Cancer Genetics 211 (2017) 18–25

Cancer Genetics

ORIGINAL ARTICLE

FAS c.-671A>G polymorphism and cervical cancer risk: a case–control study and meta-analysis

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This study aimed to investigate the association between *FAS* c.–671A>G polymorphism and cervical cancer risk in a case–control setting, followed by a meta-analysis of the published literatures. The case–control study involved genotyping of the polymorphism in 185 histopathologically confirmed cervical cancer patients and 209 cancer-free female controls utilizing PCR-RFLP technique, followed by logistic regression analyses. Meta-analysis was then conducted under homozygous, heterozygous, dominant, recessive and allele contrast models to combine data from 12 studies which consisted of 2798 cases and 3039 controls. Our case–control analysis revealed a significant association of the variant allele (G) and the homozygous variant genotype (GG) of the *FAS* polymorphism with an increased risk of cervical cancer. Subgroup analysis by ethnicity further confirmed the risk association in Malays ($P < 0.05$), but not among non-Malays ($P > 0.05$). However, results of meta-analysis suggested a lack of association between the polymorphism and cervical cancer risk in all the five genetic models analyzed. In conclusion, while the *FAS* c.-671A>G polymorphism may serve as a biomarker for cervical cancer risk prediction among the Malays, there is a limited usability of the polymorphism as a cervical cancer risk biomarker in other populations.

Keywords CD95, genetic susceptibility, genetic variation, meta-analysis, uterine cervical neoplasms

Introduction

Cervical cancer is a leading form of gynecological cancer, and was ranked the fourth most common cancer in women worldwide (1). In 2012 alone, more than half a million of new cervical cancer cases were diagnosed and approximately 266,000 cervical cancer-related deaths were reported (1). Conventionally, screening of cervical cancer has been performed by detecting abnormal cellular lesions via Papanicolaou (Pap) test. However, the lack of screening resources in developing countries has resulted in the high incidence of cervical cancer in these countries (2). This phenomenon, coupled with the fact that the Pap test has low sensitivity (3,4), has warranted the identification of additional potential biomarkers for cervical cancer detection.

Human papillomavirus (HPV) has long been recognized as the central etiologic agent for cervical cancer (5,6). However, HPV alone is not sufficient for progression of cervical cells into malignancy, and it has become more and more evident in recent years that host genetic factors could have an equally important involvement in cervical carcinogenesis (7). Among various host genetic factors, the role of single nucleotide polymorphisms in influencing cervical cancer risk has received considerable research attention and has been thoroughly investigated over the past decades (7). This is because single nucleotide polymorphisms could potentially influence the transcriptional efficiency of a gene, or affect the structure (hence, the function) of the protein product. Single nucleotide polymorphisms which occur in cancer-related genes may therefore contribute to the interindividual differences in cervical cancer susceptibility.

Received June 12, 2016; received in revised form October 16, 2016; accepted January 23, 2017.
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2210-7762/$ - see front matter © 2017 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cancergen.2017.01.004

Apoptosis represents an important anti-carcinogenic pathway that is inherent in all cell types. One of the most common pathways through which apoptosis can be initiated is the death receptor pathway (also known as the extrinsic pathway). The death receptor pathway delivers apoptotic signals from outside of the cell upon binding of death ligands to their cognate death receptors in response to various cellular stresses. The most well-characterized death receptor is the Fas receptor, also known as CD95 or Apo-1, which is encoded by the *FAS* gene on chromosome 10q24.1 in humans. Downregulation of *FAS* gene can lead to resistance to apoptosis, and this event has been reported in many types of cancer (8–10), which further highlights the importance of *FAS* in carcinogenesis.

