International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Expression and Association of MTNR1A Gene and Circadian Clock Genes in Different Stages of Breast Cancer in MCF - 7 Breast Cancer Line

Mowafag Hassan Malla Khedir[1], Amal Saeed[2], Amir Abushouk[3], Nahid A Mohammed[4], Mohammed Elshiekh[5*]

[1]Department of Basic Medical Sciences, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences (KSAU - HS) Riyadh Kingdom of Saudi Arabia

[2]Department of Physiology, Faculty of Medicine University of Khartoum, Khartoum, Sudan

[3]Department of Basic Medical Sciences, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences (KSAU - HS), Jeddah, KAIMRC), Jeddah, Kingdom of Saudi Arabia

4Department of Physiology, Faculty of Medicine, University of Gezira, WadMedani, Sudan

[5]Department of Physiology, Faculty of Medicine and Health Science, Department of Physiology, University of Dongola, Dongola, Sudan
*Correspondence: Mohammed Elshiekh, Email: *mishairmo[at]gmail.com*

Abstract: ***Background**: MTNR1A and MTNR1B are the essential receptors for melatonin physiological activity. Circadian genes expression disrupted can alter breast biology and may promote cancer. **Aim**: In this study, we investigated the expression of MTNR1A in association to circadian clock genes (Period 1, Period 2, and Cry 1) in the different stages of breast cancer according to AJCC staging system classification. **Methods**: Cell viability (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 circadian clock genes Per1, Per2, and Cry1. **Results**: Our results revealed that MTNR1A gene was significantly up - regulated in normal breast compared to the MCF - 7 breast cancer and significantly down - regulated in stage IV compared to other stages. Both Per1 and Per2 genes were significantly down - regulated in MCF - 7 breast cancer line compared to the normal breast cell. Cry1 gene was up - regulated in stage 1 breast cancer and down - regulated in stage IV breast cancer cell. In addition, our data demonstrate a strong association between MTNR1A expression and circadian genes (Per1, Per2 and Cry1) expression in different stages of breast cancer. **Conclusion**: Our data indicate that altered gene expression of MTNR1A and circadian genes in breast cancer tissue. In addition, confirmation of association between MTNR1A and circadian genes expression may point to future use of melatonin and circadian clock as molecular basis treatment of breast cancer.*

Keywords: Circadian clock genes, MTNR1A, Per1, Per2, Cry1

1. Introduction

Melatonin (N - acetyl - 5 - methoxytryptamine) is neuroendocrine hormone mainly produced by the pineal gland and other tissues (1). It has several protective roles in many physiological areas such as seasonal reproduction, circadian rhythm regulation, effective endogenous scavenger for free radical, and anti - apoptotic factor (2, 3). On the contrary, melatonin also plays a vital role as oncostatic agent in different cancers type through anti - metastatic, anti - invasive, modulation of oncogene, antiangiogenic effects, anti - proliferative and proapoptotic actions (4 - 7). Melatonin mediated its action through bind to G protein - coupled receptors to accomplish its cellular actions. Melatonin receptors are classified into type MTNR1A or MT1 and MTNR1B or MT2 with highest affinity, and are predominately mediating the downstream effects of melatonin (8). Many of the anti - cancer actions of melatonin are intermediated through two membranous G protein receptors. The two membranous G protein receptors found in mammals and humans are the MT1 melatonin receptor (formerly the Mel1a), which is expressed by the MTNR1A gene, and the MT2 melatonin receptor (formerly the Mel1B), which is expressed by the MTNR1B gene (9). It is vastly fair that melatonin largely binds to MTNR1A to mediate it anticancer actions (10). Higher MTNR1A expression was reported to be correlated with a less - malignant histologic subtype of breast cancer and a higher survival rate of breast cancer patients (11). A similar findings association was also detected in carcinoma of oral squamous cell (12). To the present, there is no research conducted to elucidate melatonin receptors expression in different breast cancer stages.

Circadian rhythm is the natural internal cyclic changes system in all living organisms everywhere on, produced by the endogenous circadian clock. Circadian rhythm plays a crucial role in the homeostasis and maintenance of genomic stability (13). The pacemaker of circadian rhythm is found in the suprachiasmatic nucleus of the hypothalamus, which synchronizes with both: endogenous (internal) and exogenous (external) factors (14). Peripheral regulator mechanisms are distributed in many organs including breast (15). Disruption of Circadian rhythm may excite various tumor - related processes (16). A relevant process for cells, such as cell cycle is indirectly controlled by clock genes. As a consequence, disruptions of this regulator mechanism are marked by abnormal cell division in many types of cancer

(17). This is supposedly produced by the disrupted synthesis of melatonin hormone, due to a permanent exposure to artificial light during the night, which particularly relates to night shift workers (18). Clock genes disruption in mammary tissue may result in cell cycle dys - regulation, which is produced by irregular cell divisions, which can be related with tumor progression and a more aggressive type of breast cancer tumors (19). However, studies investigating the association of MTNR expression with circadian clock gene expression (Period 1, Period 2, and Cry 1) in the MCF - 7 and MCF - 10 human breast cancer have not been reported. Therefore, we investigate the expression of MTNR1A in association to circadian clock genes (Period 1, Period 2, and Cry 1) in the different stages of breast cancer according to AJCC staging system classification.

