Mirna 214 and Progesterone Hormone in Breast Carcinoma

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Abstract
Background: MiRNASa (miRNAS) and progesterone have a relationship and role for progesterone receptor in breast cancers in women. Also, endocrine resistance is a problem that makes it hard for treatments to work. Our goal is to find out how the endocrine resistance can be overcome and resistance against progesterone. **Method**: The samples were taken from Al-Amel National Hospital for Cancer Treatment in Baghdad, Iraq. Their medical records and case sheets were used to find out about them. Samples were taken and split into two groups :*Group 1*, 25 samples Controls were made up of women who seemed to be healthy and were between the ages of 30 and 70. Group 2: Fifty samples from women with breast cancer who were between the ages of (30-70 years) and stages (I, II, III), patients were excluded at metastasis stage. patients divided to two groups, i) patients had not any treatments after surgery. ii) patients had many treatments after surgery. **Results** :Association between miR-214 and progesterone have role verified RT-qPCR was used to look at human breast cancer tissue samples to find the direct target of miR-214.The gene expression of miR-214 is shown as mean ± SEM value (1.12 ± 0.08 and 3.56 ± 0.21, respectively) and (p-value < 0.05) revealed a significant positive correlation between the expression level of miRNA214 and progesterone in cancer patients. (Y = 0.28, P = 0.046) **Conclusion**: MiR-214 increased sensitivity of breast cancer cells to high MiR-214 has the potential to be a new endocrine-busting drug. Technology with excessive high progesterone breast cancer.
Keywords: PR+ breast cancer, MiR-214, Protestor hormone.

1. Introduction

Cancer is one of the most dangerous diseases and one of the main reasons people die all over the world. Cancer is the growth of abnormal cells in any part of the body that can't be stopped. Cancer cells (also called malignant cells) are the name for these faulty cells (tumor cells). Cancer cells can move through the blood and lymph systems and end up in other organs, where they can continue to grow out of control and invade healthy body tissues. [1,2]. There are more than 100 types of cancer, and some are more common than others depending on things like age, gender, and ethnic . Cancers can be grouped according to the type of cells it start from. the most common type is breast cancer. aBreast cancer is the most diagnosed cancer (heterogeneous disease) among women worldwide. [3,4] Its known as abnormal growth of breast cancer cells and tumor or a lump development. aBreast cancer occurs in both males and females, but male breast cancer is very rare about only 1% of all cases of breast cancer. [5,6] In females were accounting for 1 in 4 cancer cases , There are two types of breast cancer (a) Non – invasive : cancers stay within the milk ducts or lobules in the breast . they do not grow into normal tissues or beyond the breast cells. sometimes they called carcinoma in situ or pre – cancers. (b) Invasive breast cancer stage (I –IV) : In these stages cancer calls leave their place in the ducts or lobules and spread to surrounding tissue or outside the breast cells to lymph nodes or other organs [7,8]MiRNA$_s$ are non- coding RNA$_s$ of (18 – 25) nucleotides , miRNA$_S$ bind to the three prime un- translated region (3`UTR$_S$) of the target messenger RNA$_S$ (mRNA$_S$) and control the gene expression at the post – transcriptional level [9,10,11]. Presently , The human *miRNA 214* gene is located in *DNM3* gene in the chromosomal region 1q24.3 , *miRNA214* is involved in numerous physiological and pathological functions , it coordinates the cell fate, differentiation and morphogenesis of muscles , the skeleton, nervous system , and pancreas [12,13]. *miRNA214* inhibits proliferation and increase apoptosis by targeting β – catenin. a *MiRNA214* targets RNF8, a promoter of metastatic epithelial – mesenchymal transition (EMT). [14,15]
are found in bio - Afluids such as saliva, aserum , urine , breast milk , cerebrospinal fluid , and amniotic fluid . MiRNA$_S$ can be secreted in extracellular vesicles such as exosomes. MiRNA$_S$ are stability, contrary to cellular RNA$_S$ species . MiRNA$_S$ resisting degradation at room temperature for up to 4 days and in deleterious conditions such as boiling , multiple freeze – thaw cycles and high or low PH [16,17].In cancer cells , miRNA$_S$ form central nodal points in cancer development pathways by regulate , angiogenesis , cancer stem cell biology , the epithelial – mesenchymal transition , metastasis and drug resistance ; loss

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of one miRNA can cause tumorigenesis. [18,19] MiRNAs can regulate cancer cells at different levels (i) up regulation of oncogenic miRNAs reduces expression of tumor-suppressor protein and promote tumorigenesis with highly expressed in tumor tissues. (ii) Down regulation of tumor – suppressing miRNAs results in an increased expression (production of oncogenic protein) leads to promote of tumorigenesis. [20,21,22] of function mutations in tumour – suppressing miRNAs mutation of the target section of oncogene mRNA can be caused tumorigenesis. Loss – of – function mutations in oncogenic miRNAs and mutations in tumour – suppressor mRNA would increase expression of tumour – suppressor proteins and hence reduce tumorigenesis [23,24]. Progesterone belong to an endogenous steroid hormone, progesterone is mainly secreted by the corpus luteum in the ovary. which is female sex hormone with estrogens. the main functions are regulating menstruation (when an egg is released from ovary) and supporting pregnancy in the female body. progesterone levels are measured through blood test.[25,26]

