Original Article

The role of miRNA200a as biomarker to detect breast and ovarian cancers in Iraqi women

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Abstract

MiRNASa(miRNAS)a are small non-codingaRNAS that key post- transcriptionala regulators of gene expression . Breast and ovarian cancers are most a common malignancies among females. The biological function of amiRNA200a has not been elucidated completely. miRNA200aaconsidered as a tumor-suppressor miRNA or oncogenic miRNA. The present study evaluated a the expression of miRNA200a was asignificant low expression in a breast cancer and has shown a the function of tumor-suppressor role. furthermore , the expression of miRNA200a wasa non-significant high expression in a ovarian cancer and has shown the function of a oncogenic role . The dual a role of miRNA200a in a diverse kinds of a tumor has long been attractive.

Keywords: biomarker, detect, breast.

Introduction

Cancer is one of the most dangerous a diseases and is one of a the leading causes of a death worldwide. Cancer known is the uncontrolled a growth of abnormal cells anywhere in a the body. These abnormal cells are termed cancer cells (malignant cells) or (tumor cells). Cancer cells travel through the blood and a lymph systems, and a lodge in other a organs where they can again a repeat the uncontrolled a growth cycle and these cells can in filtrate a normal a body a tissues. 1,2. There are more than 100 types of cancer , and a some are more a common than others a depending on a things like a age, agender , and a ethnic . Cancers can be grouped a according to the type of a cells it a start from . the most a common types are breast cancer and a ovarian cancer .a Breast cancer is the most a diagnosed a cancer (heterogeneous disease) a among women a worldwide . 3 its known as a bnormal a growth of breast a cancer cells and a tumor or a lump a development .a Breast cancer occur in a both males and a females , but male a breast cancer is very ar are about only 1% of all cases of a breast cancer . 4,5aIn females was a accounting for 1 in 4acanceracases , There are two a types of a breast a cancer (a)a Non – invasive :a cancers stay within the a milk ducts or lobules in the breast . they do not grow into normal tissues or a beyond the breast a cells . some times a they called a carcinoma in situ or pre – cancers . (b)a Invasive breast a cancer stage (I –IV) :a in these stages a cancer calls a leave their a place in the aducts oral obules and a spread to surrounding a tissue or outside the a breast cells to a lymph nodes or other organs . 6,7

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Ovarian a cancer (OC) is a the fifth a most common a cancer in a women worldwide ,a ovarian cancer is mostly a asymptomatic in it's a early stages , the a patients usually a present with a advanced – stage a cancer with a high mortality a rate 8.aThere are three a types of ovarian a cancer :i)a Epithelial Ovarian carcinomas: a these are the most a common a type of ovarian a cancer , about 85% -90% . ii)a Germ cell tumors :athese a make up less than 2% of all a ovarian a cancers .iii)a Stromal a cell tumors :a these represent a about 1% of all aovarian a cancers 9,10,11.

What are miRNAS

MiRNAs :are non- coding a RNAs of (18 – 25)anu cleotides , miRNAS bind to a the three a prime un-a translated aregion (3`UTRS)aof theatarget messenger RNAS (mRNAS) and control the gene expression at the post – transcriptionalalevel 12,13,14.aPresently , almost 2000 miRNAs ahad identified inahumans . MiRNAS wereaable toaregulateahumanaprotein. MiRNAs areapresentlyabeing exploredaextensively asapotential biomarkers ofanumerous diseases due to theirahigh stability and ease of detection [15,16] .aThe human amiRNA 200a is non-codingagene is located inatheachromosomal region 1p36.33,afirst reported toaplay a role in olfactoryaneurogenesis , miRNA200aasuppress cellaproliferation by inhibitingaself – renewal andadifferentiation of cancer stemacells and modulatingacell adivision and apoptosis 17,18 .AMiRNAS are found in bio -Afluids such as saliva ,aserum ,aurine , breast milk ,acerebrospinal fluid , andaamnioticafluid . MiRNAS can beasecreted in extracellular vesiclesasuch asaexosomes . MiRNAS are astability , contrary toacellular RNAS species .aMiRNAS resistingadegradation at roomatemperature for up to 4 days and inadeleteriousaconditions such asaboiling ,amultiple freeze – thawacycles and high oralow PH 19,20