2. Material and Methods

2.1 Samples

Ten identical plates cDNA which contain normal and breast cancer with different stages was purchased from OriGene Technologies, Inc.9620 Medical Center Drive Suite 200 Rockville, MD 20850 USA.

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

qRT - PCR was performed with a Rotor - Gene Q PCR (QIAGEN, German), using 2 µLcDNA, 10 µL 2X Sybergreen Master mix (150mM Tris, pH 9.2, 40mM (NH4) 2SO4, 5mMMgCl2, 0.02% Tween - 20, 0.4mM dNTPs, 1.25 Units Taq Polymerase, 1X Sybergreen) and 0.5 µL of 20µM gene - specific primers (Table 1). Primers were designed based on theoretical optimal conditions, which included primer melting temperature, primer annealing temperature, GC content, cross homology, and primer secondary structures. All primers were purchased from Bio - Basic Canada Inc. (Ontario, Canada). The specificity and size of the PCR products were tested by adding a melt curve at the end of the amplifications, analysis on a 2% agarose gel of the bands. Amplicon Bands were isolated and sequenced. The reaction protocol consisted of one activation cycle of 50◦C for 2min followed by 95◦C for 15 s. Thereafter, 40 cycles of denaturation at 95◦C for 15 s, and at 60◦C annealing/extension for 2min were performed. Although normalization to RPL13 and Ubiquitin C showed similar trends, all values were normalized to Ubiquitin C. The $2^{-\Delta\Delta CT}$ method was used for relative quantification for qRT - PCR experiments. The primers sequence for MTNR1A, MTNR1B, Period 1, Period 2, Cryptochrome 1 genes are given in table 1.

2.3 Ethical approval

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

2.4 Statistical Analysis

The results were subjected to statistical analysis using SPSS version 23 (IBM Corporation, NY, USA). Data were expressed as means with standard error or standard deviation. Gene expression levels in the breast cancer tissue samples 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. Results

To evaluate melatonin receptors M1 and M2 genes expression, encoding via MTNR1A and MTNR1B, were affected in the breast cancer, we achieved RT - PCR in the patient with breast cancer compared to normal cells. Our result showed highly significant up - regulation for MTNR1A gene in normal breast tissue compared to breast cancer tissue $P < 0.009$. Whereas MTNR1A highly significant down regulated in breast cancer tissue compared to normal breast tissue ($P < 0.009$). Fig 1A. To evaluate whether MTNR genes expression and circadian clock genes are associated with tumor stages, we investigated the MCF - 7 human breast cancer according to AJCC (American Joint Committee on Cancer Staging System for Breast Cancer) staging system classification. MTNR1A gene was up - regulated at substantial levels in normal breast tissue compared to all breast cancer stages $P < 0.05$. Furthermore, MTNR1A gene was substantial down - regulated in stage IV compared to other breast cancer stages. Fig 1B.

Per 1 and per 2 clock genes demonstrate significantly up - regulated expression in normal breast tissue compared to breast cancer tissue at $P < 0.001$ and $P < 0.000$ respectively. Furthermore, there was no significance difference in both period 1 and period clock genes between different stages of breast cancer. Fig 2A and 2B

Our result demonstrated a strong association between MTNR1A gene expression with Period clock gene at $P < 0.000$ and Period 2 clock gene at $P < 0.003$, Fig.3A and 3B, respectively.

Cry 1 gene was significantly up regulated with a higher level in stage I breast cancer compared to normal breast cancer tissue $P < 0.05$. Furthermore, Cry 1 gene was significantly down - regulated in stage IV breast cancer compared to stages II and III of breast cancer $P < 0.05$. Fig 3A. In addition, our analysis showed association between MTNR1A gene expressions with Cry 1 gene $P < 0.05$. Fig 3B.

