Let-7a Induces to Apoptosis of Breast Tumor Cells by Up-Regulation of P53

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Abstract
Let-7a is one of microRNA types which consider as suppressor of tumor in breast cancer, the low levels of let-7a in breast tumor that cause metastasis of tumor while the high expression that leads to reduce the tumor of breast cancer. In this study, we increased the expression of let-7a in MCF-7 breast tumor cell line by transfection this gene which cloned with topo-vector and detected the apoptosis by qRT-PCR for p53. We founded the apoptosis was increased in breast cancer cell line that treated with let-7a compared with the control. These data reinforce the suggestions which consider that let-7a as gene therapy in breast cancer.
Keywords: Let-7a, breast tumor, gene therapy, regulation of p53.

1. Introduction

Let-7 miRNA is one from these miRNAs which observed is repressed in multiple contrasting kinds of human cancer [1]. The decreasing of expression for *let 7* that associated with high-grade tumors, aggressive, and poor prognostic. Therefore, the high-rise of let-7 miRNA amount that correlated with prognostic and prolongate the survivalisms of patients [2]. *Let-7a* miRNA is known as tumor suppressor which contributes to apoptosis regulation, invasion and the other cellular functions of cell. One investigation showed that, *let-7a* and genes of apoptosis pathway including *p53* and *caspase-3* expression are lessened in tumoral tissues to compare with the normal tissues. Expression of *p53* is direct or indirect related to *let-7a* expression, that mean *let-7a* correlates with the pathways of apoptotic and anti-apoptotic which participate as a breast cancer regulator [3].

Much evidence that showed the important of miRNAs in breast cancer, in which observed that levels of miRNAs expression alter through cancer [4]. *Let 7* is responsible for multiple gene expression which these genes related with metastasis and stemness [5]. Several functional aspects that showed the effects of *let 7* on cancer which noticed in clinical, for vitro and for vivo observations. *Let 7* could be consider as tumor suppressor by suppress of oncogene expression in stemness [6]. Levels of *let-7* is correlate inversely with cancer stem cells percentage, when let-7a expression was increased that leads to reduce of the tumor [7]. *Let-7a* miRNA are known as tumor suppressor which participates in apoptosis regulation, offensive and the further cellular functions of cell. One explanation showed that, the expression of *let-7a* and genes of apoptosis including *caspase-3* and *p53* are reduced in tumoral tissues compared with the normal tissues. Expression of *p53* direct or indirect that related to *let-7a* expression, that mean *let-7* correlates with the pathways for apoptotic and anti-apoptotic as a breast cancer regulator [3].

Many genes of suppressive have been explored that correlated with let-7, p53 is one of these genes which related with cancer stem cells functions [8]. P53 is a key of regulators for mediating of cell death, cycle, and harm of DNA responses, that proven it is as effector on treatment of anti-cancer [9]. The correlation between let-7a and p53 can be regulated by multiple pathways, one investigates showed that the doxorubicin stimulated p53 and therefore that led to induce of let-7a expression by lessen of Lin28A [10], from this investigation can be conclude the presence of positive relationship between let-7a and p53 expression. Thus, we used in this study let-7a as anti-tumor of breast cancer by increasing the levels of this gene and analysis the effect of these increasing on p53 expression.

2. Materials and Methods

RNA Extraction and Revere Transcription. The RNAs from all samples was extracted using GENEzol™Tri RNA Pure Kit from Geneaid, according to the instruction enclosed with the kit. The RNA which extracted earlier was converted to cDNA by using of HiSenScript™ RH [-] RT PreMix Kit from INtRON.

Cloning and Transfection. *Let-7a* miRNA was amplified by PCR using cDNA as a template and primers showed in table (1) that used depending on Liu *et al.* for this purpose, exception adding start codon for the forward primer. The product of PCR was inserted in the plasmid vector according to the protocol that supplied from the Invitrogen that companied with CT-GFP Fusion TOPO® TA Expression Kit. *E. coli* HB101 competent cells which provided from Promega, were used for transformation with the recombinant vector pcDNA3.1/CT-GFP TOPO®. The recombinant vector which extracted from competent cell that transfection with MCF-7 cell line according ProFection® Mammalian Transfection System protocol from Promega.

QPCR for Detection Gene Expression assay. qPCR was used for detection of p53 expression. SYBR green-containing PCR kit (INtRON) was used for qRT-PCR, the initial qPCR step was 10 minutes at 95°C; then 40 cycles that consisted of a 15 seconds denaturation step at

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95°C followed by 1 minute annealing at 60°C and extension 30 seconds at 72°C [11]. The qPCR reactions were run on ExicyclerTM96 machine.

Table (1): Primer's sequencing		
Refrence	Sequence	Primer
[11]	F: ATGCGATTCAGTGAGGTAGTAGGTTGT R: TATGGTTGTTCTGCTCTCTGTCTC	Let-7a
[12]	F: CCTCAGCATCTTATCCGAGTGG R: TGGATGGTGGTACAGTCAGAGC	P53
[13]	F: TGCACCACCAACTGCTTAGC R: GGCATGGACTGTGGTCATGAG	GAPDH

3. Results

Acridine orange dye was used to distinguish normal tumor cells, dead cells and the early and advanced stages of programmed cell death through differential absorption of the dye components using fluorescent microscope.

