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MiR-490-5p Functions as an OncomiR in Breast Cancer by Targeting *NFATc4*

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ABSTRACT

Breast cancer is a serious health problem worldwide in women. MicroRNAs are small non-coding RNAs of 18–25 nucleotides in length that post-transcriptionally modulate gene expression. *MiR-490* has been reported as a tumor suppressor and oncomiR microRNA in breast cancer with two separate targets, NFAT and Rho. NFAT is one of the targets for miR-490 but the relationship between *hsa-miR-490* and *NFATc3*, *NFATc4* are not clear yet. Except for NFAT5, the other members of NFAT are activated by Ca^{2+} influx in the cell, either via the PLC-γ pathway or via store-operated Ca^{2+} entry, typically in T lymphoid cells. In a cross-sectional comparative study, peripheral blood samples were collected from 30 subjects with breast cancer and 30 healthy individuals as a control group. Gene expression analysis of peripheral blood mononuclear cells (PBMCs) was performed using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to study the *NFATc3*, *NFATc4*, and *hsa-miR-490-5p* gene expression alterations. As per the obtained results, a significant decrease was observed in the expression level of *NFATc4 (P<0.05)*, while *hsa-miR-490-5p* expression found to be elevated in PBMCs of breast cancer patient *(P<0.05)*. Expression changes were not significant for *NFATc3* gene *(P>0.05)*. Taken together findings of this study indicated that serum *hsa-miR-490-5p* acts as an oncomiR by direct targeting the *NFATc4*.

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Introduction

Breast cancer (BC) has an incidence of 11.6% among all types of cancer, accounting for 6.5% of mortalities worldwide (Pilevarzadeh *et al.*, 2019; Siegel *et al*., 2019). In Iran, BC is the fifth most common cause of death related to cancer comprising 24.4% of all cancers with the age-standardized rate (ASR) of 23.1 per 100,000 (Ghosn *et al.*, 2020). Therefore, early diagnosis is important to decrease mortality. There are two ways for early diagnosis, either non-invasive or invasive method. In the non-invasive methods, mammographic image analysis and blood analysis with circulating microRNA in the serum have been using in breast cancer and in the invasive method have been using surgical sampling or thick needle sampling (González-Patiño *et al*., 2019). MicroRNAs are short non-coding RNA with 18-25 nucleotides that binding to target mRNAs and play a role in post-transcriptional gene regulation (Aminisepehr *et al*., 2018). Perfect or near-perfect complementary binding of miRNAs to their target mRNAs negatively regulates gene expression in terms of accelerating mRNA degradation or suppressing mRNA translation. Many investigations have reported that miRNAs play crucial roles in numerous biological processes, such as cell cycle, cell proliferation, cell differentiation, apoptosis, metabolism, and cellular signaling (Tafrihi *et al*., 2019). There are several important microRNAs in cancer that *miR-490* is one of them. *MiR-490-3p* has been verified to suppress several cancers' proliferation, metastasis, and progression in lung cancer, colorectal cancer, and prostate cancer (Gu *et al*., 2014; Xu *et al*., 2015). In endometrial cancer, *miR-490-3p* acts as a tumor suppressor with two targets that c-Fos and TGFα as a direct

