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RESEARCH ARTICLE

T lymphocyte cell-derived nano-size exosome impedes the proliferation and induces apoptosis of breast cancer MDA-MB231 cells by negative regulation of microRNA27

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ABSTRACT

Objective(s): To study the anti-cancer effects of the human immune T lymphocyte cell derived exosomes on breast cancer MDA-MB-231 cells in vitro.

Methods: The exosome was isolated from human T cells and then characterized by western blotting respecting the expression of CD9, 63 and 81 and also morphological analysis by transmission electron microscopy (TEM). The anti-cancer effect of T cell derived exosome was assessed on MDA-MB-231 cell lines using MTT assay and Annexin V/PI staining and flowcytometry. The Bax, Bcl-2 and MiR-27a expression at mRNA levels also were estimated using real-time PCR.

Results: Exosomes inhibited the proliferation of MDA-MB-231 cell lines within 12-72 hours of treatment. Also, intervention caused an increment the apoptosis levels of treated cells within 24 and 48 hours of treatment. Moreover, MiR-27a and Bcl-2 expression was decreased while up-regulating Bax expression in MDA-MB-231 cell lines.

Conclusions: In this study, T cell derived exosome elicits a robust anti-cancer effect on MDA-MB-231 cells in part by improving Bax/Bcl-2 ratio and decreasing MiR-27a expression and triggering the apoptosis pathway.

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INTRODUCTION

Breast cancer as the most shared malignanciy in women worldwide could be treated in 70-75 percent of patients more importantly in patients with non-metastatic disease. Unfortunately, developed breast cancer with widespread metastases is mainly noticed incurable with the conventional therapies [1]. Breast cancer is an heterogeneous malignancy and includes several molecular property such as human epidermal growth factor receptor 2 (HER2, encoded by ERBB2), estrogen receptor (ER), and progesterone receptor (PR) activation in association with breast cancer gene 1 (BRCA1) mutations [2]. Based on the recent knowledge, therapeutic methods change respecting the molecular subtypes [3]. The novel treatment option needs to be evolved in a multidisciplinary setting, with respect to the tumor's molecular subtype and regional load.

Dissimilar microenvironment ingredients of breast cancer, such as soluble mediators, inhibitory immune cells, and modified extracellular matrix (ECM) may provoke breast cancer development and metastasis. These components also could avert efficient antitumor immunity. Various stromal cells which were found in breast cancer microenvironment may complicate in the prediction if clinical outcomes. They frequently are haracterized by molecular modifications and irregular signaling axis [4, 5]. Today, immune cell therapy has attracted evolving attention in the context of breast cancer therapy. Immunotherapy circumvents immune cell tolerance and exhaustion thus inspiring the anti- tumor activity to fight cancer. This therapeutic approach includes but not restricted to immune checkpoint inhibitors

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(ICIs), cytokines, adoptive T cell therapy (ACT), and cancer vaccines [6, 7]. Meanwhile, growing evidences indicate that immune cell-derived exosomes can arbitrate communication between innate and adaptive immunity and thus moderate cancer development and metastasis.

Exosomes mainly are released by human cell, specifically tumor cell and immune cells. They are 30–150 nm in diameter, and could convey RNA, proteins and other biomolecules to target cells in vivo [8-10]. Exosome generation, release, and uptake of T lymphocyte cell-derived exosomes are tightly adjusted by intracellular proteins and extracellular incentives. T cell derived exosomes was found that could prohibit tumor cell progression [11, 12]. Of course, T cell- exosomes in some case was found that inhibit anti-cancer immunity in vivo [13]. Overall, they could show great capacity tumor diagnosis and immunotherapy and also could possibly be advanced for vaccination and chemotherapy agents transport.

In the current study, we assess the anti-cancer effects of the T cell derived exosomes on human breast cancer cell line MDA-MB-231 in vitro.

MATERIALS AND METHODS

Cell culture

The human blood samples were primed from healthy human donor for efficient isolation of T cell lymphocytes. Human PBMCs were firstly isolated utilizing the centrifugation by ficoll density gradient. After that, T lymphocytes were obtained by MACS concerning the producer instructions. Separated T lymphocyte then were cultivated in RPMI-1640 media (Sigma-Aldrich, Germany) enriched with 10% FBS (Sigma-Aldrich, Germany), and 1% pen/strep (PAA, Austria).