Fluorescent test results showed the effect of let-7a in the process of cell death on MCF-7 compared with untreated MCF-7 control cells which appeared green and monolayer formation after 72 hours (figure 1-d). Early-stage of cell death can be marked on transfected MCF-7 cells by changes on the cells which were appeared as crescent shaped or yellow green acridine orange nuclear staining as appeared in figure (1-a) with 0.3 µg/ml concentration of recombinant vector, while the mid-stage of cell death the chromatin was condensed, and yellow nuclear acridine orange staining also can be seen with 0.3 µg/ml concentration of recombinant vector. In contrast, the late-stage of death can be distinguished by the fragmented chromatin with the orange nuclear ethedium bromide staining which can be seen with 0.3 µg/ml and 0.4 µg/ml of recombinant vector concentrations as shown in figures (1-a and 1-b). Necrotic cells showed uneven orange-red fluorescence at their periphery which the cells in the process were disintegrated that can be seen in all concentrations of recombinant vector but the most common with 0.6 µg/ml concentration of recombinant vector (figure 1-c).

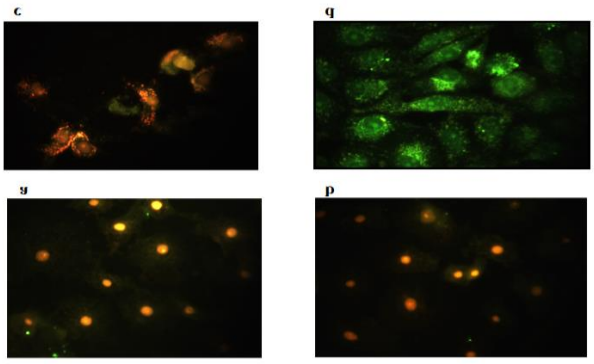

Figure 1: Elutions of transfections efficiency in MCF-7 breast cancer. (a): Represent image of MCF-7 breast cancer after 72 hours of transfection with 0.3 µg\µl of recombinant vector. (b): Represent image of MCF-7 breast cancer after 72 hours of transfection with 0.4 µg\µl of recombinant vector. (c): Represent image of MCF-7 breast cancer after 72 hours of transfection with 0.6 µg\µl of recombinant vector. (d): represent image of MCF-7 untreated control.

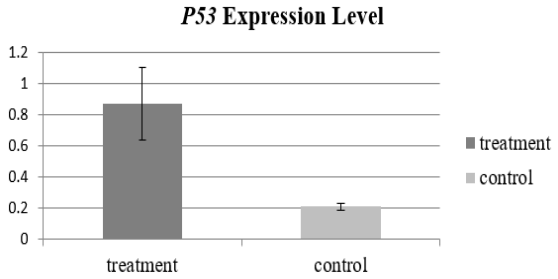

Figure 2: Expression level of p53 after 72 hours of transfection with let-7a. The expression of p53 is significantly higher in transfected MCF-7 breast cancer compared to MCF-7 untreated control.

The results of quantitative real-time PCR showed that p53 gene expression increased in transfected cells with let-7a compared with untreated control cells, using GAPDH as internal reference (figure 2).

4. Discussion

The breast cancer represents the most common types of cancers among the women in the world, therefore it is important using the development methods for diagnosis and management of this cancer. In the current study used MCF-7 cell line for the analysis of let-7a effect on breast cancer cells by using RNA as a starting point in gene expression of p53 because human breast cancer cell lines used in recent years for determine the effect of anticancer materials on the cancer as reducing of carcinoma in comparison with control cell lines [14, 15, 16]. Abnormal expression for several miRNAs plays critical role in breast carcinogenesis by the expression regulation of the target genes, which include tumor suppressor genes, differentiation, cell cycle and apoptosis [17].

Let-7 family members act seeing as tumor suppressors which considered as anti-tumor activity in the breast cancer which appear in low levels with breast cancer patients [18] therefore, high expression of let-7a in breast cancer patients that could be as useful for human cancer treatment.

Acridine orange dye was used to distinguish living and dead cells and the early and advanced stages of programmed cell death through differential absorption of dye components [19]. The stage of apoptosis can be diagnosed by the characteristic of the effected cells which in the early stage the nucleus appeared as yellow. In contrast, in the advanced stage of programmed death the nucleus appeared as red color. The figure (1) showed that some dead cells which appeared in red color and the nucleus was separated from the cytoplasm.

MicroRNA family members have been explain as regulator of p53 that lead to up-regulation of p53 then apoptotic induction [21]. The effect of let-7a miRNA on breast cancer cells growth dependent on up-regulation of p53. In the present study we raised let-7a miRNA levels in the MCF-7 cells by transfection that led to high expression of p53 in this cells compare with untreated control cells that confirms that let-7a as tumor suppressor of the breast cancer which investigated by Alexandri et al.

which explained let-7a targets more than 1000 projected genes including genes that involved in cancer apoptosis and DNA damage, and let-7a expression in the human breast tumors is correlated with apoptotic expression genes such as p53 [3]. Further studying showed that let-7a plays a critical role as a tumor suppressor in breast cancer [11], from these effect of let-7a on MCF-7 cells that can suggest using let-7a as gene therapy which agree with Esquela-Kerscher and Slack, the let-7 miRNA is consider the best example of replacement therapy using miRNA because of the expression of let-7 is reduced in cancer cell compared with the normal cell [24], while Dai et al. showed that over-expression of let-7 in animals model resulted that, reduced the size of tumor, metastasis and then prolonged survival. The other results explained in vitro functional assays, which founded let-7 decrease the proliferation and migration and invasion.

In this review we founded that high expression of let-7a in the breast cancer cell lines led to apoptosis which appear on morphological signs and high expression of p53 that indicate of let-7a as tumour suppressor in breast cancer. Therefore let-7a consider as gene therapy which that correspond with this explanation that, miRNA replacement therapy has appeared as very promising therapy approach which using miRNAs as a valued tool for cancer elimination [26]. Therefore, deeper investigate required to reconnoiter whether let-7a apply for physiological function using mouse models in breast tumor.

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