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ORIGINAL ARTICLE

Association of programmed death-1 polymorphisms with the risk and prognosis of esophageal squamous cell carcinoma

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Programmed death-1 (PD-1) is an immunoinhibitory receptor belonging to the CD28 family. This study was designed to investigate the association of *PD-1* rs36084323:A>G, rs2227981:C>T, rs2227982:C>T and rs10204525:A>G single nucleotide polymorphisms (SNPs) with the risk and prognosis of esophageal squamous cell carcinoma (ESCC) in a high-incidence population from Northern China. These four SNPs were genotyped by polymerase chain reaction ligase detection reaction (PCR-LDR) method in 584 ESCC patients and 585 healthy controls. The rs2227981:C>T SNP C/T genotype increased the risk of ESCC for the smokers (OR = 1.483, 95% CI = 1.018–2.160) and rs2227982:C>T SNP C/T genotype enhanced susceptibility to ESCC for the females (OR = 1.708, 95% CI = 1.056–2.762). For rs10204525:A>G SNP, A/A genotype was related to increased risk of ESCC (OR = 1.735, 95% CI = 1.086–2.771) overall. Among the 584 ESCC patients, the survival information of 204 ESCC patients was collected. The rs36084323:A>G SNP A/G genotype was associated with lower risk of death in ESCC patients with upper gastrointestinal cancer (UGIC) family history (HR = 0.339, 95%CI = 0.115–0.996). The rs2227982:C>T SNP C/T genotype was associated with lower risk of death in smoker ESCC patients and ESCC patients with UGIC family history (HR = 0.409 and 0.292, 95%CI = 0.194–0.863 and 0.101–0.847). *PD-1* rs2227981:C>T, rs2227982:C>T and rs10204525:A>G SNPs might be used as predictive markers of the susceptibility to ESCC for the Han nationality in a high-incidence population from Northern China. *PD-1* rs36084323:A>G and rs2227982:C>T SNPs were associated with the prognosis of the Han ESCC patients in this high-incidence region.

Keywords Programmed death-1, polymorphism, risk, prognosis, esophageal squamous cell carcinoma

Introduction

The human immune system plays a critical role in preventing the development of tumors. Circulating immune cells can identify, infiltrate and eliminate certain incipient cancer cells. However, cancer cells may escape immune surveillance and immune system-mediated cell death in cases of immune system imbalance (1). The most important anti-tumor immune response is mediated by T lymphocytes. The activation of T lymphocytes requires dual signals. The engagement of the T cell receptor (TCR) with the major histocompatibility complex (MHC) on the surface of antigen-presenting cells (APCs) regulates the initial activation of T lymphocytes (2). T lymphocytes activation also requires the stimulatory and inhibitory signals provided by the receptors and ligands of the CD28/B7 family (3,4).

The programmed death-1 (*PD-1*) gene, located on chromosome 2q37.3, encodes a 55 kDa type I transmembrane glycoprotein belonging to the CD28 family. PD-1 is expressed on activated T and B lymphocytes, natural killer T cells and monocytes and functions as an immunoinhibitory receptor (5). The binding of PD-1 with its ligands, PD-L1 or PD-L2, can activate the immune receptor tyrosine-based inhibitory motif (ITIM) of PD-1, recruit src homology 2-domain-containing tyrosine phosphatase 2 (6–8) and inhibit PI3K and AKT activation (9,10), which eventually attenuates T lymphocyte activation and proliferation, suppresses cytokine secretion, induces T lymphocytes apoptosis and maintains peripheral tolerance (6,7,11).

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PD-1 plays an important role in various diseases, such as autoimmune diseases, infectious diseases, and malignant diseases (12–15). For example, Wang et al. (15) reported that the expression of *PD-1* was significantly higher in hepatocellular carcinoma specimens than in chronic hepatitis specimens and that PD-1 may be involved in immune evasion of tumors. In addition, patients with non-small cell lung cancer, melanoma or renal cell cancer exhibited improved tumors responses when treated with a PD-1 antibody (16). Single nucleotide polymorphism (SNP) is one of the most common genetic variations. There are some SNPs within *PD-1*, such as rs36084323:A>G (PD-1.1), rs34819629:A>G (PD-1.2), rs11568821:A>G (PD-1.3), rs2227981:C>T (PD-1.5), rs10204525:A>G (PD-1.6), rs7421861:C>T (PD-1.7), rs2227982:C>T (PD-1.9). Of these SNPs, rs34819629:A>G, rs11568821:A>G and rs7421861:C>T SNPs are located in intron 2, intron 4 and intron 1, respectively. The rs36084323:A>G SNP is situated within promoter region and may affect the promoter activity (17). Although rs2227981:C>T SNP is a silent mutation in exon 5, several studies indicated that it was associated with the risk of gastric cancer, colon cancer and breast cancer et al. (18–21). The rs2227982:C>T SNP, a missense mutation in exon 5, causes the amino acid substitution from valine to alanine and possibly changes the structure and function of PD-1 (22). The rs10204525:A>G SNP is located in the 3′-untranslated region (3′-UTR). MicroRNA-4717 may regulate *PD-1* expression via specific binding to the G allele of rs10204525:A>G SNP (23). A number of studies have indicated that SNPs of the *PD-1* gene contribute to the development of autoimmune diseases and infectious diseases (24–29). Until now, only a few studies have investigated the association of *PD-1* polymorphisms with the development and prognosis of tumors (18–22,30–32).