The breast cancer cells, MDA-MB-231 (ATCC), were expanded in DMEM enriched with FBS 10% and 1% pen/strep. Then, MDA-MB-231 and also isolated T cells were kept in humidified air with 5% CO2 at 37 °C.

Exosome isolation

T lymphocyte -derived exosomes were attained from the serum-free conditioned media (CM) by the responding kit (MagCapture™ Exosome Isolation Kit) concerning the producer recommendation. For efficient isolation of the exosome, the achieved CM was centrifuged at 10000 × g for 15 min to discard the other vesicles with similar properties.

Transmission Electron Microscopy (TEM)

Briefly, the procured exosomes' morphology was analyzed by the transmission electron microscopy (FEI Tecnai 12, Philips, Netherlands).

Western blotting

For evaluation of the CD9, 63 and CD81 expression, the parental T lymphocyte and isolated exosomes were firstly lysed in RIPA buffer (BioLegend, USA) and transported to PVDF membranes. Using primary and secondary antibodies, which were acquired from (Abcam, UK), the cited proteins expression was managed.

MTT assay

The anti-cancer influences T cell- exosome on MDA-MB-231, firstly 5×10^4 cells were seeded within in each wells on the 96-well plates. MDA-MB231 cells then were treated with 25-400 ng/ml concentrations of exosomes within 12-72 hours of treatment. Following the adding of the 10 µL of 5 mg MTT/ml to wells, the OD of the well was measured at 570 nm by ELISA reader.

Flow cytometric analysis of apoptosis

Utilizing the FITC Annexin V Apoptosis Detection Kit with PI (Biolegend, USA), MDA-MB-231 cells apoptosis was assessed following treatment with 100 ng/ml T cell-exosome at 24 and 48 hours of treatment. Then, 10 µL of propidium iodide (PI) and 5 µL of fluorescein isothiocyanate (FITC)-conjugated Annexin-V were added. By a FACSCalibur machine (BD Biosciences, USA), the fluorescent emission was recognized. Outcomes were assessed by FlowJo software.

RNA extraction and cDNA synthesis and Quantitative PCR

Exploiting the RNX Plus solution kit (Sinaclon, Iran) total RNA isolation was managed from MDA-MB-231 cells. Then, the cDNA was generated by the high-capacity kit (Bioneer, USA). For evaluation of the expression of candidate genes at mRNA levels, quantitative PCR was conducted. The generated cDNA, forward and reverse primers (Table 1), distilled water, and PrimeTime™ Master Mix (Idtdna, U.S.A) were used. Used primer pairs in Real Time-PCR, have been cited in Table 1.

Statistical Analysis

Statistical analysis was managed using

Table 1. Primer pairs used for Real-time PCR

Gene	Primer Sequence (5′-3′)	
Bax	F	TTTGCTTCAGGGTTTCATCC
	R	GCCACTCGGAAAAAGACCTC
Bcl-2	F	GGATTGTGGCCTTCTTTGAG
	R	CAGCCAGGAGAAATCAAACAG
MiR-27a	F	TTCACAGTGGCTAAGTTCCGC
	R	CGAATTCTAGAGCTCGAGGCAGGCGA
GAPDH	F	GAGTCAACGGATTTGGTCGT
	R	TTGATTTTGGAGGGATCTCG

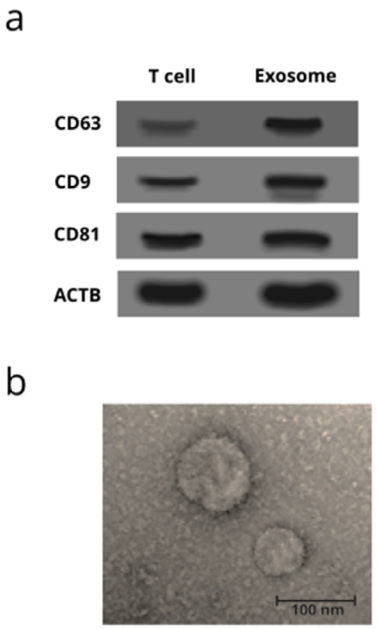

Fig. 1. The exosome characterizing by evaluation of the CD9, CD63 and CD81 expression using western blotting (a) and morphological analysis by TEM (b).

