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ARAŞTIRMA/RESEARCH

Gene expression analysis of FABP4 in gastric cancer

Gastrik kanserde FABP4 gen ekspresyon analizi

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Abstract

Purpose: Gastric cancer has high incidence and mortality rate in several countries and is still one of the most frequent and lethal disease. In this study, we aimed to determine diagnostic markers in gastric cancer by molecular techniques; include mRNA expression analysis of FABP4 gene. Fatty acid binding protein 4 (FABP4) gene encodes the fatty acid binding protein found in adipocytes. The protein encoded by FABP4 are a family of small, highly conserved, cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands. It is thought that FABPs roles include fatty acid uptake, transport, and metabolism.

Material and Methods: Total RNA were extracted from paired tumor and normal tissues of 47 gastric cancer. The mRNA expression level of FABP4 was measured employing semi- quantitative reverse transcription-polymerase chain reaction (RT- PCR).

Results: The mRNA expression level of FABP4 was significantly decreased (down- regulated).

Conclusion: Down-regulation of FABP4 gene seems to occur at the initial steps of gastric cancer development. In order to confirm the relationship between the gastric tumor and FABP4 gene, further analysis like immunohistochemistry and epigenetc techniques are necessary.

Key words: Gastric cancer, FABP4, expression analysis, semi- quantitative RT- PCR.

Öz

Amaç: Gastrik kanser birçok ülkede yüksek oran ve mortalite oranına sahiptir ve günümüzde halen en sık görülen ölümcül hastalıklarından biridir. Bu çalışmada, gastrik kanseri teşhisini için moleküler tekniklerle FABP4 geninin mRNA ekspresyon analizini içeren yeni markırlar belirlemeyi amaçladık. Yağ asidi bağlayıcı protein 4 (FABP4) geni adipositlerde bulunan yağ asidi bağlayıcı proteini kodlar. FABP4 tarafından kodlanan protein, uzun zincirli yağ asitleri ve diğer hidrofobik ligandlara bağlanan küçük, yüksek düzeyde korunmuş, sitoplazmik bir protein ailesidir. FABP'ün yağ asidi alımı, taşınması ve metabolizmayı içeren görevleri olduğu düşünülmektedir.

Gereç ve Yöntem: Total RNA, 47 gastrik kanserinin normal dokularından ve eşleştirilmiş tümörden çıkarılmıştır. FABP4 mRNA ekspresyon seviyesi yarı-kantitatif reverse transkripsiyon polimeraz zincir reaksiyonu (RT-PCR) kullanılarak ölçülmüştür.

Bulgular: FABP4'ün mRNA ekspresyon seviyesi önemli ölçüde azalmıştır (down regüle olmuştur).

Sonuç: FABP4 geninin down-regülasyonunun, gastrik kanserinin başlangıç aşamalarında meydana geldiği görünmektedir. İmmünohistokimya ve epigenetik teknikler gibi ileri analizler, FABP4 geni ve gastrik kanser arasındaki ilişkiyi doğrulamak için gereklidir.

Anahtar kelimeler: Gastrik kanser, FABP4, ekspresyon analizi, yarı-kantitatif RT-PCR.

INTRODUCTION

Gastric cancer is the second leading cause of cancer death and the fourth most prevalent malignancy worldwide, affecting about one million people per year[1]. In the United States, an estimated 21.320 cases of gastric cancer diagnosed and 10.540 patients die from this disease in 2012[2]. There is a geographic diversification in the occurrence of gastric cancer[3]. Most cases are recorded in Japan, China, South America, and significantly less in Western Europe and in the United States[3]. Gastric cancer consists of two pathological variants. The development of intestinal tumours is characterized by progression of several sequential steps that start with gastritis and then progresses to mucosal atrophy, intestinal metaplasia, dysplasia and carcinoma with subsequent metastatic

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dissemination[4]. Adenocarcinomas arising from gastric epithelium are the most common malignancies of the stomach (90% of cases)[4]. Malignancies arising from connective tissue (sarcoma) and from lymphatics (lymphoma) are less common[5]. Environmental and genetic factors are both important in gastric carcinogenesis[6]. Many studies have reflected that gastric cancer is associated with a number of risk factors; such as Helicobacter pylori infection, age, family history, smoking, alcohol consumption, obesity and diet[5].

Fatty acid binding protein 4 (FABP4) gene encodes the fatty acid binding protein found in adipocytes, it has been localized in a region of chromosome 8q21 and consists of 4 exons[7]. The protein encoded by FABP4 are a family of small, highly conserved, cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands. It is thought that FABPs roles include fatty acid uptake, transport, and metabolism[7]. Expression biomarkers can be used clinically to predict the effectiveness and toxicity of anticancer drugs and thus help to achieve individualized treatment8. Glenn et al. suggested fatty acid binding proteins, such as FABP4, that play an important role in regulating hallmarks of cancer includes gastric cancer, breast cancer and ovarian cancer[9].