4. Discussion

MCF - 7 cell line is a usually used in - vitro experimental model for evaluating the biological activity, the resistance of drug and treatment strategies in 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. Circadian clocks respond to environmental time cues to coordinate 24 - hour oscillations in almost every tissue of the body. In the breast, circadian clocks regulate the rhythmic expression of

numerous genes. Disrupted expression of circadian genes can alter breast biology and may promote cancer. In this study, we investigated the expression of MTNR1A in association to circadian clock genes (Period 1, Period 2, Cry 1) in the different stages of breast cancer according to AJCC staging system classification. In this study, a major finding was the significant association between MTNR1A gene expression and circadian clock genes expression in different stages of breast cancer. We also observed that MTNR1A was up - regulated in breast cancer stages I, II and III whereas down - regulated in stage IV. MTNR1A gene was significantly up regulated in normal breast cell compared with breast cancer in different stages. Dillon and his colleagues for the first time manifested the expression of MTN1A in normal tissue and in breast cancer in paraffin sections (18).

The direct melatonin oncostatic 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.

In addition to facilitating regulate circadian cycles, the core clock genes have been established to influence cancer related pathogenesis such astumor - suppressor genes, cell cycle, apoptosis and cell proliferation. Previous have demonstrated that overexpression of Per1 and Per2 suppresses the growth of various cancer cells. Period1 plays a role in apoptosis in human cancer cells, when its down - regulation stopping apoptosis and its over expression enhancing DNA damage and induced apoptosis (23). Both PER1 and PER2 may suppress breast cancer in vivo by inducing apoptosis (24). In the present study both Per1 and Per2 was expressed in normal breast tissue and breast cancer cell line. Expression of Per1 and Per2 genes were significantly up - regulated in non - tumor cell compared to breast cancer cell line. The first study on circadian gene expression in human breast cancer has been conducted by Chen et. al. Using the immunohistochemical assay they have found that PER proteins were down - regulated in breast cancer tissue (25). Down - regulation of Per1 and Per2 in rodent models initiates higher cancer cell growth, but on the other hand, over expressed Per2 leads to apoptosis in mouse mammary carcinoma cell lines ETM6. Per2 has a leading function in the circadian rhythm regulation. Per2 directly controls c - myc - a gene responsible for proper functioning of the cell cycle (26). The present data indicate that Per1 up - regulated in stage I, II and down - regulated in stage III, IV, whereas Per2 up - regulated in all stages of breast cancer but more up - regulated in stage I and III. Expression of core clock components, such as CRY2, PER2 and PER3, was decreased in these more aggressive tumor types (27). Our present study demonstrated strong association between MTNR1A gene expression with Per1 and Per2 gene expression in MCF - 7 breast cancer line. Expression of MTNR1A in cancer cells seems to increase the efficacy of melatonin's oncostatic activity. The expression level of MTNR1A was inversely correlated with the invasive abilities of breast cancer cell lines (28).

Several previous studies have compared the expression levels of Cry1 between cancer tissue and adjacent normal mucosa. One study found that Cry1 and other circadian gene (Cry2, Per2 and BamlI) mRNA expression levels were similar in colon cancer and adjacent normal mucosa (29). Cry1mRNA levels in CRC tissues were also significantly associated with patient age and sex (30). In contrast, our results showed Cry1 gene expression levels were higher in breast cancer MCF - 7 line cell compared to normal breast cell. Cry1 mRNA expression level was clearly higher in cancerous tissues than in normal tissues in human epithelial ovarian cancer (31). MCF - 10A breast epithelial cells express all the core clock proteins (CLOCK, BMAL1, CRY1, CRY2, PER1, and PER2), while MCF - 7 human breast cancer cells express the core clock proteins (CLOCK, BMAL1, CRY1, and CRY1), but fail to express PER1 and PER2. Cry gene impact tumorigenesis via the cell cycle. The cell cycle suppressor WEE - 1 is expressed in phase with PER, during times of day when entry to the M phase is suppressed (32). Therefore, disruption of PER and CRY causes down - regulation of growth control genes, implying a mechanistic link between the circadian system and cell proliferation. In our study, we compared expression of Cry1 gene in clinicopathological stage of tumor progression AJCC stage. A significantly up - regulated Cry1 expression level was observed in stage I, II and III, whereas Cry1 expression was significantly down - regulated in stage IV. Circadian genes have been engaged in control of cell cycle (33). The expressions of Wee1 in mice with Cry1 mutant have been raised in many tissues, including the liver. Wee1 kinase restrains cell division by suppressing the G2 - M switch. Cry1 expression elevated may suppress the ability of Wee1 kinase to reinforce cell proliferation, thereby providing a survival advantage (34).

In conclusion, our data indicate that altered gene expression of MTNR1A and circadian genes in breast cancer tissue. In addition, confirmation of association between MTNR1A and circadian genes expression may point to future use of melatonin and circadian clock as molecular basis treatment of breast cancer.