GraphPad Prism v7. The outcomes were showed as means ± SEM from 3 separate experiments and their differences were estimated using the Student's t-test. The P-value less than 0.05 were considered as statistically significant.

RESULT AND DISCUSSION

Exosome characterizing

Western blotting results verified the exosome identity based on expression of CD9, CD63 and CD81 on T cell derived exosomes (Fig. 1). Besides, morphological analysis by TEM verified the efficient isolation of the exosomes from T cells.

Exosome inhibits MDA-MB-231 proliferation

Concerning the MTT assay results, exosomes 25, 50, 100, 200 and 400 ng/ml abrogated the viability of MDA-MB-231 cell lines with 12-72 hours of exposure ($P<0.05$) (Fig. 2). Based on consequences, the suppressive impacts of exosomes on cell viability were time-dependent and dose-dependent ($P<0.05$) (Fig. 2).

Previously, it has been signified that exosomes derived from activated T-cell enhance the growth of other anti-cancer immune cells [14, 15]. Exosomes convey great quantity of microRNAs and affect the biological action of the recipient cells [16, 17]. Immunological synapses stimulate T lymphocyte cell exosome transporting between T lymphocyte and APCs. On the other hand, T cell-derived exosomes may induce ERK and NF-κB signaling axis in some cancers, such as melanoma cells,

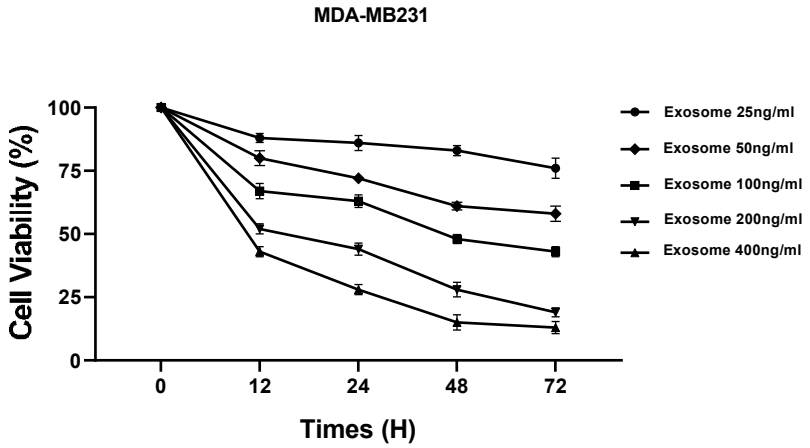

Fig. 2. MTT assay consequences based on the exosome effect on MDA-MB-231 cells within 12, 24, 48, and 72 hours of treatment. Data shows three independent tests. All values are expressed in mean ± SEM.

