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Research Article

Upregulation of Programmed Death-1 Ligand 2 (PDCD1LG2) Gene via the Effect of the Early Growth Response 2 (*Egr2*) Gene Expression in T-Lymphocyte Detected using Microarray-Technique-Based evaluation

Zainab Saad Ghafil Al-Raheem

Collage of Dentistry, University of Al-Qadisiyah, Al-Diwaniyah, Al-Qadisiyah, Iraq

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ABSTRACT

The focus, in the current work, was applied on evaluation of *Egr2*-gene-dependent effects on the expression of the programmed death-1 ligand 2 (PDCD1LG2) gene in 2 types of T-lymphocytes (MF2), one type with the *Egr2* gene (23 test samples (TS)) and other type without this gene (23 control samples (CS)). CDNA labelled with different fluorescent dyes, Cy3 and Cy5 for CSs and TSs, was mixed together and hybridized to a solid chip in a microarray-based technique. The resulted (upregulated, downregulated, and no changed) genes were categorized according to their functions using Go minor software. The results identified upregulated, downregulated, and no changed genes in the TSs compared to those in the CSs. Mainly, the PDCD1LG2, programmed death-1 ligand 2 was found to be changed in its levels. This gene is needed for T-cell-based proliferation and IFNG-based production. T-cell proliferation is inhibited via the interaction with PDCD1 via blocking cell cycle continuity. The current study presents important information that could be used as a guide for future *in vitro* and *in vivo* studies involving the PDCD1LG2 gene in links to the *Egr2* gene.

Keywords: *Egr2* gene, gene expression, microarray, PDCD1LG2 gene.

INTRODUCTION

The use of microarray was introduced by Schena and co-worker at Stanford University for measuring gene expression in which probe reaction with the targets is performed captured via imaging. Nucleic-acid and protein microarrays involving DNA and RNA and protein detections respectively. The technique is useful for detecting of thousands of genes and their activities via rapid and quantitative evaluations of these expressions. This technique is important in providing information about, for example, the cause of a certain type of cancer, aging process, hormonal activities, drug discover, and neurological conditions. Disease conditions may act on changing the expression of certain genes that could be altered back to its normal status via the activities of certain drugs, so microarrays could be useful in drug discovery to fight disease conditions an especially those with poor treatment outcomes such as viral infections and cancer. The usefulness of the microarrays could be focused on understanding some of the functionality of certain genes and via recognizing new pathways via demonstrating target genes or proteins[1-3]. The current experiment was aimed to analyse gene expression profiles in T lymphocytes with or without *Egr* gene. This gene is a coding gene for a zinc-finger-protein-based member including *Egr1*, *Egr2*, *Egr3*, and *Egr4*. This gene product, first, was revealed as a transcription factor activating early responses for early growth[4]. However, this protein also was found to be released from brain synapses in adults or via pharmacological stimulation[5]. Normally, the gene product is produced in the CD44 and T-cells controlling the proliferative and functional activities of these cells. Moreover, the gene acts via controlling self-tolerance of the T lymphocytes preventing the induction of auto-immune diseases such as lupus resulted from losing *Egr2* leading to hyperactivation of T cells[4]. On the other hand, increasing the expression of this gene decreases the activity of T cells via enhancing E3 ubiquitin ligase Cbl-b[6]. The gene also regulates the activity of invariant natural killer cells[7].

MATERIALS AND METHODS

RNA extraction

The extraction procedures were performed using a TRIzol extraction kit. Separation of the aqueous phase from the organic phase was done using the MF2 cells in 500ul TRI reagent. The extraction protocol was done following the kit instructions. The quantification and qualification of the resulted RNA were measured using a NanDrop spectrophotometer and an agarose-gel electrophoresis technique.

Primer annealing

The TSs and the CSs were exposed to this process in which 3µl of oligo-DT was added to both them. This oligo-DT, with yellow color, is attached to the poly (A) tail of the mRNA providing the first step of cDNA

