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Crosstalk between Melatonin Receptor M1 (MTNR1A) and Oxytocin Receptors (OXTR) Expression in the MCF7 Cell Line: A Novel Approach to Understand the Pathophysiology of Breast Cancer

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Abstract: ***Background: Melatonin's activity is mediated by the melatonin receptors MT1 and MT2. Melatonin promotes apoptosis, regulates pro-survival signaling and tumor metabolism, and suppresses angiogenesis and metastasis. Oxytocin works as a tumor growth regulator by activating the oxytocin receptor, a particular G-coupled transmembrane receptor. Furthermore, an oxytocin-inhibitory impact in mouse and rat breast carcinomas was examined and verified in vivo. Oxytocin secretion follows a circadian rhythm, which is especially noticeable during birth and breastfeeding since most labor phases begin at night when melatonin levels are high.Aim In this study, we will investigate the relationship between melatonin receptors expression M1 MTNR1A and Oxytocin Receptors (OXTR), to understand role of Melatonin Oxytocin correlation in pathogenesis of Breast cancer. Methods: (MCF - 7) breast cancer line and normal breast cell were studied through Real - Time Polymerase Chain Reaction (RT - PCR) to evaluate gene expression of melatonin receptor (MTNR1A) and oxytocin receptors (OXTR) expression and its correlation before and after MTNR1A plasmid transfection. Results: Our results revealed that mRNA MTNR1A expression was significantly low in patient with breast cancer compared to normal subjects also at the same samples the Oxytocin receptors (OXTR) is corelated with melatonin receptors expression as melatonin receptor M1 was high in normal subject the oxytocin Receptors is also was high and low when melatonin receptors was low is liner relationship which confirmed after melatonin receptor up regulated via plasmid transfection and level of apoptotic factors (P53,Bcl2and Caspase 3) Conclusion: Our data indicate that low gene expression of MTNR1A and Oxytocin Receptor (OXTR) in breast cancer tissue. In addition, confirmation of association between MTNR1A and Oxytocin Receptor (OXTR) expression may point to future use of melatonin and Oxytocin Receptor (OXTR) as molecular basis treatment of breast cancer.***

Keywords: MTNR1A, OXTR, P53, Bcl2, Caspase 3

1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is an indolic chemical released largely by the human and mammalian pineal gland in response to darkness(1). Melatonin production is found in various organs other than the pineal, including the retina, gastrointestinal system, skin, bone marrow, and lymphocytes(2). Melatonin production and secretion are controlled by the' master biological clock' situated in the hypothalamic suprachiasmatic nucleus (SCN) (3). Melatonin is regulated by the central circadian clock, but it may also modify the central circadian clock and peripheral oscillators in tissues and organs, making it a marker of circadian rhythms (4). Melatonin levels rise at night and fall throughout the day. Increased nighttime melatonin levels in the blood have been demonstrated in studies to deliver messages to the body's cells and organs that it is evening and to assist arrange target organs and organ systems into optimal homeostatic metabolic cycles (5). As a result, light at night (LAN) may disturb the circadian rhythm and melatonin production(6), which may play a role in the genesis, promotion, and progression of cancer Melatonin may have an oncostatic effect on certain kinds of cancers, according to epidemiological research. Furthermore, experimental investigations have shown that melatonin can prevent the development of various human tumor cells in vitro and in animal models. Antioxidant action, modulation of melatonin receptors MT1 and MT2, promotion of apoptosis, control of pro-survival signaling and tumor metabolism, suppression of angiogenesis, metastasis, and production of epigenetic changes are among the underlying processes. Melatonin might potentially be used as an adjuvant in cancer treatments, increasing the therapeutic effects while decreasing the negative effects of chemotherapies or radiation. Melatonin has the potential to be a great option for the prevention and treatment of numerous malignancies, including breast cancer and prostate cancer.

The hypothalamic nonapeptide oxytocin is involved in a range of reproductive and behavioral activities. However, oxytocin has recently been revealed to have a unique function in neoplastic disease. Oxytocin acts as a growth regulator in tumors by activating a specific G-coupled

transmembrane receptor, the oxytocin receptor. Oxytocin inhibits the development of epithelial (mammary and endometrial), neuronal, and bone neoplastic cells that express the oxytocin receptor in vitro. Furthermore, an oxytocin-inhibiting effect in mouse and rat breast carcinomas was investigated and validated on vivo (7).

In contrast to the impact previously seen in all other neoplastic oxytocin-responsive cells, oxytocin was shown to increase cell proliferation in neoplastic cells derived from two additional oxytocin target tissues, trophoblast and endothelium (7).