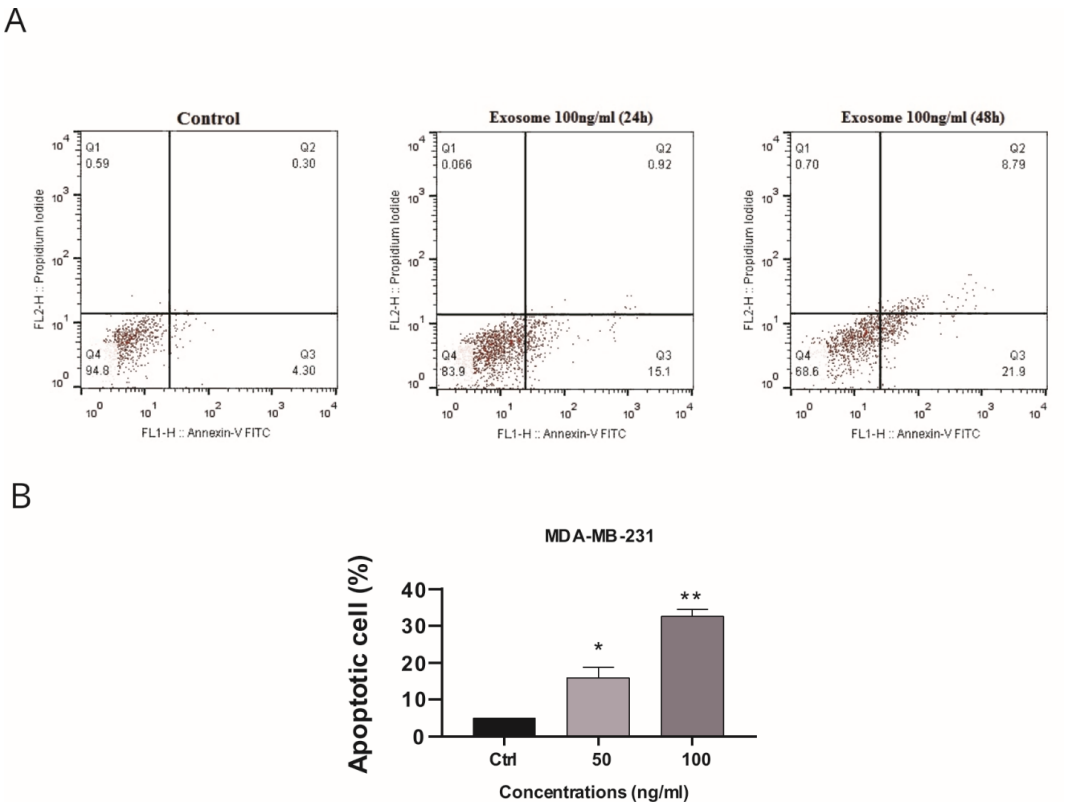

Fig. 3. The apoptosis percentages in MDA-MB-231 cells treated with exosome 100 ng/ml within 24 and 48 hours of treatment. The percentage of cells in each quadrant is demonstrated (viable cells are in Q4).

leading to enhanced metastases of cancerous cells in part by up-regulating matrix metalloproteinase-9 (MMP9) expression [18].

Exosome stimulated MDA-MB-231 cell apoptosis

The apoptosis percentages of MDA-MB-231 cells were treated with exosome 100 ng/ml within 24 and 48 hours of treatment with Annexin-V/PI staining and FACS analysis. Accordingly, exosoms 100 ng/ml resulted in a significant enhancement in the apoptosis percentages of MDA-MB-231 cells within 1 and 2 days of exposure ($P<0.05$) (Figs. 3A, B). The apoptosis

percentages in MDA-MB-231 cells (control) and MDA-MB-231 cells upon exposure with 100 ng/ml at 1 and 2 days of treatment were 5.31±1.08, 17.78±2.98, 31.07.14±2.06, respectively (Figs. 3A, B).

Exosome inhibited MiR-27a expression in MDA-MB-231 cells

The expression levels of MiR-27a were estimated by real-time PCR in MDA-MB-231 cells upon treatment with exosomes 50 and 100 ng/ml during the 24 and 48 hours of treatment (Fig. 4A). Accordingly, exosomes at 50 and 100 ng/ml concentrations down-regulated MiR-27a expression in MDA-MB-231 cell at 24 and 48 hours of treatment (P<0.05) (Fig. 4A). The suppressive effects were more evident at 100 ng/ml concentration and 48 hours of treatment.

Culminating evidences indicated the key role for miR-27a in adjusting cancer growth, metastasis along with chemotherapy resistance [19, 20]. The miR-27a is drastically related with the clinical phase and survival breast cancer patients. In this light, the improved levels of the miR-27a sustain breast cancer progression. Mechanistically, various tumor suppressor genes like ZBTB10, FOXO1 and prohibitin could be affected by miR-27a [21]. The miR-27a induces cancer development as well as metastasis through affecting zinc finger and BTB domain containing 10 (ZBTB10) [22]. Also, it reduces the expression of F-Box and WD Repeat Domain Containing 7 (FBXW7) to inspire epithelial-mesenchymal transition (EMT) and human breast tumor cell migration [23].

Exosome promoted Bax/Bcl-2 expression ratio in MDA-MB-231 cells

The expression levels of Bax and Bcl-2 were estimated by real-time PCR in MDA-MB-231 cells