**Author for Correspondence: zainab.al-raheem@qu.edu.iq*

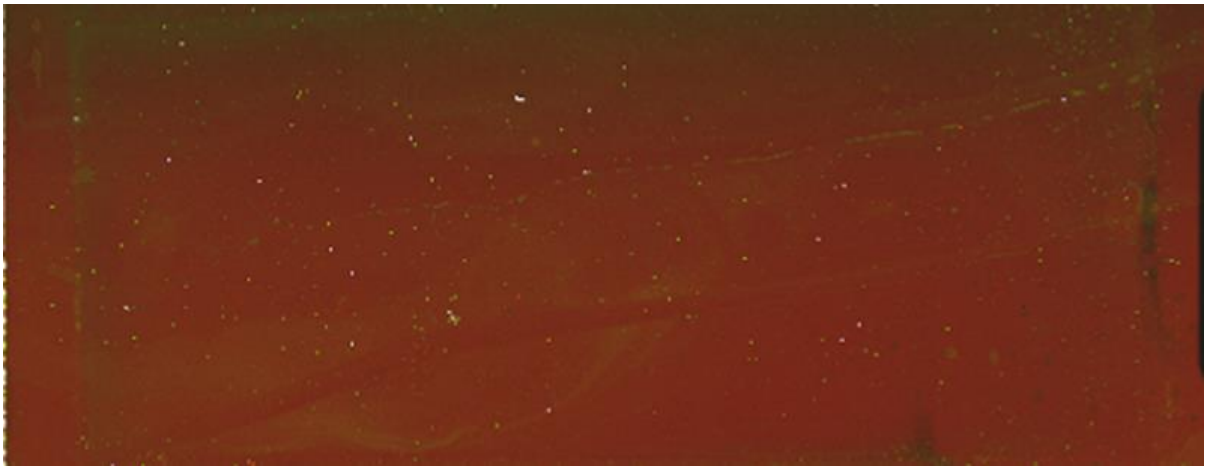

Figure 1: Image of microarray hybridization of G8. It shows the results of a hybridized microarray slide after scanning. Red spots represent high gene expression. Green spots represent low gene expression. Yellow spots represent equal gene expression.

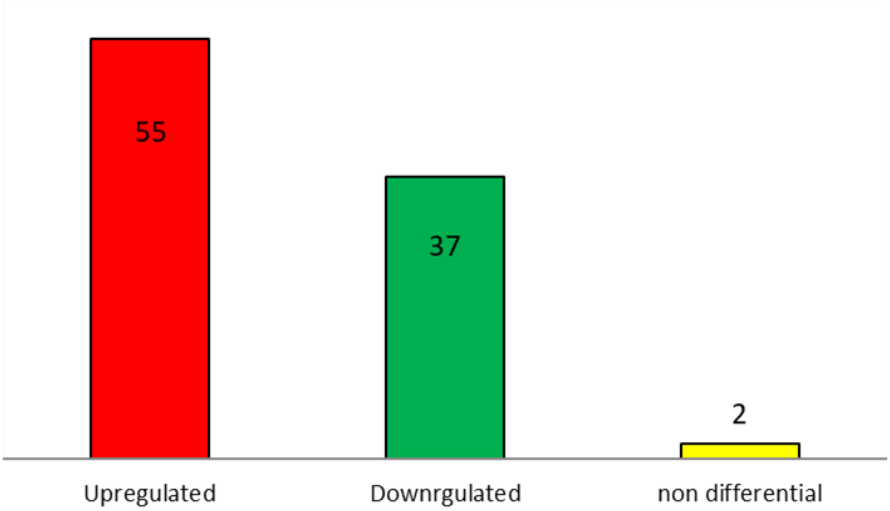

Figure 2: Go minor analysis (intensity measurements) of upregulated and downregulated genes. Red color represents the upregulated genes, green color represents the downregulated genes, and yellow color represents non differences.

synthesis. These samples were then mixed together and incubated at 70^0C for 5 minutes. Then, the reaction was cooled down for 10 mins followed by a centrifugation step.

Extension reaction

Cyc-3mix at 9µl was added to the CSs while, and cy-5 at 9µl was added to the TSs. Both mixtures contained 5x cyscribe buffer 4µl, 0,1 MDTT 2µl, dCTP nucleotide 1ul, dCTP cye-Dye-labelled Nucleotide 1µl , Cyscribe RT enzyme 1ul. The dyes in the mixtures were pipetted and centrifuged for mixing purposes for 30s and incubated at 42^0C for 1.5hr. In this step, RNA was labelled. The rest of unlabelled RNA was degraded using water bath at 73^0C and 2µl M NAOH. Vortexing and spinning down steps were induced for 15s and incubated at 73^0C for 15 mins. Then, 10ul of 2MHEPES was used to make the condition at a neutralization status. Finally, the samples were vortexed for 15s.

Purification of labelled cDNA

Removing the Cyc Dye nucleotides and prime was done using a purification step for cleaning the signal background using a 1GF-column-based purification kit and following its instructions.

Hybridization of the chip

The hybridization step was performed on15,000 genes provided by Brunel university, Department of Bioscience. The instruction of the kit and the instruments were followed. Finally, the reactions were scanned using a spectrophotometer in the Microarray Lab (Brunel University).

RESULTS

According to the scanned images, group 8 (G8) showed the highest in the presence of red spots, figure 1.

The resulted (upregulated, downregulated, and no changed) genes were categorized according to their functions using Go minor software, figure 2.