target in the 3′ UTR (Qu *et al*., 2017). The expression of *miR-490-5p* was gradually downregulated and transfection with *miR-490-5p* lentivirus reversed the differentiation ability of the human adipose-derived stem cells (*hADSCs*). Thus, *miR-490-5p* inhibits *hADSC* differentiation by suppressing bone morphogenetic protein receptor type II (*BMPR2*) expression (Yang *et al*., 2015). *MiR-490-3p* has been validated to act as a regulator of cell proliferation, migration, invasion, or in the EMT in hepatocellular carcinoma cells and vascular smooth muscle cells (Chen *et al*., 2014). Another recent study shows that the expression of *miR-490-5p* was significantly down-regulated in neuroblastoma (NB) tissues and cell lines that significantly decreased *miR-490-5p* levels were correlated with lymph-node metastasis stage and poor survival prognosis in NB patients (Wang *et al*., 2020). *MiR-490-5p* has been proven to act as an oncomiR, promotes cell proliferation and inhibits apoptosis in hepatocellular carcinoma by targeting *miR-490-5p*/*SOX2* signaling pathway (Cai *et al*., 2018). Previous studies have identified various miRNAs functioning as tumor suppressors in bladder cancer, including *miR-409-3p* that regulate the proliferation, migration, and invasion of bladder cancer cells by downregulating various oncogenes (Liang *et al.,* 2017). The relationship between *miR-490-5p* and Roundabout homolog 1(*ROBO1*) has been verified in Hepatocellular Carcinoma. *MiR-490-5p* inhibited cell proliferation, migration, and invasion, but promoted cell apoptosis of *Hep3B* cells by inhibiting *ROBO1* (Chen *et al*., 2019). Another study showed that *miR-490-5p* inhibits the proliferation of bladder cancer cells by targeting c-Fos (Mao *et al.,* 2015). Evaluation of *miR-490* was detected as a biomarker of disease activity among patients with Focal segmental glomerulosclerosis (FSGSClin) (Chung *et al.,* 2017). There are five members for the nuclear factor of activated T-cells (*NFAT*) family, that two members are very important in cancer and tumor progression (Hoey *et al*., 1995; Ho *et al*., 1995). *NFAT3(NFATc4)* and *NFAT4(NFATc3)* are activated by Ca^{2+} influx in the cell, either via the phosphoinositide phospholipase C (PLC-γ) pathway or via store-operated Ca^{2+} entry, typically in T lymphoid cells (Luo *et al.,* 1996; Mancini *et al.,* 2009). It has been reported that *NFAT* isoforms are overexpressed in human solid tumors (Pan *et al*., 2013). *NFAT4* activates transcription of downstream gene targets, thus directly linking calcium signaling to gene expression (Rao *et al.,* 1997). The pro-angiogenic role of *NFAT* signaling was first demonstrated in *NFAT3/NFAT4* null mice and the calcineurin B (*CNB1*) knockout mice (Graef *et al.,* 2001). Mice lacking *CNB*1 or both *NFAT3/NFAT4* genes die at mid-gestation due to disorganized vasculature and increased and deregulated expression of vascular endothelial growth factor A (*VEGFA*) (Maillet *et al.,* 2010). *NFAT* appears to modulate the expression of *VEGF* by regulating the transcription of *VEGF* receptor 1 (*VEGFR1*). *VEGF* stimulates PLC-γ receptor-mediated activation, increasing intracellular calcium levels that activate calcineurin to cause *NFAT* nuclear translocation (Schulz *et al.,* 2004). Though *NFAT* has an inhibitory effect on *VEGF* expression, *VEGF* can induce *NFAT* transcriptional activity by mediating its nuclear translocation (Jinnin *et al.,* 2008). *NFAT* activation by *VEGF* in endothelial cells also induces the pro-angiogenic factor granulocyte-macrophage colony-stimulating factor (GM-CSF) (Cockerill *et al.,* 1995). Inhibition of *NFAT4* reduces the secreted frizzled-related protein 2 (SFRP2)-stimulated angiogenesis in vitro, and inhibition of calcineurin with tacrolimus also blocks SFRP2-stimulated angiogenesis and angiosarcoma growth (Siamakpour-Reihani *et al.,* 2011). Moreover, proteins belonging to this family play a central role in inducible gene transcription during the immune response and T-cell activation, which are important in breast cancer (López-Rodríguez *et al., 2004).* Thus, this study aimed to evaluate the alteration of *hsa-miR-490-5p (mature microRNA), NFATc3* and *NFATc4* expression levels in peripheral blood mononuclear cells (PBMCs) obtained from breast cancer patients that relationship between the two groups (*miR-490* and *NFATc3*, and *NFATc4*) have not been investigated in breast cancer until now.

Materials and Methods

Samples collection

Venous blood samples (5 ml) were obtained from patients (n = 30) with breast cancer and control subjects (n = 30). The age of the patients was 29 to 61 years old. All of the healthy controls had no history of breast cancer diseases. Patient consent for all samples was obtained (ethics committee: IR.IAU.QOM.REC.1397.011); all pathological information of patients was gathered from the pathology department of the academic Imam Reza Hospital - 501(Tehran).

