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Association analysis of ERBB2 amplicon genetic polymorphisms and STARD3 expression with risk of gastric cancer in the Chinese population

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ABSTRACT

The purpose of this study was to investigate whether risk of gastric cancer (GC) was associated with single nucleotide polymorphisms (SNPs) in a gene cluster on the chromosome 17q12-q21 (ERBB2 amplicon) in the Chinese Han population. We detected twenty-six SNPs in this gene cluster containing steroidogenic acute regulatory-related lipid transfer domain containing 3 (STARD3), protein phosphatase 1 regulatory subunit 1B (PPP1R1B/DARPP32), titin-cap (TCAP), per1-like domain containing 1(PERLD1/CAB2), human epidermal growth factor receptor-2 (ERBB2/HER2), zinc-finger protein subfamily 1A 3 (ZNFN1A3/IKZF3) and DNA topoisomerase 2-alpha (TOP2A) genes in 311 patients with GC and in 425 controls by Sequenom. We found no associations between genetic variations and GC risk. However, haplotype analysis implied that the haplotype CCCT of STARD3 (rs9972882, rs881844, rs11869286 and rs1877031) conferred a protective effect on the susceptibility to GC ($P = 0.043$, odds ratio [OR] = 0.805, 95% confidence intervals [95% CI] = 0.643–0.992). The STARD3 rs1877031 TC genotype endued histogenesis of gastric mucinous adenocarcinoma and signet-ring cell carcinoma ($P = 0.021$, OR = 2.882, 95% CI = 1.173–7.084). We examined the expression of STARD3 in 243 tumor tissues out of the 311 GC patients and 20 adjacent normal gastric tissues using immumohistochemical (IHC) analysis and tissue microarrays (TMA). The expression of STARD3 was observed in the gastric parietal cells and in gastric tumor tissues and significantly correlated with gender ($P = 0.004$), alcohol drinking ($P < 0.001$), tumor location ($P = 0.007$), histological type ($P = 0.005$) and differentiation ($P = 0.023$) in GC. We concluded that the combined effect of haplotype CCCT of STARD3 might affect GC susceptibility. STARD3 expression might be related to the tumorigenesis of GC in the Chinese population.

1. Introduction

Gastric cancer (GC) is the one of the most prevalent cancers worldwide and is the second leading cause of cancer-related death (Jemal et al., 2011). The occurrence and development of GC may result from both genetic and environmental changes. Although a variety of clinical modalities such as gastrectomy, chemotherapy or radiotherapy are applied, the prognosis of advanced GC is still poor.

Epidermal growth factor receptor-2 (ERBB2) amplicon is located in human chromosome 17q12-21 gene-rich region, in which there are more than 20 genes. These co-located genes may influence clinical phenotype of ERBB2 via affecting its expression and amplification (Glynn et al., 2010). The correlation analysis between this gene cluster and diseases has been hot point to investigate. Recent studies demonstrated that gene coamplification at this locus might play a crucial role and be associated with the clinical and biological implications in breast cancer

Abbreviations: CI, confidence interval; EDTA, ethylenediaminetetraacetic acid; ERBB2, epidermal growth factor receptor-2; GC, gastric cancer; GRB7, growth factor receptor-bound protein 7; HapMap, International HapMap Project; HWE, Hardy–Weinberg equilibrium; IHC, immunohistochemistry; LD, linkage disequilibrium; MAF, minor allele frequency; MIEN1, migration and invasion enhancer 1; OR, odds ratio; PBS, phosphate buffer saline; PCR, polymerase chain reaction; PERLD1, per1-like domain-containing protein 1; PNMT, phenylethanolamine N-methyltransferase; PPP1R1B, protein phosphatase 1 regulatory subunit 1B; START, steroidogenic acute regulatory-related lipid transfer; STARD3, steroidogenic acute regulatory-related lipid transfer domain-containing 3; SNP, single nucleotide polymorphism; TBE, Tris/borate/EDTA; TCAP, titin-cap; TMA, tissue microarray; TOP2A, topoisomerase (DNA) II alpha; UTR, untranslated regions; ZNFN1A3, zinc-finger protein subfamily 1A3.