The signal transduction pathways associated with oxytocin's biological effects differ in oxytocin-inhibited and oxytocin-stimulated cells and may be altered by the position of the oxytocin receptor on the membrane. The inhibitory effect of oxytocin appears to be mediated through cAMP-protein kinase activation. In contrast to 'traditional' oxytocin transducers, a nonconventional oxytocin signaling route in which the mitogenic impact is connected to an increase in intracellular [Ca 2+] and tyrosine phosphorylation. Furthermore, the presence of the oxytocin receptor in caveolin-1-rich lipid rafts changes cell growth inhibition into a proliferative response, resulting in distinct patterns of epidermal growth factor receptor/mitogen-activated protein kinase activation.This surprising involvement of oxytocin (and oxytocin analogues) in controlling cell proliferation, as well as the ubiquitous expression of oxytocin receptors in neoplastic tissues of various origin, sheds fresh light on the oxytocin-oxytocin receptor system's biological significance in cancer (7).

Melatonin synergizes with OT to promote myometrial cell contractions and to facilitate gap junction activity in vitro. Such a synergy in vivo would promote coordinated and forceful contractions of the late term pregnant uterus necessary for parturition (8).

The oxytocin secretion has a circadian rhythm, which is extremely obvious in instances of labor and lactation because most labor stages begin at night, when melatonin levels are high. In this study, we will investigate the relationship between melatonin receptors expression M1 MTNR1A and Oxytocin Receptors (OXTR), to understand how melatonin may be anti and prevent breast cancer through Oxytocin action.

2. Material and Methods

2.1 Samples and materials

Ten identical plates cDNA which contain normal and contain normal sample breast cancer stage I, stage IIA, stage IIB, stage IIIA, stage IIIB, Stage IIIC, Stage IV, MTNR1A (GFP-tagged) - Human melatonin receptor 1A (MTNR1A) was purchased from OriGeneTechnologies, Inc.9620 Medical Center Drive Suite 200Rockville, MD 20850 USA. Primers see table 1, MCF-7 cells (HTB-22). Organism is Homo sapiens, human Cell type epithelial cell, Morphology Epithelial, Tissue Breast; Mammary gland disease is Adenocarcinoma.

2.2 Transformation of E. coli with Plasmid MTNR1A DNA

One microcentrifuge tube is labeled "+DNA," while the other is labeled "-DNA." Then, using a sterile 1 ml pipet, put 500 µl of ice-cold cacl2 solution into the "– DNA" tube. The next step was to use a toothpick to transfer 15 well-isolated colonies, each around 1-1. 5 mm in size, from the e. coli source plate to the "-DNA" tube. To liberate the cells, a toothpick was twirled between the fingers. Re-suspend the bacterial cells in the $Cacl_2$ solution by aggressively vortexing until no clumps of cells are visible and the cell suspension appears hazy. 250 µl of the cell suspension should be transferred to the tube labeled "+ DNA."

I put the tubes on ice. 10 µl plasmid MTNR1A DNA was added to the tube labeled "+ DNA." The plasmid was not inserted to the "-DNA" tube. For 10 minutes, place the tubes on ice. For 90 seconds, immerse the transformation tubes in a 42° C water bath. Return the tubes to the ice bucket immediately and incubate for two minutes. Using a sterile 1 ml pipet, 250 µl of recovery broth was added to each tube. and gently blended it with a flick of the tube The cells were then incubated for 30 minutes in a 37° C water bath while the cells recovered, with four agar plates labeled at the bottom as instructed by the procedure.250 µl of recovered cells were transferred using a sterile 1 ml pipette from the tube labeled "– DNA "to the middle of the specific plates 1 and 2 as described in the protocol, and 250 µl of recovered cells were transferred from the tube labeled "+DNA "to the middle of the specific plates 3 and 4 as described in our protocol. Using an inoculating loop, the cells spread throughout the entire plate. The plates were then covered for five minutes to allow the cell suspension to absorb by the agar. We stack the plates on top of each other and tape them together before placing them inverted (agar side up) in a 37° C bacterial incubation oven for overnight incubation (16-18 hours).

2.3 Transfection MCF7 by plasmid MTNR1A

We transplanted our cells the day before transfection such that they were 70% to 90% confluent at the time of your experiment. The Lipofectamine® LTX &PlusTM Reagent technique will be followed in a 24-well plate format. We produced four 50-µl tubes of Opti-MEM® Medium and labeled them 1 through 4. 2 µl Lipofectamine® LTX Reagent was added to Tube 1, 3 lµl to Tube 2, 4 lµl to Tube 3, and 5µl to Tube 4. We thoroughly mixed each tube by moving or whipping it. We created a tube with 250 µl of Opti-MEM® medium and 5 µg of plasmid DNA. We will add 5 µl of water because our DNA content is 1 µg per µl.
Then we add 5µl of Plus ™ reagent and thoroughly mix it together. 50 l of the diluted DNA should be added to each Lipofectamine® LTX dilution in tubes 1–4. We incubate the compound at room temperature for 5 minutes. We withdrew the 24-well plate holding our cells from the incubator after 5 minutes and transported them to the workstation in the lid. We transferred 50 µl of the DNA reagent complex from tubes 1 to 4 to wells 1 to 4 of a 24-well plate. After that, we returned our 24-well plate to the incubator and cultured cells for one day at 37 ° C. After incubating our cells, use GFP

fluorescence to determine transfection effectiveness in each well.