Isolation of peripheral blood mononuclear cells

PBMCs were isolated using Ficoll density-gradient centrifugation (Baharafshan, Tehran, Iran) from the whole blood. The tubes were centrifuged at 800*g* for 20 min. The PBMCs layer was transferred to a new canonical tube. The cells were washed twice in PBS (for 10 min at 400*g* followed by 200*g* for 5 min) and the supernatant was separated. Finally, the mixture was then transferred to a 1.5 ml microcentrifuge tube and kept at -70 °C for further testing.

RNA extraction and cDNA synthesis

RNA isolation was performed immediately after PBMC preparation. The mRNA from PBMCs was isolated using the RNA extraction kit (RiboEX Gene All, England) based on the manufacturer's instructions. To synthesize a cDNA from a cDNA synthesis kit (Fermentase, Germany) according to the manufacturer's instructions, which uses 1 µg of RNA and kept frozen at -20 °C until use.

Real-time PCR

For real-time PCR, Mic real-time PCR cycler instrument was used. The designed primer pairs for *NFATc3*, *NFATc4*, *hsa-miR-490-5p*, *RNU6* (internal control) were utilized (Table 1).

Table 1. Primers used in RT-qPCR

Locus	Primer (5'→3')	Amplicon size (bp)	Accession number
NFATc4	F: GCACCGTATCACAGGCAAGATG R: TCAGGATTCCCGCGCAGTCAAT	131	NM_001136022.2
NFATc3	F: CGGTTCCTGGTGCTGCTCG R: GAAGTCGAGCTCGTCGTGGG	246	NM_004555.4
MiR-490-5p	F: TGTTTTTGCCATGGATCTCCAG R: GTGCAGGGTCCGAGGT	74	MIMAT0004764
RNU6	F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTTGCGT	94	NR_002439.1

Cyber green fluorogenic nucleotide (Roche kit, Germany) was used for monitoring the cDNA amplification in the process of real-time PCR. Thermal cycling consisted of an initial denaturation step at 95 °C for 10 minutes followed by an amplification program repeated for 45 cycles. The amplification was done at 95 °C for 10 seconds, 61 °C (*NFATc3*), 61 °C (*NFATc4*), 62 °C (*RNU6*) and 59 °C (*hsa-miR-490-5p*) for 10 seconds, and 72 °C for 20 seconds with a single fluorescence acquisition at the end of the elongation step. The amplification specificity of each primer set was also controlled by a melting curve and the amount of mRNA target was assessed via the comparative cycle threshold ($2^{-\Delta\Delta Ct}$) method (Fig. 1).

Statistical analysis

Analysis of variance (ANOVA) test was applied to determine genes that were differentially expressed on one or more of the groups. Real-time PCR data were analyzed by $2^{-\Delta\Delta Ct}$ and using Excel (ver.2010), GraphPad PRISM (ver. 5.04) software for the correlation between the changes in *NFATc3*, *NFATc4*, and *hsa-miR-490-5p* expression levels in PBMCs. In the current study, the *p*-value less than 0.05 (p<0.05) was statistically considered significant.

Results

Gene expression analysis

Analysis of Real-time PCR data indicated that there are differences in the level of expression of genes among the groups. The expression of *NFATc4* in breast cancer patients was lower than the control group, and this was significant (p<0.05), but the expression of *NFATc3* was not significantly different (p>0.05). Also, changes in the expression of *hsa-miR-490-5p*, in the patients compared to the control group, was significant (p<0.05). However, *NFATc4* mRNA levels were significantly decreased by about 2-fold in all

patient samples as compared with the control group. The level of *NFATc3* did not differ significantly between the control and patient samples. The expression of *hsa-miR-490-5p* was significantly increased in patient samples as compared with the control group (Fig. 2).

