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Expression analysis of C-FOS and XRCC3 Thr241Met polymorphism in gastric cancer

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Abstract

Gastric cancer is one of the causes of deaths related to cancer across the globe and both genetic and environmental factors are the most prominent. Causes of its pathogenesis. This paper researches the expression of the C-FOS gene. and Thr241Met talking in the XRCC3 gene in patients with gastric cancer and healthy individuals. Controls, in an attempt to clarify their behavior as possible disease susceptibility molecular markers. A total of 100 gastric cancer patients and 100 matched healthy individuals were enrolled, with genomic DNA and RNA extracted from blood samples. Quantitative real-time PCR was used to assess C-FOS expression, while PCR-RFLP determined XRCC3 Thr241Met genotypes. The C-FOS and the Thr/Met XRCC3 genotypes, 12 genotypes revealed that C-FOS was significantly overexpressed in patients than in controls ($P < 0.001$). The XRCC3 Thr/Met genotype was very frequent in patients, as well ($P = 0.0020$). Also, blood type A, family history of gastric cancer, and residing in the country were shown to be categorized as major factors of the risk, and were not significant factors. These results indicate that upregulation of C-FOS and the XRCC3 Thr241Met variant are risk factors of gastric cancer and that blood type and familial history are additional risk factors. We present in our findings that molecular profiling coupled with demographic profiling is highly relevant in risk assessment and early detection techniques in gastric cancer. The study contributes to the further comprehension of the molecular pathogenesis of gastric carcinogenesis and suggests C-FOS and XRCC3 as possible clinical and epidemiological markers.

Keywords: c-FOS expression, Gene expression profile, Molecular biomarkers, Genetic vulnerability, Blood type A, Risk factors.

1. Introduction

The cancer, as it is currently manifested, is a significant population health problem. The etiology can be greatly explained by a mix of predispositions that are genetic and environmental exposures [1,2]. Gastric cancer (GC) is one of the aggressive solid tumours. Unfortunately, most of the patients are diagnosed at late stages and thus portray bad prognostic indicators. Treatment approaches to GC include radiotherapy, immunotherapy, targeted agents, chemotherapy, and interventions [3]. GC is especially common in males and older people. Many risk factors have been named that contribute to GC development, among them being of ethnic origin, gastroduodenoscopy reflux disease, dietary habits of high salt and low fruit and vegetable consumption, tobacco consumption, family history of the disease, socioeconomic differences, and the relationships with specific blood types [4-6].

Genetically, mutations are crucial to GC. The FOS gene (also known as Fos proto-oncogene, AP1 transcriptional factor subunit, with other names being p55, AP1, or C-FOS) is found on chromosome 14q24.3. It covers about 10.5 kb and is most profuse expressed in the bone marrow. This gene is responsible for encoding the AP-1 transcription factor complex, which emerges through the dimerization of proteins with a leucine zipper structure. The pivotal role of the AP-1 protein in cellular activities has elevated the importance of FOS proteins in regulating cellular transformation, differentiation, and proliferation. Notably, an association between C-FOS expression and apoptosis has been observed [7,8]. The X-ray repair cross-complementing group 3 (XRCC3) gene also holds considerable importance, primarily for its vital function in DNA repair processes. A notable mutation in this gene's exon 7.0 causes an amino acid substitution at codon position 241, where methionine takes the place of threonine, denoted as the Thr241Met polymorphism [9,10]. Although many studies have delved into the relationship between this polymorphism and GC susceptibility, findings, especially concerning Asian populations, have been somewhat inconclusive [11].

The objective of this study is to shed light on the relative expression levels of the CFOS gene in patients with GC, and to discern the possible links between specific demographic variables, the polymorphism Thr241.0Met and the probability of GC.

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2. Materials and Methods

2.1. Sample collection

Samples were collected between February 2022 and March 2023 from several hospitals and clinics in the sacred city of Karbala, Iraq. The patient cohort consisted of 100 individuals diagnosed with stomach cancer by experienced surgeons. This cohort was compared against a control group of 100 healthy individuals.

2.2. DNA extraction and quantification

For the extraction and quantification of DNA, genomic DNA was isolated utilizing kits provided by Geneaid (Taiwan), suitable for both blood and tissue samples, in strict adherence to the manufacturer's guidelines. Prior to commencing the RT-PCR (using Execycler 96, BIONNER) and PCR-RFLP analyses, a vital step was undertaken to ascertain the DNA concentration, utilizing horizontal agarose gel electrophoresis. Upon confirmation of the DNA concentration, we carefully stored the samples at −20°C to maintain their integrity, thus preparing them for their integral role in the subsequent phases of our research.

