

## Studying The Expression of Human TGFB1 Gene in Patients Carrying Breast Cancer

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### Abstract:

TGFB1 gene encodes a member of the transforming growth factor beta (TGFB) family of cytokines, that play multifunctional roles in regulation the proliferation, differentiation, and other functions in different cell types. Therefore, we collected thirty samples from Yarmouk hospital in Baghdad ,Iraq.

Twenty- nine from women patients carrying Breast cancer and the last one was from healthy woman. and the samples that we have taken were embedded in paraffin wax, The extracted RNA from each samples was used to check the expression of TGFB1 gene via Real time PCR.

We designed primers of TGFB1 gene on encoded sequence (Exon), to make sure generation a specific PCR product of TGFB1 gene by converting mRNA to cDNA then PCR Product , The expression of each samples was fluctuated between 0.1 fold to 0.2 fold compared with the control ( the healthy sample). The highest expression showed was samples 14 with 0.2 fold , and the lowest expression showed was samples 27 with approximately 0.1 fold that is similar relatively compared with control expression .

The results indicate that tumor may affects on a TGFB1 gene and can increase the gene expression, and eventually can increase the TGFB1 protein that can be used for upregulation of the cells that carried the cancer.

### الخلاصة:

هذا الجين ((TGF-beta1 يمثل عامل النمو التحولي لمجموعة بيتا لعائلة السايوتوكاينات المهمة في المناعة ويفرز في الخلية على شكل بروتين يؤدي وظائف متعددة خلوية من اهمها السيطرة على نمو الخلية والتضاعف والتميز والموت المبرمج ولاجل ذلك تم جمع 30 عينة تسعة وعشرون منها لاشخاص مصابين بسرطان الثدي وعينة واحدة لمرأة سليمة تم جمعهم من مستشفى اليرموك في بغداد . كل العينات كانت مطمورة بشمع البارافين.

تم استخلاص الرنا من كل العينات وتم قياس التعبير الجيني ((TGF-beta1 لها عن طريق استخدام جاز الريل تايم. تم تصميم البرايمرات على منطقة الاكزون لجين ((TGF-beta1 وتم عمل سي دي ان اي ثم تضخيم جيني بواسطة الريل تايم.

اظهرت نتائج التعبير الجيني تباين بين (0.1) و(0.2) فولد مقارنة بالسيطرة . حيث اظهرت اعلى العينات (عينة رقم 7) (0.2) فولد وكانت اقل العينات تعبيراً العينة رقم (27) ب (0.1) فولد. تشير النتائج الى ان السرطان قد يؤثر على زيادة البروتين او بالاحرى زيادة التعبير الجيني والمطلوب في تنظيم فعالية الخلايا المصابة بالسرطان.

### Introduction:

This gene encodes a member of the transforming growth factor beta (TGFB) family of cytokines, which are multifunctional peptides that regulate proliferation, differentiation, adhesion, migration, and other activities in many cell types[1-9]. Many cells have TGFB receptors, and the protein positively and negatively regulates many other growth

factors. The secreted protein is cleaved into a latency-associated peptide (LAP) and a mature TGFB1 peptide, and is found in either a latent form composed of a TGFB1 homodimer, a LAP homodimer, and a latent TGFB1-binding protein, or in an active form composed of a TGFB1 homodimer. The mature peptide may also form heterodimers with other TGFB family members. This gene is frequently upregulated in tumor cells, and mutations

in this gene result in Camurati-Engelmann disease TGF- $\beta$  is secreted by many cell types, including macrophages, in a latent form in which it is complexed with two other polypeptides, latent TGF-beta binding protein (LTBP) and latency-associated peptide (LAP). Serum proteinases such as plasmin catalyze the release of active TGF- $\beta$  from the complex. This often occurs on the surface of macrophages where the latent TGF- $\beta$  complex is bound to CD36 via its ligand, thrombospondin-1 (TSP-1). Inflammatory stimuli that activate macrophages enhance the release of active TGF- $\beta$  by promoting the activation of plasmin. Macrophages can also endocytose IgG-bound latent TGF- $\beta$  complexes that are secreted by plasma cells and then release active TGF- $\beta$  into the extracellular fluid.[3]