To make sure the transfection was done in right way we are going to measure MTNR1A before and after transfection. Figure 4 and 5

2.4 Real - Time Polymerase Chain Reaction (RT - PCR)

The qRT - PCR was carried out using an Applied Biosystems® 7900HT Real-Time PCR System with 2 LcDNA, 10 L 2X Sybergreen Master mix (150mM Tris, pH 9.2, 40mM (NH4) 2SO4, 5mMMgCl2, 0.02 percent Tween - 20, 0.4mM dNTPs, 1.25 Units Taq Polymerase, 1X Sybergreen) and 0.5µL of 20 µM gene (Table 1). Theoretical optimal conditions were used to design primers, which comprised primer melting temperature, primer annealing temperature, GC content, cross homology, and primer secondary structures. Bio – Basic Canada Inc. provided all primers (Ontario, Canada). The specificity and size of the PCR products were evaluated by including a melt curve at the conclusion of the amplifications and analyzing the bands on a 2 percent agarose gel. Isolation and sequencing of amplicon bands the reaction procedure included one activation cycle at 50°C for 2 minutes, followed by one at 95°C for 15 seconds. Following that, 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 2 minutes were conducted. Despite showing comparable trends when normalized to RPL13 and Ubiquitin C, all results were normalized to Ubiquitin C. For relative quantification in qRT-PCR studies, the 2−ΔΔCT method was used. The primers sequence for MTNR1A, Oxytocin Receptor genes aregiven in table 1.

Table 1: The primers sequence for MTNR1A, Oxytocin Receptor genes are

Primers	Forward	Reverse	Accession number
MTNR1A	GGGAATCCAAGCGGGCTC	GACATCAGCACCAACGGGTA	NM_005958
Oxytocin	TCCTGTACCCATCCAGCGA	TCCGCAGGCGAACCTAAAG	NM_000916
RPL13	CTTTCCGCTCGGCTGTTTTC	GGACTCCGTGGACTTGTTCC	NM_000977.4
P53	GAACAAGTTGGCCTGCACTG	GAAGTGGGCCCCTACCTAGA	NM_000546
Caspase3	TGGAACCAAAGATCATACATGGAA	TTCCCTGAGGTTTGCTGCAT	NM_004346
Bcl2	CTTTGAGTTCGGTGGGGTCA	GGGCCGTACAGTTCCACAAA	NM_000633.3

2.4 Ethical approval

This research was approved by the post graduate board of University of Gezira, Sudan, in 2018.

2.5 Statistical Analysis

The results were subjected to statistical analysis using SPSS version 24 (IBM Corporation, NY, USA). Data were expressed as means with standard error or standard deviation. Gene expression levels in the breast cancer tissuesamples were compared with the non - tumorous tissue samples (normal tissue), by using the paired t - test. Comparison between the multiple groups was performed by using the analysis of variance (ANOVA), followed by the in between - group comparisons with the Tukey HSD test if ANOVA yielded a significant difference. Correlation between variables was performed by using the Spearman test. Significance was considered for p levels less than 0.05.

3. Result

We used RT-PCR to compare melatonin receptor M1 encoding through MTNR1A and Oxytocin receptor mRNA expression in breast cancer patients with breast cancer to the to normal subject. Our results showed a higher significant upregulation of the MTNR1A gene in normal breast tissue when compared to breast cancer tissue ($P < 0.003$). MTNR1A, on the other hand, was significantly down regulated in breast cancer tissue compared to normal breast tissue ($P < 0.003$). Post hoc comparisons using the LSD test indicated that the mean mRNA MTNR1A receptor expression in normal subject (M = 16.1440, SD = 5.78660) is significantly different than stage I (M = -1.8636, SD = 3.69456), stage IIA (M = -5.9388, SD = 14.53858),stage IIB, (M = .4150, SD = 3.10389) ,stage IIIA (M = -.0163, SD = .65585) , stage IIIB (M = 1.3350, SD = .37477), stage IIIC (M = 1.4025, SD = 1.24936) stage IV (M = -3.3250, SD = 5.90826) and there is no significantly different between stages. **see figure 1**

Our findings revealed a more significant increase of the Oxytocin receptors mRNA gene expression in normal breast tissue than in breast cancer tissue ($p<.05$ level for [F] = 9.933, p = 0.000). Oxytocin receptor mRNA gene expression, on the other hand, was significantly lower in breast cancer tissue than in normal breast tissue ($p<.05$ level for [F] = 9.933, p = 0000].” According to Post hoc comparisons using the LSD test indicated that the mean mRNA Oxytocin receptor Expression in normal subject (M = 50.9860, SD = 37.05136) is significantly different than stage I (M = -2.8855, SD = 4.24135), stage IIA (M = 1.3550, SD = 2.16454),stage IIB, (M = -4.7200, SD = 8.59074) ,stage IIIA (M = -1.5688, SD = 3.26146) , stage IIIB (M = -1.6850, SD = .09192), stage IIIC (M = -.8875, SD = 1.46618) stage IV (M = -16.8075, SD = 25.55033) and there is no significantly different between stages **See figure 2**

