Circulating microRNA-182 overexpression as a biomarker for breast cancer

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الخلاصة: يهدف البحث الى دراسة التعبير المعلمة الجزيئية (miR-182) في نسيج الثدي السرطاني وفي مصل الدم لنفس لمريضة لمعرفة (زيادة اونقصان) على اعتبارها متغير حقيقي عن النسيج الطبيعي وعوامل التنبؤ لسرطان الثدي ولمعرفة ترابط هذا التغير مع ثوابت أخرى مثل عمر المريض وماركرات اخرى . هذه الدراسة هي دراسة تجريبية من الشهر الثالث سنة 2013الى الشهر الاول سنه 2015. تم اخذ اربعين زوجا من عينات سرطان الثدي ومن النسيج الطبيعي حول سرطان الثدي من النساء اللواتي تم استئصال الورم بسبب السرطان وهذه العينات تم اخذها في صالة العمليات وكانت العينات طازجة وتم اخذ مصل الدم من نفس المريضات ومن نساء سليمات . تم استخلاص شريط ال RNA ثم بعد ذلك تم فحص جزيئة ال (miR-182) وتم تطبيق الواسم المناعي النسيجي الكيمياوي IHC لهرمون الاستروجين و البروجستين مع her-2 في العينات التي تم وضعها في بلوكات الشمع . جميع الحالات كانت جزيئة ال (miR-182)مرتفعة في النسيج السرطاني عن ماهو في النسيج الطبيعي وكانت هذه الجزيئة عالية في مصل الدم مريضة سرطان الثدي بلمقارنة مع النساء السليمات كما انه توجد علاقة واضحة مع التعبير الجيني لهرمون الاستروجين والبروجستين مما يدل على ان المعلمة الجزيئية (miR-182) تلعب دور أساسي في تشخيص سرطان الثدي وتشخيص تغير الجيني لهذه المعلمة الجزيئية التي تحتاج الى علاج جيني .

Background:- MiR-182 is one of the most frequently studied cancer-related gene miRs and plays a crucial role in tumorigenesis , progression and may become a potential therapeutic target and biomarker of tumor diagnosis and prognosis.

Aim of study:-Estimation of miR-182 gene expression levels in both fresh tissues and serum of same breast cancer patients by using stem-loop follow by Taq-Man real time PCR (RT-PCR) technique and correlate the miR-182 gene expression with ER,PR and Her-2 by IHC technique .

Material and methods:- Stem-loop RT-PCR was performed to identify the level of miR-182 gene expression in both fresh tissues and serum of same breast cancer patients . The expression levels of miR-182 relative to mRNA of GAPDH were determined using the livak method . IHC were done for ER,PR and HER-2 .

Results:- Mean fold change of miR-182 was statistical significantly higher in breast cancer from paraneoplastic tissues , mean fold change of miR-182 was statistical significantly higher in serum of patients from apparently healthy control and miR‑182 serum level of patients with ER,PR positive was statistical significantly lower compared with the negative patients.

Conclusion:- The miR‑182 as a new original diagnostic and prognostic biomarker for breast cancer .

Key word:- Breast cancer, miR-182 , RT-qPCR ,ER,PR and HER-2,IHC

Introduction

Breast cancer is the most common type of cancer in females worldwide [1] . MicroRNAs (miRs) are small non protein coding RNAs involved in gene regulation through binding to the 3' un-translated region of target messenger RNAs (mRNAs) and down-regulate their translation to protein or degrade the mRNAs . Thus, miRs play critical biological roles in many different cellular processes including metabolism, development , differentiation ,proliferation and apoptosis. They are also linked to human diseases, including cancer [2] .The

