1). Unfortunately there is not clear agreement on the genetic changes present in this type of malignancy. Conventional cytogenetic studies of gastric cancer have shown simple abnormalities, such as polysomy X, trisomy 8, 9 and 19, del (7q), i(8q), as well as complex abnormalities involving chromosomes 1, 3, 6, 7, 11, 13 and 17 (2-7). However the detection of recurrent genetic changes by conventional cytogenetic studies is particularly problematic because of the complex nature of the chromosomal aberrations and the difficulty in preparing metaphase spread of adequate quality and quantity. Studying a few cases by conventional staining techniques, we found that numerical aberrations of chromosome 8 are frequent in gastric cancer (3). Furthermore, we described in one gastric cancer case with very long survival an i(8q) as the sole anomaly (5). Xiao et al. (4) have also observed trisomy 8 in a case with minimal chromosomal changes, suggesting that this abnormality might be a non-random event in gastric tumorigenesis.

Molecular cytogenetic techniques have proven valuable in the study of primary solid tumors. By these techniques a complex pattern of gains and losses of many chromosomes was observed in gastric cancer. Thus DNA copy number gains were reported to be frequent on 20q, 17q, 8q and 1p, whereas loss of 4q, 5q, 3p, 18q and 17p genetic material seemed to be common (8-13).

The fluorescence in situ hybridization (FISH) technique with centromeric specific DNA probes allows the rapid detection of numerical aberrations in interphase nuclei of tumor cells. FISH studies have shown numerical aberrations 7, 9, 17 and Y to be common in gastric cancer. However, a few studies only using FISH techniques were focused on chromosome 8 (14-17).

Genetic changes involved in gastric tumorigenesis or progression and their clinical significance for different subtypes of gastric cancer have not been clearly defined. This might partly be due to the fact that most of the studies focused on the analysis of selected genes or chromosomal regions, while studies simultaneously comparing various clinicopathological parameters and genetic alterations remain limited (11-13, 18).

The aim of this study was to evaluate, by FISH technique, the numerical aberrations of chromosome 8 in gastric...
cancer. Correlation of these abnormalities with certain histopathological characteristics, representing prognostic parameters in this type of malignancy, was also estimated.

Materials and Methods

Thirty-seven patients with gastric cancer, who underwent gastrectomy, were included in this study. None of the patients had ever received chemotherapy or radiation prior to surgery. Tissue specimens were collected from fresh surgically resected tumors and a routine histopathological examination followed. The tumors were classified histopathologically according to the World Health Organization guidelines (19) and Lauren (20).

A small portion of each resected tumor was directly processed for cytogenetic study as described elsewhere (8). FISH was applied on recently made slides from the methanol/acetic acid-fixed cells of all patients. An a-satellite DNA probe D8Z2 specific for chromosome 8 (chromosome region 8p11.1-q11.1) was used. The slide was washed in 2xSaline Sodium Citrate (SSC) buffer and dehydrated in a sequence of 70%, 80% and 95% ethanol. Both slide and hybridization solution containing the chromosome 8 a-satellite DNA probe were prewarmed at 37°C for 5 minutes. Next, 10 µl of the hybridization mixture was added to the slide under a glass cover-slip. Denaturation was performed at 75°C on a heating plate for 2 minutes. In situ hybridization was carried out for 1 hour at 37°C in a humidified lightproof chamber. Post hybridization washings were done and the nuclei were counterstained with DAPI antifade. The hybridization of the probe with the cellular DNA site was visualized by fluorescence microscopy NICON E 600 with a triple filter DAPI/FITC/Texas RED. Positive chromosome signals appeared as red spots in the nuclei. A minimum of 200 cells from each slide were evaluated for each case. Signals were scored using the criteria of Hopman et al. (21). To avoid misinterpretation due to technical error, normal lymphocyte nuclei were used as a control. Approximately 97% of control lymphocyte nuclei showed two signals for probe specific for chromosome 8. A case was counted as aberrant if more than 10% of cell nuclei showed loss or gain of signals for chromosome 8.

For statistical evaluation the Chi-square test and Kruskal-Wallis test were used.
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<th>Table II. Copy number of chromosome 8 and histopathological parameters in gastric cancer cases (n=37).</th>
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**Results**

Histopathological characteristics of resected gastric tumors are shown in Table I. A representative example of FISH analysis is shown in Figure 1. Numerical aberrations of chromosome 8 were observed in 23 out of 37 tumors (62.16%). The vast majority of these aberrations involved an increase in the number of chromosome 8. Trisomy 8 was detected in 16 cases (43.24%) and tetrasomy in 4 cases (10.81%). One copy of chromosome 8 was observed only in 3 cases (8.10%). Table II shows the numerical aberrations of chromosome 8 in association with the histopathological characteristics of resected gastric tumors. Statistical analysis using the Chi-square test showed that there was no association of chromosome 8 copy number with the histological type according to WHO or Lauren’s classification and the lymphatic vessel or venous invasion. A Kruskal-Wallis statistical test did not show any correlation between chromosome 8 copy number and the stage of tumor invasion, $p=0.14$.