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(Jacot et al., 2013; Kauraniemi et al., 2001). Such an invariant coamplification was observed in other types of cancers, including pancreatic (Mahlamaki et al., 2002), prostate (Chan et al., 2012) and gastric cancers (Vidgren et al., 1999). Varis et al. conducted a genetic analysis of copy number and overexpression of the 17q amplicon in GC and found that ERBB2 and DNA topoisomerase 2-alpha (TOP2A) genes were frequently amplified and overexpressed (Varis et al., 2002). Maqani et al. identified a 168-kb region from this gene-rich cluster and found that the expression levels of protein phosphatase 1 regulatory subunit 1B (PPP1R1B), steroidogenic acute regulatory-related lipid transfer domain containing 3 (STARD3), titin-cap (TCAP), phenylethanolamine N-methyltransferase (PNMT), per1-like domain containing 1(PERLD1), ERBB2, migration and invasion enhancer 1 (MIEN1) and growth factor receptor-bound protein 7 (GRB7) genes were associated with each other in the upper gastrointestinal adenocarcinomas using quantitative real-time reverse transcription-PCR (Maqani et al., 2006). The synergetic expression of these nonhomologous clustered genes indicated that the genes in this region might intricately interact with each other in GC. However, no risk associations between the genetic variations and breast cancer of British women were found (Benusiglio et al., 2006), though the associations of genetic variation with benign diseases such as asthma and primary biliary cirrhosis were observed (Marinho et al., 2012; Tanaka et al., 2011). It was supposed that genetic variation of adjacent gene loci in a cluster was a potential mechanism for their altered expression levels (Hines et al., 2003). So far, the association study of genetic variants in this region and risk as well as clinical parameters of GC remains not yet reported.

STARD3, also known as metastatic lymph node protein 64 (MLN64), was originally identified in the metastatic lymph node from breast cancer (Tomasetto et al., 1995). STARD3 gene encodes a 445 residue protein consisting of two distinct domains. The N-terminus of the protein contains a MENTAL (MLN64 N-Terminal) domain including four potential transmembrane regions and targets the protein to the membrane of late endosomes (Alpy et al., 2001). The N-terminus plays a part in the particular cytoplasmic localization of MLN64 (Moog-Lutz et al., 1997). The C-terminus of the protein consists of a steroidogenic acute regulatory (StAR)-related lipid transfer (START) domain that is homologous to StAR protein (Moog-Lutz et al., 1997). START domain binds and transports cholesterol from endosome to cytoplasmic acceptor (Alpy and Tomasetto, 2005; Alpy et al., 2001). STARD3 captures cholesterol by MENTAL domain and promotes production of steroid in the mitochondria by START domain (Zhang et al., 2002). Therefore, STARD3 is involved in cellular cholesterol transport and steroid production (Akiyama et al., 1997; Moog-Lutz et al., 1997; Tomasetto et al., 1995). In steroid hormone-responsive cancers, such as breast and prostate cancers, STARD3 contributes to tumor growth and progression through increased intratumoral steroidogenesis (Alpy et al., 2003; Cai et al., 2010; Stigliano et al., 2007). High expression level of STARD3 was associated with high TNM stage, poor prognosis and short overall survival in breast cancer (Cai et al., 2010) as well as was significantly correlated with clinical stage and short tumor recurrence time in prostate cancer (Stigliano et al., 2007). On the other hand, increasing evidences demonstrated that there was increased cholesterol metabolism in cancer cells and low serum cholesterol levels in a variety of cancers, indicating that cholesterol accumulated in tumor tissues (Benn et al., 2011). Higher cholesterol which is particularly found in the mitochondria membrane of cancer cells reduced membrane fluidity (Montero et al., 2008). Therefore, mitochondrial permeability transition and release from mitochondria of cell-death-promoting molecules such as cytochrome c were suppressed. The inhibited effect facilitated survival of cancer cells (Montero et al., 2008; Smith and Land, 2012). Hence, the expression of STARD3 might be a potential indicator for tumorgenesis (Cai et al., 2010; Stigliano et al., 2007).