apoptotic and necrotic primary tumor discharges miRs into the blood circulation, known as circulating miRs. Therefore, blood contains circulating miRs from numerous cells (including tumor cells), which makes it possible to detect miRs from specific organs, tissues or cells using surface markers for proper quantification [3]. The circulating miRs, resistant to RNase activity, are rare and extremely stable in serum and plasma [4]. This stability translates into consistent miR expression levels among individuals, which makes serum miRs attractive biomarkers for the diagnosis of breast cancer patients . However, there have been only a few previous publications investigating circulating miRs in the peripheral blood of breast cancer patients [5,6] . MiR-182, a member of the miR-183 family located on chromosome 7q31-34, is one of the most frequently studied cancer-related gene miRs and miR-182 is involved in several key steps of tumorigenesis, including epithelial-mesenchymal transition, cell cycle regulation, proliferation, survival, migration, aggressiveness, and drug resistance . MiR-182 plays a crucial role in tumorigenesis and progression, and miR-182 may become a potential therapeutic target and biomarker of tumor diagnosis and prognosis [7,8] . However, other studies have reported that miR-182 is downregulated in gastric adenocarcinoma and that increased miR-182 levels are correlated with clinical treatment benefits [9,10] . A novel miR quantification method has been established using stem–loop RT followed by TaqMan PCR analysis in tissue or cultured cells [11,12] . The present study estimated the levels of miR‑182 in the tissue and serum of breast cancer patients to identify the potential of serum miRs as biomarkers for breast cancer.

Materials and methods

Patients selection: The study was conducted during the period from March 2013 to January 2015 . This is a prospective study, where by 40 patients with newly diagnosed breast cancer, aged 30-65 years, were recruited at the Surgical Department/AL-Diawania Teaching Hospital in Diawania City .The patients had not received neoadjuvent therapy , all of whom underwent lumpectomy . Apparently healthy controls (n=10) were diagnosed without any tumor or physical illness. Serum sample were collected from breast cancer patients and apparently healthy controls . Forty-pairs of fresh tissues from same cases of breast cancer and paraneoplastic tissues (which consider as internal control) , for total RNA extraction and for RT-qPCR . Another 40 pairs specimens of both breast cancer and paraneoplastic tissues for histopathological examination and after proved the diagnosis, tumor marker were done for ER ,PR ,Her-2 by Immunohistochemistry (IHC) . The histopathological classification was performed according to the WHO classification (2013) ,the grading system was carried out according to modified Bloom and Richardson system (2009), tumor staging was carried out according to AJCC, 2010 .

Immunohistochemistry: Histological sections (4‑μm) were deparaffinized in xylene and rehydrated. Antigen retrieval was performed by water bath the sections in 10 ᴍm citric acid monohydrate. Endogenous peroxidase activity was blocked by 0.5% H2O2 treatment. The slides were incubated with appropriate dilutions of the primary antibodies [anti‑ER, 1:200; anti‑progesterone receptor (PR), 1:200 and anti-Her-2,1:200 Dako] at 4˚C overnight. The same procedure was performed for negative controls which were incubated overnight in 1X PBS without antibody. The reaction was

visualized by the ABC Kit (Dako) , positive ER and PR (nuclear staining of >10%) according to allred scoring system while positive Her-2 (complete membrane staining) according to HercepTest scoring system .

MiRNA isolation from serum and tissue:-Serum samples were collected between 8:00 and 9:00 a.m. following centrifugation for 30 min at 2,650 g ,then serum samples were stored at 80^{o} c .Tissue samples were homogenized in adenaturing lysis solution and dissolved RNA was stored at -20°C before use . Isolation of total RNA (RNA was extracted from serum and fresh tissues using the Trizol reagent (Bioneer ,Korea) according to the manufactures instructions. .RNA quality was assessed with a NanoDrop 1000 spectrophotometer.

