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Molecular analysis of PTEN gene in some Iraqi colorectal cancer patients

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Abstract

PTEN gene is refer to Phosphatase and TENs in homolog deleted on chromosome 10 which is a tumor suppressor gene located at chromosome 10q23.31, encoding for protein that have both lipid and protein phosphatase activities. The essential function of PTEN is to block the PI3K pathway by dephosphorylating phosphatidylinositol (PI) 3,4,5-triphosphate to PI-4,5-bisphosphate thus neutralize PI3K function. Genetic alterations of the genes related to PI3K/Akt pathway, including mutations of PI3K and PTEN, facilitate tumor genesis and are common in human cancers. The aim of this study is to assess a molecular analysis of PTEN gene in some colorectal Iraqi patients. Twenty-two patients, 11 colorectal cancers and 11 with bowel inflammation from Iraqi patients were screened in exon 7 of PTEN gene using PCR and sequencing analysis. The results showed 23% (3 out of 22) patients were detected mutations. All 45% (10 out of 22) PTEN mutations in 3 colorectal patients were single base substitutions. In this study there are 60% (6 out of 10) transition mutations, 30% (3out of 10) transversion mutations and 10% (1 out of 10) insertion mutation. In conclusion PTEN gene mutations may cause the etiology of CRC as there were genetic alterations in bowel inflammation and colorectal patients.

Keywords: PTEN; Colorectal cancer; PI3K

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Introduction

It is well known that one of the most important kinase cascades is the (PI3K)/Akt signaling pathway which interfere with different cellular functions such as survival, proliferation, migration, differentiation and angio-genesis [1]. PI3Ks is a family of lipid kinases having the ability of phosphor-rylating the 3'OH of the inositolring of phosphoinositides, which are energized by receptor tyrosine kinases include epidermal growth factor receptor (EGFR), insulin-like growth factor receptor (IGFR) and vascular endothelial growth factor receptor

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(VEGFR) [2]. PTEN gene is refer to Phosphatase and TENsin homolog. It was Identified at 10q23.31 as tumor suppressor gene, PTEN protein have dual of lipid and protein phosphatase activities. The essential task of PTEN protein is to neutralize PI3K by dephosphorylating phosphatidylinositol (PI) 3,4,5-triphosphate to PI-4,5-bisphosphate driving block of the PI3K signal pathway [3] Genetic modification of the genes concerning PI3K/Akt pathway, like mutations of PI3K and PTEN genes, leads to tumorigenesis in many human cancers [4]. Loss of PTEN expression may be results from mutation, promoter hypermethylation , a loss of heterozygosity at the PTEN locus which is a recurrent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers [5]. The aim of this study was to assess the molecular analysis of exon 7 in PTEN gene in some colorectal Iraqi patients.

Method

Tissues' samples were obtained from twenty Iraqi patients [11] suffering colorectal cancer and 11 having bowel inflammation who were attending the Gastroenterology and Hepatology Diseases Center in Baghdad between October, 2011 and June, 2012. The Diagnosis and selection of patients were assessed under the supervision of pathologist committee. Ethical permission to conduct the research was obtained from this hospital and from all participants in this study, in the previously mentioned patients treated with radiotherapy or chemotherapy were excluded.

DNA Extraction

The extraction of DNA from fresh frozen tissue were performed using QIAamp kit (Germany).

PCR

PCR reaction were started through a former denaturation at 94°C for 10 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 51°C for 30s, and 72°C for 30s, after that extension at 72°C for 10 min followed by a final extension at 72°C for 10 min and hold at 4°C. The forward primer was 5´TTGACAGTTAAAGGCATTTC-3´ and the reverse primer was 5´-CCTATTTTGGATATTTCTCCC-3 [6]. Each 25µl reaction mixture for PTEN amplification include 12.5 µl of master mix and 1.5 µl of each primer and 9.5 µl of genomic DNA PCR amplifications were achieved in an Applied Biosystem 96 thermocycle.

Sequencing

All DNA templates were sent to Microgene company (Koria). It was processed for the DNA sequencing reaction. Each sample was amplified in a new 25 µl PCR reaction and sequenced using the same forward and reverse.