TGF- $\beta$  exists in at least three isoforms called TGF- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3. Until the three isoforms were discovered, TGF- $\beta$  referred to TGF- $\beta$ 1, as it was the first member of this family to be discovered. The TGF- $\beta$  family is part of a superfamily of proteins known as the transforming growth factor beta superfamily, which includes inhibins, activin, anti-müllerian hormone, bone morphogenetic protein, decapentaplegic and Vg-1. [10-14]

Most tissues have high expression of the genes encoding TGF- $\beta$ . In contrast, other anti-inflammatory cytokines such as IL-10 show minimal expression in unstimulated tissues and seem to require triggering by commensal or pathogenic flora.[4]

TGF- $\beta$  acts as an antiproliferative factor in normal epithelial cells and at early stages of oncogenesis.[5,16-21] Some cells that secrete TGF- $\beta$  also have receptors for TGF- $\beta$ . This is known as autocrine signalling. Cancerous cells increase their production of TGF- $\beta$ , which also acts on surrounding cells.

## Materials and Methods:

### Primer design

The primer was designed specifically on (TGFB) gene (NCBI Reference Sequence: NM\_000660.6) and the sequence of nucleotides was synthesized in IDT company, (USA), (Table1).

### RNA isolation, transcriptional profiling, and quantitative RT-PCR

RNA was isolated using Total RNA Mini Kit (Tissue), Geneaid ,Thailand. The purified RNA was quantified and equalated in all RNA samples using Nanodrop (Thermo fisher, USA) One-step PCR , was used for quantitative RT-PCR, universal sybr master mix(2x) (Kappa biosystems , South Africa) was used with the following reaction(20 $\mu$ l)

- 1) 10 P mole Primer F (TGFB) 1 $\mu$ l
- 2) 10 P mole Primer R (TGFB) 1 $\mu$ l
- 3) 2X Kapa one step master mix 10 $\mu$ l
- 4) RT. Enzyme 0.4  $\mu$ l
- 5) RNA (100ng) 5 $\mu$ l
- 6) dNTP ( 10mM) 1 $\mu$ l
- 7) D.W 1.6 $\mu$ l

<b>PCR condition:</b>		
Reverse transcription	5 min	42°C Heat
inactivation	3 min	95°C
Initial Denaturation	3 min	94°C
Denaturation	18sec	94°C
Annealing	0.30 min	56°C
Polymerization	0.30 min	72°C

Standard dissociation curve was used with this condition .

**Results And Discussion:**

A total of 29 clinical isolates of patient women carrying breast cancer disease from Yarmook hospital in Baghdad were confirmed with the standard diagnostics . The gene is currently available in the GenBank. Initially, TGFB1 gene was thought to specifically work as producing cytokines, and subsequently was found to affect on cancer cell [9].

The map of TGFB1 gene was designed with its specific primers in order to determine all nucleotides that participate in forming a PCR product ( Figure 2) . A study of the gene expression is a broadly recommended in checking the gene activity of suspected gene in cancer researches. The gene expression was measured by statistical calculation mentioned in (figure 3).