2.3. Quantitative Real-Time PCR (qRT-PCR)

In our lab, we meticulously adhered to a strict protocol for the Quantitative Real-Time PCR process. The first step was to isolate the total RNA from the blood samples using GENEZOL TM TriRNA Pure Kit (Geneaid, Taiwan) following the manufacturer's protocol strictly. This step was essential to maintain the integrity and purity of the RNA.

Following the RNA extraction, cDNA was synthesized using Pioneer, Korea-made AccuPower Rocket Script TM RT Master Mix, RNase H Minus kit. This intermediate stage of converting RNA to a more stable DNA form is very important for the next amplification.

The major aim of the current study was to determine the relative expression ratio of the C-FOS gene among the samples under investigation. To this end, gene-specific primers have been used with a SYBR Green master mix, which has been found to have higher sensitivity and specificity in transcriptomic quantification. Relative up- or down-regulation of the target gene was determined by comparative quantification of expression level between experimental samples and a control cohort.

The qRT-PCR procedure was initiated by a 10-minute denaturation period at 95 o C to ensure the total denaturation of DNA. This was combined with amplification (40 cycles), 15 seconds denaturing at 95 °C and a subsequent annealing/extension step (gene-specific phase) at 58.2 °C of the C-FOS amplicon. Confirmation of product specificity was done by means of a melting-curve analysis, starting at 58.2 o C and gradually heating to 95 o C. Gene expression was evaluated quantitatively by using 2 -Delta delta Ct method. Primers that target the C-FOS gene were as shown below:

F: CACTCCAAGCGGAGACAGAC

Complementary (C): TCGCATGCTACCGATCTTGCAG

The product with the amplification was of 193 base pairs and the melting temperature (Tm) was 58.2 °C.

2.4. Statistical analysis

To interpret our information, we used strong statistical tools. In a more specific manner, paired t-tests and chi-square tests were applied to estimate the statistical significance of our results, whose values were expressed as mean ± standard deviation (SD). There was a pre-set level of p-value, which was used to conclude statistical significance; thus, this determines the reliability and validity of our data. It is a very systematic method of data analysis as the data was presented clearly and in an organised manner and helped in the correct interpretation of the results in the light of genetic and molecular studies.

3. Results

As can be seen in Table 1, the data proved that the difference in the levels of C-FOS gene expression was statistically significant between patients with gastric cancer compared to the control group ($P < 0.001$).

Both the patient group and the healthy control group are examined in Table 2 of the study regarding the Thr-241Met gene polymorphisms.

Observable among the results of the table is the dominance of the ThrMet genotype of the XRCC3 gene Thr-241Met polymorphism in the population of the patients, which comprises 54.9 percent. Furthermore, the statistical analysis's findings showed that there were notable variations in the distribution of the three genotypes between the sick and healthy groups.

A significant difference was observed in the distribution of XRCC3 Thr241Met genotypes between gastric cancer patients and healthy controls ($p = 0.0020$), with the Thr/Met genotype being more prevalent among patients.

The results presented in Table 3 demonstrate the asso-

Table 1. Gastric cancer patients and the control group relative gene expression of C-FOS gene.

Group Name	N	Mean±SD	P value
CT of C-FOS	100	12.495±2.637	**<0.001**
CT of control	100	23.307±3.307	

N = number* = significant less than 0.001

Table 2. Distribution of XRCC3 Thr241Met gene polymorphism in gastric cancer patients and control group.

Genotype	Patients (n, %)	Controls (n, %)	Total (n, %)
Thr/Thr	45 (43.3%)	59 (56.7%)	104 (100.0%)
Thr/Met	50 (54.9%)	41 (45.1%)	91 (100.0%)
Met/Met	5 (100.0%)	0 (0.0%)	5 (100.0%)

Chi-square value: 7.775, p-value: 0.0020.

Table 3. Risk factors associated with gastric cancer in patients and control groups.

Risk Factor	Category	Patients n (%)	Controls n (%)	Chi-square	p-value
Gender	Male	59 (55.8%)	52 (55.2%)	0.8365	0.3604
	Female	42 (44.2%)	48 (44.8%)		
Residence	Urban	28 (39.3%)	47 (58.3%)	10.9023	0.00096
	Rural	72 (60.7%)	49 (41.7%)		
Family history	Yes	58 (58.0%)	32 (32.0%)	13.6566	0.000219
	No	42 (42.0%)	68 (68.0%)		
Blood group	A	45 (45.0%)	15 (15.0%)	28.8619	0.00001
	B	19 (19.0%)	21 (21.0%)		
	AB	22 (22.0%)	18 (18.0%)		
	O	15 (15.0%)	46 (46.0%)		

Note: Percentages are calculated within each group.

ciation between various risk factors and gastric cancer. No statistically significant difference was observed between male and female patients and controls (p = 0.3604). However, significant differences were found between patients and controls with respect to place of residence (urban vs. rural; p = 0.00096) and family history of gastric cancer (p = 0.000219). Additionally, blood type A was significantly more frequent among patients compared to controls (p = 0.00001), whereas no significant differences were observed for other blood groups (B, AB, O) between the two groups. These findings highlight the importance of certain demographic and genetic risk factors in the susceptibility to gastric cancer.

