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Role Of Let-7ain Breast Tumor Proliferation By Target Of PIK3CA And AKT1 Expression

Suhyila Fadhil Ali[1] , MohammedAl-Hajaj[2] and Rafid Adel Abood[3]

1&2: Department of Biology, College of Science, Basrah University.

3: Medical College, Basrah University.

Abstract

Let-7a is one of micro RNA family members and it consider as oncogene or tumor suppressor in different cancers. Breast cancer is one of these cancers which is effected by let-7a levels that founded, the levels of let-7a is down-expression in the breast tumor compare with healthy women. In this study, we determined thelet-7alevel in the breast tumor patients and compare with healthy women and increased the level of let-7a in the MCF-7 breast tumor cell line by transfection this gene which cloned with topo-vector and detected the proliferation by q RT-PCR for PIK3CA and AKT1.We founded that, the decrease of proliferation of breast cancer cell line compared with untreated control. These data reinforce the suggestions which consider that let-7a as tumor suppressor in the breast cancer and using as replacement therapy for breast cancer.

Introduction

Micro RNAs are known asa small non-coding regions in RNA which play a critical role as regulator in the expression of multiple genes at the post transcription [1]. Mi RNAs have important functions and effect on many functional processes including proliferation, cell differentiations, migration by act as regulators of gene expression [2]. When a single of mi RNA is deregulated, that effect on pattern of expression for several hundred mi RNAs [3], which cause the transformation of the cells [4]. Mi RNAs are encoded within the genomes of all multicellular organisms ranging from plants to animals, which mi RNAs became as an essential feature for development [5], thus mi RNAs are often correlated with diseases of human including diabetes [6], muscular dystrophy [7] and several types of cancer [8].The information about miR NAs are increased continuously now hold onto information for 1917 of human precursors and about 2656 of mature miRNAs [9].

Let-7miRNA is one of these mi RNAs which observed is reduced in multiple divergent kinds of human cancer [10]. The decreasing of expression for let-7 that associated with high-grade tumors, aggressive, and poor prognostication; therefore, the high-riseof let-7 mi RNA levels that correlated with prognostication and prolon gate patients survivalist [11], these studies encouraged the researchers to use let-7 mi RNA as replacement therapy in cancer.

Let-7 down-regulation promotes the proliferation of cells by activation of a variety signaling for cellular proliferation. Studies showed effect of let-7 as inhibitor of cell proliferation which occur transcriptional factors regulation, as STAT3, CBFB, GTF2I, YAP1 and ARID3A[12]. Let-7 also suppress proliferation of cells by regulation of Wnt signaling pathway [13]. Let-7miRNA effects on cellular proliferation rate that, decrease it by decreased proliferation of the cells in S phase of cell cycle [14,15].Let-7a decrease cell proliferation by inhibition CCR7 in the breast tumor cells [16], and also have various contributions in beginning and advancement of KRAS. The understanding the function of plethora mi RNAs associated with KRAS will grant insight for other therapeutic target as treatment[17]. Other studies showed that let7a inhibit the proliferation of cell by targets EZH2 and PKM2 [18]

Materials and Methods

RNA Extraction. The RNAs from breast cancer patients and healthy women and MCF-7 cell culture were extracted using GENEzol^{TM}Tri RNA Pure Kit from Gene aid, according to the instruction enclosed with the kit.

Revere Transcription. The RNAs which extracted earlier were converted to c DNA by using of Hi SenScript^{TM} RH[-] RT PreMix Kit from INtRON.

Let-7a Expression level. let-7amiRNAexpression detection in breast cancer patients and normal women by using primers showed in table (1), depending on Liuet al. for this purpose, exception adding start codon for the let-7aforward primer.

Detection proliferation in Cell Culture. Let-7atransfected with MCF-7 breast tumor cell line by topo-cloning vector, then detected proliferation of transfected MCF-7 breast tumor cell line and untreated control cells after 72 hours for treatment by q PCR according of the primers showed in table (1).SYBR green- containing PCR kit (INtRON) was used for q RT-PCR, the initial q PCR step was 10 minutes at 95ºC ; then 40 cycles that consisted a 15 seconds denaturation step at 95ºC then1 minute at60ºC and 30 seconds at 72ºC the q PCR reactions were run on Exicycler^{TM}96 machine.