Real-time RT-PCR for miR-182 quantification: The Primers and probes for miR-182 were design in this study by using (The Sanger Center miR database Registry) to selected miR-182 sequence and using miR Primer Design Tool .The cDNAs were synthesized by stem-loop primer, 5'-AACATGTACAGTCATG-GATG -3'. MiR-182 was then analyzed by qPCR and the primer used was: forward, 5' -GGCAATGGTAGAACTCACACT-3' and reverse , 5'-AACAT GTACAGTCCATGGATG-3'. PCR analysis was performed using Taq-Man probe. initial denaturation at 95˚C for 5 min, followed by 40 cycles of 95˚C denaturation for 20 sec, 52˚C annealing for 20 sec and extension at 72˚C for 30 sec. Reverse Transcription and real-time PCR was subsequently performed in duplicate . All miR-182 quantification data were normalized to housekeeping gene like Glycer aldehyde 3-phosphate dehydrogenase (GAPDH) . The mRNA of GAPDH gene primers and probe were designed by using NCBI- Gene Bank data base and Primer 3 plus design online. The cDNAs primer of GAPDH as design as Random Hexamer primer and the primer used in qPCR was: forward, TCAGCCGCATCTTCTTTTGC and reverse, TTAAAAGCAGCCCTGG-TGAC Taq-Man probe for mGAPDH was: FAM-CCAGCCGAGCCACATCGC-TC-TAMRA .These primers and probe of miR-182 and GAPDH were provided by (Bioneer company, Korea) .The data results of RT-qPCR for miR-182 and GAPDH were analyzed by the relative quantification gene expression levels (fold change) were based on Ct values by using the Livak method (Fold change = $2^{-\Delta\Delta CT}$) that described by (Livak and Schmittgen, 2001) [13] .

Statistical analysis :SPSS version 16 and Microsoft Office Excel 2007 were using in analysis of these data ,Chi-square test and Fisher exact test were used to study association between any two nominal variables. P-value of less than or equal to 0.05 was considered significant.

Results

1- Clinicopathological characteristics of patients:- A total of (n=40) breast cancer patients , all breast cancer cases with invasive ductal carcinoma (IDC), and mean age was (45.20±7.55) years old. Out of 40 cases of breast cancer, 25(62.5%) were ER(+ve) , 23(57.5%) +ve for PR and 10(25%) +ve for Her-2 by IHC.

2-Comparison the miR-182 in breast cancer and paraneoplastic tissues

Mean cancer tissue of miR-182 was statistical significantly higher from paraneoplastic tissue , 5±0.4 versus 2.5±0.090,respectively (P<0.001) ,figure (1)

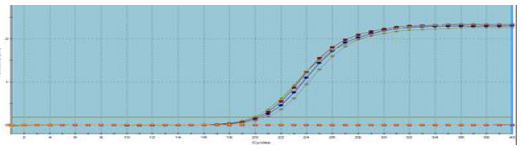


Figure (1): Stem- Loop qRT-PCR amplification plots for miR-182 cDNA in breast cancer tissue patients by using Taq-Man probe .

3-comparison the miR-182 gene expression between serum of breast cancer patients and apparently healthy control .

The mean serum level of miR-182 in breast cancer patients was statistical significantly higher from apparently healthy control , (5.130×10^{3}) versus (0.002×10^{3}) , respectively (P<0.001) , as table (1), figure (2) .

Table (1) : Comparison the mean serum level between control and patients' groups

Group	Median*	Mean±SD*	P-value
Control (n =10)	0.003	0.002±0.001	<0.001
Patients (n = 40)	4.950	5.130±0.996	

*** Numbers are expressed $X10^{3}$**

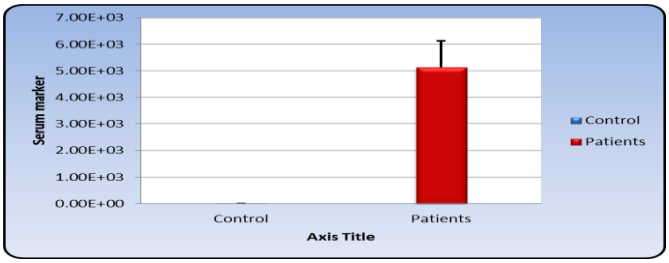


Figure (2): Comparison the circulating miR-182 between control and patients' groups .