Results

Patients and disease

Twenty-two colorectal diseased patients were investigated, including 11 samples with CRC and 11 samples with bowel inflammation. The mean age of the colorectal cancer patients was 50 years the average ages were (38-62) years,

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54.50% (5 cases) were females and 45.5% (6 cases) represented males, the severity of the tumor was shown to be moderately differentiated adeno-carcinoma 63.6% (7 cases), well differentiated adenocarcinoma well 27.3% (3 cases) and 1% represented the poor differentiated adenocarcinoma the other specific characters of samples are shown in **Tables 1, 2**.

Table 1.
Distribution of patients with colorectal cancer

Characterization	Total no. (%)
All patients	11 (100)
Mean age 50 years	
> 50	5(45.5)
< 50	6(54.5)
Gender	
Male	5(45.5)
Female	6(54.5)
Site of tumor	
Colon	5(45.5)
Rectum	4(36.4)
Recto sigmoidal	2(18.2)
Differentiation	
Moderately	7(63.6)
Well	3(27.3)
Poorly	1(1)

Table 2.
Distribution of patients with bowel inflammation

Characterization	Total no. (%)
All patients	11 (100)
Mean age 51 years	
> 50	4 (36.4)
< 50	7 (63.6)
Gender	
Male	6 (54.5)
Female	5 (45.5)

PCR

Amplified exon seven of the PTEN gene using PCR technique and screened for the presence of mutations in 22 colorectal disease patients, showed that the product size was 267 bp **Figure 1**.

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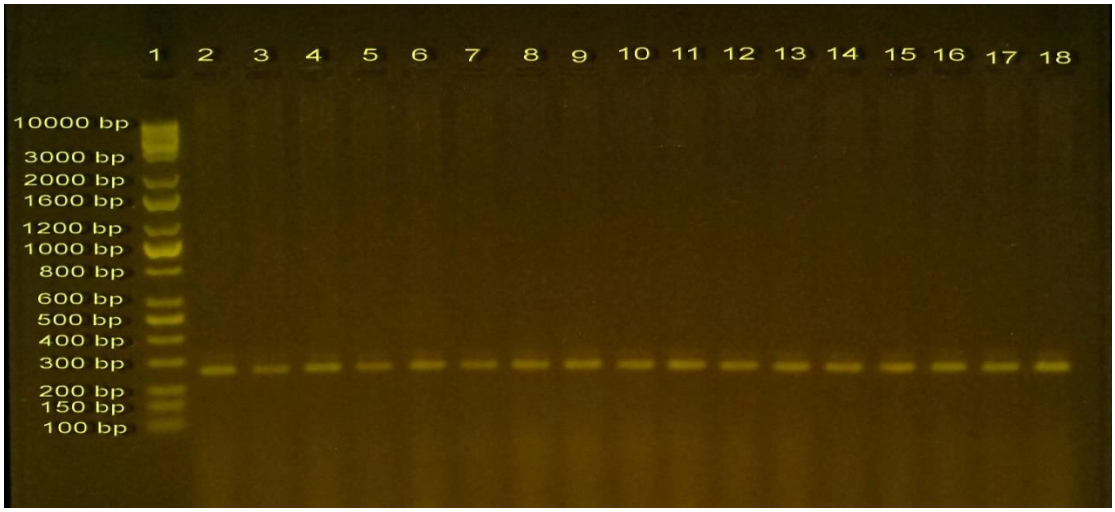

Figure 1.
PCR product for PTEN gene for DNA samples of colorectal disease on 1.2 % agarose gel. Molecular marker (1) and (2-18) samples.

Sequences analysis results

In a series of 11 colorectal carcinomas and 11 bowel inflammation, 23% (3 out of 22) patients were detected to have mutations in exon 7 when comparing all sequence variants with NCBI data base accession no. AF067844. All 45% (10 out of 22) PTEN mutations in 3 colorectal patients showed single base substitutions. Two sample of inflammation patients 18% (2 out of 11) showed one single base substitutions T > C transition mutation was seen in one showed sample, on the other hand there were 4 single base substitutions seen in the other sample A>G and 2(G>A) transition mutations, while the fourth was transversion mutation T>G.

Table 3.
PTEN gene mutations in colorectal patient.

No. Case	*PTEN* Mutations Position
*N 25	c. 1158918 T>C
N 6	c.115873 A>G c. 115884G>A, c.115892 G>, c.115932 T>G
*T 7	c.115893_115894 Insertion A duplication c.115882 A>C, c.115884G>A, c. 115892G>A, c.115932 T>G

*N=Normal (inflammation Patient), *T=Tumor.