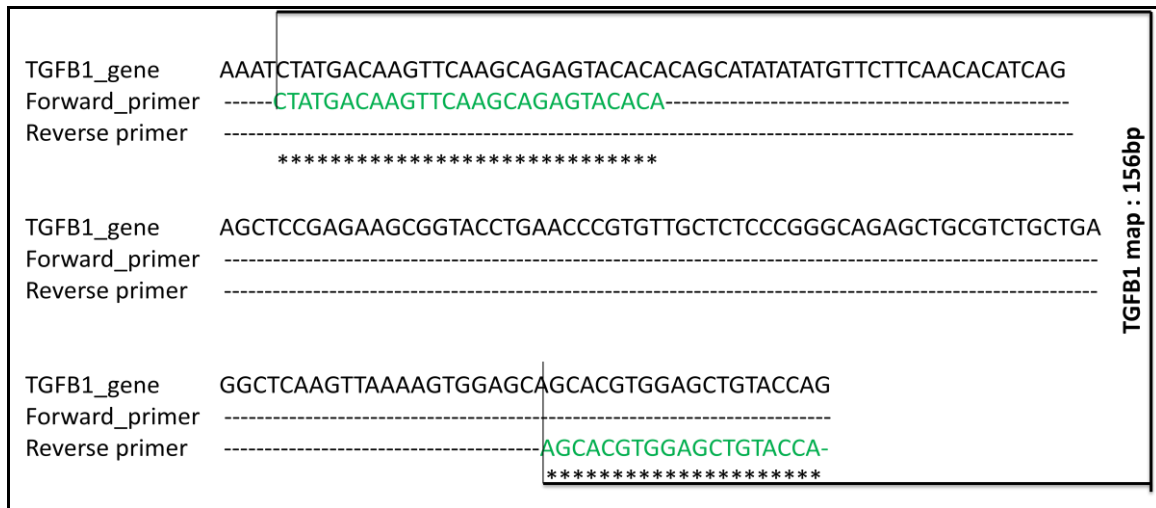
In this study the important measurement for us was quantitative Polymerase Chain Reaction (qPCR) technique that used two enzymes: RT and Taq DNA polymerase mixed with sybr dye and other components

such as the specific primers targeting to the specific sequences of the *TGFB1* mRNA, The *TGFB1* gene is found in all of the total genome sequences of currently available in the GenBank. Initially, *TGFB1* gene was thought to specifically work as transforming growth factor, and subsequently was found to affect on cancer cell , The expression of each samples was fluctuated between 0.1 fold to 0.2 fold compared with the control ( the healthy sample). The highest expression showed was samples 14 with 0.2 fold , and the lowest expression showed was samples 27 with approximately 0.1 fold that is similar relatively compared with control expression .

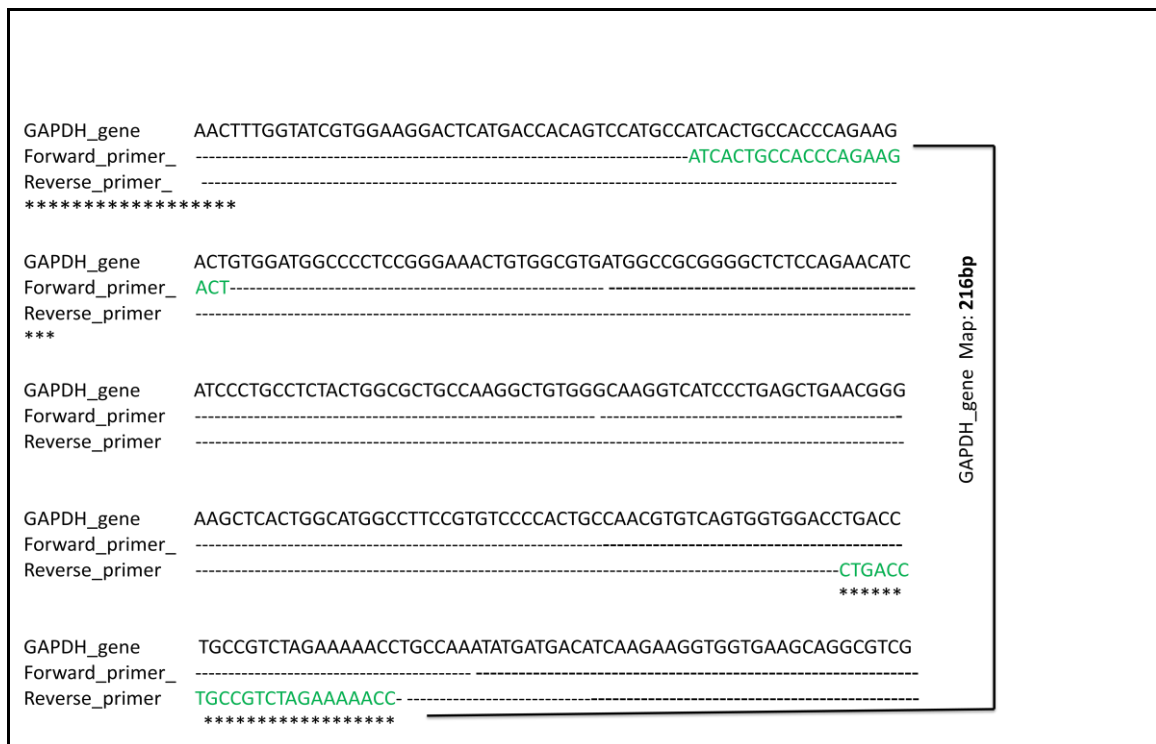
The results indicate that tumor may affects on a TGFB1 gene and can increase the gene expression, and eventually can increase the TGFB1 protein that can be used for upregulation of the cells that carried the tumor.

