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RESEARCH ARTICLE

Genetic Analysis of SPP1 Polymorphism in Samples of Iraqi Colon Cancer Patients

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ABSTRACT

Cancer is a multistage defect and very complex disease. It is distinguished by particular genetic mutations that lead to a loss of management of cellular functions during uncontrolled growth of normal cells leading to malignant cells. CA18 occurs by the abnormal growth of cells in colon. SPP1 is multifunctional protein. The aim of this study is to study genetic polymorphism of SPP1gene in CA18 patients along with healthy individuals to find the relationship with CA18 and compare both globally. DNA was extracted and the genetic polymorphism of the SPP1gene was determined. Genetic analysis of SPP1 (in rs11730582 T/C region) polymorphism for the two groups showed of (CC) 1 (6.7%) in healthy control and 6 (20.0%) in patients. The results of (TC) showed 8 (53.3%) in healthy control and 14 (46.7%) in patients. The result of (TT) was 6 (40.0%) in healthy control and 10 (33.3%) in patients, it indicates no significance between the two groups in this region at ($p > 0.05$). The results showed SPP1 (in rs11439060 GG/G-region) for the two groups 2 (13.3%) dilation/dilation in healthy control and 14 (46.7%) in patients, while they gave 8 (53.3%) insertion/dilation in healthy control and 12 (40.0%) insertion/dilation in patients. A result of insertion/insertion was 5 (33.3%) in healthy control, while 4 (13.3%) in patients. These results mean that there was no significance between two groups in this region at ($p > 0.05$). The result of rs11730582 SNP of SPP1 gene was close to the results of East Asian.

Keywords: CA18 colon cancer, CC homozygous genotype, PCR polymerase chain reaction, SPP1 osteopontin, TC heterozygous genotype

Introduction

Cancer is a very complex and multi-stage defect disease. Uncontrolled development of normal cells leading to malignant cells is the cause of this disease. In growth signals, these cells are self-sufficient, insensitive to anti-growth signals, overcome proliferative aging, avoid apoptosis, and stimulate angiogenesis and active invasion leading to disease spread.[1–4] Cancer is treated with chemotherapy, radiation, and surgery.[5] Colon cancer (CA18) in the United States is the third most prevalent type of cancer in men and women. It results from the unusual growth of colon cells and that can invade other organs.[6] Symptoms of colon cancer include blood in the patient's stool, a difference in bowel movement, weight loss, and symptoms of fatigue throughout the day.[7,8] Osteopontin (OPN) protein is a multifunctional protein which has emerged as a potential biomarker for cancer diagnosis. In the various signaling pathways within cells, OPN plays an important role, as in cancer, as it mediates critical processes for the disease to develop, such as the immune response, tumor formation, and others.[9–11]

This research studying genetic polymorphisms (rs11730582 and rs11439060) of SPP1gene in CA18 patients with healthy individuals to find the relationship of cancer and compare it with several published studies internationally.

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Materials and methods

Choosing samples

Thirty samples of patients with colon cancer (all patients exposed to chemotherapy) from both sexes of range age (30–65) years were recruited from the Oncology Teaching Hospital/Medical city at February 2020. Fifteen samples of healthy individuals, without any sign and symptoms of colon cancer and other types of cancer, with age range for both genders (25–45) years, were selected as a control. The samples were divided into two groups: Control group consisted of 15 healthy people from both genders and patients' group consisted of 30 (male and female patients). Blood samples were drawn from patients and non-sick people intravenously using a 3 ml syringe. Then the samples were placed in EDTA tube (Whole Blood), and stored in freeze until work time. Genomic DNA isolated from entire blood in accordance with the protocol ReliaPrep™ Blood gDNA Miniprep System from Promega in USA.[12]

Statistical analysis

The genomic DNA was isolated and purified from whole blood using (ReliaPrep™ Blood gDNA Miniprep System kit, Promega, USA) for patients of CA18 and control. Quantus Fluorometer Device was utilized to determine the concentration of extracted DNA to detect the quality of samples for subsequent applications.

Results and discussion

The sequencing of SPP1 gene

PCR product was dispatched for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation – Korea. The range of DNA concentration values was 30–50 ng/μl. Using the Gel imaging system, the colored bands were imaged with the Ethidium bromide in the gel for PCR product, Fig. 1. The result of rs11730582 SNP of SPP1 gene have been presented in Fig. 2.

After performing the statistical analysis using Chi Square, the results are recorded in the following Table 1.