When study the correlation between Melatonin M1 receptors expression and Oxytocin receptors expression we find that strong correlation. the Correlation is significant at the 0.01 level (2-tailed). There is correlation between MTNR1A and Oxytocin receptors expression r =0.601, N = 48 P = .000 **figure 3**

To confirm the correlation between MTNR1A and Oxytocin receptors expression we up regulated M1 receptors using plasmid transfection MTNR1A then study the effect of Transfection MTNR1A on Oxytocin receptors expression and apoptotic factors (P 53, Bcl2 and Caspase3) **see figure 4 and 5**

4. Discussion

Melatonin is essential for oxytocin action and potentate oxytocin activity, which in turn in preventing and anti-breast cancer.Melatonin hormone antagonizes cancer occurrence and cancer cell progression in several cancer types in vitro and in vivo. MTNR1A and MTNR1B are the essential receptors for melatonin exerting physiological activity. Oxytocin action exhibit circadian rhythm like melatonin secretion which indicated the possibility of relation between Melatonin oxytocin action. we investigated the expression of MTNR1A in association to OXTR expression in the different stages of breast cancer according to AJCC staging system classification Our results finding low melatonin and oxytocin receptors (OXTR) expression at the same cDNA Breast cancer samples. the correlation was confirmed via MTNR1A plasmid transfection after MTNR1A transfection Oxytocin receptors (OXTR) expression, P53, Caspase3 was increase and Bcl2 decrease

The direct melatonin on costatic effect might mediate through its binding with MTNR1A and MTNR1B specific G protein receptors in the cell membrane (19). Expression of MT1 melatonin receptor in human breast cancer cells both declines the basal in vitro proliferative rate and enhances the responsiveness of MCF - 7 cells to melatonin (20). MCF - 7-line breast cancer cells express low levels of the MTR1A receptor, they show a growth suppressive response to melatonin whereas the MDA - 231-line cells, which express low levels of the receptor are unresponsive to melatonin (21). In the presented results, MTNR1A was expressed in MCF - 7 breast cancer line in stage I, II and III whereas down - regulated in stage IV. Previous data also demonstrated the positive association between MTR1A gene expression and grade of malignancy in breast cancer (18). The MTNR1A gene expression was negatively correlated with the invasive abilities of breast cancer cell lines (22). Expression of the MTNR1A gene may raise the level of constitutively active receptors, result in a higher suppression of cellular proliferation malignant tumor.

4.1 Oxytocin and breast cancer

The neuropeptide hormone oxytocin, which is produced by the posterior pituitary gland, has a role in a variety of physiological activities. New study in this field is gradually enhancing our understanding of its impacts. While oxytocin is well known as a hormone that governs the reproductive system, it also affects the brain and cardiovascular system. Recent study has focused on elucidating its role in cancer, and preliminary evidence suggests that oxytocin could be employed as a cancer biomarker.

Following research have revealed that oxytocin reduces the proliferation of breast cancer cell lines such MDA-MB231, MCF7, and T47D(9, 10), well as the canine mammary cell line CMT-U27(11), mouse mammary carcinoma cell line TS/A, and rat mammary carcinoma cell line D-R3230AC(12). In human cell lines, this action was revealed to be mediated by the cyclic adenosine monophosphate protein kinase A(13). importantly, both oxytocin and its counterpart F314 were found to have anti-proliferative and tumor suppressive characteristics in vivo in both rat and mouse experimental models (12). Exercise training has recently been suggested to lower the expression of particular signaling proteins linked in breast cancer by promoting oxytocin production (14).

Lactation has long been associated with a lower risk of canc er, with studies reaching back to the 1950s (15, 16). Breast feeding has been demonstrated to reduce the risk of both breast and uterine cancer around the world, with longer periods of breastfeeding (typically involving many children breastfed) corresponding with a gradual reduction in both breast and uterine cancer risks (17, 18). The function of oxytocin as a paracrine and endocrine hormone in lactation could explain the link to uterine cancer.

While the links between oxytocin, lactation, and breastfeeding and a lower risk of breast and uterine cancer have all been well established, further research is needed to determine whether the link between oxytocin, lactation, and breastfeeding and a lower risk of breast and uterine cancer is causative. The discovery of such a link could lead to the development of new cancer treatment targets (19).