In this study, for the first time we aimed to determine and measure exon 2 for FABP4 gene among gastric cancer patients in Erbil province using semi-quantitative reverse transcription-polymerase chain reaction and image software.

MATERIALS AND METHODS

Sample collection

The samples were collected from the Tumor unit at Rizgary hospital in Erbil, Iraq. A total of 94 samples were analyzed. The study included 47 paired normal and tumor samples of patients that were grouped according to the types of gastric cancer and the clinical characteristics of the patients, including gender and average of age (Table 1). The tissue samples of the gastric stored at -80˚C until further analysis. The study was approved by the local Ethics Committee and was conducted in accordance with the guidelines of the Declaration of Helsinki.

Table 1. Number of patient samples according to type of stomach cancer and gender

Cancer types	Male/pair	Female/pair	Total	Ages
Gastric Adenocarcinoma	24	8	32	57 - 68
Gastric diffuse cancer	7	3	10	44 - 58
Lymphoma gastric cancer	2	3	5	53 - 63
Total	33	14	47	

RNA extraction and cDNA converted

RNA extraction was carried out upon confirmation by histopathological analysis. RNA from gastric tissue was extracted with RNeasy Total RNA Kit (Qiagen, Hilden, Germany). The tissue was homogenized in 700 µl lysis buffer RLT using a Polytron rotor-stator followed by aspiration 10 times through a 21 gauge syringe needle to shear chromosomal DNA.

Cell pellets were resuspended in 700 µl lysis buffer RLT by aspiration 10 times through a 21 gauge syringe needle. RNA was eluted from spin columns with 40 µl diethylpyrocarbonate treated water. Quantification and qualification of total RNA concentration was performed using NanoDrop (ND- 1000, USA). Samples with (A260 – A320) / (A280 – A320) ratios less than 1.7 and/or yields less than 0,5 µg total RNA were excluded from subsequent analysis. Complementary DNA (cDNA) is synthesized from a messenger RNA (mRNA) template in a reaction catalyzed by the enzymes reverse transcriptase and DNA polymerase. In this study, ProtoScript First Strand cDNA Synthesis Kit (BioLabs, England) was utilized. A control reaction without reverse transcriptase is recommended to examine the DNA contamination in the samples. Variable amount of total RNA were employed for each sample since quality and quantity of total RNA are not equal.

RT-PCR amplification and gel discrimination

Semi-quantitative reverse transcriptase-PCR technique was used in this research to amplify the mRNA sequence of exon 2 in FABP4. A pairs of primer were designed by SDSC workbench online primer design program (Table 2) and a pair primer

of housekeeping gene; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization10. PCR reaction and condition was performed by using MJ Research, AB Applied Biosystem thermal cycler (Eppendorf, Germany). 50 µL reaction mixture was prepared in PCR tubes containing 1.5 µL cDNA template, 25 µL OnePCRTM master mix (GeneDirex, Korea), 1 µL forward primer, 1 µL reverse primer and 21.5 µL ddH2O. The cycling conditions comprised of initial denaturation at 95˚C for 5 min, 35 cycles of denaturation at 95˚C for 30 sec, annealing temperatures in Table 2 for 30 sec and extension at 72˚C for 30 sec, and final extension at 72˚C for 3 min.

Expression alterations were discriminated using agarose gel electrophoresis (2%) in the presence of ethidum bromid. The image of agarose gel was captured and quantitated mRNA expression level by image J software program ((version 1.46r, downloaded from http://imagej.nih.gov/ij)11 The statistical analysis of mRNA expression was carried out using Wilcoxon signed rank test. Significance was assumed for values $p \leq 0.05$.

Table 2. Primer sequences, PCR product size of FABP4 and GAPDH genes and optimal annealing temperature

Primer	Sequence	Site	PCR product (bp)	Annealing temperature (˚C)
FABP4 gene Forward Reverse	5'- TGGCATGGCCAAACCTAACA -3' 5'- CTTCGTCAAATTCCTGGCCC -3'	173- 192 291- 272	119	54.1
GAPDH gene Forward Reverse	5'-GGTCCACCACCCTGTTGCTGT-3' 5'-AGACCACAGTCGATGCCATCAC-3'	Random region	456	59.4

RESULTS

Gene expression analysis

Fatty acid binding protein 4 (FABP4) gene was amplified. The FABP4 mRNA sequences of exon 2 analyzed and separated by agarose gel electrophoresis and normalized with (GAPDH) as shown in Figure 1.