Interestingly, ~1939 genes were upregulated, and 1459 genes were downregulated. The diversity of gene types was 55 and 37 types for upregulated and downregulated genes respectively. Genes such as ANXA5, ATP6V0D,

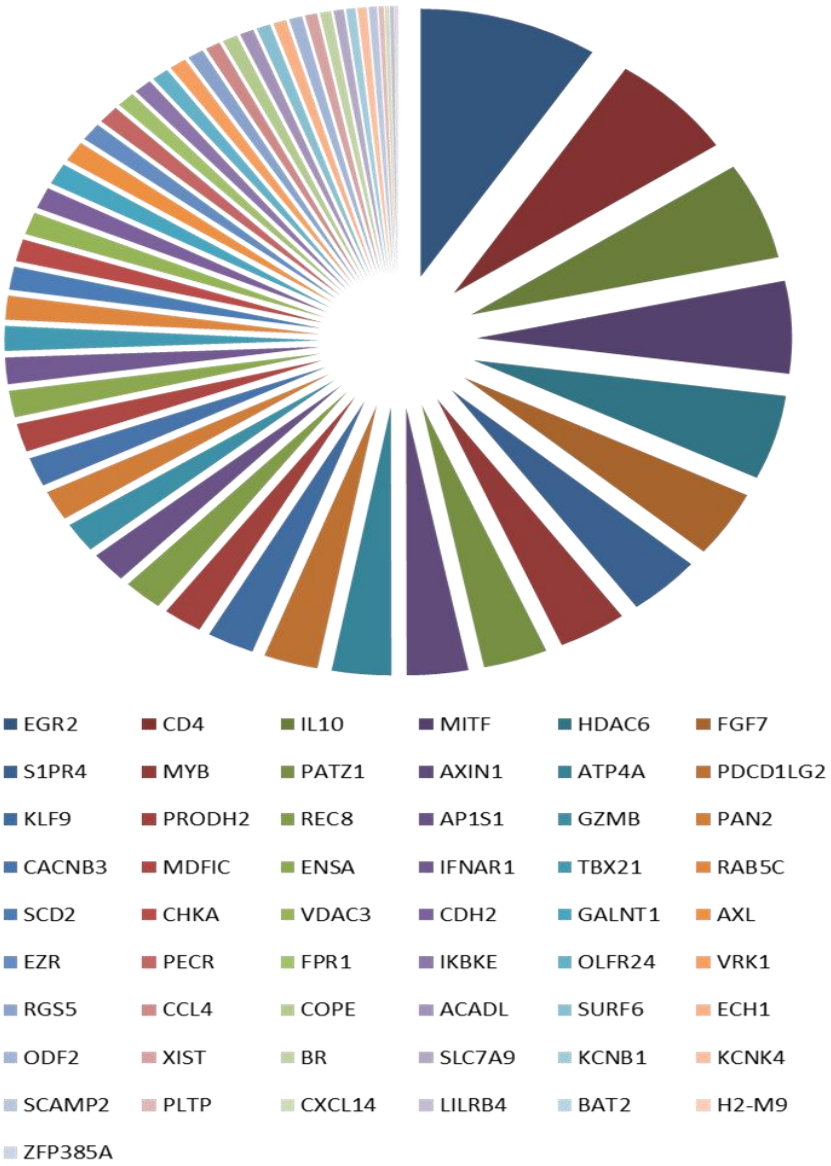

Figure 3: The rate of incidence for the upregulated genes.

CNTN2, CTSE, SOD1 were repeated about 253 times, figure 3 for the upregulation of the PDCD1LG2.

DISCUSSION

It is well known that microarrays are used as a standard technology for measuring the expression of thousands of genes. After analyzing the data, a ratio of 1, yellow spot, represents equal in the expression levels between the TSs and the CSs. However, a ratio greater than 1, red spot, reveals higher expression in the CSs. Moreover, a ratio less than 1, green spot, identifies low expression. The microarray reactions are affected by factors such as concentration of salts, temperature of hybridization, hybridization length, pH, and denaturant presence such as formaldehyde present in the hybridization buffer.

In the current work, the PDCD1LG2, programmed death-1 ligand 2 was found to be changed in its levels. This gene is needed for T-cell-based proliferation and IFNG-based production. T-cell proliferation is inhibited via the interaction with PDCD1 via blocking cell cycle continuity. PDCD1LG1 and PDCD1LG2 are shown as inhibitory receptors in adaptive immunity. The PD-1, PD-L1, and PD-L2 genes were up-regulated in patients with tuberculosis indicating important roles in this disease via the activity of NK cells due to expression gamma-IFN. When the ligands and the PD-1 bind to each other, inhibition of the cell proliferation and cytokine production is performed[8-10]. Moreover, PD-1LG1 and PD-1LG2 were linked to upregulation occurred in CD11c, CD25, CD40, CD80, CD86, and MHC II via maturation of murine myeloid dendritic cells (DCs)[11].

In conclusion, the current study presents important information that could be used as a guide for future *in vitro* and *in vivo* studies involving these genes in links to the *Egr2* gene and the PD-1LG2 gene.

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