4-The correlation of miR-182 gene expression pattern between breast cancer tissues and paired serums

The results showed a statistical significant correlation of miR-182 gene expression in the breast cancer tissues with those in the paired serums, with **r = 0.41** (p<0.001)

5-Correlation between microRNA-182 gene expressions with age.

The result of present study showed that there was no statistical significance correlation between serum level of miR-182 and age of patients (P>0.005) , as table (2)and figure (3) .

Table (2) : The mean age in patients' and control groups

Age	N	Mean	SD	Minimum	Maximum	P-value
Control	10	44.10	8.58	30	56	0.758
Patients	40	45.20	7.55	30	65	

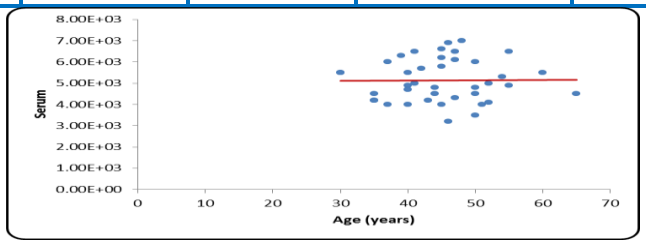


Figure (3): Spearman correlation between age of patients and serum of miR-182 .

When divided in two group (<50 and ≥50) , no statistical significant between mean fold change of miR-182 serum levels in breast cancer patients and age of patients (p>0.05) ,as table (3) and figure (4) .

Table (3): Association between mean serum level of miR-182 and age of patients

Age2	Median*	Mean±SD*	P-value
<50 years (n =28)	5.250	5.236±1.047	0.344
≥50 years (n =12)	4.850	4.883±0.855	

*** Numbers are expressed X10^3**

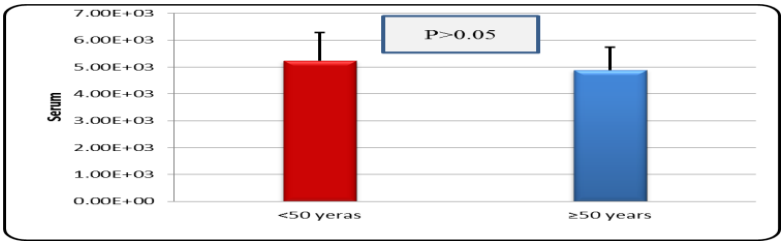


Figure (4): Association between mean serum level of miR-182 and age of patients.

6-Correlation of serum miR-182 in patients with ER , PR and Her-2 .

The mean serum level of miR-182 in patients with ER positive was statistical significantly lower than the ER-negative patients , (4.504x10 ^{3}) copies/ml , (P<0.001) and the mean serum level of miR-182 in patients with PR positive was statistical significantly lower than the PR-negative patients , (4.304 x10 3) copies/ml, (P<0.001) .While the mean serum level of miR-182 in patients with Her-2 positive was no statistical significantly from the Her-2 negative patients , (P>0.005) . As shown in table (4) .

Table (4) : Association between serum level of miR-182 in patients and receptor status

Marker		N	Median	Mean	SD	P-value
ER	Positive	25	4.500	4.504	0.399	<0.001
	Negative	15	6.200	6.173	1.131	
PR	Positive	23	4.200	4.304	0.01	<0.001
	Negative	17	6.100	5.976	0.2	
her2neu	Positive	10	4.900	5.020	0.02	0.888
	Negative	30	5.000	5.166	0.01	

*** Numbers are expressed X10^3**

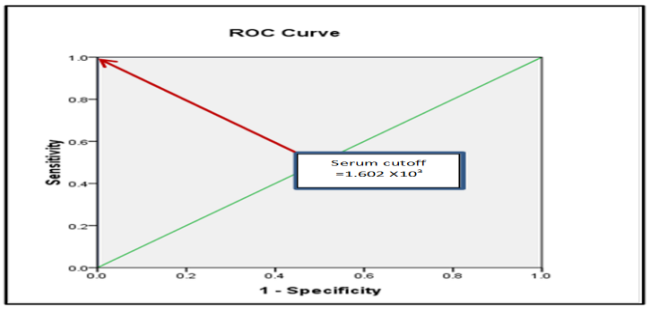
7-Validity of serum microRNA-182 gene expression as gene aberration

