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Concurrent expression of aryl hydrocarbon receptor and CYP1A1 but not CYP1A1 MspI polymorphism is correlated with gastric cancers raised in Dalian, China

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Abstract

The frequency of cancer-associated m2m2- (C-) genotype of CYP1A1 and the factors contributing to the increased CYP1A1 expression in gastric cancers (GCs) are largely unknown. To address theses issues, PCR-restriction fragment length polymorphism (PCR-RFLP) was performed to elucidate the MspI polymorphism in 60 GC cases and 57 normal donor samples. The frequencies of m1m1-, m1m2- and m2m2-genotype were 43.3, 45 and 11.7% among GC patients and 45.6, 49.1 and 5.3% among the normal donors respectively, demonstrating no significant difference of them between cancer and control groups ($\chi^2=0.343$, $P=0.558$). The correlation of Aryl hydrocarbon receptor (AhR) with the frequent CYP1A1 expression in stepwise gastrocarcinogenesis was determined by RT-PCR, immunohistochemical staining (IHC) and Western blotting, using GC samples as well as their pre-malignant and non-cancerous counterparts. RT-PCR revealed that the AhR detection rates were 100, 94.12 and 85.17% in GC, pre-malignant and non-cancerous mucosa ($P>0.05$) respectively but the level of AhR expression in GCs was much higher than that of non-cancerous tissues. IHC showed that the frequencies of AhR detection were 94.87% (37/39) in GCs, 94.12% (16/17) in pre-malignant lesions and 50% (3/6) in non-cancerous mucosa, revealing significant difference in frequencies of AhR detection and levels of AhR expression between GC or pre-malignant group and non-cancerous one ($P<0.05$). The frequency of AhR nucleus translocation was significantly high in GCs (94.87%; 37/39) than that in pre-malignant (70.59%; 12/17) and especially in non-cancerous group (16.67%; 1/6). Co-existence of AhR nuclear translocation and CYP1A1 expressions were found in 82.70% (43/52) of GCs ($r_s=0.437$, $P<0.01$). Our results suggest (1) that CYP1A1 MspI polymorphism may not contribute to the high gastric cancer risk in Dalian region and (2) that enhanced AhR expression and especially its nuclear translocation may be a favorable factor for GC formation presumably via up-regulating CYP1A1 expression.

Keywords: CYP1A1 MspI polymorphism; AhR; Gene expression; Gastric cancer; Susceptibility

1. Introduction

Gastric cancer (GC) is one of the commonest malignancies in China, especially in Dalian region

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[1]. This provides a unique resource for investigating the association of genetic and/or environmental factors with the high GC risk. Epidemiological studies demonstrated that people in this area had the habit to eat toasted and preserved seafood, meat and vegetables that were rich in gastro-carcinogens such as heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) [2,3]. However, most of those compounds are pro-carcinogens and need to be metabolized into bioactive forms in the affected cells before playing oncogenic roles [4]. It would be of significance to shed light on the essential element(s) that regulate the interaction(s) between carcinogens and its activators.

CYP1A1 is an important member of CYP superfamily and acts actively in metabolic activation of pro-carcinogens including HCAs and PAHs [5]. The correlation of CYP1A1 with cancer formation has been investigated at structural and transcriptional levels [6,7]. It was found that single nucleotide polymorphisms (SNPs) were frequent in CYP1A1, especially at 3′ non-coding region. The individuals with m2m2 genotype were susceptible to lung, colorectal and coetaneous basal cell carcinomas [8–10]. However, the studies on gastric cancers yielded conflicting results because m2m2 frequencies were almost similar between gastric cancer and control groups in Japanese population [11]. Although it has been proposed but not yet ascertained whether this discrepancy is due to epigenetic reasons [12].

Frequent CYP1A1 expression could be found in a variety of human cancers [6,7,13]. In the case of stomach, the basal CYP1A1 level is negligible in normal epithelium of humans and rodents, but become increased in GCs induced by PAHs such as benzo[a]pyrene (BaP) [14]. Our recent study demonstrated frequent CYP1A1 up-regulation in GCs and their related lesions [15], suggesting the potential role(s) of this gene in the stepwise gastrocarcinogenesis. It was generally considered that CYP1A1 expression was induced through aryl hydrocarbon receptor (AhR)-dependent pathway [16]. Many PAHs compounds were ligands of AhR and stimulate CYP1A1 expression. However, the status of AhR in different gastric tissues has not yet been documented.

To clarify the above-mentioned uncertainties, the genotypes of CYP1A1 MspI polymorphism of GC patients and normal donors and potential link of AhR and CYP1A1 expression in different gastric tissues were investigated in this study.

2. Material and Methods

2.1. Establishment of frozen gastric tissue and blood banks

After getting patients' consents, gastric cancer specimens were collected from the operation room of the affiliated Hospitals of Dalian Medical University (DMU) within 20 min after removal. The tissue samples were incised from cancer, tumor surrounding region (1 cm apart from cancer border) and, where possible, grossly normal-looking mucosa, respectively. The tissue samples were cut into appropriate size and stored separately in −80 °C and liquid nitrogen until use. The blood samples were obtained from corresponding GC patients before and after operation. Fifty-seven cases of blood samples were collected from healthy unrelated Han (Chinese) donors as control.

2.2. DNA preparation and PCR-RFLP assay

0.5 ml anti-agglutinating blood samples from 57 normal donors and 60 GC patients were treated with proteinase K (terminal concentration 100 μg/ml) at 37 °C overnight. Genomic DNAs were isolated by standard phenol/chloroform method as described previously [17]. DNA pellet was dissolved in 1×TE buffer (pH 8.0), adjusted to suitable concentration and stored at −20 °C until use.

The region from 266 to 605 of CYP1A1 gene was amplified by PCR in a total reaction volume of 10 μl containing 1 μl 10×PCR buffer (Mg^{2+} Plus), 1 μl 2.5 mM dNTP Mixture, 0.5U Taq DNA polymerase (TaKaRa Co. In. Dalian Branch, China), 50–100 ng DNA template, each of 0.4 μl (20 μM) primers, appropriate amount of purified water. A pair of primers was synthesized according to the published sequences (Forward: 5′-CAG TGA AGA GGT GTA GCC GCT-3′; Reverse: 5′-TAG GAG TCT TGT CTC ATG CCT-3′) [18]. The PCR proceeded under the following conditions: pre-denaturing at 95 °C for 5 min, 30 cycles with 94 °C for 1 min, 61 °C for 1 min and 72 °C for 1 min, and at 72 °C for 10 min. PCR products were digested with excess MspI restriction enzyme (TaKaRa Biotech. (Dalian) Co. Ltd., Dalian Branch, China) at 37 °C overnight in water bath, and then electrophoresized in 1.8% agarose gels and visualized by ethidium bromide staining on UV transilluminator (M-20, UVP, USA).