Research Article

Study the Expression of Human P53 Gene in Patients with Breast Cancer

Dawood S. Edan¹, Mohammed F. Al-Marjani², Nahi Y. Yaseen³

¹Department of Molecular biology, Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University, IRAQ.
²Department of Biology, College of Science, Mustansiriyah University, IRAQ.
³Head of Iraqi center for cancer and medical genetics research, Mustansiriyah University, IRAQ.

*Correspondence email: david80altaii@yahoo.com

Abstract

The gene P53 can activate DNA repair proteins when DNA has sustained damage. So, it is an important factor in aging. Also, P53 gene can also be modified by mutagens (chemicals, radiation, or viruses), increasing the likelihood for uncontrolled cell division. For this importance, 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 sample was used to check the expression of a suppressor gene (P53) via Real time PCR. We designed primers of P53 gene on encoded sequence (Exon), to make sure generation a specific PCR product of P53 gene by converting mRNA to cDNA then PCR Product. The expression of each sample was fluctuated between 0.1 fold to 0.2 fold compared with the control (the healthy sample). The highest expression showed was samples 7 (0.2 fold), and the lowest expression showed was samples 27 (0.1 fold) that compared with control expression (sample 19). While the control expression was the highest (0.25 fold) among all samples. The results indicate that tumor may affects on a suppressor gene (P53) and can decrease the gene expression, and eventually can decrease the P53 suppressor protein that can be used it in the cell protection against cancer.

Keywords: P53 gene, Gene expression, Breast cancer.

Introduction

Tumor protein P53, known as phosphoprotein P53, a cellular tumor antigen, tumor suppressor protein 53, or the transformation related protein 53 (TRP53). It is any isoform of a protein encoded by the homologous genes in many different organisms such as TP53 (humans) and Trp53 (mice). This homolog (originally thought to be as, a single protein) is crucial in multi-cellular organisms, it prevents cancer development. Thus, functions as a tumor suppressor [1]. P53 was described as "the guardian of the organism genome" because of its role in conserving stability in the eucaryotic cells by preventing genome mutations [2]. Hence, TP53 is named as a tumor suppressor gene. [3] [4] [5] [6] [7] The name p53 was called in 1979 describing the apparent of molecular mass; SDS-PAGE analysis was indicated that it was a 53-kilodalton (kDa) protein. Nevertheless, the actual mass of the full length p53 protein (p53α) based on the sum of masses of the amino acid residues
was only 43.7kDa. This difference was due to the high number of proline residues in p53 protein, which slow its migration on SDS-PAGE, thus making it appear heavier than it real appears. [8] In addition to the full length protein, the human TP53 gene encoded at least 15 protein isoforms, among 3.5 to 43.7 kDa. All these p53 proteins were called the p53 isoforms [1]. The International Cancer Genome Consortium has based that the TP53 gene was the most frequently mutated gene (>50%) in human cancer, indicating that the TP53 gene was played a crucial role in blocking cancer formation [1]. TP53 gene encodes proteins that bound to DNA and regularized gene expression to prevent mutations of the genome.

In human beings, a common polymorphism involved the substitution of an arginine for a proline at codon position 72. A lot of studies have investigated a genetic link between this variation and cancer susceptibility. A study in 2011 found that the TP53 proline mutation did have a profound effect on pancreatic cancer risk among males [9]. A study of Arab women found that proline homozygosity at TP53 codon (72) is associated with a decreased risk for the breast cancer [10] [11] [12]. One study suggested that TP53 codon 72 polymorphisms, p53 mutation still the most common genetic change named in human neoplasia. In the breast cancer, p53 mutation was associated with more aggressive disease and worse overall survival. The relative frequency of mutation in p53 is, however, lower in breast cancer than in other solid tumors. Change both epigenetic and genetic have been identified in regulators the activity of p53 and in some downstream transcriptional targets of p53 in breast cancers that expressed wild type p53. Molecular pathological analysis of the structure and expression of constituents of the p53 pathway is likely to have value in diagnosis, in prognostic assessment and ultimately in treatment of breast cancer [13]. Another study found the suppressor protein 53 homozygous (Pro/Pro) genotype was associated with a significantly increased risk for renal cell carcinoma [10] [14] [15] [16]. According to recent researches, P53 mutations are highly correlated in human breast cancer. The aim of this study was to find the expression of P53 gene of each patient carrying the breast cancer compared with the expression of P53 gene of healthy person.