5. Conflicts of Interest

The authors confirm that there are no conflicts of interest.

6. Acknowledgements

The authors appreciated and acknowledged the technical and advisory support of the members of Department of Basic

Medical Sciences King Saud Bin Abdulaziz University for Health Sciences (KSAU - HS) Riyadh, Kingdom of Saudi Arabia.

7. Funding

This research is conducted by a full personal funding from first author Mowafag Hassan MallaKhedir.

Authors' Contributions

Mowafag HMKhedir, Amal Seedand Amir Abushouk designed the research proposal, planned, the experimental work, data collectionand analysis. ***MowafagHM Khedir***conducted the laboratory work, ***MowafagHM Khedirand Mohammed Elshiekh*** preparedthe initial draft of the manuscript according to the journal format (table and graph format). ***MowafagHMKhedir, Amal seed, Amir Abushouk, Mohammed Elshiekh, Nahid A Mohammed, Mohand HM Khder and Abderrhman AM Ismeil*** provided critical feedback throughout all the research steps and reviewed the manuscript.

References

[1] Barrett P, Bolborea M. Molecular pathways involved in seasonal body weight and reproductive responses governed by melatonin. J Pineal Res.2012; 52: 376 - 88.
[2] Reiter RJ, Tan DX, Terron MP, Flores LJ, Czarnocki Z. Melatonin and its metabolites: new findings regarding their production and their radical scavenging actions. ActaBiochim Pol.2007; 54: 1 - 9.
[3] Um HJ, Kwon TK. Protective effect of melatonin on oxaliplatin - induced apoptosis through sustained Mcl - 1 expression and anti - oxidant action in renal carcinoma Caki cells. J Pineal Res.2010; 49: 283 - 90.
[4] Su SC, Hsieh MJ, Yang WE, Chung WH, Reiter RJ, Yang SF. Cancer metastasis: Mechanisms of inhibition by melatonin. Journal of pineal research.2017; 62.
[5] Reiter RJ, Rosales - Corral SA, Tan DX, Acuna - Castroviejo D, Qin L, Yang SF, et al. Melatonin, a Full Service Anti - Cancer Agent: Inhibition of Initiation, Progression and Metastasis. International journal of molecular sciences.2017;
[6] Liu J, Clough SJ, Hutchinson AJ, Adamah - Biassi EB, Popovska - Gorevski M, Dubocovich ML. MT1 and MT2 Melatonin Receptors: A Therapeutic Perspective. Annu Rev PharmacolToxicol.2016; 56: 361 - 83.
[7] Dubocovich ML, Delarange P, Krause DN, Sugden D, Cardinali DP &Olcese J 2010 International union of basic and clinical pharmacology. LXXV Nomenclature, classification, and pharmacology of G - protein - coupled melatonin receptors. Pharmacological Reviews 62 343–380.
[8] Tam CW, Mo CW, Yao KM, Shiu SY. Signaling mechanisms of melatonin in antiproliferation of hormone - refractory 22Rv1 human prostate cancer cells: implications for prostate cancer chemoprevention. J Pineal Res.2007; 42: 191 - 202.
[9] Jablonska K, Pula B, Zemla A, Owczarek T, Wojnar A, Rys J, et al. Expression of melatonin receptor MT1 in cells of human invasive ductal breast carcinoma. Journal of pineal research.2013; 54: 334 - 45.
[10] Nakamura E, Kozaki K, Tsuda H, Suzuki E, Pimkhaokham A, Yamamoto G, et al. Frequent silencing of a putative tumor suppressor gene melatonin receptor 1 A (MTNR1A) in oral squamous - cell carcinoma. Cancer science.2008; 99: 1390 - 400.
[11] Kettner NM, Katchy CA, Fu L. Circadian gene variants in cancer. Ann Med.2014; 46 (4): 208±20. Epub 2014/06/05.
[12] Weaver DR. The suprachiasmatic nucleus: a 25 - year retrospective. J Biol Rhythms.1998; 13 (2): 100±12.
[13] Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol.2010; 72: 517±49. Epub 2010/02/13.
[14] Kelleher FC, Rao A, Maguire A. Circadian molecular clocks and cancer. Cancer Lett.2014; 342 (1): 9±18. Epub 2013/10/04.
[15] Filipski E, King VM, Li X, Granda TG, Mormont MC, Liu X, et al. Host circadian clock as a control point in tumor progression. Journal of the National Cancer Institute.2002; 94 (9): 690±7. PMID: 11983758.
[16] Stevens RG. Artificial lighting in the industrialized world: circadian disruption and breast cancer. Cancer causes & control: CCC.2006; 17 (4): 501±7.
[17] Blakeman V, Williams JL, Meng QJ, Streuli CH. Circadian clocks and breast cancer. Breast cancer research: BCR.2016; 18 (1): 89. Epub 2016/09/02.
[18] Dillon DC, Easley SE, Asch BB et al. Differential expression of high - affinity melatonin receptors (MT1) in normal and malignant human breast tissue. Am J ClinPathol 2002; 118: 451–458.
[19] Vanecek J: Cellular mechanisms of melatonin action. Physiol Rev 78: 687 - 721, 1998.
[20] Yuan L, Collins AR, Dai J, Dubocovich ML and Hill SM: MT1 melatonin receptor overexpression enhances the growth suppressive effect of melatonin in human breast cancer cells. Mol Cell Endocrinol 192: 147 - 156, 2002.
[21] Blask DE, Sauer LA and Dauchy RT: Melatonin as a chronobiotic/anticancer agent: cellular, biochemical and molecular mechanisms of action and their implications for circadianbased cancer therapy. Curr Topics Med Chem 2: 113 - 132, 2002.
[22] Mao L, Yuan L, Slakey LM, Jones FE, Burow ME, Hill SM. Inhibition of breast cancer cell invasion by melatonin is mediated through regulation of the p38 mitogen - activated protein kinase signaling pathway. Breast Cancer Res.2010; 12: R107.
[23] Gery S, Komatsu N, Baldjyan L, Yu A, Koo D, Koeffler HP. The circadian gene per1 plays an important role in cell growth and DNA damage control in human cancer cells. Molecular cell.2006; 22: 375–382.
[24] Gery S, Koeffler HP. Circadian rhythms and cancer. Cell Cycle Georget Tex.2010; 9: 1097–103.
[25] Chen ST, Choo KB, Hou MF, Yeh KT, Kuo SJ, Chang JG. Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. Carcinogenesis.2005; 26 (7): 1241±6.
[26] Fu L, Pelicano H, Liu J, Huang P, Lee C. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. Cell.2002; 111 (1): 41±50.