**Discussion**

Cyto genetic studies focused on the evaluation of recurrent chromosomal aberrations in tumor cells are of great importance, providing entry points for molecular studies to identify genes involved in the pathogenesis of human cancer. However, in solid tumors, the detection of recurrent genetic alterations by conventional cytogenetics was hampered by the complexity of the chromosomal abnormalities and the difficulty in preparing adequate metaphase spreads. Molecular cytogenetic techniques have been valuable in solving some of these problems.

Abnormalities of chromosome 8 are a frequent finding in gastric cancer. Whether they result in loss or excess of chromosome 8 material is still in dispute (4, 10, 12, 22, 23).

In the present study we evaluated the numerical aberrations of chromosome 8 in a total of 37 gastric adenocarcinomas. We used the FISH technique, which is considered a valuable method for the detection of numerical chromosomal aberrations. In most specimens (54.05%) an increase in the copy number of chromosome 8 was detected, while loss of this chromosome was found in only 8.10% of the tumors. Han et al. (15), by FISH studies of paraffin-embedded tumor cells of 18 gastric adenocarcinomas, detected polysomy 8 in 5 cases (27.8%) and monosomy in one case (5.5%). In another FISH study (16), with centromeric probes specific for chromosomes 7, 8, 11, 17 and Y on deparaffinized tissue sections from 40 gastric tumors, polysomy 8 was found in 62.5% of the cases.

Comparative genomic hybridization (CGH) studies have shown gains on 8q material in approximately 18-55% of the cases studied (11, 13, 18).

Numerical abnormalities involving chromosome 8, in which c-myc is located, have been suggested as a mechanism accounting for the increase of c-myc copy number. Amplification leading to activation of c-myc have been observed in various solid tumors including gastric cancer (24-27). However, other investigators did not find concordance of c-myc overexpression with amplification in gastric cancer (28). The possibility that numerical aberrations of chromosome 8 might reflect alterations of other genes implicated in genesis and progression of gastric cancer could not be excluded. Our study has not focused on the c-myc alterations reflected by polysomy 8.

Aberrations in certain chromosome copy numbers may be related to tumor aggressiveness and many studies have investigated the relationship between numerical chromosomal abnormalities and clinicopathological parameters of various cancers (29, 30). As far as we know there are no previous reports on the association between chromosome 8 numerical aberrations detected by FISH and the clinicopathological characteristics of gastric tumors. However a few studies by CGH have been reported on the association between excess on 8q material and the clinicopathological characteristics of gastric cancers with different results. Koo et al. (12) reported amplifications on the 8q material predominantly in lymph node metastatic of diffuse type gastric adenocarcinomas. Also Wu et al. (11) by CGH studies showed gains on 8q to be higher in advanced gastric cancer of intestinal type. On the other hand, Wu et al. (13), in a series of 62 gastric adenocarcinomas...
studied by CGH, did not find any correlation between excess on 8q material and the clinicopathological characteristics of the disease.

Although the number of cases we studied was not so large, a correlation of chromosome 8 numerical aberrations with certain histopathological characteristics representing prognostic factors in gastric cancer was evaluated. Our results did not reveal any association of chromosome 8 copy number with the histological type, the tumor aggressiveness and the tumor invasion. Thus, despite the notion that tumors accumulate more genetic abnormalities as they progress, in the present study advanced gastric cancer did not harbor polysomy 8. Our findings are in agreement with those of Wu et al. (13) regarding correlation between excess on 8q material by CGH studies and the clinicopathological characteristics of gastric tumors.

Gastric cancer is a genetically heterogeneous disease proceeding via different pathways of tumorigenesis and/or progression, but the possible genetic causes of this heterogeneity have not been thoroughly investigated. Stocks et al. (18), by CGH studies of junctional and distal gastric tumors, showed that DNA aberrations may distinguish distinct tumor subtypes among histologically identical tumors.

It is clear from all the above that further studies at molecular and clinical level must be carried out to identify the gene alterations reflected by polysomy 8 and to contribute to the classification of this disease.

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References


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