The progesterone levels fluctuate through the menstrual cycle. High levels of progesterone are responsible for symptoms such as breast tenderness, feeling bloated and mood swings (during pregnancy). In the breast, a progesterone stimulates normal human breast epithelium hrough paracrine mechanism, a so progesterone level can increase, decrease or have mitotic activity and proliferation in breast epithelial cells .[27,28] In breast cancer progesterone promotes pre – neoplastic progression via stimulation of cyclical proliferation of mammary stem cell pools or occult initiating cells in the mature breast epithelium . In abreast cancer is result of progesterone signaling and switch from paracrine to autocrine regulation of proliferation. Thea key mediators of biologic effects of progesterone are nuclear receptor. [29,30] . In all invasive breast cancer and DCIS are tested for hormone (progesterone receptors) . which are :i) Hormone receptor – positive .ii) Hormone receptor – negative .[31]

2. Materials and Methods

Patients

The total number of participants in the study was 75 individuals**,** study groups included the following: -

Group 1: 25 samples of apparently healthy individuals of women, aged between (30-70 years) obtained for controls.

Group 2: Fifty patient's samples of women diagnosed with breast cancer, aaged between (30-70 years) stages (I, II, III) , patients were excluded at metastasis stage . patients divided to two groups, i) patients had not any treatments after surgery. ii) patients had many treatments after surgery. The samples were collected at Al-Amel National Hospital for Cancer Treatment in Baghdad/Iraq, their clinical information was obtained from their hospital files and case-sheet records.

MiRNAs Extraction from Blood Samples

The miRNA extraction from whole blood of both patient and apparently healthy by using protocol in *EasyPure*® Blood Genomic miRNA Kit (Transgen, China) . Catalog Number (ER601-1). First (1 ml) of Lysis Buffer 10 (LB10) on eppendrof tube and tack (250 µl) of hole blood from EDTA tube and mix thoroughly. a Incubate for 5 minutes at room temperature. Then, for the previous components, chloroform was added. a Shake the tube briskly by hand for 3 minutes at roomatemperature, thenacentrifuge the samples for 15aminutes at 10,000 rpm . To create optimal binding conditions for all *miRNA* molecules , ethanol was added to the separated aqueous phase . After that, miRNA was eluted in RNase-free water and kept at (-20 °C) until further processing.

Primers

Primers used in this study with their sequences are shown in (Table 1).

Table 1. Primers used in the study with their sequences.

Primers	Sequence (5`→3` direction)	Ref.
miRNA 214	ACAGCAGGCACAGACAGGCAG	32
F.P. miRU6 (housekeeping gene)	AGAGAAGATTAGCATGGCCCCT	33
Univ.miRNA-qPCR-R	CAGTGCAGGGTCCGAGGT	34
miRNA-universe R.P.	GCGAGCACAGAATTAATACGAC	35

Real-time PCR Run

Real-time PCR (RTq-PCR) was used to measure the expression of the *214* gene. *miRNA214* was extracted from the blood of both patients and healthy controls *EasyPure*® miRNA Kit (Transgen, China). Gene polymorphism was quantified by probe color reaction (Master Mix Kits components) as shown in (Table2). The expression was reverse transcribed for cDNA synthesis using the *EasyScript*® One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgen, China) kit. RTq-PCR analysis was performed using the *TransStart*® Top Green qPCR SuperMix for gene expression Mix (Transgen, China). According to the manufacturer's procedure . To assess the expression. (Tables 3,4) arespectivel.

Table 2. Reaction Components and volume for gene expression .		
No.	Component	Volume (20µl)
1	Master mix Syper Green	10
2	Forward primer	1
3	Reverse primer	1
4	cDNA	3
5	Nuclease free water (N.F.W)	5

Table 3. Thermal profile of *miRNA214* gene expression.			
Step	Temperature/°c	Duration /sec	Cycles
Enzyme activation	94	30	1
Denature	94	5	35
Anneal	64	15	
Extension	72	20	
Dissociation	55 °C-95 °C		1

Table 4. Thermal profile of *miR6* gene expression			
Step	Temperature/°c	Duration /sec	Cycles
Enzyme activation	94	30	1
Denature	94	5	35
Anneal	64	15	
Extension	72	20	
Dissociation	55 °C-95 °C		1

To assess the effect of *miRNA214* on breast cancer development; used RT-PCR with SYBR green to quantify the expression level. Additionally, aU6 was used as control normalize variance the fold changes were calculated via normalization technique used during processing of Cycle Threshold (C_T) readings from RT-PCR software. The fold changes were calculated via relative quantification ($2^{-\Delta CT}$). To compare the transcript levels between different samples, the $2^{-\Delta\Delta CT}$ technique was utilized.