[27] Engelen E, Janssens RC, Yagita K, Smits VA, van der Horst GT, Tamanini F. Mammalian TIMELESS is involved in period determination and DNA damagedependent phase advancing of the circadian clock. PloS One 2013; 8: e56623
[28] Oshima T, Takenoshita S, Akaike M, Kunisaki C, Fujii S, et al. (2011) Expression of circadian genes correlates with liver metastasis and outcomes in colorectal cancer. Oncol Rep 25: 1439–1446.
[29] Mazzoccoli G, Panza A, Valvano MR, Palumbo O, Carella M, et al. (2011) Clock gene expression levels and relationship with clinical and pathological features in colorectal cancer patients. ChronobiolInt 28: 841–851.
[30] Tokunaga H, Takebayashi Y, Utsunomiya H, Akahira J, Higashimoto M, et al. (2008) Clinicopathological significance of circadian rhythm - related gene expression levels in patients with epithelial ovarian cancer. ActaObstetGynecolScand 87: 1060–1070.
[31] Matsuo T, et al. Control mechanism of the circadian clock for timing of cell division in vivo. Science.2003; 302: 255–9.
[32] Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, et al. (2003) Control mechanism of the circadian clock for timing of cell division in vivo. Science 302: 255–259.
[33] Gauger MA, Sancar A (2005) Cryptochrome, circadian cycle, cell cycle checkpoints, and cancer. Cancer Res 65: 6828–6834.
[34] Stevens RG, Brainard GC, Blask DE et al. Breast cancer and circadian disruption from electric lighting in the modern world. CA Cancer J Clin 2014; 64: 207–218.

Figure 1: Gene expression of MTR1A (A) MTR1A gene expression in MCF - 7 breast cancer cell line and normal cell (B) MTR1A gene expression in different stages of breast cancer MCF - 7 line and normal cell. Data represent as mean ± SEM. #p < 0.009*vs* MCF - 7 breast cancer cell line, **p < 0.05 *vs* other stages of breast cancer.

Figure 2: Gene expression of Period clock gene in normal cell and different stages of breast cancer (A) Period 1 clock gene expression in MCF - 7 breast cancer cell line and normal cell, #p < 0.001 vs breast cancer stages (B) Period 2 clock gene expression in MCF - 7 breast cancer and normal cell, #p < 0.000 vs breast cancer stages. Data represent as mean ± SEM.

Figure 3: Association between MTR1A gene and Period clock genes expression in MCF - 7 breast cancer cell line and normal cell. (A) Association between MTR1A gene and Period 1 clock gene (B) association between MTR1A gene and Period 2 clock gene.