Many investigations have backed up our findings of a substantial link between melatonin and oxytocin. Melatonin (MEL), a monoamine hormone secreted by the epithalamic pineal gland, is a key molecule in the light-dark cycle's nocturnal phase. MEL communicates with the world through two G protein-coupled receptors, MEL receptor 1 (MT1R) and MEL receptor 2 (MT2R) (20). We previously demonstrated that the human myometrium is a MEL target and expresses both MEL receptors (21). These findings suggested that the OT and MEL signaling pathways may interact in some way. MEL potentiates norepinephrine-induced contractility in human myometrial strip in a dose-dependent manner, according to a previous study. The median serum melatonin concentrations in late-term and post-term pregnancies with and without labor were substantially lower than in term pregnancies (p0.05). The highest median melatonin concentration was found in term pregnancies with labor (p0.05). With the measurement of serum melatonin in women with term pregnancies, it is feasible to forecast the development of late-term and post-term pregnancies, as well as whether these pregnancies will go into spontaneous labor (22).OXTR expression is considerably reduced in breast cancer tissues when compared to non-cancerous tissues(23-25).Overexpression of OXTR activated the prolactin (PRL)/p-STAT5 pathway, resulting in a milieu that encourages breast cancer. Bromocriptine (Br), a PRL inhibitor, may slow the growth of OXTR-driven mammary tumors. The study reveals Oxtr is an oncogene and a potential therapeutic target for HER2-type breast cancer (26).

Social bonding, stress, maternal behavior, sexual activity, uterus contraction, milk ejection, and cancer are all biological responses elicited by the cyclic neuropeptide oxytocin (OT) via the oxytocin receptor (OTR) in both the central and peripheral neural systems. OTR is an appealing target for cancer therapy because it is a typical member of the G protein-coupled receptor family. OTR appears to play a role in the genesis and progression of breast cancer, and various breast cancer cell lines express it. Despite evidence

that OT reduces the incidence of breast cancer, its molecular role in breast cancer formation and related signaling pathways remains unknown (27).

The status and expression of the ER influence the level of OXTR expression in breast tumor tissues. Furthermore, OXTR expression is linked to the degree of HER2 expression in luminal BC. The lack of an increase in the OXTR mRNA level in ER-negative MDA-MB-231 cells and an increase in the OXTR mRNA level in MCF-7 cells suggest that the shift in the expression of OXTR in BC tissues may be driven by enhanced ER expression. To validate or disprove the link between OXTR and the presence of metastases, more research with a bigger sample of patients is needed. (28).

We can say now according to our data and previous study melatonin can prevent and inhibit breast cancer through up regulation oxytocin receptors expression in breast tissue and low melatonin hormone and its receptors is risk factor for breast cancer development

5. Conclusion

Our data indicate that low gene expression of MTNR1A and Oxytocin Receptor (OXTR) in breast cancer tissue. In addition, confirmation of association between MTNR1A and Oxytocin Receptor (OXTR) expression may point to future use of melatonin and Oxytocin Receptor (OXTR) as molecular basis treatment of breast cancer.

6. Conflicts of Interest

The authors confirm that there are no conflicts of interest.

7. Acknowledgements

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9. Authors' Contributions

Mowafag HMKhedir, Amal Saeeddesigned the research proposal, planned, the experimental work, data collectionand analysis. Mowafag Khedirconducted the laboratory work, Mowafag Khedirinitial draft of the manuscript according to the journal format (table and graph format). Amal Saeed provided critical feedback throughout all the research steps and reviewed the manuscript.

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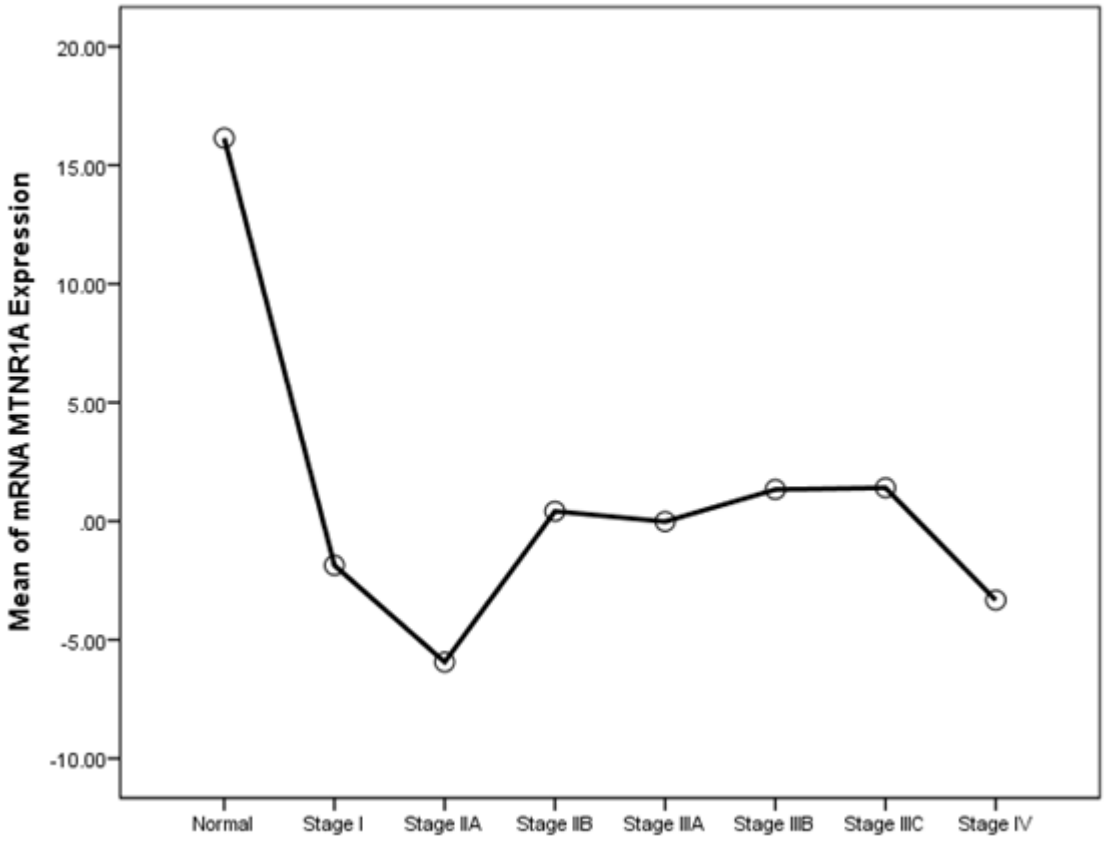
Figure 1 Melatonin receptor MTNR1A expression in patient with breast cancer compared to normal subject.High mRNA MTNR1A gene in normal breast tissue when compared to breast cancer tissue ($P < 0.003$). Post hoc comparisons using the LSD test indicated that the mean mRNA MTNR1A receptor expression in normal subject (M = 16.1440, SD = 5.78660) is significantly different than stage I (M = -1.8636, SD = 3.69456), stage IIA (M = -5.9388, SD = 14.53858),stage IIB, (M = .4150, SD = 3.10389) ,stage IIIA (M = -.0163, SD = .65585) , stage IIIB (M = 1.3350, SD = .37477), stage IIIC (M = 1.4025, SD = 1.24936) stage IV (M = -3.3250, SD = 5.90826) and there is no significantly different between stages.