**Table(1): Primer Sequencing of genes that were studied for gene expression of TGFB1 gene .**

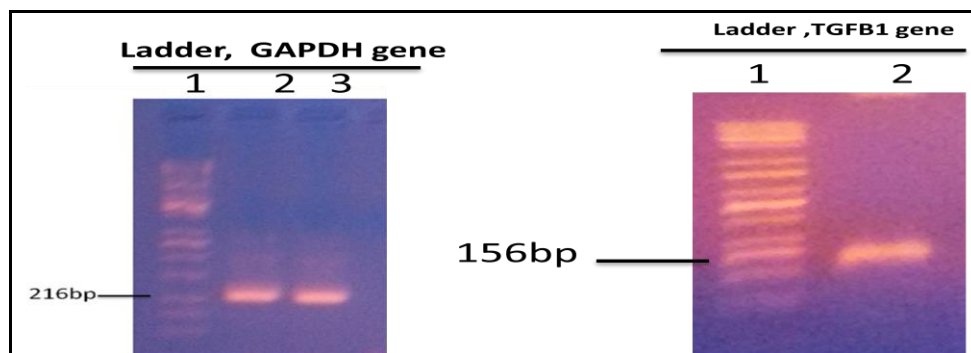
Primers	Sequences	PCR product	References
TGFB1 gene -F	CTATGACAAGTTCAAGCAGAGTACACA	bp156	Current study
TGFB1 gene -R	TGG TAC AGC TCC ACG TGC T		Current study
F-GAPDH- Human	5-ATCACTGCCACCCAGAAGACT-3	216bp	Current study
R-GAPDH – Human	5-GGTTTTTCTAGACGGCAGGTCAG-3		Current study