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Figure 2.
Representative data of sequences analysis of the PTEN gene in colorectal patients: A tumor sample sequence, B and C inflammation samples sequences, D reference sequence.

One colorectal cancer patient sample 9% (1 out of 11) has 4 single base substitutions 2G>A transition mutations, two transversion substitution (A>C and T>G). Insertion A base mutation 115893-115894 also was seen in the tumor sample. The present results revealed the existences of 60% (6 out of 10) transition mutations, 30% transv-ersion mutations and 10% (1 out of 10) insertion mutation **Table 3**.

Discussion

The main tumor suppressor gene included in the PI3K-AKT mTOR pathway is the PTEN gene. Many articles refer to inactivation of PTEN phosphatase deregulates the PI3K pathway. Such studies indicate approximately 20%-40% of colorectal cancer harbor PTEN loss (7, 8). The present study assessed a relationship between the mutations in exon 7 and colorectal diseases depending on PTEN gene using PCR and DNA sequencing analysis in 22 colorectal disease patients.

The results revealed 23% (3 out of 22) patients have PTEN gene mutation, 45%(10 out of 22) PTEN mutations showed single base substitutions point mutations in PTEN gene, 50% of this mutations were in the inflammation patients, this indicate that PTEN gene may play a crucial role in processes of colorectal cancer development and its etiology because of the presence of 3 similar mutations c.115882 G>A, c.115892 G>A c.115932 T>G in both samples of inflammation and colorectal cancer . The results also found insertion A base (115894-115894) mutation in tumor sample also there were 60% transition mutation. These data in keeping with results of A Northern India

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population study which found the insertion of "G" and A in two different codon of exon 7 in PTEN gene. These findings may have confirmed the activity of PTEN gene in CRC. The outcome proposed that PTEN gene mutation and lack of PTEN expression may provide useful prognostic data to aid treatment strategies for CRC (9). Han and his colleagues found a significant association between genotypes of variants in rs3830675 and increased CRC risk [10]. The data of the present study is inconsistent with results obtained by other studies which reported that PTEN gene mutations are infrequent events in sporadic CRC [11, 12]. PTEN crystal structure comprise of two domains (phosphatase and a C2), both have a great value for tumor suppressor function. Exon 7 consists of CBR3 loop, which is thought to have a main role in phospholipid membrane bound of the C2 domain. The mutation on the CBR3 loop reduce the affinity for membranes in vitro (13). Despite the truth that N-terminal Phosphatase domain is responsible for PTEN physiological activity, about 40% of PTEN tumorigeneses mutation may occur in the C- terminal C2 domain (exons 6, 7, and 8) and in the tail sequence (exon 9) encoding for tyrosine kinase phosphorylations it is significant for protecting PTEN function and protein stability (14,15). lack of PTEN work therefore cause increased PIP3 and persistent activation of PIK3 effector, which has multiple effect of tumor development such as cell proliferation apoptosis resistance, angiogenesis, genomic inconstancy and metastasis (16,17). Other studies indicate that distal (left-side) CRC has lower rate of PTEN expression and mutational in comparison to proximal (right-sided) (18,19). Lin et al., (2015) found that polymorphism in the PTEN gene is connected with increased risk to CRC, these results indicate that rs701848 polymorphism in the 3'UTR region of PTEN gene might be a candidate pharmacogenomics factor to assess the susceptibility and prognosis in CRC patients even this region is not able to change the encoded amino acids it may cause genetic splicing, protein expression, regulation of cell cycle or alter the microRNAs binding site [20]. Other study reported PTEN gene alterations lead to genome instability and describe as biomarkers prognostic value usually found in colorectal cancer [21]. Other reports investigated the association between PTEN mutations and microsatellite instabilities, proposing that the PTEN gene causing genomic instability in microsatellite instabilities colorectal tumorigenesis [22, 23].

In conclusion, this paper suggests that PTEN gene may have an important role in etiology of colorectal cancer as there are similar mutations in inflammation bowel and colorectal cancer patients. Future studies with larger patient's groups and investigate the relation between BRAF gene and clinicopathological parameter may give more clear picture regarding the molecular mechanism.

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Competing interests

Authors declare that We have no competing interests

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