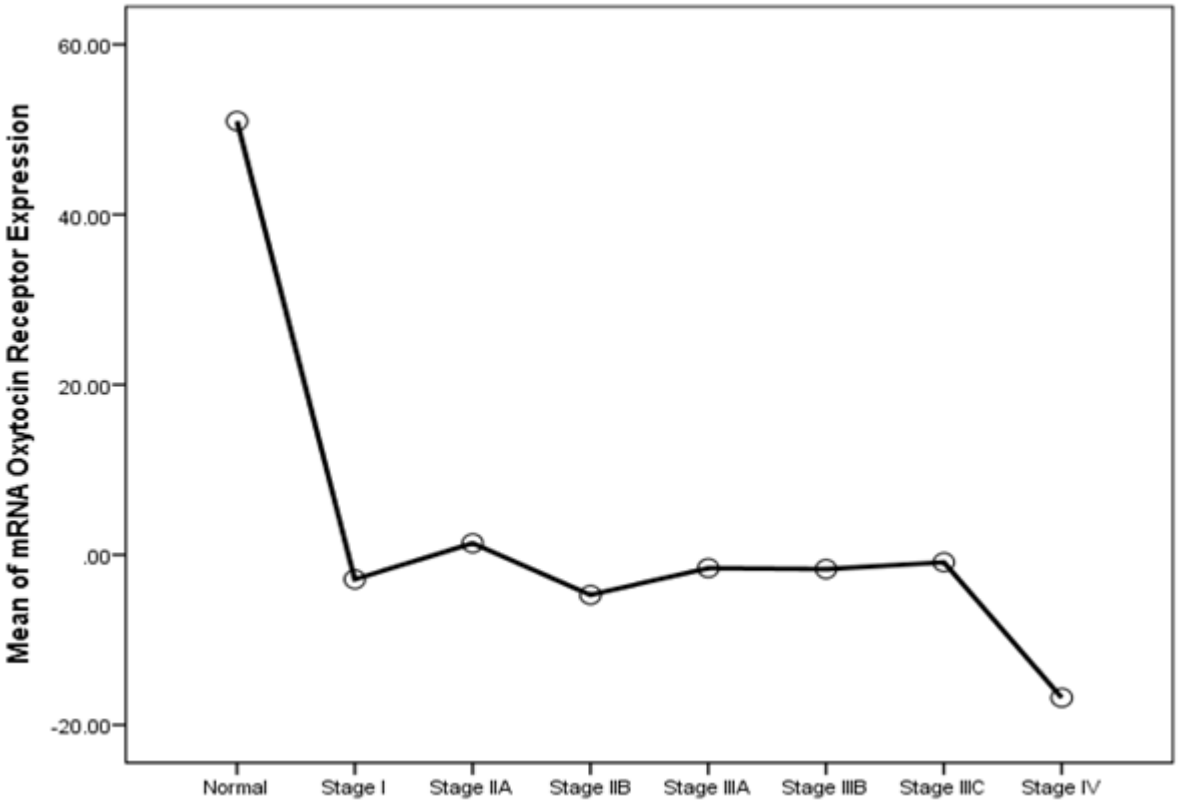
Figure 2 High Oxytocin receptors mRNA gene expression in normal breast tissue than in breast cancer tissue ($p<.05$ level for [F] = 9.933, p = 0.000). Oxytocin receptor mRNA gene expression. According to Post hoc comparisons using the LSD test indicated that the mean mRNA Oxytocin receptor Expression in normal subject (M = 50.9860, SD = 37.05136) is significantly different than stage I (M = -2.8855, SD = 4.24135), stage IIA (M = 1.3550, SD = 2.16454),stage IIB, (M = -4.7200, SD = 8.59074) ,stage IIIA (M = -1.5688, SD = 3.26146) , stage IIIB (M = -1.6850, SD = .09192), stage IIIC (M = -.8875, SD = 1.46618) stage IV (M = -16.8075, SD = 25.55033) and there is no significantly different between stages.