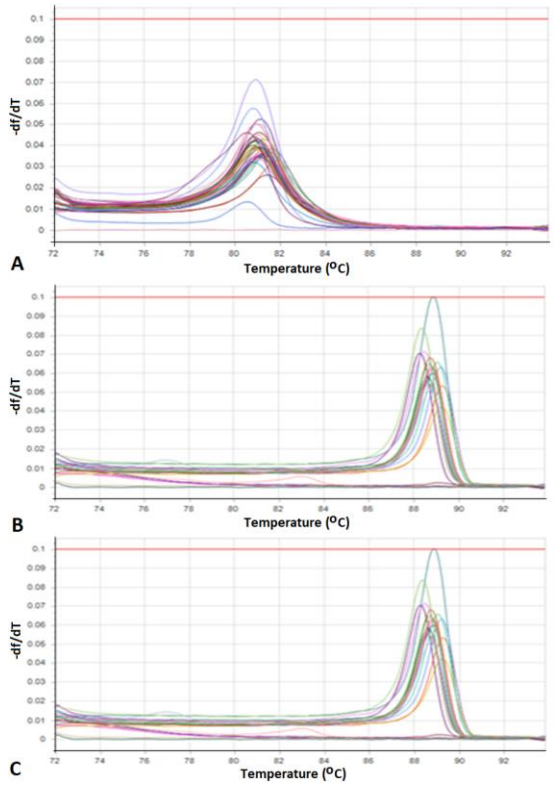

Fig. 1. Melting curves of Real-time PCR: A) *hsa-miR-490-5p*; B) *NFAT*; C) *RNU6*.

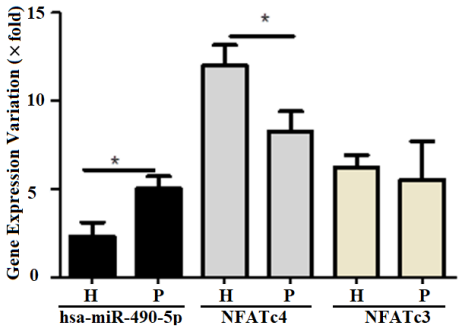

Fig. 2. The gene expression of *hsa-miR-490-5p* and *NFATc3*, *NFATc4*.

Our result showed that the expression of *hsa-miR-490-5p* and *NFATc3*, *NFATc4* were changed in PBMC (Fig. 2). The expression of *NFATc4* in breast cancer patients was lower than the control group, and this was significant (p<0.05). The expression of *NFATc3* was not significant (p >0.05). Also, changes in the expression of *hsa-miR-490-5p* in the patients compared to the control group, which was significant in patients (p<0.05).

Discussion

Men can get breast cancer, too, but they account for less than 1% of all breast cancer cases (Becker *et al*., 2010). Among women, breast cancer is the most second most common cancer diagnosed in women after skin cancer and the second leading cause of cancer deaths after lung cancer (Paluch-Shimon *et al*., 2020). On average, 1 in 8 women will develop breast cancer in her lifetime. About two-thirds of women with breast cancer are 55 or older. Most of the rest are between 35 and 54. Breast cancer usually begins in a small area of either produce milk (lobular carcinoma) or the ducts (ductal carcinoma), which carry it to the nipple (Wijayabahu *et al*., 2020). Some women will get breast cancer even without any other risk factors. Most women have some risk factors, but most women do not get breast cancer (Key *et al.*, 2001). There are several ways for early detection of cancer that blood analysis and circulating microRNA in the serum is the easy way (Shimomura *et al.*, 2016). There are two important roles in cancer for microRNA, either tumor suppressor or oncomiR. Tumor suppressor miRNAs exhibit their role by inhibiting × the expression of target mRNA (Jiang *et al.*, 2020). One of the microRNAs is *miR-490* with an important role in some cancer. The increase of *miR-490-5p* expression was showed to decrease EGFR expression to suppress bladder cancer (Wu *et al.*, 2019). *MiR-490-5p* on renal cancer cell was verified to directly bind to the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mRNA and reduce the expression of *PIK3CA* and inhibits phosphatidylinositol 3-kinase/Akt signaling pathway (Chen *et al.*, 2016). In the case of breast cancer, a significant decrease in *NFAT5* gene expression with *miR-490-5p* was observed and it is concluded that *hsa-miR-490-5p* acts as oncomir in serum (Nikfarjam *et al.*, 2019) while the relationship between *hsa-miR-490-5p* and *NFATc3*, *NFATc4* have not studied in breast cancer. *NFAT* is a family with five members that

