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Expression of FGFR2 in breast cancer Iraqi patients

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Abstract

The FGFR2 gene produces a protein called fibroblast growth factor receptor 2 (FGFR2), which is involved in cell growth. Expression of FGFR2 in Iraqi breast cancer patients was the subject of this research. It was observed that FGFR2 was overexpressed in breast cancer tumors and cell lines. On the 10q26 chromosome, the FGFR2 gene has 20 exons. The FGFR2 gene has been linked to a higher risk of breast cancer in a number of studies. The relationship was confirmed by polymorphisms in FGFR2 intron 2, which increased BC susceptibility by 5−10%. The CC genotype of the SNP rs1219648 showed the lower level of the FGFr2 gene expression in both patient s and control (143.3±117.0 and 1.2±2.7, respectively) and the TT genotype showed the higher level (203.9±145.0 and 11.6±18.7). The results are shown in the table (4) and include the patients and control with the genotype rs2981582 and those with the SNP rs4563158.

Keywords

Expression, FGFR2, breast cancer, Iraqi patients.

Breast cancer (BC) is the most common cancer in women across the world. In 2018, Belgium has the highest incidence of breast cancer in the world. Following lung cancer, it is the second leading cause of death. Diets, on the other hand, are most likely to improve the quality of life of BC patients. Physical exercise appears to lower the risk of recurrence and death in BC patients, according to research[1]. The tyrosine kinase receptor fibroblast growth factor receptor 2 (FGFR2) regulates cell differentiation, proliferation, and death. FGFR2 has an important function in a variety of malignancies. Overexpression of FGFR2 in breast cancer tumors and cell lines was discovered. The FGFR2 gene has 20 exons and is found on the 10q26 chromosome [2]. Several investigations have demonstrated that the FGFR2 gene has a significant role in breast cancer risk. Polymorphisms in FGFR2 intron 2 validated the link, and susceptibility to BC was enhanced by 5−10%. Several genetic mutations in FGFR2 have been found to activate FGFR2 signaling pathways up and/or downstream in breast cancer. Two genome association analyses found five single nucleotide polymorphisms (SNPs) inside FGFR2, rs2981579, rs11200014, rs1219648, rs2981582, and rs2420946, which were linked to BC. The polymorphisms in intron 2 stayed inside the linkage disequilibrium block. s rs298158 2Gastric cancer, endometriosis, pancreatic cancer, squamous cell carcinoma of the lung, and ovarian cancer have all been linked to the FGFR2 gene[3,4]. Several studies have been conducted in many groups to corroborate the function of the FGFR2 gene in susceptibility, including Chinese, North Indians, European Americans, and African Americans. In some groups, variations in the FGFR2 gene intron 2 have been linked to BC. rs2981582 is one of the most significant SNPs in intron 2 that has been linked to the risk of breast cancer. FGFR2 gene polymorphisms rs2981582 have been linked to BC risk in several studies. However, no study has looked into the relationship between the rs2981582 gene polymorphism and BC susceptibility in Iraqi women. The tyrosine kinase fibroblast growth factor receptor 2 (FGFR2) is one of four membrane-bound receptor tyrosine kinases (RTKs)[5]. Although FGFR5, a new FGF receptor, has been discovered, it lacks the

intracellular tyrosine kinase domain. FGFs are a gene family with at least 22 members that have a variety of important functions, including developmental induction, pattern formation, cell growth and differentiation, as well as survival and death[6,7]. Mutations in FGFRs have been demonstrated to increase tumor growth by increasing cell proliferation and survival, but they can also decrease tumor growth[8]. Studies of animal models have consistently indicated that FGFR2 is both an oncogenic and a tumor suppressor gene. The aim of the study researches the expression of FGFR2 in breast cancer Iraqi patients.

Material and method

Gene expression

RNA extraction

RNA become confined from the example utilizing the TRIzolTM Reagent (Thermo Fisher Scientific, USA) in accordance with the maker's convention, which included the following steps:

Sample lysis

The lysate transformed into homogenized by involving pipetting all over a few examples in each tube, which contained 0.25 mL of blood and 0.75 mL of TRIzolTM Reagent.