The role of miRNAS inahumanacancer

In cancer cells ,amiRNAS form centralanodalapoints in cancer developmentapathways byaregulate ,aangiogenesis ,acancerastem cell biology,athe epithelial – mesenchymalatransition ,ametastasis and drug resistance ;aloss of oneamiRNA can causeatumorigenesis. 21,22 MiRNAS can regulate cancer cells at different levels (i) up regulation of oncogenic miRNASareducesaexpression of tumor –asuppressoraprotein and promoteatumorigenesis withahighlyaexpressed inatumoratissues.(ii) Down regulation of tumor – suppressing miRNAS results in an increased expressiona(production of oncogenic protein) leads to promote of tumorigenesis . 23,24aLoss of functionamutations inatumour – suppressing miRNAS mutation of the target section of oncogene mRNA can be caused tumorigenesis . Loss – of – function mutations in oncogenic miRNAS andamutations in tumour – suppressoramRNA would increaseaexpression of tumour – suppressoraproteins and henceareduce tumorigenesis .25

Materials And Methods

Patients and Methods

The total number ofaparticipants in the study was 150 individuals, study groups included the following: -

Group 1: Fifty samples ofaapparentlyahealthyaindividuals of women, aged between (20-70 years)awere obtained foracontrols.

Group 2: Fifty patient'sasamples ofawomen diagnosedawith Breast cancer ,aaged between (30-70 years)aand stages (I,II,III) , patients wereaexcluded at metastasis stage . The samples were collected at Al-Amel National Hospital for Cancer Treatment in Baghdad/Iraq, theiraclinical information was obtained afrom their hospital files and case-sheet records.

Group 3: Fifty patient's samples of Iraqi women diagnosed with Ovarian cancer, patients agedabetween (20-50 years) and stages (I,II,III) , patients wereaexcluded at metastasis stage ,athe samples were collected at Al-AmelaNationalaHospital foraCancer Treatment in Baghdad/Iraqa,theiraclinical information was obtained fromatheir hospital files and case-sheetarecords.

MiRNAs Extraction from Blood Samples

The miRNAaextractionafrom wholeablood of bothapatient and apparently healthy byausing protocol in EasyPure® Blood Genomic miRNA Kit (Transgen, China) .aCatalog Number (ER601-1) First (1 ml) of Lysis Buffer 10 (LB10) on eppendrof tube andatack (250 µl) of hole bloodafrom EDTA tubeaand mix thoroughly.aIncubate for 5 minutes at room temperature. Then, for the previous components,achloroform was added .aShake the tube briskly by hand for 3 minutes at roomatemperature, thenacentrifuge the samples for 15aminutes at 10,000 rpm . To createaoptimal bindingaconditions for all miRNAamolecules, ethanol was added toathe separatedaaqueous phase .aAfter that, miRNA was eluted in RNase-free wateraand kept at (-20 °C) until further processing.

Primers

Primers used in this studyawith theirasequences are shown in (Table 1).

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

Primers	Sequence (5`→3` direction)	Ref.
miRNA200a	TAACACTGTCTGGTAACGATG	26
F.P. miRU6 (housekeeping gene)	AGAGAAGATTAGCATGGCCCCT	27
Univ.miRNA-qPCR-R	CAGTGCAGGGTCCGAGGT	28
miRNA-universe R.P.	GCGAGCACAGAATTAATACGAC	26

Real-time PCR Run

Real-time PCR (RTq-PCR) was used to measure the expression of the miRNA200a gene . miRNA200a wasaextracted from theablood of both patientsaand healthy controls EasyPure® miRNA Kit .aGene polymorphism wasaquantified by probeacolor reaction (MasteraMix Kits components) as shown in (Table2). The expression was reverse transcribed for cDNAasynthesis using the EasyScript® One-Step gDNA Removalaand cDNAaSynthesis SuperMix (Transgen, China) kit. RTq-PCR analysisawas performedausing the TransStart® Top Green qPCRaSuperMix for geneaexpression Mix (Transgen, China).aAccording to the manufacturer's procedure . To assess theaexpression. (Tables 3,4,5),arespectively.