except *NFAT5*, another member is activated either PLC-γ pathway or via store-operated Ca^{2+}. Phospholipase C gamma (PLC-γ) is one of the important signaling pathways in T-cell. There are several steps for Activate of PLC-γ. The first step is preferentially hydrolyzing of the membrane phospholipid phosphatidylinositol 4, 5-bisphosphate (*PIP2*) to generate the second messenger's diacylglycerol and inositol 1, 4, 5-trisphosphate (*IP3*). Diacylglycerol is retained within membranes where it recruits and activates numerous proteins including conventional isoforms of protein kinase C. In contrast, IP3 diffuses throughout the cytosol where it binds to IP3 receptors embedded in endoplasmic reticulum leading to mobilization of sequestered calcium. PLC-mediated depletion of PIP2 also modulates the activities of several ion channels and proteins with phosphoinositide-binding domains. Thus, the PLCs coordinate fluctuations in PIP2 levels and the bifurcating signaling pathways emanating from PIP2 hydrolysis to regulate numerous cellular processes, including fertilization and embryogenesis, cell proliferation and differentiation, as well as various types of cell migration (Mark *et al.*, 2006). Also, the overall structure is highly electronegative, and this property will inhibit lipase activity by disfavoring interactions with negatively charged membranes. In particular, the overall negative charge of the PH domain indicates that it is unlikely to bind phosphatidylinositol 3, 4, 5-trisphosphate as previously reported. For PLCs to hydrolyze membrane-embedded PIP2, the hydrophobic ridge of the catalytic TIM barrel must insert into lipid bilayers. However, in the case of Phospholipase C-gamma1 (*PLC-γ1*), the hydrophobic ridge interacts with portions of the serine protease homologs (sPH) domain in the regulatory array; this arrangement is expected to effectively block membrane engagement. The active site sits beneath the hydrophobic ridge and is readily located by the bound Ca^{2+} cofactor. As implied by the visibility of the Ca^{2+} cofactor, the active site is fully solvent-exposed and could presumably hydrolyze soluble substrates not embedded in lipid bilayers. Two major interfaces lock the regulatory array on top of the catalytic core. The first is the aforementioned sPH domain interacting with the hydrophobic ridge of the TIM barrel. A second interface is formed between loops of the cSH2 domain and the C_2 domain of the catalytic core. The pinched region of the C_2 domain is an additional membrane anchor point in the Phospholipase C- δ (PLC-δ) isozymes, where Ca^{2+} mediates between the C_2 domain and negatively charged membranes (Nishimura *et al.*, 2011; Lomasney *et al.*, 2012). Based on sequence conservation and overall charge distribution, this region of the C_2 domain of *PLC-g1* also seems likely to interact with Ca^{2+} and membranes. PLC-g2 is anticipated to engage Ca^{2+}, similarly. Therefore, the PLC-γ pathway and Calcium ion enter the nucleus causes T-cell activation. *NFAT* acts as a calcium sensor, integrating calcium signaling with other pathways involved in development and growth, immune response, and inflammatory response (Nishida *et al.*, 2003). *NFATC4*, a member of the nuclear factor of activated T cells (*NFAT*) family of transcription factors that are involved in immune cell signaling, survival, and angiogenesis (Mancini *et al*., 2009). *NFATc4* is anti-apoptotic and mediates cell survival in some tissues, such as neurons (Benedito *et al.*, 2005). In renal tubular cells, *NFATc4* was induced by carboplatin leading to increased apoptosis, which is assumed to mediate carboplatin-induced renal toxicity (Vashishta *et al*., 2009). Moreover, it may be due to activation of the calcium/calcineurin signaling pathway, which activates *NFATc4* and leads to upregulation of FasL and inducing the activation of caspase-8, leading to the activation of caspase-3, -6 and -7, and therefore apoptotic cell death (Kalivendi *et al*., 2005). Here, our investigation showed significantly high serum levels of *hsa-miR-490-5p* in breast cancer patients compared to controls. The obtained results illustrated that although *hsa-miR-490-5p* expression level is reduced, down-regulation was statistically significant. The findings also showed a significantly low serum level *NFATc4* in breast cancer patients. *NFAT* is important for the expression of Interleukin-2 (*IL-2*) and *IL-2* is necessary for activation of T-cell. Therefore, with decrease expression of *NFATc4* and disorder PLC-γ, FasL pathways, expression of T-cell, and apoptosis are decreased in the cell leading to promoting tumor progression, migration. Nevertheless, this is a preliminary