The results in Table 3 demonstrate the association of various risk factors with gastric cancer. No statistically significant difference was observed between male and female patients and controls (p = 0.3604). However, significant associations were found between gastric cancer and both rural residence (p = 0.00096) and a positive family history of the disease (p = 0.000219). Blood group A was significantly more prevalent among patients than controls (p = 0.00001), whereas no significant differences were observed for blood groups B, AB, or O. These findings highlight the importance of demographic and genetic factors in the susceptibility to gastric cancer.

4. Discussion

Genes hold significant sway in the onset of cancerous diseases, including gastric cancer. The C-Fos gene, for instance, has demonstrated variable expression between patients and healthy individuals. While numerous studies have consistently reported an increase in C-FOS expression across various diseases and cancers [12,13], a Korean research group found decreased C-FOS protein expression in GC, especially in criteria like shorter survival, lymphatic invasion, lymph node metastasis, and advanced cancer stages. They inferred that C-FOS might act as a tumor suppressor in GC, potentially due to its pro-apoptotic activity [14].

The influence of the Thr241Met polymorphism on GC's etiology has been the subject of considerable research, yielding diverse findings. Some studies, for instance, identified a significant correlation between the Thr241Met polymorphism and GC, with patients having a greater frequency of the ThrMet genotype than controls [15]. However, our findings contrast with some research [16], which indicated a higher ThrMet genotype prevalence in the control group.

In terms of risk factors, our study explored their association and influence on GC's onset. While our data found no significant gender-based differences in GC incidence, corroborating some findings [17], other studies suggest males are at a heightened risk. This gender disparity in GC incidence could be attributed to various causes, including socioeconomic influences on body mass index (BMI) changes. Notably, males with a higher socioeconomic standing have shown faster BMI growth rates [18].

Furthermore, our findings emphasize the pronounced difference in GC incidence between rural and urban populations. Rural dwellers face distinct challenges, such as longer travel times, limited access to oncology specialists, and a greater likelihood of being uninsured, possibly exacerbating their risk [19].

Our results also underscore the significant role of familial history in GC susceptibility. Individuals possessing a family history of cancer exhibited an elevated incidence rate of gastric cancer (GC), indicating a potential hereditary component or increased genetic susceptibility in the context of this malignancy. Affirming existing literature that identifies an elevated risk among those with affected first-degree relatives [20].

Moreover, a remarkable correlation was observed between blood type A and GC susceptibility in our study, as opposed to other blood types. It's worth noting that gastric carcinoma cells and blood type A share similar antigens. Hence, individuals with blood types A and AB, who lack antibodies against the antigen A, are potentially more vulnerable. On the other hand, the blood group O individuals possess a protective system antigen A, which delays the growth of tumors and their metastases [21].

This paper has illustrated that the C-FOS gene expression on the one hand and the XRCC3 Thr241Met polymorphism on the other are quite important in determining the predisposition to the development of gastric carcinogenesis. These results suggest that molecular and demographic risk factors profiling including assessing the levels of C-FOS expressions and XRCC3 genotyping was done may improve early detection and risk management of gastric cancer. The identification of these genetic and environmental factors provides valuable insights into the molecular mechanisms underlying gastric carcinogenesis and supports the potential utility of C-FOS and XRCC3 as biomarkers for clinical and epidemiological applications. Future studies with larger, multi-ethnic cohorts are war-

ranted to validate these associations and to explore their implications for targeted prevention and personalized therapeutic approaches in gastric cancer management.

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Declaration

Ethics approval and consent to participate
All procedures performed in this study involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved by the relevant ethics committee. Written informed consent was obtained from all individual participants included in the study.

Consent for publication
All authors have reviewed the manuscript and consent to its publication.

Availability of data and materials
All data generated or analyzed during this study are included in this published article. Additional datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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Authors' contributions
All authors contributed substantially to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting of the article, and critical revision for important intellectual content. All authors read and approved the final manuscript.

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