STAT3= Signal transducer and activator of transcription 3, CBFB= Core-binding factor subunit beta, GTF2I= General transcription factor II-I, YAP1= yes-associated protein 1, ARID3A=AT-rich interactive domain-containing protein 3A, CCR7= C-C chemokine receptor type 7

Table (1): Primers Sequences

Primers	Sequences	References
Let-7a	F: ATGCGATTCAGTGAGGTAGTAGGTTGT R: TATGGTTGTTCTGCTCTCTGTCTC	[19]
U6snRNA	F: ATTGGAACGATACAGAGAAGATT R: GGAACGCTTCACGAATTTG	
PIK3CA	F: GGTTGTCTGTCAATCGGTGACTGT	[20]

	R: GAACTGCAGTGCACCTTTCAAGC	
AKT1	F: TTCTGCAGCTATGCGCAATGTG R: TGGCCAGCATACCATAGTGAGGTT	
GAPDH	F: TGCACCACCAACTGCTTAGC T R: GGCATGGACTGTGGTCATGAG	[21]

Results

The results of quantitative real-time PCR showed that let-7amiRNA were downward of regulate in breast cancer samples in compare with the normal women using U6sn RNA as internal reference figure (1).

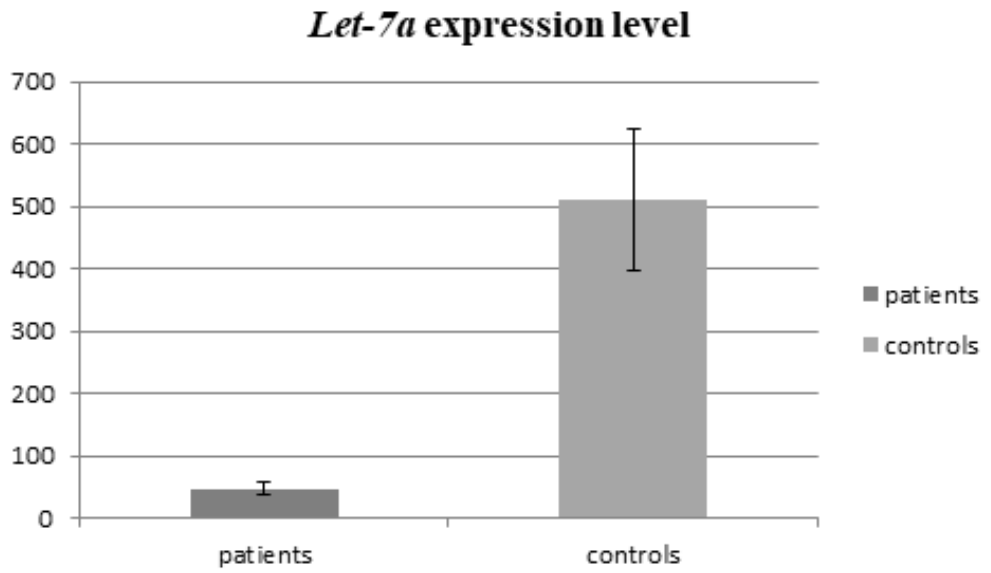

Figure 1: Expression level of *let-7a* in patients with breast cancer and normal women. The expression of *let-7a* is significantly lower in breast cancer patients compared to normal women.

The results of proliferation for transfected MCF-7 breast cancer cell line and untreated control showed decreased proliferation of transfected MCF-7 compared to control by using PIK3CA and AKT1, while GAPDH as internal reference figures (2,3).