The cutoff value for serum miR-182 gene expression fold change that predict gene expression aberration in breast cancer patients by using the RT-qPCR technique , an Receiver Operator Characteristic (ROC) curve analysis was done that showed the following results:

The best cutoff value for serum miR-182 in patients was (1.602 x10^3) with a specificity of 100%, sensitivity of 100% and accuracy excellent ,table (5) and figure (5) .

Table (5) : ROC curve for cutoff value of serum miR-182 in patients and control group

Parameter	Value	Interpretation
Cut off value	1.602 $X10^3$	
AUC (accuracy)	1.000(100%)	Excellent
Sensitivity	100%	Excellent
Specificity	100%	Excellent



Figure(5): ROC curve for cutoff value of serum miR-182 in patients and control group

8-Validity of serum microRNA-182 gene expression as prognostic marker

A-The cutoff value of fold change for serum miR-182 gene expression, that predict breast cancer patient with negative ER , an ROC curve analysis was performed. The best cutoff value of fold change for serum miR-182 was 5.600 $x10^3$ with a specificity of 100%, sensitivity of 87% and an accuracy excellent , as table (6) and figure (6).

Table (6) : ROC curve for cutoff value of serum miR-182 in patients with negative ER

Parameter	Value	Interpretation
Cut off value	5.600 $X10^3$	
AUC (accuracy)	0.984(98.4%)	Excellent
Sensitivity	87%	Very good
Specificity	100%	Excellent

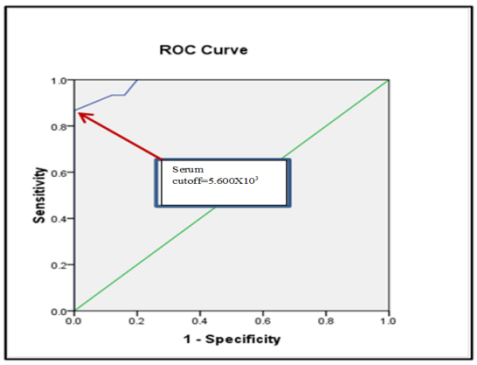


Figure (6) : ROC curve for cutoff value of serum miR-182 that predict with negative ER status

B-The cutoff value of fold change for serum miR-182 gene expression, that predict breast cancer patient with negative PR , an ROC curve analysis was performed . The best cutoff

value of fold change for serum miR-182 was 5.600 x10^3 with a specificity of 100%, sensitivity of 77% and accuracy excellent , as table (7) and figure (7).

Table (7) : ROC curve for serum cutoff the miR-182 that predict with negative PR status

Parameter	Value	Interpretation
Cut off value	5.600 X10^3	
AUC (accuracy)	0.926 (92.6%)	Excellent
Sensitivity	77%	Good
Specificity	100%	Excellent

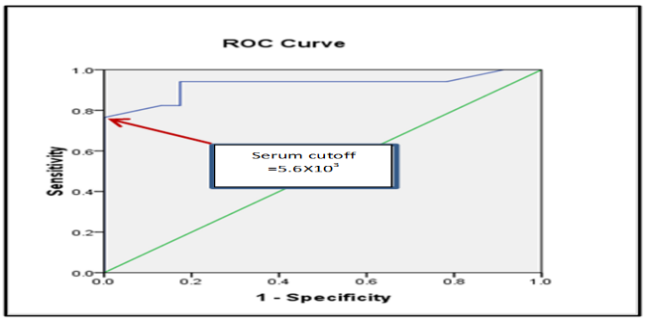


Figure (7) : ROC curve for cutoff value of serum miR-182 that predict negative PR status