**Figure (1):Alignment of Human TGFB1 gene with primers that shows the PCR product size (156) bp.**



**Figure (2):Alignment of Human GAPDH gene with primers that shows the PCR product size (216) bp.**



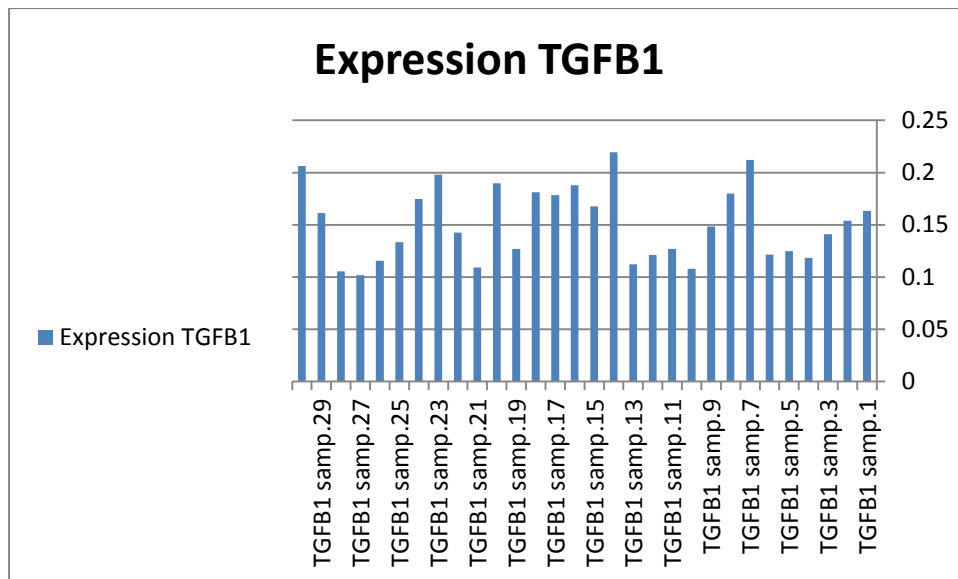
**Figure (3):PCR products on 2% Agarose gel with 100 volt for 40 minutes with different Kappa ladders .Right gel: 5  $\mu$ l of GAPDH PCR product loaded. Left gel: 5  $\mu$ l of TGFB1 PCR product was loaded .**

(A)

Sample no.	$\Delta$ Ct-value ( $s=\sqrt{(s_{\text{Target}}^2+s_{\text{Normalization}}^2)}$ )	$\Delta\Delta$ Ct= $\Delta$ CtTest sample- $\Delta$ CtCalibrator sample
TGFB1 samp.1	0.71886	2.446667
TGFB1 samp.2	0.635819681	2.443333
TGFB1 samp.3	0.81882029	3.17
TGFB1 samp.4	0.605695193	3.076667
TGFB1 samp.5	0.987387124	3.003333
TGFB1 samp.6	0.816496581	3.04
TGFB1 samp.7	0.628755384	2.236667
TGFB1 samp.8	0.757869822	2.473333
TGFB1 samp.9	0.764155307	2.753333
TGFB1 samp.10	0.596601486	3.21
TGFB1 samp.11	0.555757741	2.976667
TGFB1 samp.12	0.860426251	3.043333
TGFB1 samp.13	0.848331696	3.153333
TGFB1 samp.14	0.60855019	2.313333
TGFB1 samp.15	1.124737006	2.576667
TGFB1 samp.16	0.825085854	2.266667
TGFB1 samp.17	0.562820279	2.486667

TGFB1 samp.18	0.805770852	2.463333
TGFB1 samp.19	0.697949377	2.976667
TGFB1 samp.20	0.624393039	2.396667
TGFB1 samp.21	0.792927067	3.193333
TGFB1 samp.22	0.774166218	2.32
TGFB1 samp.23	0.70969477	2.336667
TGFB1 samp.24	0.949929822	2.516667
TGFB1 samp.25	0.757869822	2.6413
TGFB1 samp.26	0.764155307	3.12321
TGFB1 samp.27	0.596601486	2.4756667
TGFB1 samp.28	0.555757741	3.1133
TGFB1 samp.29	0.860426251	3.187
TGFB1 samp.30	0.848331696	2.3091

(B)



**Figure (4): Gene expression of TGFB1 gene measured by real time PCR using Syber filter (A) Expression of TGFB1 gene compared with control (GAPDH) Each sample has been replicated triple times and the average calculated using the formula:  $\Delta\Delta Ct = (\Delta Ct_{\text{Test sample}} - \Delta Ct_{\text{Calibrator}})$ , relative quantification ( $\Delta\Delta Ct$  method), (B): The fold-change has been calculated by the formula  $2^{-\Delta\Delta Ct}$  with  $\Delta\Delta Ct+s$  and  $\Delta\Delta Ct-s$**

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