Introduction and any other front-matter sections are unnumbered.

Keeping the concept of the introduction the same, different documents have different styles to introduce the written text. For example, the introduction of a Functional Specification consists of information that the whole document is yet to explain. If a User guide is written, the introduction is about the product. In a report, the introduction gives a summary about the report contents.

**Material and Methods**

**Primer Design**

The primers were designed specifically on MGMT gene (NCBI Reference Sequence: NM_001276761.1 and the sequence of nucleotides were synthesized in IDT company, USA). The sequences of primers (forward and reverse) were shown in Table 1.

**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 equivalented 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 (20µl): 10 P mole Primer F (MGMT) (1µl); 10 P mole Primer R (MGMT) (1µl); 2X Kapa one step master mix (10µl); RT. Enzyme (.4 µl); RNA (100ng) (5µl); dNTP (10mM) (1µl) and D. W (1.6µl). The PCR condition that used: Reverse transcription (5min, 42°C); Heat inactivation (3min, 95°C); Initial Denaturation (3min,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 (mx3000 Agilent).

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
<th>PCR product</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>d53 gene-F</td>
<td>GC1 TGC CAA GTA ATC GAT</td>
<td>273bp</td>
<td>Current study</td>
</tr>
<tr>
<td>d53 gene-R</td>
<td>GAA GAT GAC AGG CCC GAG</td>
<td>215bp</td>
<td>Current study</td>
</tr>
<tr>
<td>F: GAPDH Human</td>
<td>5'-GCC TCT TCA GAG GAG GAC-3'</td>
<td>152bp</td>
<td>Current study</td>
</tr>
<tr>
<td>5'-GCC TCT TCA GAG GAG GAC-3'</td>
<td>152bp</td>
<td>Current study</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Primer Sequencing of genes that were studied in gene expression of P53.
Results and Discussion

A total of 29 samples of women carrying breast cancer disease from Yarmook hospital in Baghdad were confirmed with the standard diagnostics. The \( p53 \) gene sequence is found in the GenBank. Initially, \( p53 \) gene was thought to specifically work as a suppressor protein, and subsequently was found to effect on cancer cell [3] [4] [6] [7].

The map of \( p53 \) gene was designed with its specific primers in order to determine all nucleotides that participate in forming a PCR product (Figures 2 and 3). 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 4.

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 \( P53 \) mRNA, the results showed that \( P53 \) expression depending on the cycle threshold measurement in each samples. The GAPDH gene was used as calibrator for subscription the endogenous expression from each sample. The P53 gene expression of each sample was between 0.1 fold to 0.2 fold compared with the control 0.25 fold (the healthy sample). The highest expression showed was samples seven with 0.2 fold, and the lowest expression showed was samples twenty seven with approximately 0.1 fold that compared with control expression. While the control expression was the highest (0.25 fold) among all patient samples. The results elucidate that tumor may effects on a suppressor gene (\( P53 \)) and can decrease the gene expression, and eventually can decrease the \( p53 \) suppressor protein that can be used in the cell protection against cancer, or may clear the mutations in suppressor protein (\( P53 \)) can prevent protection the cell from tumor formation [1].
compared with control (GAPDH). Each sample has been replicated triple times and the average calculated using the formula: ΔΔCt=(ΔCt Test sample-ΔCt Calibrator), relative quantification (ΔΔCt method), and finally as showed the fold-change has been calculated by the formula 2^ΔΔCt with ΔΔCt+s and ΔΔCt-s.

Acknowledgments
This work has been implemented in Iraqi research center of Mustansiriyah University.

References