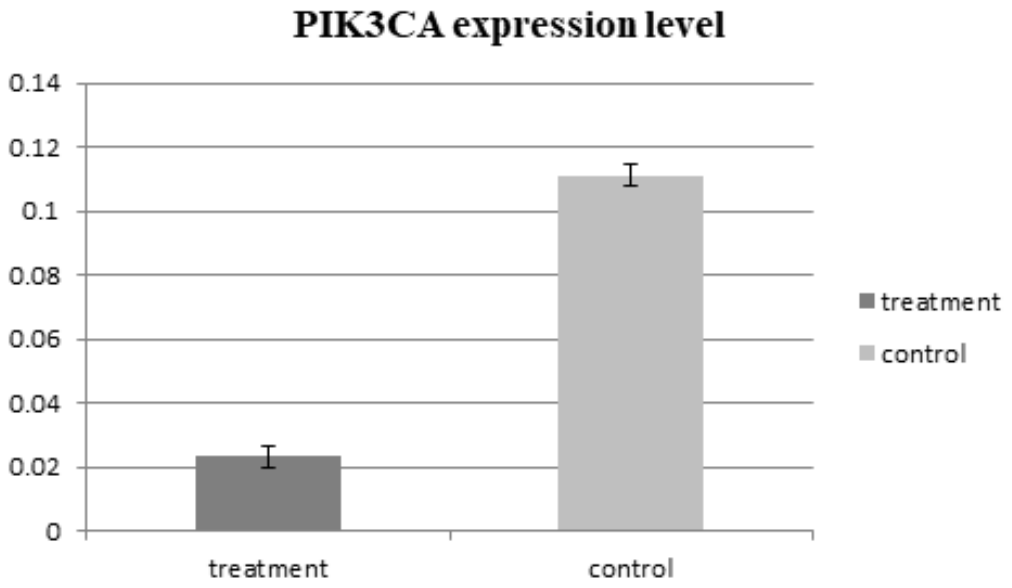

Figure 2: Expression level of PIK3CAafter 72 hours of transfection with *let-7a*. The expression of PIK3C is significantly lower in transfected MCF-7 breast cancer compared to MCF-7 untreated control.

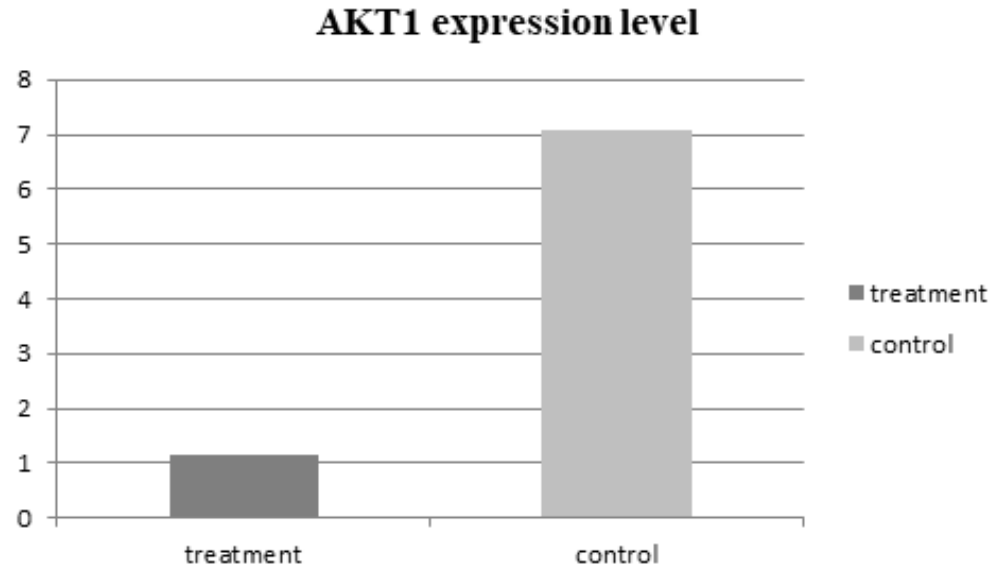

Figure 3: Expression level of AKT1 after 72 hours of transfection with *let-7a*. The expression of AKT1 is significantly lower in transfected MCF-7 breast cancer compared to MCF-7 untreated control.

Discussion

Thelet-7aamount decrease in patients with breast tumor, meaning that the let-7a could be a novelette biomarker of breast tumor [22], this explain agree with the present study which let-7a appear in high level in healthy women compare with breast cancer patients, also other studies suggested the low amount of let-7a in breast tumor such as Mansooriet al. appeared that reduce of let-7a expression in the breast tumoral tissue compare with the normal margin tissue.