Serum test

Blood samples collected in gel tubes 2ml to obtain serum to determine progesterone, this test applied by system called (cobas e 411), test number 1380. This system intended to use immunoassay for the vitro quantitation of progesterone in human serum.

Statistical Analysis

Data analysis was done by utilizing IBM SPSS for Windows, version 26 (SPSS Inc. Chicago, Illinois, United States) . Data were shown as variables were expressed as mean ± standard error mean (SEM) and media. And p <0.05 was considered to be statically significant. Kruskal-Wallis H test was used to evaluate median differences from more than two groups followed Dunn's post-hoc for statistically significant pairwise values. Coefficient (r) that measures the association between the miRNAs expression and progesterone hormone ranked variables. Graphics were drawn using the SPSS software.

3. Results

MiRNA214 expression in breast cancer: Thea study include 75 patients and 25 apparently healthy people (controls), the data revealed that *miRNA214* was significant high expression level. It shown as mean value ± SEM (1.12±0.08 and 3.56±0.21, respectively) and (p value 0.001<0.05) when breast cancer vs. controls . (Table 5)

Table 5. *miRNA214* expression in control and breast cancer patients.		
Disease	Mean± SEM edian	*P* value
Breast cancer (N=50)	3.56± 0.21 3.64	0.001
Control breast (N=25)	1.12± 0.08 1.25	

Correlation between *miRNA214* expression and serum progesterone: Altered *miRNA 214* expression has been associated with several types of human cancer. One of these was breast cancer. progesterone hormone was important sex hormone in women. In this study, it revealed positive significant correlation between the expression level of *miRNA214* and progesterone hormone in breast cancer patients. (r = 0.28, P = 0.046). (Figure 1)

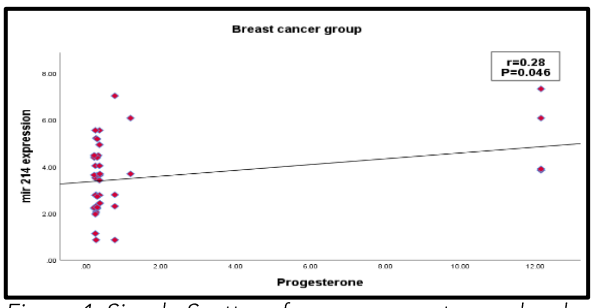

Figure 1. Simple Scatter of serum progesterone levels and miRNA-214 expression in breast cancer patients.

4. Discussion

The purpose of this review is to describe the role of *miRNA214* in breast cancer patients with different aging ,aclinical data and tumor subtypes and clarify its effect on progesterone hormone .How resistance to endocrine therapy has grown over timeThe hardest part of treating breast cancer isHaving a lot of progesterone.A link was found between several factors, such as the loss of How well cisplatin works on anaplastic thyroid cancer cells MiR-101 stopped autophagy caused by breast cancer cells becoming more sensitive to Tamoxifen [19]. miR-214 was found in this study.And how it affects the progesterone hormone by stopping the process of autophagy.Studies done over time have shed light on the possible miR-214 is used as a way to treat cancer. Upregulation of MiR214 has been found in breast cancers [22].MiR-214 may play important roles in both resistance and sensitivity in different types of cancer. These roles could go in different directions. out of the way By going after p53/Nanog, a high level of miR-214 made ovarian cancer cells mature and resistant to chemotherapy. MiR-214 may be to blame for chemoresistance [33].For cisplatin or gefitinib in non-small cell carcinomas and ovarian cancer Lung cell carcinoma [32, 31]. miR-214, on the other hand,cisplatin-induced cell death was made worse by Bcl2l2 is turned down in cervical cancer cells [34]. In this study, it was found that miR-214 was turned down in human breast cancer tissues.To answer 4-OHT/FUL. Based on what we found, Having less miR-214 was linked to having more autophagy and resistance to hormones, while too much miR-214 shuts down both norm and Autophagy was caused by OHT/FUL. The second study showed that miR-214 may stop autophagy directly Targeting UCP2.UCP2 is a member of a family of proteins called uncoupling proteins that are found in the inner membrane of the mitochondria. UCP2 is Take part in separating oxidative phosphorylation from Make it easier for energy to be lost as heat [35]. Chemotherapy drugs became less effective when UCP2 was overexpressed. and better chances of living by turning down ROSin this study , the targeted gene *miRNA214* shown the function as oncogenic role. Moreover, *miRNA214* had significant positive correlation with progesterone hormone. These results appeared based on the statistical testa type, different clinicopathologic parameters tests types and breast tumor subtypes that used in our study.

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