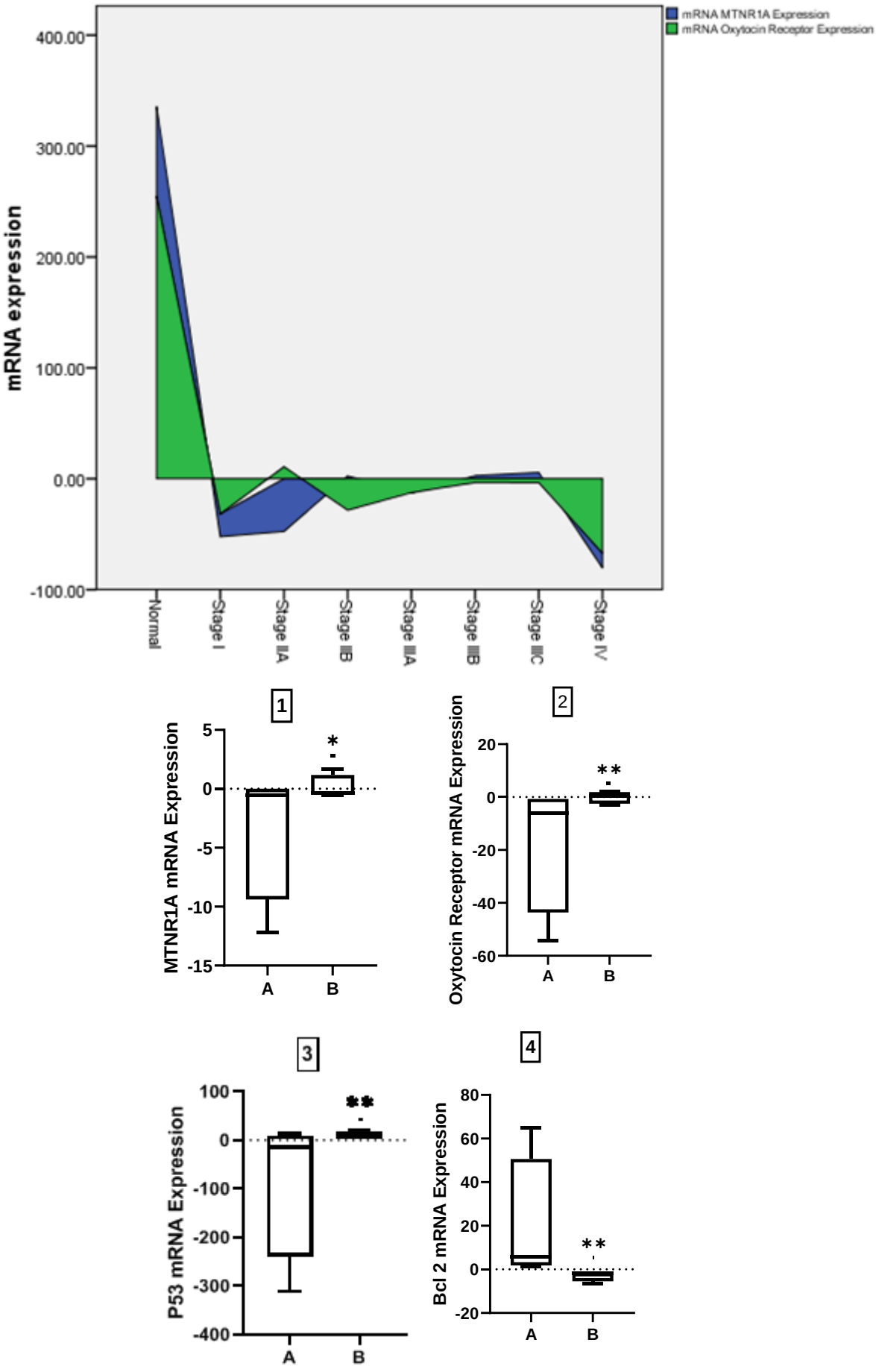
Figure 2 Correlation between mRNA MTNR1A Expression and mRNA Oxytocin Receptor Expression. the Correlation is **significant at the 0.01 level (2-tailed). There is correlation between MTNR1A and Oxytocin receptors expression r =0.601, N = 48 P = .000