In this study, we performed a genetic analysis with regard to the association of the ERBBR2 amplicon, including STARD3/MLN64, PPP1R1B/DARPP32, TCAP, PERLD1/CAB2, ERBB2/HER2, ZNFN1A3/IKZF3 and TOP2A, with GC susceptibility. The position relationship of the seven genes in the cluster was shown in Fig. 1. In particular, we investigated the potential associations of the STARD3 genetic variants with clinical characteristics of GC and demonstrated the STARD3 expression in tumor tissues.

2. Materials and methods

2.1. Study population

Three hundred and eleven cases with GC and 425 cancer-free controls were investigated in this study. The patients with GC were collected from the First Affiliated Hospital of Anhui Medical University between March 2008 and July 2009 and received no chemotherapy or radiotherapy before surgical gastrectomy. Table 1 showed basic patient characteristics. The patients were comprised of 246 men and 65 women with an average age of 60.4 ± 10.4 years (ranging from 24 to 83 years). Two hundred and forty-three cases with full clinical pathological data were used for IHC analysis, including 187 men and 56 women with an average age of 60.3 ± 10.2 years (ranging from 24 to 83 years). Additional 20 normal gastric tissues over 4 cm away from boundary of tumors out of the patients were used as controls. All the tissue samples were morphologically identified by two pathologists. In addition, a total of 336 men and 89 women control subjects were matched with an average age of 60.6 ± 8.4 years (ranging from 30 to 86 years). The control subjects without a history of cancers were recruited from patients who visited hospital for a conventional cancer-screening program. Information on demographic characteristics, such as gender, age, smoking habits, alcohol consumption and family history of cancer was obtained from a personal interview administered by trained personnel. Smoking habit was defined as non-smoker and smoker. Individuals who smoked one cigarette per day for over 1 year were defined as smokers. Alcohol consumption was defined as non-drinker and drinker. Individuals who consumed more than 200 mL alcohol per day were defined as drinker. The intake of salted food was defined as no or occasional and yes. Individuals who took salt in diet over 6 gram per day were defined as high salt. Clinical diagnosis and staging of GC were assessed by two pathologists according to the World Health Organization classifications and tumor-node-metastasis (TNM) classifications issued in 2006. Informed consents were obtained from all the participants. This study was approved by the ethics committee for genome research of the Anhui Medical University of China.

2.2. Extraction of peripheral blood DNA

Total blood samples were collected from each individual before treatment and stored at −80 °C until analysis. Genomic DNA was extracted from the peripheral blood by using QIAamp DNA Blood Midi Kit (Qiagen Inc., Germany) according to the manufacturer's protocol. The quantitative concentration of DNA was measured by the Nanodrop Spectrophotometer (ND-1000, USA) of full wavelength and standardized to 50 ng/μl.

2.3. SNP selection and sequenom assay

Based on Hapmap CHB data, we selected 26 SNPs of the ERBB2 amplicon in the gene cluster region on the chromosome 17q12-21, including STARD3/MLN64, PPP1R1B/DARPP32, TCAP, PERLD1/CAB2, ERBB2/HER2, ZNFN1A3/IKZF3 and TOP2A. All of the SNPs selected by the Haploview software were in Hardy–Weinberg equilibrium (HWE) and their minor allele frequencies (MAF) were over 0.05. Genotyping of the SNPs for fast-track validation analysis was performed using the Sequenom Mass Array system.15 ng of genomic DNA was standardized for genotyping of each sample. Locus-specific PCR and detection