Purification

- 0.2 mL of chloroform become added to each container of lysate, and the cylinder cap changed into then screwed on firmly.
- In the wake of hatching for 2−3 minutes at room temperature and centrifuging for 10 mins at 12,000 rpm, the total become isolated into 3 stages: a lower normal stage, an interphase, and a dreary top fluid stage.
- The watery portion containing the RNA was moved to a spic and span tube and the way become rehashed.

Precipitation

- A total of 0.5 mL of isopropanol became introduced to the aqueous phase, which was incubated for 10 mins earlier than being centrifuged for 10 minutes at 12,000 rpm for 10 minutes.
- Total RNA become brought about, resulting in a white gel-like pellet at the lowest of the tube.
- The supernatant become discarded following this technique.

D-Washing

- To each cylinder, 0.5mL of 70 rate ethanol changed into presented and vortexed in short sooner than centrifuging for five minutes at 10,000 rpm for 5 minutes to get rid of any garbage.
- After that, the pellet was aspirated and dried with air.

Solubility

Hatching become cultivated at 55-60°C in a water bath or warmth block for 10−15 minutes after the pellet had been rehydrated in 20−50 mL of Nuclease Free Water.

Determine RNA concentration.

While deciding the centralization of separated RNA with the goal that it will conclude the extraordinary of tests for downstream bundles, a Quant's Fluorometer changed into used to find the consciousness of extricated RNA. A general of 199 mL of weakened QuantiFlour Dye become blended in with 1 mL of RNA. After 5 mins of hatching at room temperature in a darkish environmental element, the RNA not set in stone.

primers

Primer and probe design

The cDNA groupings for the (FGFR2) quality were gotten from the National Center for Biotechnology Information GenBank data set. RT-qPCR preliminaries were planned the use of the Primer Premier three programming program with liquefying temperatures beginning from 58 to 62 levels Celsius, preliminary lengths going from 18 to 23 nucleotides, and PCR amplicon lengths going from 75 to one hundred fifty base sets, with the groundwork and test groupings displayed inside the table as specific illustrations.

Table (1) show primer and probe sequence

APrimer name	ASequence	AAnnealingA Temp. (ϵC)
TEGT-F	5`-TGCTGGATTTGCATTCCTTACA-3`	60
TEGT-R	5`-ACGGCGCCTGGCATAGA-3`	
FGFR2_ex1-F	5`-AGAGCCTTCGGTCATTT-3`	55
FGFR2_ex1-R	5`-TCCTCCACAATGCTAGTC-3`	
FGFR2_exp.-P	FAM--3`TGATGCATGAGCCATCTCAGCAGA-3`	

Primer preparation

These preliminaries were outfitted with the guide of the Macrogen Company in a lyophilized shape to your comfort. As a stock arrangement, lyophilized groundworks have been broken up in nuclease-detached water to an absolute keep going consideration of 100pmol/l, with a last attention to 100pmol/l. To get a functioning preliminary arrangement with 10pmol/l leisure activity, 10mL of groundwork stock response (saved at - 20 C) transformed into brought to 90mL of nuclease-detached water to procure a working preliminary arrangement with 10pmol/l distraction (see Methods).

Response Setup and Thermal Cycling Protocol

Table (2): component of real time PCR reaction

Master mix componentsA	AStock	UnitA	FinalA	UnitA	VolumeA
					1 Sample
qPCR Master Mix	2	X	1	X	5
RT mix	50	x	1	x	0.25
MgCl2					0.25
Forward primer	10	µM	1	µM	0.5
Reverse primer	10	µM	1	µM	0.5
Nuclease Free Water					2.5
RNA		ng/µl		ng/µl	1
Total volume					10
Aliquot/single rxn	9µl of Master mix/tube & add 1µl of Template				

Real Time PCR programe

Table (3): qPCR program

AStepsA	A°C	Am: sA	AcycleA
RT. Enzyme Activation	37	15:00	1
Initial Denaturation	95	05:00	
Denaturation	95	00:20	40
Annealing	60	00:20	
Extension	72	00:20	

Analysis Gene Expression using Pfaffi Method

Relative quantification

$$\text{Folding} = 2^{-\Delta\Delta CT}$$

$$\Delta CT = CT_{\text{gene}} - CT_{\text{House Keeping gene}}$$

$$\Delta\Delta CT = \Delta CT_{\text{Treated or Control}} - \Delta CT_{\text{Average Control}}$$

Statistical Analysis

The statistical analysis of this study have been done by SPSS version 24.2 and Microsoft excel has also used. The numerical data have represented as mean ± Standard error (S.E), t-student test has been used to compare two numerical data. ANOVA test used for numerical data more than two. Chi-square has used for two categorical parameters.