Figure 4 Demonstrated fivefigures (1-5) each figures contain (A) &(B): - (**A) Represent the MCF7 Before MTNR1A transfection (B) Represent the MCF7 After MTNR1A Transfection. (1)** Unpaired t test was significant Differences between MTNR1A expression Before and After transfection MTNR1A P value = 0.0172 **(2)** Unpaired t test was significant Differences between Oxytocin Receptors expression Before and After transfection MTNR1A P value = 0.0025**(3)**Unpaired t test was significant Differences between **P53** mRNA expression Before and After transfection MTNR1A P value = 0.0035**(4)**Unpaired t test was significant Differences between **Bcl2** mRNA expression Before and After transfection MTNR1A P value = 0.0016**(5)**Unpaired t test was significant Differences between **Caspase 3** mRNA expression Before and After transfection MTNR1A P value = 0.0004

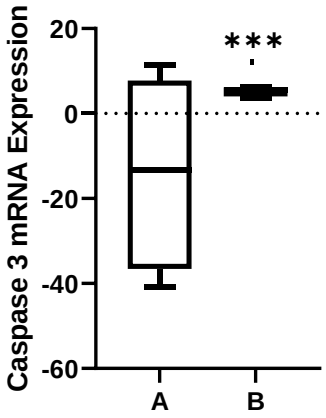
Figure 3 contain two figures (**A)** represent MCF7 Before MTNR1A Transfection and (B**)** represent MCF7 After MTNR1A transfection



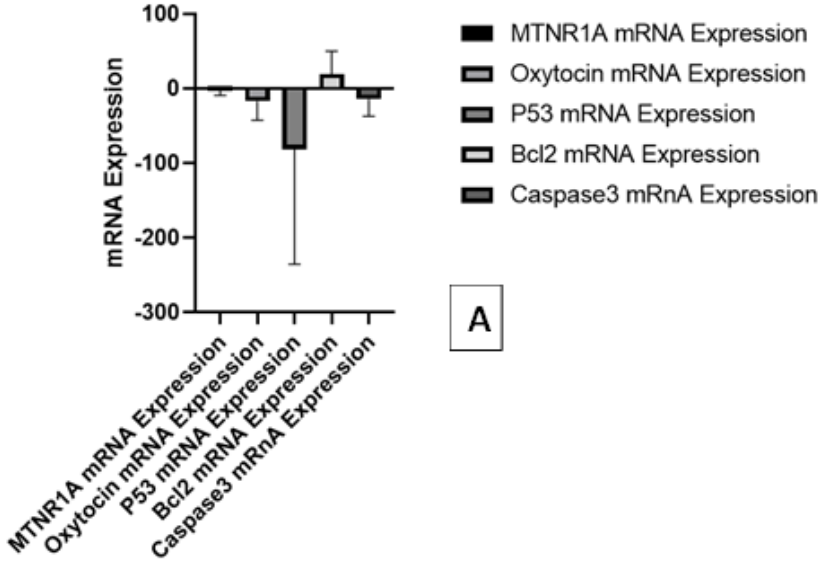




5

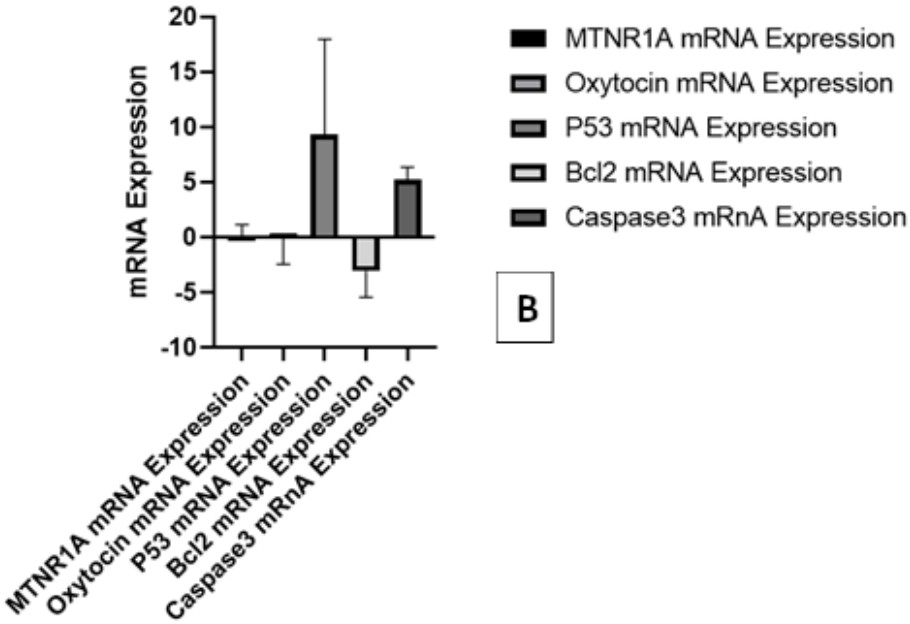


cDNA MCF7 Before MTNR1A Transfection



A

cDNA MCF7 After MTNR1A Transfection



B