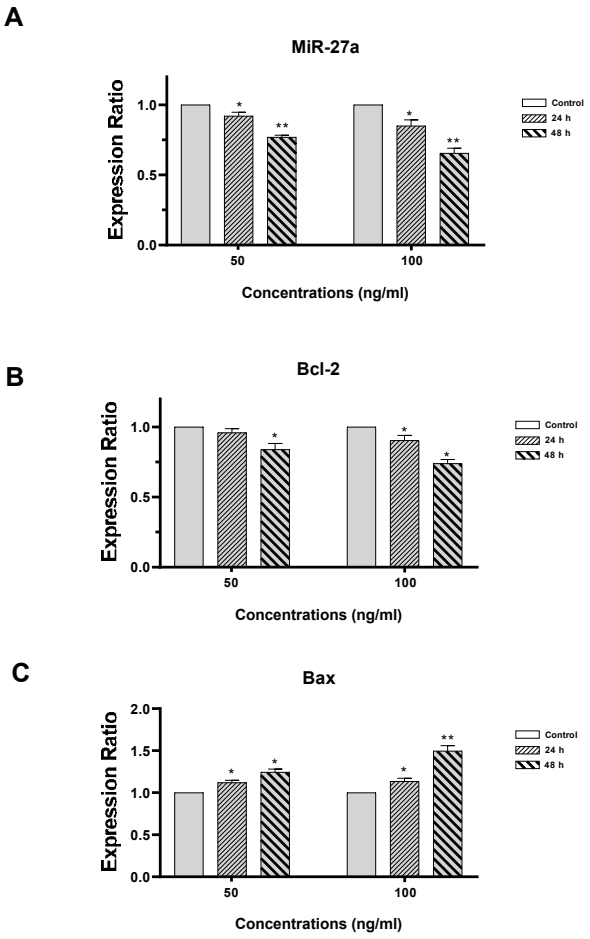

Fig. 4. The real time-PCR consequences for MiR-27a (A), Bcl-2 (B) and Bax (C) expression in MDA-MB-231 cell lines. Data is demonstrated as means ±SEM from three independent tests. P values <0.05 were noted as statistically significant. (* ; $p < 0.05$, ** ; $p < 0.01$)

upon treatment with exosomes 50 and 100ng/ml during the 24 and 48 hours of treatment (Fig. 4B, C). Accordingly, exosomes at 50 ng/ml concentration did not modify Bcl-2 expression in MDA-MB-231 cell at 24 hours of treatment ($P<0.05$) (Fig. 4B). However, exosome 50 ng/ml within 48 hours of treatment and exosome 100 ng/ml within 24 and 48 hours of treatment reduced Bcl-2 expression ($P<0.05$) (Fig. 4B). Besides, exosomes 50 and 100 ng/ml during the 24 and 48 hours of treatment improved Bax expression ($P<0.05$) (Fig. 4C). This effect was time and dose-dependent.

It seems that improved expression of Bax in association with down-regulated Bcl-2 expression was ensured as result of miR-27a down-regulation by T cell derived exosome.