report demonstrating the release of *hsa-miR-490-5p* and its correlation with *NFATc4* in the serum of breast cancer patients.

Conflicts of interest

The authors have no competing interests.

References

Aminisepehr F, Babaei E, Hosseinpour Feizi MA. 2018. Study of the expression of miR-4270 in plasma of patients with breast invasive ductal carcinoma. *J Genet Resour* 4:85-89.

Becker TS, Moreira MA, Lima LA, De Oliveira ELC, Freitas-Júnior R. 2010. Pilomatrixoma mimicking breast cancer in man. *Breast J* 16:89-91.

Benedito AB, Lehtinen M, Massol R, Lopes UG, Kirchhausen T, Rao A, Bonni A. 2005. The transcription factor NFAT3 mediates neuronal survival. *J Biol Chem* 280:2818-2825.

Cai H, Hu B, Ji L, Ruan X, Zheng Z. 2018. Hsa_circ_0103809 promotes cell proliferation and inhibits apoptosis in hepatocellular carcinoma by targeting miR-490-5p/SOX2 signaling pathway. *Am J Transl Res* 10:1690-1702.

Chen K, Zeng J, Tang K, Xiao H, Hu J, Huang C. 2016. *miR-490-5p* suppresses tumour growth in renal cell carcinoma through targeting PIK3CA. *Biol Cell* 108: 41-50.

Chen S, Chen X, Xiu Y-L, Sun K-X, Zong Z-H, Zhao Y. 2014. microRNA 490-3P enhances the drug-resistance of human ovarian cancer cells. *J Ovarian Res* 7:84. doi: 10.1186/s13048-014-0084-4.

Chen W, Ye L, Wen D, Chen F. 2019. *miR-490-5p* inhibits hepatocellular carcinoma cell proliferation, migration and invasion by directly regulating ROBO1. *Pathol Oncol Res* 25:1-9.

Chung Y-H, Li S-C, Kao Y-H, Luo H-L, Cheng Y-T, and Lin P-R. 2017. MiR-30a-5p inhibits epithelial-to-mesenchymal transition and upregulates expression of tight junction protein claudin-5 in human upper tract urothelial carcinoma cells. *Int J Mol Sci* 18: 1826. doi:10.3390/ijms18081826.

Cockerill G, Bert A, Ryan G, Gamble J, Vadas M, Cockerill P. 1995. Regulation of granulocyte-macrophage colony-stimulating factor and e-selectin expression in endothelial cells by cyclosporin A and the T-cell transcription factor NFAT. *Blood* 86(7):2689-2698.

Ghosn B, Benisi-Kohansal S, Ebrahimpour-Koujan S, Azadbakht L, Esmaillzadeh A. 2020. Association between healthy lifestyle score and breast cancer. *Nutr J* 19:1-11.

González-Patiño D, Villuendas-Rey Y, Argüelles-Cruz A-J, Karray F. 2019. A novel bio-inspired method for early diagnosis of breast cancer through mammographic image analysis. *Appl Sci* 9:4492. doi:10.3390/app9214492.

Graef IA, Chen F, Chen L, Kuo A, Crabtree GR. 2001. Signals transduced by Ca2+/calcineurin and NFATc3/c4 pattern the developing vasculature. *Cell* 105: 863-875.