Promoter polymorphisms in the *FAS* gene may potentially influence the transcriptional regulation of the gene, which in turn affects the risk of cancer among their carriers. One candidate promoter polymorphism of the *FAS* gene which has been commonly postulated to affect cervical cancer risk is the c.–671A>G polymorphism (conventionally known as the -670A>G polymorphism) (7). The *FAS* c.–671A>G polymorphism is located within a STAT-1 transcription factor binding site of the gene, and different alleles of the polymorphism have been shown to result in differential apoptosis sensitivity (11). Numerous studies have investigated the role of the *FAS* c.–671A>G polymorphism in cervical carcinogenesis across different populations (12–16). However, limited data are available on the possible involvement of the *FAS* c.–671A>G polymorphism in cervical carcinogenesis in Malaysian population. In addition, the results obtained from studies in different populations have been inconsistent and often contradicted with one another (12–16). To address the inconsistency, several meta-analyses have been performed in the past. Nevertheless, the most recent meta-analysis was performed in 2014 (17) and a few additional reports have been available since then (18,19). Furthermore, methodological flaws were noted in the previous meta-analysis (20). This study therefore aimed to investigate the association between the *FAS* c.–671A>G polymorphism and cervical cancer risk in a case–control setting in the Malaysian population, followed by a meta-analysis of published literatures.

Materials and methods

Ethical approval

The study was approved by the Human Research Ethics Committee (HREC) of Universiti Sains Malaysia (Ref. No: USMKK/PPP/JEPeM[253.3.(7)]) and Medical Research and Ethics Committee (MREC) of Ministry of Health, Malaysia (Ref. No: (2) dlm. KKM/NIHSEC/08/0804/P12-380). Written informed consent was obtained from all subjects prior to recruitment into the study.

Study subjects

A total of 185 cervical cancer patients and 209 cancer-free female controls were recruited from (1) Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, (2) Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan, and (3) Hospital Sultan Ismail, Johor, Malaysia. Cases composed of histopathologically confirmed cervical cancer patients, while controls were cancer-free healthy female volunteers who were biologically unrelated to the cases and tested negative on HPV and Pap tests. Cases and controls were frequency-matched in terms of age (±5 years), and subjects with a history of any other malignancy were excluded from the study.

Genotyping of *FAS* c.-671A>G polymorphism

Genomic DNA was extracted from peripheral blood specimens of the study subjects by using QIAamp DNA Blood Mini Kit (QIAGEN, Germany). Genotyping of the polymorphism was subsequently performed via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Briefly, the 5′-CTA CCT AAG AGC TAT CTA CCG TTC-3′ and 5′-GGC TGT CCA TGT TGT GGC TGC-3′ primers were used for amplification of a 332 bp fragment containing the polymorphism of interest. The PCR products were then digested with BstNI restriction enzyme at 60 °C, and the products of restriction digestion were electrophoresed on an agarose gel for identification of polymorphic genotypes. The homozygous wild type (AA) genotype was identified by the presence of two bands of 231 bp and 101 bp on agarose gel, while the homozygous variant (GG) genotype was identified by the presence of three bands (184 bp, 101 bp and 47 bp) on agarose gel. Bands of all the above sizes were displayed for heterozygous samples. Approximately 10% of the samples were randomly chosen and sequenced to verify the genotyping results, and a 100% concordance rate was observed.

Statistical analysis

IBM® SPSS® Statistics (version 22) was used for all statistical analyses. The difference in demographic characteristics between the cases and controls were compared by using chi-square test and Student's t-test, whenever appropriate. Departure of the genotypic distribution from Hardy–Weinberg equilibrium (HWE) was assessed using a chi-square goodness-of-fit test. Besides, chi-square test of association was used to compare the difference in the distribution of genotype and allele frequencies between cases and controls. Univariate and multivariate binary logistic regression analyses were then used to calculate the crude and adjusted odds ratios (ORs) respectively, along with the corresponding 95% confidence intervals (95% CIs), for estimating the risk of cervical cancer. For the overall assessment, the ORs were adjusted for age, ethnicity, smoking status, number and circumcision status of the sexual partner, parity, duration of oral contraceptive use and age at first sexual intercourse, whereas for ethnically-stratified analyses, the ORs were adjusted for all the parameters above except ethnicity. The wild type genotype (AA) or allele (A) served as the reference for all analyses. All statistical tests were two-sided and statistical significance was assumed at $P < 0.05$.

Meta-analysis

A systematic literature search was carried out in PubMed, Web of Science, Scopus, Ovid MEDLINE, China National Knowledge Infrastructure (CNKI), Wanfang databases to identify relevant studies up to March 2016. No language restriction was applied in the searches. The PubMed search terms used