Table 1. shown that the frequency of homozygous genotype (CC) shows 1 (6.7%) in healthy controls and 6 (20.0%) in patients with CA18, the results of rs11730582 heterozygous (TC) genotype showed 8 (53.3%) in healthy controls and 14 (46.7%) in patients with CA18. The frequency of homozygous (TT) genotype was 6 (40.0%) in healthy controls and 10 (33.3%) in patients with CA18. These results showed that there is no significance between controls and patients groups at ($p < 0.05$).

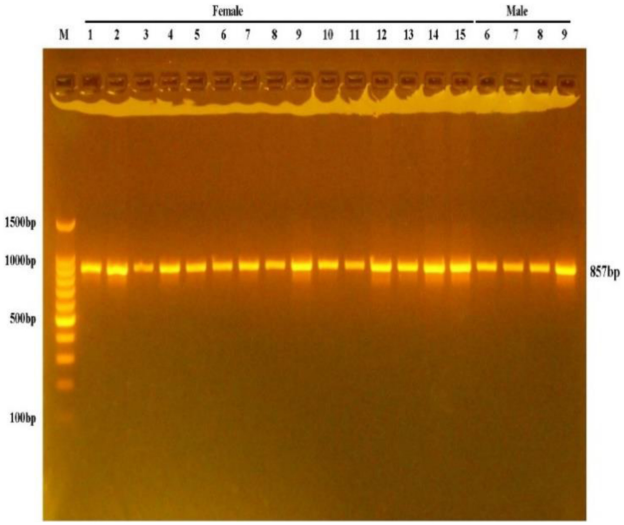

Fig. 1. Results of the amplified of SPP1 gene of human samples were fractionated on 1.5% gel electrophoresis colored with Eth.Br. M: 100bp ladder marker. Lanes 1-9 resemble 857bp PCR products.

Table 1. Association between arm of study and rs11730582 T/C region.

	rs11730582 T/C						
	CC		TC		TT		
	N	%	N	%	N	%	P value
arm							
Controls	1	6.7%	8	53.3%	6	40.0%	0.507
Patients	6	20.0%	14	46.7%	10	33.3%	

This region of SPP1 gene was studied in patients with breast cancer (BRC) in Guangxi, China. The results of this study had genotype (CC) and (C) allele decreasing in BRC patients in clinical stages I-III which contrast with clinical stage IV; therefore, this study suggests that SNP of rs11730582 may enhance the occurrence and develop of BRC during regulating SPP1expression.[13]

There is no study of this region of SPP1 gene and its relationship to CA18. The analysis of rs11439060 SNP of SPP1 gene using Sanger sequencing is manifested in Fig. 3:

Table 2. Association between arm of study and rs11439060 GG/G- region.

	rs11439060 GG/G-						
	del/del		ins/del		ins/ins		
	N	%	N	%	N	%	P value
Arm							
Controls	2	13.3%	8	53.3%	5	33.3%	0.063
Patients	14	46.7%	12	40.0%	4	13.3%	

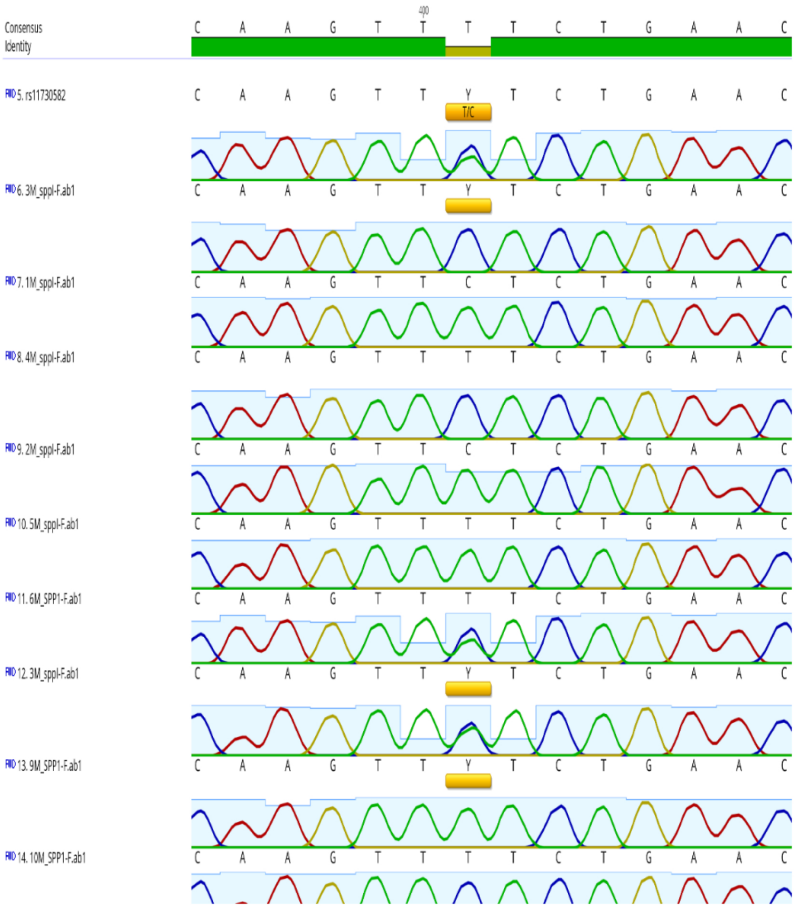

Fig. 2. The result of rs11730582 SNP of SPP1 gene.