The FABP4 expression level of mRNA of 47 cancer patients were obtained from normal controls and tumors. Different expression level of each patients were observed. The mRNA expression level of all tumors cancer patients according to normal controls were highly decreased. The expression level of FABP4 gene was obtained from 47 pairs; cancer and normal samples

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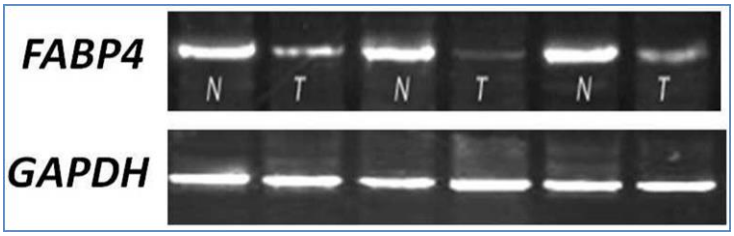


Figure 1. The mRNA expression results of 2% agarose gel electrophoresis staining with ethidium bromide; FABP4 and GAPDH genes expression in normal controls and tumor samples in GC. The base pairs of the mRNA fragments; FABP4= 119 and GAPDH= 456. N= normal sample: T= tumor sample.

The agarose gel electrophoresis results were measured by ImageJ software program, quantity of mRNA expression of FABP4 tumor samples were highly decreased according to expression level of normal control samples, p=0.0001 and statistically it is significant counted on (Wilcoxon signed rank test; $p<0.05$). The mRNA expression level for both normal controls and tumors are shown in Figure 2.

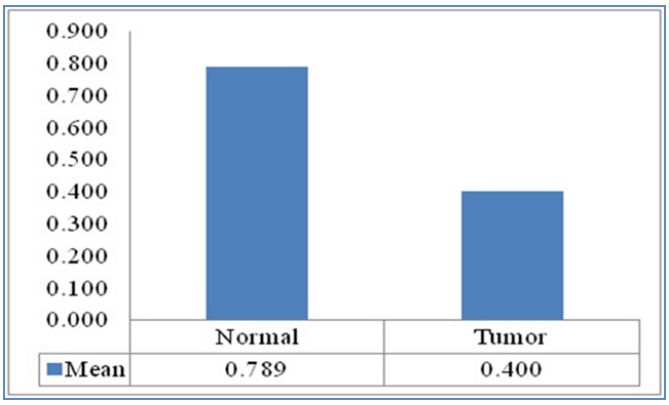


Figure 2. The quantitative of the mRNA expression level of the FABP4/GAPDH gene of both normal controls and tumors
(Wilcoxon signed rank test; p= 0.000).

Table 3. The statistical results of gastric cancer types.

Type of gastric cancer	Total number	p value
FABP4 gene		
Gastric Adenocarcinoma	32	<0.0001
Gastric diffuse cancer	10	<0.0001
Lymphoma gastric cancer	5	<0.001

DISCUSSION

Recently, Glenn et al., (2015) suggested and reported fatty acid binding proteins, such as FABP4, that play an important role in regulating hallmarks of cancer includes gastric cancer. In the current study, the first time we aimed to investigate mRNA expression level of FABP4 gene among Gastric cancer patients in Erbil province. The protein encoded by FABP4 are a family of small, highly conserved, cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands, it roles in fatty acid uptake, transport, and metabolism[7]. Lots of expression profiling researches have suggested the potential of gene expression models to distinguish between histologic subtypes of cancers, and also gene expression analysis has showed many specific diagnostic markers among cancer patients[12].

In our study, the mRNA expression level of FABP4 gene was loss and decreased (down-regulated) was shown in figure 2. However, statistically was significance counted on (Wilcoxon signed rank test; $p < 0.05$). Yong et al. (2013) was reported the FABP4 expression down regulated is compatible with our results were down-expressed[13]. Expression level of all gastric cancer types of FABP4 gene is significantly decreased among patients (Table 2). According to our data, reduced gene expression of FABP4 might be a useful markers for diagnosis in Gastric Adenocarcinoma, Gastric diffuse cancer and Lymphoma gastric cancer types of GC patients.

Recently in cancer study, observation has been focused on modifications in the gene expression regulation that do not contribute a change in the sequence of DNA of the cell[14]. These are named to as epigenetic alterations, and the most prominent involves changes in DNA methylation[14]. A microRNA expression study; involved in tumor development and tumor progression including metastasis[12]. Junker et al. (2013) analyzed distant metastases with primary tumors and found a distinct miRNA signature at metastases. Some of the primary tumor samples clustered together with the distant metastasis, suggesting that these primary tumors have a metastasis-specific signature[12].

In conclusion, reduced mRNA expression of FABP4 gene might be a risk factor for GC development, and suggesting that mRNA expression alteration in FABP4 gene modify individual

susceptibility to GC. In order to understand molecular mechanism of GC further analysis warranted such as; DNA sequencing, miRNA expression analysis, protein expression analysis and epigenetic analysis.

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