Figure 4: Gene expression of Cry 1 clock in normal cell and MCF - 7 breast cancer cell line in different stages of breast cancer. Data represent as mean ± SEM. #p < 0.05 vsnormal cell, **p < 0.05 vs other stages of breast cancer.

Figure 5: Association between MTR1A gene and Cry 1 clock gene expression in MCF - 7 breast cancer cell line in different stages of breast cancer and normal cell.

Table 1: Primer sequences for MTNR and circadian clock genes

Primers	Forward	Reverse
MTNR1A	CTGTCGGTGTATCGGAACAAG	CCAACGGGTACGGATAAATGG
Period 1	GCCAACCAGGAATACTACCAGC	GTGTGTACTCAGACGTGATGTG
Period 2	GCCAACCAGGAATACTACCAGC	AGGCTAAAGGTATCTGGACTCTG
Cryptochrome 1	CTCCTCCAATGTGGGCATCAA	CCACGAATCACAAACAGACGG

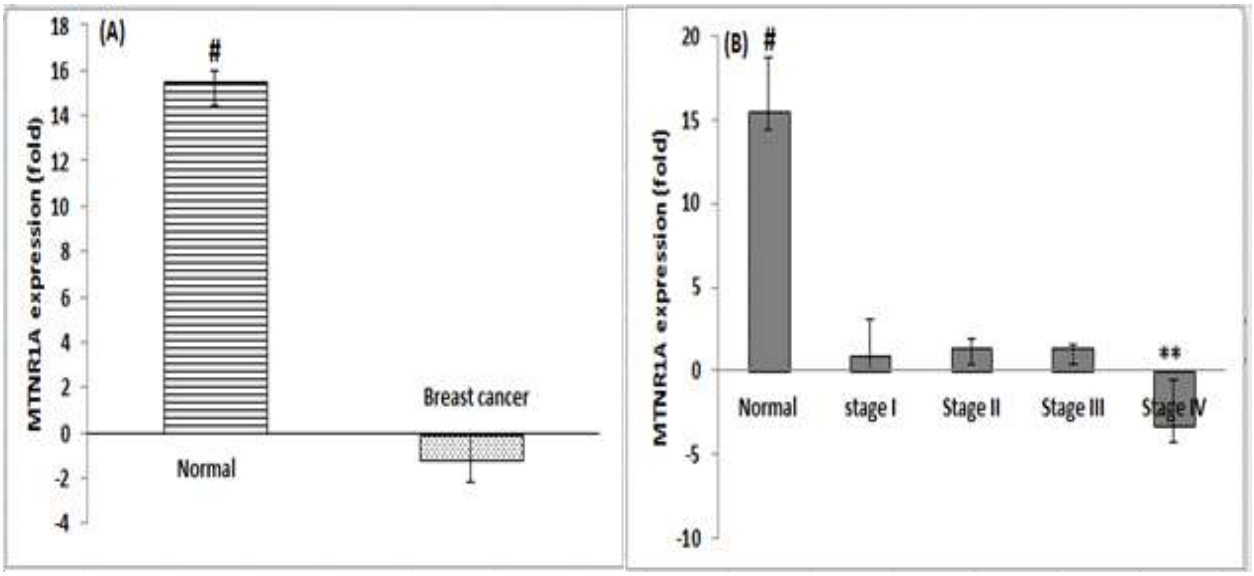

Figure 1

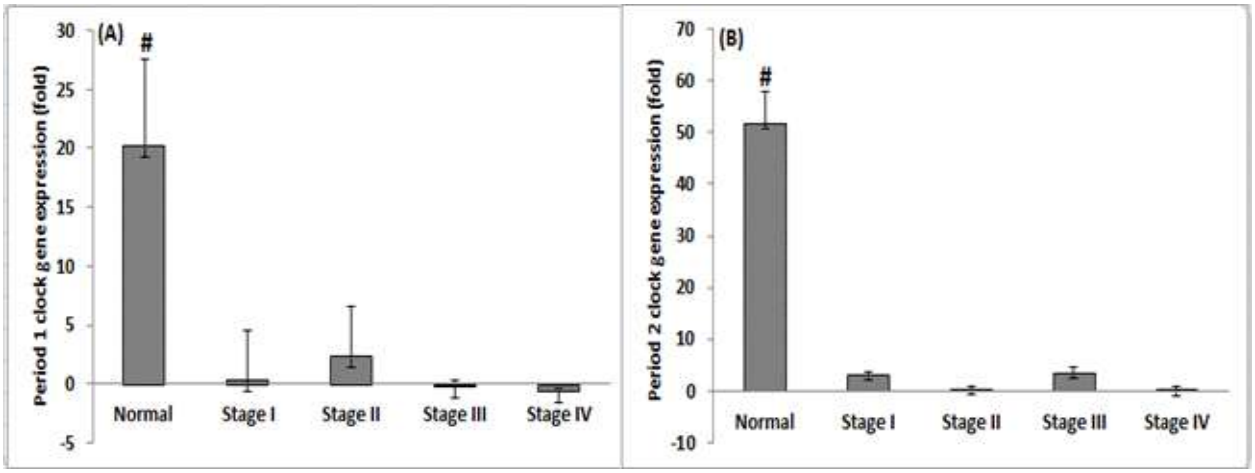

Figure 2

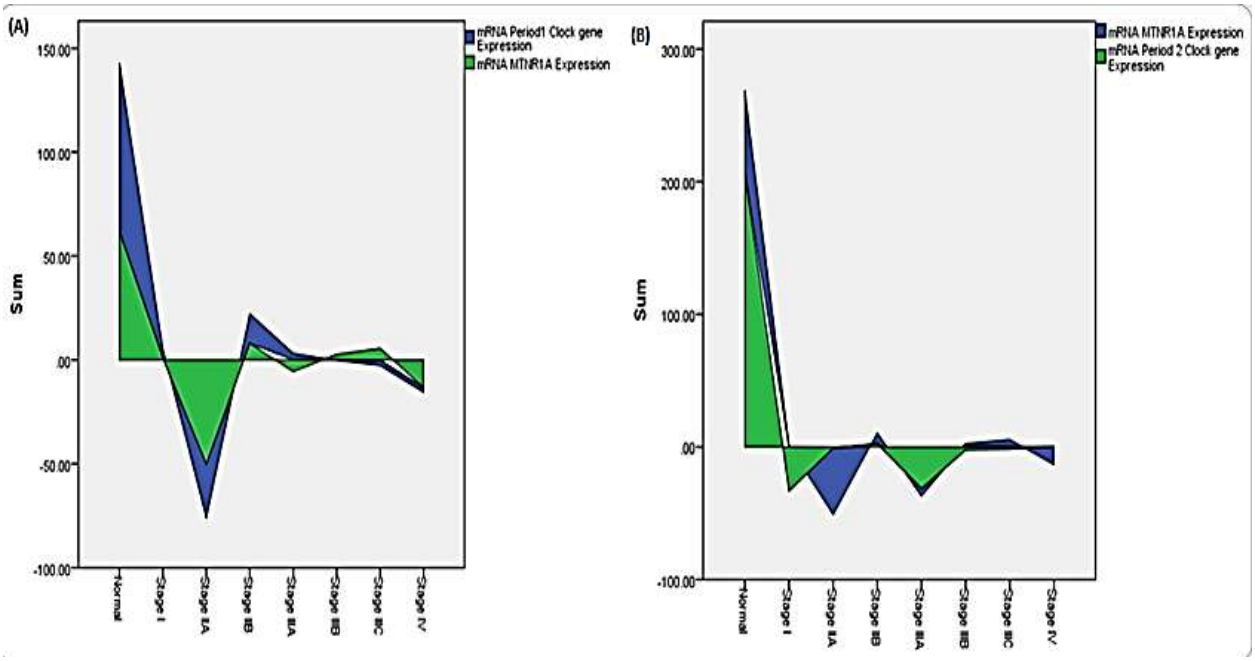

Figure 3

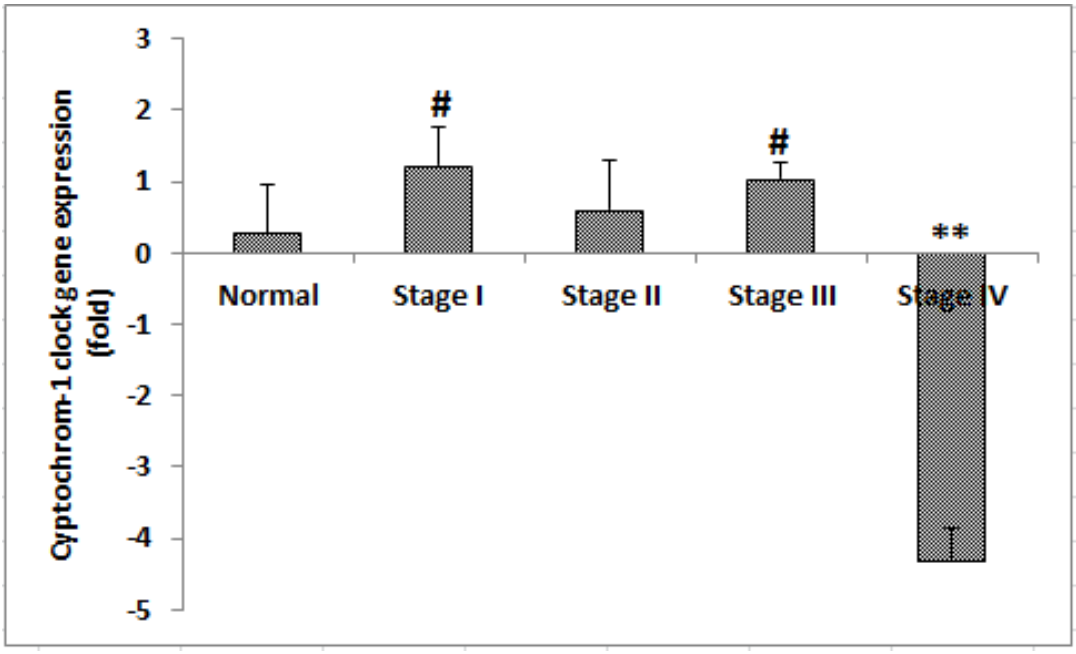

Figure 4

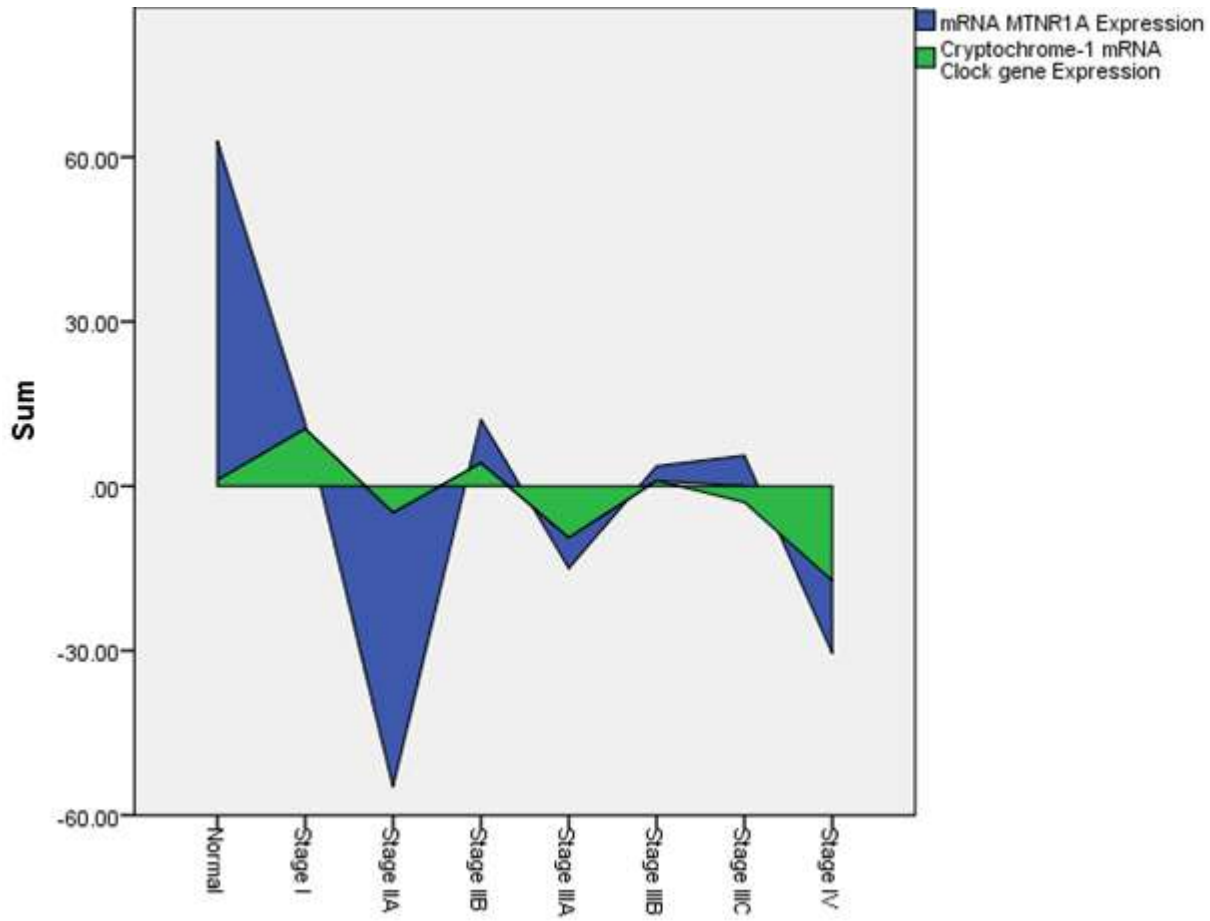

Figure 5