Gu H, Yang T, Fu S, Chen X, Guo L, Ni Y. 2014. MicroRNA-490-3p inhibits proliferation of A549 lung cancer cells by targeting CCND1. *Biochem Biophys Res Commun* 444:104-108.

Ho SN, Thomas DJ, Timmerman LA, Li X, Francke U, Crabtree GR. 1995. NFATc3, a lymphoid-specific NFATc family member that is calcium-regulated and exhibits distinct DNA binding specificity. *J Biol Chem* 270: 19898-19907.

Hoey T, Sun Y-L, Williamson K, Xu X. 1995. Isolation of two new members of the NF-AT gene family and functional characterization of the NF-AT proteins. *Immunity* 2: 461-472.

Jiang L, Cao S. 2020. Role of microRNA-26a in cartilage injury and chondrocyte proliferation and apoptosis in rheumatoid arthritis rats by regulating expression of CTGF. *J Cell Physiol* 235: 979-992.

Jinnin M, Medici D, Park L, Limaye N, Liu Y, Boscolo E. 2008. Suppressed NFAT-dependent VEGFR1 expression and constitutive VEGFR2 signaling in infantile hemangioma. *Nat Med* 14: 1236-1246.

Kalivendi SV, Konorev EA, Cunningham S, Vanamala SK, Kaji EH, Joseph J, Kalyanaraman B. 2005. Doxorubicin activates nuclear factor of activated T-lymphocytes and Fas ligand transcription: role of mitochondrial reactive oxygen species and calcium. *Biochem J* 389:527-539.

Key TJ, Verkasalo PK, Banks E. 2001. Epidemiology of breast cancer. *Lancet Oncol* 2:133-140.

Lewis BP, Burge CB, Bartel DP. 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120:15-20.

Liang Z, Wang X, Xu X, Xie B, Ji A, Meng S. 2017. MicroRNA-608 inhibit proliferation of bladder cancer via AKT/FOXO3a signaling pathway. Mol Cancer 16: 96. doi: 10.1186/s12943-017-0664-1.

Lomasney JW, Cheng H-F, Kobayashi M, King K. 2012. Structural basis for calcium and phosphatidylserine regulation of phospholipase C δ1. *Biochemistry* 51: 2246-2257.

López-Rodríguez C, Antos CL, Shelton JM, Richardson JA, Lin F, Novobrantseva TI. 2004. Loss of NFAT5 results in renal atrophy and lack of tonicity-responsive gene expression. *Proc Natl Acad Sci* 101: 2392-2397.

Luo C, Shaw K, Raghavan A, Aramburu J, Garcia-Cozar F, Perrino BA. 1996. Interaction of calcineurin with a domain of the transcription factor NFAT1 that controls nuclear import. *Proc Natl Acad Sci* 93: 8907-8912.

Maillet M, Davis J, Auger-Messier M, York A, Osinska H, Piquereau J. 2010. Heart-specific deletion of CnB1 reveals multiple mechanisms whereby calcineurin regulates cardiac growth and function. *J Biol Chem* 285: 6716-6724.

Mancini M, Toker A. 2009. NFAT proteins: emerging roles in cancer progression. *Nat Rev Cancer* 9: 810-820.

Mao XP, Zhang LS, Huang B, Zhou SY, Liao J, Chen LW. 2015. Mir-135a enhances cellular proliferation through post-transcriptionally regulating PHLPP2 and FOXO1 in human bladder cancer. *J Transl Med* 13: 86. doi:10.1186/s12967-015-0438-8.

Mark M, Ghyselinck NB, Chambon P. 2006. Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signaling pathway during mouse embryogenesis. *Annu Rev Pharmacol Toxicol* 46: 451-480.

Nikfarjam A, Pornour M, Sohrabi M, Vaseghi H. 2019. Correlation between Expression of hsa-miR-490-5p and NFAT5 in Peripheral Blood Mononuclear Cell Obtained from Breast Cancer Patients. *J Genet Resour* 5: 31-37.

Nishida M, Sugimoto K, Hara Y, Mori E, Morii T, Kurosaki T. 2003. Amplification of receptor signaling by Ca^{2+} entry-mediated translocation and activation of PLCγ2 in B lymphocytes. *EMBO J* 22: 4677-4688.