Discussion

Breast cancer is the most common cancer in women, and early diagnosis and treatment of breast cancer has important prognostic significance. Serum markers have become increasingly important to diagnosis of breast cancer for its simple collection and less invasiveness. CA15-3 and CEA are the most widely used markers in monitoring patients with breast cancer, and elevated serum levels are associated with breast cancer relapse, but both lack of sensitivity for early-stage disease and specificity [14] . What is more, CEA is a non-specific tumor marker and has a lower positive rate in early diagnosis of breast cancer. It cannot serve as an indicator of early diagnosis of breast cancer [15] .

In recent years, miRs have attracted increasing interest among investigators, particularly cancer researchers, as vital cellular molecules involved in normal and pathological states. Many studies have demonstrated that miRs are aberrantly expressed in different classes of cancers and can be used as novel biomarkers of tumor identification and prognosis [16, 17,18] . Among these miRs,miR-182 is considered a micro-oncogene. Extensive profiling studies over the past several years have linked the dysregulated expression of miR-182 to several cancer types, including colorectal cancer, lung cancer, glioma, bladder cancer, endometrial carcinoma, prostate cancer, and ovarian cancer [19, 20, 21] . The ideal biomarkers for breast cancer diagnosis should be easily accessible in order that they may be sampled relatively non-invasively. In addition, biomarkers must be sensitive enough to be detected in early stage tumors in almost all patients, while absent or minimal in healthy control individuals [22] .

In present study, the miR-182 gene expression was statistically significant higher in the breast cancer tissues compared with the paraneoplastic tissues. These results consist with results of Ping *et ,al* [23] ,whom were found that miR-182 gene expression was statistical

significantly increased (>4-fold higher) in breast cancer tissues compared with paraneoplastic tissues . This result is consistent with the oncogenic role of miR-182 in various types of cancer. Several studies support that miR-182 acts as an oncogene in the development of breast cancer [21,24,25,26]. MiR-182 is overexpressed in human breast cancer tissues and cell lines and β-catenin binds to the promoter to increase the expression of miR-182 [24].

The miR-182 gene expression levels in the serum of breast cancer patients in present study were also statistically significant higher than those of the healthy controls and the best cutoff value for gene aberration was (1.602 $X10^3$ fold change) ,in which can detect normal gene expression from gene aberration and also can detect normal cases from malignant one , indicating miR−182 is a useful biomarker for breast cancer diagnosis. These results were accepted with other study reported by Ping *et ,al* [23] , whom were found that the serum miR-182 levels in breast cancer patients (7.075x103 copies/ml) , which were significantly higher compared with the serum of healthy controls (0.003x103 copies/ml) .

In order to determine the correlation of miR expression in tissue and the matched serum samples, the results of present study shown a significant correlation of miR-182 gene expression in the tissues with those in the serums (Spearman-Rho test r = 0.41, p< 0.01), which suggests that miR-182 isolated from serums could reflect most of the characteristic expression patterns of their tissue counterparts and further show promise for miR as blood-based biomarkers for detecting and screening breast tumors .

In the present study, there is no statistical significance between age of patients and fold change of miR-182 gene expression. These findings are accepted with other studies [23,24,25,26] .

The prognostic and therapeutic roles of ER or PR in breast cancer have been studied extensively and are well established [27, 28]. Significant associations have been found between ER and PR positive rates with menopausal status, tumor size or the presence of distant metastases in breast cancer [29] . In present study found that the miR-182 gene expression level in serum of breast cancer patients was significant lower in ER and PR positive than ER and PR negative by IHC. These results were accepted with other study reported by Ping *et ,al* [23]. The results indicated that there is a close correlation between serum level of miR-182 and ER, PR negative expression in breast cancer patients.

