RESEARCH ARTICLE

Is Mitochondrial DNA Copy Number Associated with Clinical Characteristics and Prognosis in Gastric Cancer?

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Abstract

Alterations in mitochondrial DNA (mtDNA) have been studied in various cancers. However, the clinical value of mtDNA copy number (mtCN) alterations in gastric cancer (GC) is poorly understood. In the present study, we investigated whether alterations in mtCNs might be associated with clinicopathological parameters in GC cases. mtCN was measured in 109 patients with GC by quantitative real-time PCR. Then, correlations with clinicopathological characteristics were analyzed. mtCN was elevated in 64.2% of GC tissues compared with paired, adjacent, non-cancerous tissue. However, the observed alterations in mtCN were not associated with any clinicopathological characteristics, including age, gender, TN stage, Lauren classification, lymph node metastasis, and depth of invasion. Moreover, Kaplan-Meier survival curves revealed that mtCN was not significantly associated with the survival of GC patients. In this study, we demonstrated that mtCN was not a significant marker for predicting clinical characteristics or prognosis in GC.

Keywords: Gastric cancer - mitochondrial DNA - copy number - prognosis

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Introduction

Mitochondrial DNA (mtDNA) is a 16,569 bp, circular, double-stranded DNA molecule, and multiple copies of mtDNA are present in each mitochondrion. The frequency of mutations in mtDNA is 10 to 100-fold higher than that of nuclear DNA because of high concentrations of reactive oxygen species (ROS) in the mitochondrial inner membrane, fewer repair mechanisms, and no mtDNA-coating proteins such as histones in the nucleus (Howell et al., 1996; Paabo, 1996; Zhu et al., 2004). Therefore, mitochondrial genetic studies performed in various cancers demonstrated that most alterations were found in the D-loop, which is a hot spot region (Stoneking, 2000; Bianchi et al., 2001; Lievre et al., 2005; Wang et al., 2005; Jeong et al., 2010; Lee et al., 2011). Because the D-loop, containing the H-strand replication origin, is an essential element for mtDNA replication, mutations in the D-loop may cause a decrease in mtDNA copy number (mtCN) or altered mtDNA gene expression (Shadel, 2008). It has been hypothesized that mutations or decreases in mtCN could lead to a deficiency in oxidative phosphorylation and enhanced generation of ATP by glycolysis. Therefore, mtCN changes may be of clinical significance in cancers (Lee et al., 2004; Yin et al., 2004; Wu et al., 2005; Guo et al., 2013).

Gastric cancer (GC) is highly prevalent in Asia and is the leading cause of death worldwide. Gastric carcinogenesis is a multi-step process that begins with chronic gastritis, which leads to atrophy, intestinal metaplasia, dysplasia, and finally, invasive cancer (Correa, 1992; Correa and Shiao, 1994). Our previous study also suggested that alteration in mtDNA is an early and important event in gastric carcinogenesis (Jeong et al., 2010). In addition, Wu et al. (2005) suggested that somatic mutations and depletion of mtDNA occurs in GC and that mtDNA depletion is involved in carcinogenesis. It has been reported that mtCN was associated with prognosis in some cancers (Yu et al., 2007; Lin et al., 2008; Cui et al., 2013). However, mtDNA copy number has yet to be studied in a large sample of patients with GC. In the present study, we examined mtCN in GC and then analyzed the clinicopathological characteristics and prognostic value.

Materials and Methods

Patients and DNA extraction

We recruited 109 patients who underwent gastrectomy for treating gastric adenocarcinoma from archives of paraffin blocks at Keimyung University Dongsan Hospital from October 1999 to December 2001. Tissue samples were fixed in formalin and embedded in paraffin. All cases were reviewed by an expert panel of two pathologists according to the current criteria of the WHO classification. The clinical data and pathological reports of the patients

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with gastric adenocarcinoma were collected from the medical records. Tumor area and adjacent normal mucosa were selected from slide according to hematoxylin and eosin stained sections by pathologists. Subsequently, the selected areas from paraffin embedded tissues were used for DNA extraction. DNA was isolated by using DNA extraction Kit (Absolute™ DNA extraction Kit, BioSewoom, Korea) according to the manufacturer’s instructions.