Nishimura K, Sano M, Ohtaka M, Furuta B, Umemura Y, Nakajima Y. 2011. Development of defective and persistent Sendai virus vector a unique gene delivery/expression system ideal for cell reprogramming. *J Biol Chem* 286: 4760-4771.

Paluch-Shimon S, Cardoso F, Partridge AH, Abulkhair O, Azim JH, Bianchi-Micheli G, *et al*. 2020. ESO-ESMO 4th International Consensus Guidelines for Breast Cancer in Young Women (BCY4). *Ann Oncol* S0923-7534(20)36363-8. doi: 10.1016/j.annonc.2020.03.284.

Pan M-G, Xiong Y, Chen F. 2013. *NFAT* gene family in inflammation and cancer. *Curr Mol Med* 13: 543-554.

Pilevarzadeh M, Amirshahi M, Afsargharehbagh R, Rafiemanesh H, Hashemi SM, Balouchi A. 2019. Global prevalence of depression among breast cancer patients: a systematic review and meta-analysis. *Breast Cancer Res Treat* 176(3):519-533.

Qu M, Li L, Zheng W. 2017. Reduced miR-490-3p expression is associated with poor prognosis of Helicobacter pylori induced gastric cancer. *Eur Rev Med Pharmacol Sci* 21:3384-3388.

Rao A, Luo C, Hogan PG. 1997. Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* 15: 707-747.

Schulz RA, Yutzey KE. 2004. Calcineurin signaling and NFAT activation in cardiovascular and skeletal muscle development. *Dev Biol* 266: 1-16.

Shimomura A, Shiino S, Kawauchi J, Takizawa S, Sakamoto H, Matsuzaki J, Ochiya T. 2016. Novel combination of serum microRNA for detecting breast cancer in the early stage. *Cancer Sci* 107: 326-334.

Siamakpour-Reihani S, Caster J, Nepal DB, Courtwright A, Hilliard E, Usary J. 2011. The role of calcineurin/NFAT in SFRP2 induced angiogenesis-a rationale for breast cancer treatment with the calcineurin inhibitor tacrolimus. *PLoS One* 6(6):e20412. doi: 10.1371/journal.pone.0020412.

Siegel RL, Miller KD, Jemal A. 2019. Cancer statistics, 2019. *CA Cancer J Clin* 69:7-34.

Tafrihi M, Hasheminasab E. 2019. MiRNAs: Biology, Biogenesis, Their Web-based Tools, and Databases. *MicroRNA* 8:4-27.

Vashishta A, Habas A, Pruunsild P, Zheng J-J, Timmusk T, Hetman M. 2009. Nuclear factor of activated T-cells isoform c4 (NFATc4/NFAT3) as a mediator of antiapoptotic transcription in NMDA receptor-stimulated cortical neurons. *J Neurosci* 29:15331-15340.

Wang J, Zhang X, Yao H, Le Y, Zhou W, Li J, Li X. 2020. MiR-490-5p functions as tumor suppressor in childhood neuroblastoma by targeting MYEOV. *Hum Cell* 33:261-271.

Wijayabahu AT, Egan KM, Yaghjyan L. Uterine cancer in breast cancer survivors: a systematic review. Breast Cancer Research and Treatment. 2020:1-19.

Wu L, Zhang M, Qi L, Zu X, Li Y, Liu L. 2019. ERα-mediated alterations in circ-0023642 and miR-490-5p signaling suppress bladder cancer invasion. *Cell Death Dis* 10: 1-11.

Xu X, Chen R, Li Z, Huang N, Wu X, Li S, Wu S. 2015. MicroRNA-490-3p inhibits colorectal cancer metastasis by targeting TGFβR1. *BMC Cancer* 15:1023. doi: 10.1186/s12885-015-2032-0.

Yang Z, Hao J, Hu ZM. 2015. MicroRNA expression profiles in human adipose-derived stem cells during chondrogenic differentiation. *Int J Mol Med* 35:579-586.