Table 3. 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 4. Thermal profile of miRNA200a geneaexpression.

Step	Temperature/oc	Duration /sec	Cycles
Enzyme activation	94	30	1
Denature	94	5	
Anneal	58	15	40
Extension	72	20	
Dissociation	55 ºC-95 ºC		1

Table 5. Thermal profile of miR6 gene expression

Step	Temperature/oc	Duration /sec	Cycles
Enzyme activation	94	30	1
Denature	94	5	
Anneal	64	15	35
Extension	72	20	
Dissociation	55 ºC-95 ºC		1

To assess the effecta of miRNA200a onabreast and ovarian cancers development ; used RT-PCR with SYBRagreen to quantify the expressionalevels . Additionally ,aU6 was used as a controlanormalize variance the fold changes were calculated via normalization technique used duringaprocessing of CycleaThreshold (CT) readings from RT-PCR software .aThe fold changes werea calculated via relativeaquantification (2-ΔCT).

Statistical analysis

Data analysis a was done by a utilizing IBM SPSS for Windows ,a version 26 (SPSS Inc. Chicago, Illinois, United States).a Data were shown as variables were expressed as mean ± standard error mean (SEM) and median. And $p < 0.05$ was considered to be statically significant .

5. Results Discussion

The data revealed that miRNA200a in breast cancer a was low expression levels and mean ± SEM vs. control breast (2.17±0.60 and 0.92±0.16 , respectively) and shown a significan ta differences (p value 0.01<0.05) . Further more,a in previous study , it has been shown that miRNA200a over expressions in ovarian cancer ,amean vales of miRNA200a ±SEM vs. controls aovarian (7.18±1.31) and (2.72±0.55) , respectively . and shown non-significant a differences (p value 0.10>0.05),a(Table 6).a Because of their accessibility ,are markable stability and a specific a expression a patterns in association a with diseases.aMiRNA200aaexpressionawasahelpful molecular to diagnosis ofabreast and a ovariana cancers ,a with a good specificity and a sensitivity. Expression a levels of miRNA200a can be used as molecular a markers for early a diagnosis .

Table 6. Comparison of the studding miRNA200a expressions in breast and ovarian cancers vs controls.

Disease	Mean± SEM Median	P value
Breast cancer (N=50)	0.92± 0.16 0.24	
Control breast (N=25)	2.17± 0.60 1.13	0.01
Ovarian cancer (N=50)	7.18± 1.31 3.22	
Ovarian Control (N=25)	2.72± 0.55 1.01	0.10

Conclusions

The discovery of a miRNAs in general and a particularly of circulating miRNAs is one a of the majora scientific breakthroughs in the a modern era and has are volutionized our a understanding of current a cell biology and medical science .a Therefore, it had a enough evidence to a understand the biology of a circulating miRNAs and confidently a conclude that circulating miRNAs are a potential a regulator of developmental a processes and could be a promising a biomarker for several a diseases including cancer.

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Compliance with Ethical Standards statements

Ethical approval :AppliedaScienceaDepartment /aBiotechnology division /aUniversity of Technologyaand Iraqi HereditaryaCompanya(IHC) and Al-AmelaNational Hospital foraCanceraTreatment in Baghdad/Iraq Iraq / certifies the ethical approval, Funding details (In case of Funding) :I am responsible for paying the financing, Conflict of interest :There is no conflict of interest, Informed Consent: AppliedaScienceaDepartment /aBiotechnology division /aUniversity of Technologyaand Iraqi HereditaryaCompanya(IHC) and Al-AmelaNational Hospital foraCanceraTreatment in Baghdad/Iraq Agreed

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