Mitochondria copy number

The mtCN was examined using real-time quantitative PCR (qPCR). For the quantitative determination of mtDNA content relative to nuclear DNA (nDNA), primers for specific amplification of the mtDNA COX1 and nDNA-encoded β-actin genes were selected according to previous studies with minor modifications (Lee et al., 2004; Yu et al., 2007; Cui et al., 2013). [12, 17, 19]. Real-time qPCR was then performed using a LightCycler 480 II system (Roche Diagnostics, Germany) with a total reaction volume of 20μl, which contained 10μl SYBR Green Master MIX (Takara, Japan), 8 pmol of each primer, and DNA (50ng). The PCR conditions were 95°C for 1min, followed by 40 cycles of 95°C for 15s and 60°C for 30s. The threshold cycle number (Ct) values of the β-actin gene and the mitochondrial COXI gene were determined. The mtCN in each tested specimen was then normalized against that of the β-actin gene to calculate the relative mtCN. Each measurement was repeated in triplicate, and five serially diluted control samples were included in each experiment.

Statistical analysis

The SPSS statistical package, version 19.0 for Windows, was used for all statistical analyses. Correlation between mtCN change and clinicopathological characteristics was analyzed by Fisher’s exact test or Pearson’s Chi square test. Disease-free and overall survivals were measured according to the Kaplan Meier method. Disease free survival was measured from the date of diagnosis to the date of recurrence or the last follow-up. Overall survival was measured from the date of diagnosis to the date of death or the last follow-up visit. Differences between curves were analyzed using the log-rank test. p values <0.05 were considered to indicate statistically significant results.

Results

The mean age of the 109 patients with gastric adenocarcinoma was 56.2 years (range, 25-82 years). There were 82 (75.2%) male patients and 27 (24.8%) female patients. Early gastric carcinoma that invaded the mucosal or submucosal layer was observed in 46 (42.2%) patients, and advanced gastric carcinoma, which invaded the proper muscle or a deeper layer, was observed in 63 (57.8%) patients. According to the Lauren classification, 

![Table 1. Clinicopathological Characteristics of Mitochondrial Copy Number in Gastric Cancers](image)

|                      | mtCN     |
|----------------------|----------|
|                      | Low      | High     |
|                      | N        | p        |
| Total                | 109      |          |
| Age                  |          |          |
| < 60                 | 54       | 39 (51.3) |
| ≥ 60                 | 55       | 37 (48.7) |
| Gender               |          |          |
| Male                 | 82       | 56 (73.7) |
| Female               | 27       | 20 (26.3) |
| pT                   |          |          |
| 1                    | 46       | 33 (43.4) |
| 2                    | 26       | 16 (21.1) |
| 3                    | 2        | 2 (2.6)   |
| 4                    | 35       | 25 (32.9) |
| pN                   |          |          |
| 1                    | 67       | 45 (59.2) |
| 2                    | 21       | 15 (19.7) |
| 3                    | 11       | 9 (11.8)  |
| 4                    | 10       | 7 (9.2)   |
| Lauren classification*|        |          |
| Diffuse              | 26       | 17 (22.7) |
| Intestinal           | 82       | 58 (77.3) |
| Lymph node metastasis|          |          |
| No                   | 76       | 45 (67.2) |
| Yes                  | 33       | 22 (32.8) |
| Depth of invasion    |          |          |
| Early                | 46       | 33 (43.4) |
| Advanced             | 63       | 43 (56.6) |

*One case with mixed type was excluded; mtCN, mitochondrial DNA copy number

![Figure 1. Survival Analysis by Mitochondrial Copy Number in Gastric Cancers. (A) Overall survival; (B) Disease-free survival](image)
processes that promote cancer, such as cell growth, cell changes in mtDNA content may affect various cellular polymerase γ (POLG), or oxidative stress. Moreover, According to these studies, alterations in mtCN originate et al., 2001; Cook and Higuchi, 2012; Dai et al., 2013). In GC, there were few studies about the association between mtDNA content and prognosis, with only one case-control study being performed (Liao et al., 2011). These studies showed no association between mtDNA copy number levels and risk of developing GC. However, low mtDNA content may have an early effect on the development of GC. To the best of our knowledge, this is the first study to evaluate GC prognosis according to mtDNA copy number. Both overall and disease-free survivals in GC were analyzed with long term follow-up (80.4 months). As a result, we observed no association between survival and mtDNA copy number in GC. Through stratified analyses, we also observed no clinical prognostic value of mtCN in GC. However, we cannot rule out a possible association between mtCN and GC prognosis because there was no study that evaluated mtCN in precancerous lesions of GC. Considering the rapid progression of gastric dysplasia to GC (Rugge et al., 1994), future studies should focus on the role of mtDNA content in GC development.

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