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Coding region polymorphisms in the *CHFR* mitotic stress checkpoint gene are associated with colorectal cancer risk

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Abstract

CHFR was recently identified as an early mitotic checkpoint that delays transition to metaphase in response to mitotic stress. Although studies have shown that CHFR is relevant to tumorigenesis, no previous report has investigated whether polymorphisms in the *CHFR* gene are associated with the risk of cancer development. Here, we genotyped polymorphisms in the *CHFR* gene and analyzed the possible associations of single polymorphisms and haplotypes with the risk and clinicopathological characteristics of colorectal cancer. Six coding SNPs in the *CHFR* gene were genotyped in 462 colorectal cancer patients and 245 healthy normal controls, using either the TaqMan assay or direct sequencing. Our results revealed that the V539M polymorphism was significantly associated with a lower risk of colorectal cancer ($P = 0.03$; OR, 0.533; 95% CI, 0.302–0.94), and significantly correlated with no distant metastasis (M0 stage), different TNM stage, and microsatellite instability (MSI) among the colorectal cancer patients. Among the five tested haplotypes, hap 10 (TGACTA) was significantly associated with a lower risk of colorectal cancer ($P = 0.017$; OR, 0.496; 95% CI, 0.279–0.883), and colorectal cancer patients carrying this haplotype showed no distant metastasis, different TNM stage, and microsatellite instability at a significantly higher frequency. These results reveal for the first time that polymorphisms in the *CHFR* gene are associated with colorectal cancer susceptibility.

Keywords: *CHFR*; Polymorphism; Colorectal cancer; MSI; Haplotype

1. Introduction

Cells are controlled by a series of checkpoints responsible for monitoring normal cell division. The mitotic checkpoint, which is the major cell cycle control mechanism during mitosis, is responsible for safeguarding the production of identical daughter cells by ensuring accurate chromosome segregation [1]. Abnormalities in this checkpoint can result in aneuploidy, which contributes to cancer development and drug resistance against some chemotherapeutic agents [2]. Mitotic checkpoint defects facilitate tumorigenesis in association with impaired tumor suppressor expression and increased avail-

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ability of oncogenic proteins. It has been suggested that changes in the levels of mitotic checkpoint proteins are associated with a weakened mitotic checkpoint response in tumor cells [2].

While spindle assembly checkpoints such as Bub1, BubR1, Mad1 and Mad2 are mostly known mitotic checkpoints, evidence for the existence of an additional checkpoint called CHFR (checkpoint protein with FHA and RING domains) has been suggested by Scolnick et al. [3] The CHFR protein, which is ubiquitously expressed in normal human tissues, was shown to delay centrosome separation and chromosome condensation during early prophase under conditions of mitotic stress [3]. *CHFR* expression loss has been observed in many human cancers, suggesting that *CHFR* might be a tumor suppressor gene, and aberrant promoter methylation was found to be a major inactivation mechanism of CHFR in human cancers [4]. We have previously reported that *CHFR* expression is frequently lost in human gastric cancers due to epigenetic inactivation involving promoter hypermethylation and histone deacetylation [5]. Recently, Yu et al. suggested that CHFR is required for tumor suppression in mice and maintains chromosomal stability by regulating the expression level of Aurora A, a putative oncoprotein involved in mitosis [6].

Several single nucleotide polymorphisms (SNPs) have been identified in the coding region of *CHFR*, along with inactivating mutations affecting normal CHFR function [3,7]. However, although multiple studies have shown that the CHFR mitotic checkpoint protein is functionally important in tumorigenesis, no previous study has investigated whether SNPs in the *CHFR* gene are associated with the risk of cancer development.

In the present study, we genotyped six coding SNPs (cSNPs) in the *CHFR* gene and analyzed the association of individual cSNPs as well as haplotypes with colorectal cancer risk and clinicopathological characteristics.

2. Materials and methods

2.1. Study subjects

Four hundred and sixty-two colorectal cancer samples collected between 1990 and 2003 were obtained from the Seoul National University Hospital and the National Cancer Center of Korea. Clinical characteristics, including age, sex, tumor location and TNM stage were obtained by chart review. The normal controls consisted of 245 healthy Korean individuals selected from cancer-free samples enrolled at the Cancer Cohort Study Branch of the National Cancer Center. The mean ages of the colorectal cancer patients at diagnosis and the normal controls were 58.9 years (58.9 ± 12.9) and 57.3 years (57.3 ± 9.5), respectively.

2.2. DNA preparation and microsatellite instability (MSI) analysis

For colorectal cancer samples, DNA was extracted from normal colorectal tissues surgically dissected with tumor specimens stored at -70 °C in a liquid nitrogen tank. For normal controls, DNA was isolated from blood samples. Total genomic DNA was extracted using the Trizol reagent, according to the manufacturer's instructions (Invitrogen, San Diego, CA, USA). This study was approved by the by the institutional review board of the National Cancer Center, and informed consent was obtained from all subjects prior to enrollment. MSI status was determined by PCR amplification of two microsatellite markers (BAT-25 and BAT-26), followed by DHPLC (denaturing high-performance liquid chromatography) analysis as previously described [8]. DNA was extracted from frozen tumor specimens (containing >50% cancer cells) and matched normal tissues using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. The MSI results were displayed by the WAVEMAKER software (Transgenomic, Omaha, NE, USA). Cancer samples were classified as MSI when MSI was evident in at least one of the two markers.

2.3. CHFR genotyping

Six cSNPs in the *CHFR* gene were genotyped by either TaqMan assay [413C > T (P138L), 1582T > C (L528L), and 1615G > A (V539M)] or direct sequencing [685G > A (G229R), 762A > C (P254P), and 1367C > T (A456V)]. For the TaqMan assay, one allelic probe was labeled with FAM dye and the other with VIC according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). PCR was performed using the TaqMan Universal Master Mix without UNG in a 7900HT fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). The resulting data were analyzed using the SDS2.1.1 software package (Applied Biosystems, Foster City, CA, USA). Direct sequencing analysis was performed with the BigDye Terminator kit and an ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Samples were amplified in a final volume of 15 μl containing 10 ng genomic DNA, 10 pmol of each primer (forward, 5′-CTT GGT TAC GAC AGA ACA CA-3′ and reverse, 5′-AAG AAC CTG CAG TGC CAT-3′ for 685G > A (G229R) and 762A > C (P254P);