After performing the statistical analysis using Chi Square, the results are recorded in the following Table 2.

The results of Table 2 demonstrated that 2 (13.3%) deletion/deletion in healthy controls and 14 (46.7%) in patients with CA18, while 8 (53.3%) insertion/deletion in healthy controls and 12 (40.0%) insertion/deletion in patients with CA18. Also, a result of insertion/insertion was 5 (33.3%) in healthy controls, while 4 (13.3%) in patients with CA18. The result was, there is no significance between controls and patients group at ($p < 0.05$). The results of these analytical statistics indicated that there is no genetic cause associated with colon cancer. Due to a lack of research sources, this gene's region and its connection to colon cancer have not been studied, but there are

Table 3. Lists the results of rs11730582 of SNP for SPP1 gene in globally, these results were published in a report on the NCBI website.

Population	Group	Sample size	Ref Allelle	Alt Allele
Total	Global	94736	T = 0.53516	C = 0.46484
European	Sub	80398	T = 0.51063	C = 0.48937
African	Sub	5658	T = 0.8782	C = 0.1218
African Others	Sub	204	T = 0.961	C = 0.039
African American	Sub	5454	T = 0.8751	C = 0.1249
Asian	Sub	240	T = 0.633	C = 0.367
East Asian	Sub	170	T = 0.676	C = 0.324
Other Asian	Sub	70	T = 0.53	C = 0.47
Latin American 1	Sub	556	T = 0.642	C = 0.358
Latin American 2	Sub	5412	T = 0.5078	C = 0.4922
South Asian	Sub	66	T = 0.39	C = 0.61
Others	Sub	2406	T = 0.5790	C = 0.4210