Loss of the tumor suppressive essmi RNAs or obtain of oncogenic mi RNAs that cause to generate tumor and tumour development. In the rear decade mi RNAs have considered as a basis of regulators of a broaddomain of genes and signsthat participated inregulate of EMT/CSC features, like the PI3K/AKT pathways [24]. Therefore, we used PI3K and AKT in this study for determination the proliferation in transfected MCF-7 cell lines and compare with untreated control, genetical and tumor biology evidences give a demonstration that PI3K have certain outcomes on the tumor cell proliferation and survivabilities [25]. We founded the expression ofPI3K decreased in transfected cell lines compare with untreated cells that indicate of the growth of breast tumor cells decreased compare with control cell lines when this cells transfected with let-7amiRNA. This finding agree with suggestion that high amount of let-7a decrease the proliferation,let-7a is decreased the cells growth in breast cancer by inhibition of CCR7 [26] while Wang et al. founded that let-7a inhibit cells growth in breast cancer by targeting Lin28. AKT1 also showed in the present study decreasing level in transfected MCF-7 cell lines compare with untreated control, this is as indicator for proliferation decreasing in transfected cells with let-7a in comparison with untreated cell line. We choose PI3KAC/AKT1 as indicator for proliferation in breast cancer because it have important role in critical activity of cells such as metabolism, growth, proliferation angiogenesis and apoptosis [28] and it is normally this pathways changed in breast cancer this is agree with the findings explain that 30-40% of breast cancer patients present mutations in PIK3CA [29]. We conclude in this studying that let-7a mi RNA low amount in breast tumour compare with the normal women and when raise let-7a expression in breast cancer cells that lead to decrease of proliferation of this cancer cells, therefore investigate of adding studies showed other mi RNAs effects to other pathways of breast and other cancers.

References

1-O′Brien, j.; Hayder, H.; Zayed, Y and Peng, C.(2018). Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front. Endocrinal. 9: 402.

2-Lozano, C.; Duroux-Richard, I.; Firat, H.; Schordan, E. and Apparailly, F. (2019). MicroRNAs: Key Regulators to Understand Osteoclast Differentiation?. Front. immunol. 10: 375.

3-Jeansonne, D.; De Luca, M.; Marrero, L.; Lassak, A.; Pacifici, M.; Wyczechowska, D.; Wilk, A.; Reiss, K. and Peruzzi, F. (2015). Anti-Tumoral Effect of miR-3189-3p in Glioblastoma. J Biol Chem. 290:8067-80.

4-en-Hamo, R. and Efroni, S. (2015). Micro RNA regulation a molecular pathway as a genetic mechanism and as a core disease phenotype. Oncotarget. 6(3): 1594-604.

5-Dexheimer, P. J. and Cochella, L.(2020). Micro RNAs: From Mechanism to Organism. Front. Cell Dev. Biol. 8:409.

6-Bielska, A.; Niemira, M. and Kretowski, A. (2021). Recent Highlights of Research on miRNAs as Early Potential Biomarkers for Cardiovascular Complications of Type 2 Diabetes Mellitus. Int. J. Mol. Sci. 22:3153.

7-Aranega, A.E.; Lozano-Velasco, E.; Rodriguez-Outeirino, L.; deAcuna, F. R.; Franco, D. and Hernandez-Torres, F. (2021).Mi RNAs and Muscle Regeneration: Therapeutic Targets in Duchenne Muscular Dystrophy. Int J Mol Sci. 22(8): 4236.

8-Inoue, J. and Inazawa, J. (2021). Cancer-associated miRNAs and their therapeutic potential. Journal of Human Genetics. 66: 937-945.

9-Kozomara, A.;Birgaoanu, M. and Griffiths-Jones, S. (2018). miRBase: from microRNA sequences to function. Nucleic Acids Res. 47:D155–D162.

10-Ma, Y.; Shen, N.; Wicha, M.S. and Luo, M. (2021).The Roles of the Let-7 Family of MicroRNAs in the Regulation of Cancer Stemness. Cells. 10: 2415.

11-Zhang, W.; Liu. X.; Liu, S.; Qin, Y.; Tian, X.; Niu, F.; Liu, H.; Liu, N. and Niu, Y. (2017) Androgen receptor/let-7a signaling regulates breast tumor- initiating cells. Oncotarget 9:3690–3703.

12-Barh, D.; Malhotra, R.; Ravi, B.and Sindhurani, P. (2010). MicroRNA let-7: an emerging next-generation cancer therapeutic. CurrentOncology. 17: 70–8.

13-Jin, B.; Wang, W.;Meng, X.; Du, G.; Li, J.; Zhang, S.; Zhou, B. and Fu,Z. (2016). Let-7 inhibits self-renewal of hepatocellular cancer stem-like cells through regulating the epithelial-mesenchymal transition and the Wnt signaling pathway. BMC Cancer. 16:863.

14-Sun, H.; Ding, C.; Zhang, H. and Gao, J. (2016). Let7 mi RNAs sensitize breast cancer stem cells to radiationinduced repression through inhibition of the cyclin D1/Akt1/Wnt1 signaling pathway. Mol Med Rep. 14:3285–3292.