Odds ratio and their corresponding fisher value for the genetic polymorphism have been calculated by using the program Wini-pipi [14].

Results and Discussion

The effect of the SNP genotypes on the FGFr2 gene expression level are shown in the table (4).

The CC genotype of the SNP rs1219648 showed the lower level of the FGFr2 gene expression in both patient s and control (143.3±117.0 and 1.2±2.7, respectively).

The CC genotype of SNP rs2981575 showed the higher level of FGFR2 im both patients and control (203.9±145.0 and 11.6±18.7, respectively) and the TT genotype showed the lower level (163.1±141.2 and 1.2±2.7).

SNP rs4563158 showed lower level of FGFR2 gene expression level within the genotype TT (157.7±158.0 and 1.0±0.2), also the SNP rs7579866 showed the lower level within the TT genotype (195.3±151.7 and 3.7±8.8).

The results showed the patients and control with CC genotype of rs2981582 have the lower level of the FGFR2 gene expression (143.3±117.0 and 1.2±2.7).

Table 4; comparison between the gene expression level of FGFR2 in relation to polymorphism

Group	rs1219648		
	TT	TC	CC
patients	176.2±143.4	245.2±169.1	143.3±117.0
Control	10.2±17.7	2.7±3.5	1.2±2.7

Group	rs2981575		
	TT	TC	CC
patients	163.1±141.2	215.9±165.1	203.9±145.0
Control	1.2±2.7	2.6±3.4	11.6±18.7
Group	rs4563158		
	TT	TC	CC
patients	157.7±158.0	213.4±155.9	194.1±154.9
Control	1.0±0.2	3.0±5.0	4.1±10.1
Group	rs7579866		
	TT	TC	CC
patients	195.3±151.7	232.7±211.2	0
Control	3.7±8.8	1.0±	0
Group	rs2981582		
	TT	TC	CC
patients	220.3±145.7	218.2±172.5	143.3±117.0
Control	10.2±17.7	2.7±3.5	1.2±2.7
Group	rs2162540		
	TT	TC	CC
patients	157.7±125.1	224.1±174.2	203.9±145.0
Control	1.1±2.5	3.0±3.6	10.2±17.7

It is, as yet, uncertain which polymorphism is genuinely responsible for the increased risk, or whether several polymorphisms in cis act in concert. Comparison with other polymorphisms in FGFR2 might demonstrate a better correlation with protein expression.

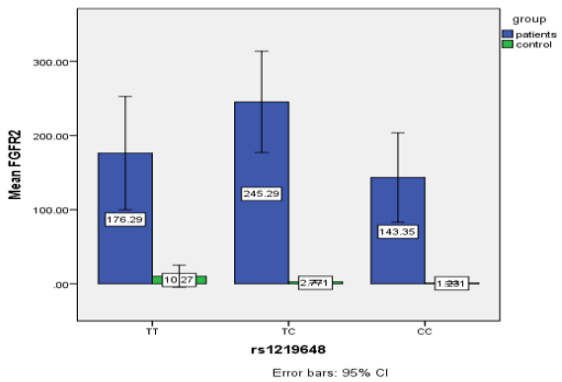

Figure 1; comparison among the genotypes of the rs1219648 SNP in response to the FGFR2 gene expression

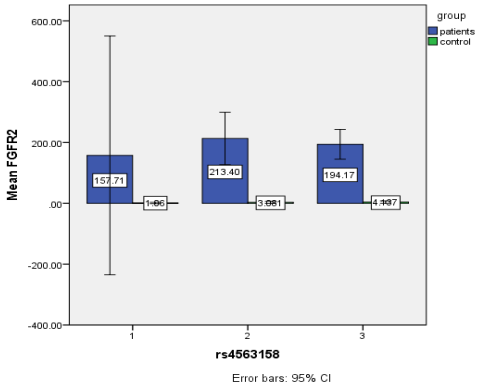

Figure Error! No text of specified style in document.; comparison among the genotypes of the rs4563158 SNP in response to the FGFR2 gene expression

Single Nucleotide Polymorphisms (SNPs) in intron 2 of the Fibroblast Growth Factor Receptor Type 2 (FGFR2) gene, including rs2981582, contribute to multifactorial breast cancer susceptibility. The high risk polymorphism haplotype in the FGFR2 gene has been associated with increased mRNA transcription and altered transcription factor binding but the effect on FGFR2 protein expression is unknown.