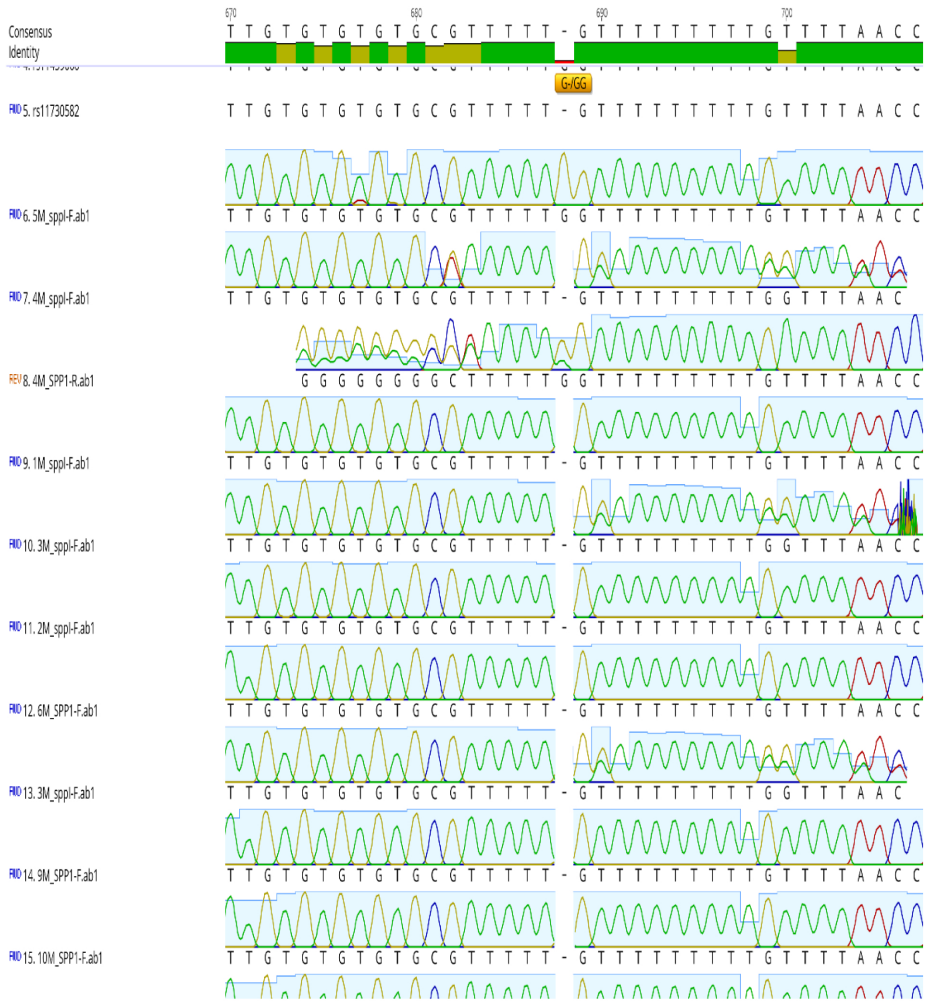

Fig. 3. The result of rs11439060 SNP of SPP1 gene.

some studies to study this region of SPP1 gene and the relationship to high-risk nephrolithiasis, one of which was a study in china on the effect of the G allele (insertion) where it was found to be a possible marker for predicting the risk of kidney stones.[14,15] Through Table 3, the results of East Asian were T = 0.676 and C = 0.324 (NCBI website); therefore, it close to the results of Iraq which were T = 0.733 and C = 0.267.

Conclusion

The SPP1 gene's rs11730582 SNP and CA18 were not found to be associated because the results for this region indicated no significant between the patient and control groups at ($p < 0.05$).

There is no association between rs11439060 SNP of SPP1 gene and CA18 because the result showed no significance between controls and patients group at ($p < 0.05$).The results of these analytical statistics indicated that there is no genetic cause associated with colon cancer. Insufficient research sources have prevented any investigation into this gene's region and its connection to colon cancer. The result of rs11730582 SNP of SPP1 gene was close to the results of East Asian.

Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the figures and tables in the manuscript are ours. Furthermore, any figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- No animal studies are presented in manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Mustansiriyah University.

Authors' contribution statement

The idea of this research was conceived by A Y. A. He supervised the research and provided all advice, while G K. T. collected the samples, separated the DNA, and did PCR, and the samples were sent to do DNA Sequencing.

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دراسة التحليل الجيني لتعدد الأشكالSPP1 بسرطان القولون عند مرضى عراقيين

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المستخلص

السرطان خلل متعدد المراحل ومرض معقد للغاية. انه يتميز بطفرات جينية معينة تؤدي إلى فقدان التحكم بالوظائف الخلوية أثناء النمو غير المنضبط للخلايا الطبيعية مما يؤدي إلى الخلايا الخبيثة. CA18يحدث بسبب النمو غير الطبيعي للخلايا في القولون. SPP1 هو بروتين متعدد الوظائف, الهدف من هذه الدراسة هو دراسة تعدد الأشكال الجيني لـ SPP1gene في مرضىCA18 مع افراد اصحاء لإيجاد العلاقة مع CA18 ومقارنتها عالميًا. تم استخلاص الحمض النووي وتم تحديد تعدد الأشكال الجيني للجين SPP1. أظهر التحليل الجيني في تعدد الاشكال لجين SPP1في منطقة (rs11730582 T / C) للمجموعتين أن (CC) يظهر 1 (6.7%) في مجموعة الأصحاء و 6 (20.0%) في المرضى ، أظهرت نتائج ان 53.3 8 (TC))% (في مجموعة الأصحاء و 14 (46.7%) في المرضى. ظهر) 40.0% 6 (TT) في مجموعة الأصحاء و 10 (33.3%) في المرضى . هذه النتائج تعني عدم وجود فرق ذو قيمة معنوية بين المجموعتين في هذه المنطقة عند .(p> 0.05) أظهرت النتائج في جين SPP1في المنطقة rs11439060GG/G-)) للمجموعتين 2 (13.3%) حذف / حذف في مجموعة الأصحاء و 14 (46.7%) في المرضى ، بينما كانت 8 (53.3%) ادراج / حذف في مجموعة الأصحاء و 12 (40.0%) ادراج / حذف في المرضى . أيضا ، نتيجة ادراج / ادراج كانت 5 (33.3%) في مجموعة الأصحاء ، بينما 4 (13.3%) في المرضى . هذه النتائج تعني عدم وجود فرق ذو قيمة معنوية بين المجموعتين في هذه المنطقة عند.(p>0.05) كانت نتيجة SNP rs11730582 لجين SPP1 قريبة من نتائج شرق آسيا.

الكلمات المفتاحية: CA18سرطان القولون، CC النمط الجيني المتماثل، PCRتفاعل البلمرة المتسلسل، SPP1 الاوستيوبونتين، TC النمط الجيني المتغير.