15-Liu, Y.; Yin, B.; Zhang, C.; Zhou, L. and Fan, J. (2012) Hsa-let-7a functions as a tumor suppressor in renal cell carcinoma cell lines by targeting c-myc. BiochemBiophys Res Commun. 417:371–375.

16-Shi, Y.; Cao, T.; Huang, H.; Lian, C.; Yang,Y.; Wang, Z.; Ma, J. and Xia, J. (2017). Arsenic trioxide inhibits cell growth and motility via up-regulation of let-7a in breastcancer cells. CELL CYCLE. 16: 2396–2403.

17-Ibrahim, H. and Lim, Y. C. (2020). KRAS-associated micro RNAs in colorectal cancer. Oncol Rev. 14(2): 454.

18-Tang, R.; Yang, C.; Ma, X.; Wang, Y.; LUO, D.; Xu, Z.; Liu, P. and Yang, L.(2016). MiR-let-7a inhibits cell proliferation, migration, and invasion by down-regulating PKM2 in gastric cancer. Oncotarget. 7:5972-84.

19-Liu, K.; Zhang, C.; Li, T.; Ding, Y.; Tu, T.; Zhou, F.; Qi, W.; Chen, H. and Sun, X. (2015). Let-7a inhibits growth and migration of breast cancer cells by targeting HMGA1. INTERNATIONAL JOURNAL OF ONCOLOGY. 46: 2526-2534.

20-Riquelme, I.; Tapia, O.; Espinoza, J.A.; Leal, P.; Buchegger, K.; Sandoval, A.; Bizama, C.; Araya, J.C.; Peek, R.M. and Roa, J. (2016). The Gene Expression Status of the PI3K/AKT/mTOR Pathway in Gastric Cancer Tissues and Cell Lines. PatholOncol Res. 22(4): 797-805.

21-Pattyn, F.;Robbrecht, P.; De, P.A.; Speleman, F.and Vandesompele, J. (2006).RTPrimer DB: the real-time PCR primer and probe database, major update. Nucleic Acids Res.34:D684–D688.

22-Heneghan, H.M.; Miller, N.; Lowery, A.J.; Sweeney, K.J.; Newell, J. and Kerin, M.J.(2010). Circulating micro RNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg. 251:499–505.

23-Mansoori, B.; Mohammadi, A.; Shirjang, S.; Baghbani, E. and Baradaran, B.(2016). Micro RNA 34a and let-7a expression in human breast cancers is associated with apoptotic expression genes. Asian Pac J Cancer Prev.17:1887–1890.

24-Luo, X.; Dong, Z.; Chen, Y.; Yang, L. and Lai, D.(2013). Enrichment of ovarian cancer stem-like cells is associated with epithelial to mesenchymal transition through an miRNA-activated AKT pathway. Cell Prolif. 46:436–446.

25-Molina, J.R.; Hayashi, Y.; Stephens, C. and Georgescu, M.M. (2010). Invasive glioblastoma cells acquire stemness and increased Akt activation. Neoplasia. 12:453–463.

26-Kim, S.J.; Shin, J.Y.; Lee, K.D.; Bae, Y.K.; Sung, K.W.; Nam, S.J. and Chun, K.H. (2012). MicroRNA let-7a suppresses breast cancer cell migration and invasion through downregulation of C-C chemokine receptor type 7. Breast Cancer Res. 14:R14.

27-Wang, L.; Wang, Y.X.; Zhang, D.Z.; Fang, X.J.; Sun, P.S. and Xue, H.C. (2016)**.** Let-7a mimic attenuates CCL18 induced breast cancer cell metastasis through Lin 28 pathway. Biomed Pharmacother. 78:301–307.

28-Lim, W.; Mayer, B. and Pawson, T.(2015). Cell Signaling: Principles and Mechanisms; Garland Science. New York, NY, USA.

29-Martínez-Sáez, O.; Chic, N.; Pascual, T.; Adamo, B.; Vidal, M.; González-Farré, B.; Sanfeliu, E.; Schettini, F.; Conte, B.; Brasó-Maristany, F.; Rodriguez, A.; Martinez, D.; Galvan, P.; Rodriguez, A.B.; Martinez, A.; Munoz, M. and Prat, A.(2020). Frequency and spectrum of PIK3CA somatic mutations in breast cancer. Breast Cancer Res. 22:45.