There are many published reports concerning the expression of FGFR2 in various cancers. Previous reports have shown overexpression of the C3 isoform in gastric cancer cell lines and the C2 and C3 isoforms in breast cancer cell lines (Itoh H. et al., 1994), suggesting that aberrant expression of C2 or C3 splicing variants may contribute to cancer development.

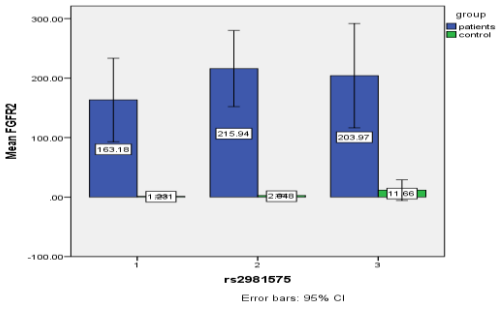

Figure 2; comparison among the genotypes of the rs2981575 SNP in response to the FGFR2 gene expression

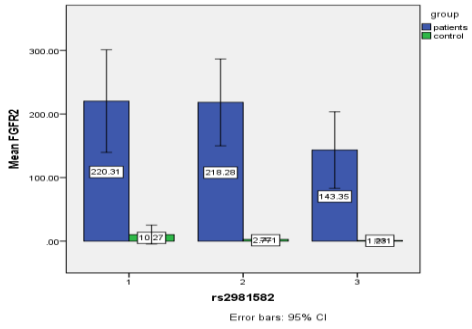

Figure 4; comparison among the genotypes of the rs2981582 SNP in response to the FGFR2 gene expression

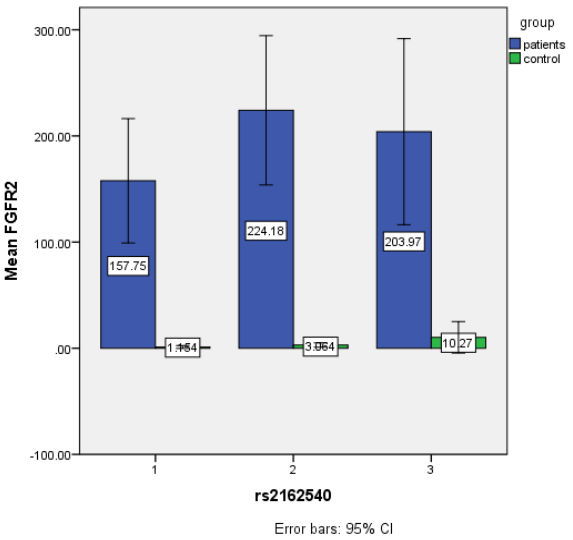

Figure 5; comparison among the genotypes of the rs2162540 SNP in response to the FGFR2 gene expression

Anomalous FGF signaling is associated with cancer development and progression. Gene amplification or missense mutations of FGFR2 occur in gastric, lung, breast, ovarian, and endometrial cancers and melanomas (Zu. Et al., 2009). SNPs of intron 2 in FGFR2 are associated with an increased risk of breast and endometrial cancers (Gartside et al., 2009). Furthermore, activating mutations of FGFR2 have been identified in approximately 10% of endometrial cancers, and inhibition of activated mutations of FGFR2 induced apoptosis and growth inhibition of endometrial carcinoma cells (Chaffer et al., 2007). In contrast, loss-of-function mutations of FGFR2 have been reported in melanomas. These findings suggest that FGFR2 can play a context-dependent, opposing roles in various cancers.

Conclusions

From the results obtained from real PCR and fgfr2 analysis we concluded that theirs high gen expression in the genotype but in different value especially in the patient with breast cancer.

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