CONCLUSION

In this study, T cell derived exosome elicits a robust anti-cancer effect on MDA-MB-231 cells in part by improving Bax/Bcl-2 ratio as result of MiR-27a up-regulation and triggering the apoptosis pathway. Also, study of the detailed underlying mechanism is urgently required.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Harbeck, N. and M. Gnant, Breast cancer. Lancet, 2017. 389(10074): p. 1134-1150. https://doi.org/10.1016/S0140-6736(16)31891-8
2. Katiyar, P., et al., Regulation of progesterone receptor signaling by BRCA1 in mammary cancer. Nucl Recept Signal, 2006. 4: p. e006. https://doi.org/10.1621/nrs.04006
3. Harbeck, N., et al., Breast cancer. Nat Rev Dis Primers, 2019. 5(1): p. 66. https://doi.org/10.1038/s41572-019-0111-2
4. Soysal, S.D., A. Tzankov, and S.E. Muenst, Role of the Tumor Microenvironment in Breast Cancer. Pathobiology, 2015. 82(3-4): p. 142-52. https://doi.org/10.1159/000430499
5. Hassanzadeh, A., et al., Mesenchymal stem/stromal cell-based delivery: a rapidly evolving strategy for cancer therapy. Frontiers in Cell and Developmental Biology, 2021. 9. https://doi.org/10.3389/fcell.2021.686453
6. Baxevanis, C.N., S.A. Perez, and M. Papamichail, Cancer immunotherapy. Critical reviews in clinical laboratory sciences, 2009. 46(4): p. 167-189. https://doi.org/10.1080/10408360902937809
7. Kouhbanani, M.A.J., et al., The inhibitory role of synthesized nickel oxide nanoparticles against Hep-G2, MCF-7, and HT-29 cell lines: The inhibitory role of NiO NPs against Hep-G2, MCF-7, and HT-29 cell lines. Green Chemistry Letters and Reviews, 2021. 14(3): p. 444-454. https://doi.org/10.1080/17518253.2021.1939435
8. Beheshtkhoo, N., et al., Green synthesis of iron oxide nanoparticles by aqueous leaf extract of Daphne mezereum as a novel dye removing material. Applied Physics A, 2018. 124(5): p. 363. https://doi.org/10.1007/s00339-018-1782-3
9. Mosleh-Shirazi, S., et al., Biosynthesis, simulation, and characterization of Ag/AgFeO2 core-shell nanocomposites for antimicrobial applications. Applied Physics A, 2021. 127(11): p. 1-8. https://doi.org/10.1007/s00339-021-05005-7
10. Nasirmoghadas, P., et al., Nanoparticles in cancer immunotherapies: An innovative strategy. Biotechnology progress, 2021. 37(2): p. e3070. https://doi.org/10.1002/btpr.3070
11. Zhang, X., et al., Administration of mircoRNA-135b-reinforced exosomes derived from MSCs ameliorates glucocorticoid-induced osteonecrosis of femoral head (ONFH) in rats. Journal of Cellular and Molecular Medicine, 2020. 24(23): p. 13973-13983. https://doi.org/10.1111/jcmm.16006
12. Xie, J.-y., et al., Angiopoietin-2 induces angiogenesis via exosomes in human hepatocellular carcinoma. Cell Communication and Signaling, 2020. 18(1): p. 1-13. https://doi.org/10.1186/s12964-020-00535-8
13. Zhou, Y., et al., Exosomes released by human umbilical cord mesenchymal stem cells protect against cisplatin-induced renal oxidative stress and apoptosis in vivo and in vitro. Stem Cell Res Ther, 2013. 4(2): p. 34. https://doi.org/10.1186/scrt194
14. Tavasolian, F., et al., The Impact of Immune Cell-derived Exosomes on Immune Response Initiation and Immune System Function. Curr Pharm Des, 2021. 27(2): p. 197-205. https://doi.org/10.2174/1381612826666201207221819
15. Yan, W. and S. Jiang, Immune Cell-Derived Exosomes in the Cancer-Immunity Cycle. Trends Cancer, 2020. 6(6): p. 506-517. https://doi.org/10.1016/j.trecan.2020.02.013
16. Patwardhan, S., et al., ECM stiffness-tuned exosomes drive breast cancer motility through thrombospondin-1. Biomaterials, 2021. 279: p. 121185. https://doi.org/10.1016/j.biomaterials.2021.121185
17. Ni, C., et al., Breast cancer-derived exosomes transmit lncRNA SNHG16 to induce CD73+γδ1 Treg cells. Signal Transduct Target Ther, 2020. 5(1): p. 41. https://doi.org/10.1038/s41392-020-0129-7
18. Cai, Z., et al., Activated T cell exosomes promote tumor invasion via Fas signaling pathway. J Immunol, 2012. 188(12): p. 5954-61. https://doi.org/10.4049/jimmunol.1103466
19. Lu, X., et al., MiR-27a-3p Promotes Non-Small Cell Lung Cancer Through SLC7A11-Mediated-Ferroptosis. Front Oncol, 2021. 11: p. 759346. https://doi.org/10.3389/fonc.2021.759346
20. Yao, X., et al., Endoplasmic reticulum stress-induced exosomal miR-27a-3p promotes immune escape in breast cancer via regulating PD-L1 expression in macrophages. J Cell Mol Med, 2020. 24(17): p. 9560-9573. https://doi.org/10.1111/jcmm.15367
21. Tang, W., et al., MiR-27 as a prognostic marker for breast cancer progression and patient survival. PLoS One, 2012. 7(12): p. e51702. https://doi.org/10.1371/journal.pone.0051702
22. Sun, B., et al., Adipose tissue-secreted miR-27a promotes liver cancer by targeting FOXO1 in obese individuals. Onco Targets Ther, 2015. 8: p. 735-44. https://doi.org/10.2147/OTT.S80945
23. Ben, W., et al., MiR-27a-3p Regulated the Aggressive Phenotypes of Cervical Cancer by Targeting FBXW7. Cancer Manag Res, 2020. 12: p. 2925-2935. https://doi.org/10.2147/CMAR.S234897