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
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.