Esophageal cancer is the sixth most common cancer worldwide (33). Ci county of Hebei province is one of the high-incidence regions for esophageal cancer in China. In this region, the incidence of esophageal cancer was $98.67/10^5$ in males and $84.21/10^5$ in females in 2011 (34), and esophageal squamous cell cancer (ESCC) is the dominant histological type. Most patients have advanced disease at the time of diagnosis. Despite the rapid development of surgical techniques, the prognosis is still poor because of tumor resistance to radiotherapy, chemotherapy or immunotherapy. Therefore, it is necessary to identify the risk factors and prognostic markers of esophageal cancer for the population in this high-incidence region. In view of the crucial role of PD-1 in malignant diseases, we conducted a population-based case-control study including 584 ESCC patients and 585 healthy controls to investigate whether four potentially functional polymorphisms (rs36084323:A>G, rs2227981:C>T, rs2227982:C>T and rs10204525:A>G SNPs) could predict susceptibility to ESCC in a high-incidence population in Ci county of Hebei province in Northern China. Simultaneously, a survival analysis was conducted in 204 ESCC patients with follow-up information.

Materials and methods

Study subjects

The study consisted of 584 ESCC patients and 585 healthy controls. All of the study subjects were ethnically homogeneous (of Han descent) and permanent residents of Ci county. All of the patients and healthy controls were recruited during an endoscopic screening campaign between 2009 and 2014. The patients had histologically confirmed ESCC; self-reported, cancer-free subjects who were confirmed to be without upper gastrointestinal cancer (UGIC) by endoscopy were selected as healthy controls. Information on the sex, age, smoking habits and family history of UGIC from the cancer patients and healthy controls were obtained by two professional interviewers directly after blood sampling. Eventually, 585 healthy controls that were sex and age-matched to the ESCC cases were enrolled in the study. Smokers were defined as those who formerly or currently smoked no less than five cigarettes per day for at least 2 years. Individuals who had at least one first-degree relative or at least two second-degree relatives who had esophageal/cardiac/gastric cancer were defined as having a family history of UGIC. In addition, the ESCC patients were followed based on survival information. Two hundred and four ESCC patients with survival information were included in the survival analysis. The study was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University. Informed consent forms were obtained from all recruited subjects.

DNA extraction

Five milliliters of venous blood was drawn from each subject in Vacutainer tubes containing ethylene diamine tetra acetic acid and stored at 4 °C. After sampling, genomic DNA was extracted within 1 week by proteinase K (Merck, Darmstadt, Germany) digestion, followed by a salting out procedure according to the method published by Miller et al. (35).

Polymorphism genotyping

The genotypes of *PD-1* polymorphisms were determined by the Shanghai Generay Biotech Co., Ltd. (Shanghai, China) using the polymerase chain reaction ligase detection reaction (PCR-LDR) method. At first, target DNA sequences including the SNP locus were amplified using a multiplex PCR method. Then, with the help of the Taq DNA ligase, the ligation reaction occurred when the two oligonucleotide probes were complete complementary with the target DNA sequences and no gap existed between the two probes. At last, the genotypes of PD-1 polymorphisms were determined through fluorescence scan of LDR products length. The primers for amplification were shown in Table 1. PCR reactions were carried out in a total volume of 15 μl including 50 ng of genomic DNA, 1.5 μl of 10× PCR buffer, 0.3 μl of 10 mM dNTPs, 0.25 μl of 10 pmol/μl of each primer, and 1.25 U of Taq DNA polymerase (TaKaRa, Biotechnology CO. LTD, Dalian, China). The cycling parameters were as follows: 94 °C for 2 min, 35 cycles of 94 °C for 15 s, 55 °C for 15 s, 72 °C for 25 s and a final extension step at 72 °C for 3 min. The probes for LDR were shown in Table 2. LDR reactions were performed in a 10 μl reaction volume containing 3 μl of PCR product, 1 μl of 10× Taq DNA ligase buffer, 0.01 μl of 10 pmol/μl of each probe and 5 U of Taq DNA ligase (New England Biolabs LTD, Beijing, China). The LDR parameters were as follows: 25 cycles of 94 °C for 30 s and 56 °C for 1 min. After the LDR reaction, 1 μl of LDR reaction product was mixed with 10 μl of loading