The best for your knowledge, this study could be the first study of its type to be conducted in Iraq, evaluating tissue and serum level of miR-182 gene expression in same patients by RT-qPCR , in a sample of Iraqi female patients . There was no baseline study regarding tissue and serum level of miR-182 gene expression stratification in apparently healthy control in Iraqi individuals. Although, similar studies were conducted abroad to stratify tissue and serum level of miR-182 in breast cancer patients in other countries [21, 23, 24, 25 , 26] .

In summary, the key finding of the present study is that miR-182 gene expression in the serum of breast cancer patients were markedly up-regulated compared with healthy controls while the levels of miR-182 in the serum of patients with positive ER,PR was lower compared with the negative patients and RT- PCR is a original and useful means for investigation of serum miR samples .In general, the present study tourist attractions for miR-182 as a new

diagnostic and prognostic biomarker for breast cancer and miR-182 may sever as a novel therapeutic target for the treatment of breast cancer. However, studies with larger number of patients and healthy controls are needed to validate our findings and to investigate whether other miRs are also capable of indicating breast cancer progression and more importantly whether miR-182 is the best choice among potential breast cancer biomarkers .

References

1- Sariego J. Breast cancer in the young patient. Am Surg 76: 1397‑1400, 2010.

2- Huang G, Zhang X , Guo G ,et al . Clinical significance of miR-21 expression in breast cancer: SYBR-greenI-based real-time RT-PCR study of invasive ductal carcinoma. Oncol Rep 2009; 21: 673e679.

3-Hunter M , Ismail N, Zhang X, et al . Detection of microRNA expression in human peripheral blood microvesicles. PLoS One 3: e3694, 2008.

4-Mitchell P, Parkin R, Kroh E, et al . Circulating microRNAs as stable blood‑based markers for cancer detection. Proc Natl Acad Sci USA 105: 10513‑10518, 2008.

5-Heneghan H , Miller N, Lowery A, et al . Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg 251: 499‑505, 2010.

6- Schrauder M, Strick R, Schulz‑Wendtland R, et al. Circulating micro‑RNAs as potential blood‑based markers for early stage breast cancer detection. PLoS One 7: e29770, 2012.

7-Wang M, Wang Y, Zang W, et al . Downregulation of microRNA-182 inhibits cell growth and invasion bytargeting programmed cell death 4 in human lung adenocarcinoma cells. Tumour Biol 2014, 35(1):39–46.

8- Pignot G, Cizeron-Clairac G, Vacher S, et al. MicroRNA expression profile in a large series of bladder tumors: identification of a 3-miRNA signature associated with aggressiveness of muscle-invasive bladder cancer. International Journal of Cancer, vol. 132, no. 11, pp. 2479–2491, 2013.

9- Kong W, Bai R , Liu T, et al . MicroRNA-182 targets cAMP-responsive element-binding protein 1 and suppresses cell growth in human gastric adenocarcinoma . The FEBS Journal, vol. 279, no. 7, pp. 1252–1260, 2012.

10-Rodr´ıguez-Gonz´alez F , Sieuwerts A, Smid M ,et al. MicroRNA-30c expression level is an independent predictor of clinical benefit of endocrine therapy in advanced estrogen receptor positive breast cancer .Breast Cancer Research and Treatment, vol. 127, no. 1, pp. 43–51, 2011.

11-Bustin S, Benes V, Garson J, et al. Minimum information for publication of quantitative real-time PCR experiments . Clinical Chemistry 2009; 55:1–12.

12- Ridzon D, Chen C, Broomer A, et al. Realtime quantification of microRNAs by stem-loop RT-PCR . Nucleic Acids Res 2005; 33: 179.

13- Livak and Schmittgen . The 2–ΔΔCT (Livak) Method Real-Time PCR. Applications Guide 2001: 41.

14-Samy N, Ragab HM, El Maksoud NA, et al. Prognostic significance of serum Her2/neu, BCL2, CA15-3 and CEA in breast cancer patients: A short follow-up. Cancer Biomark 2010;6:63-72.

15-Wu ZC, Zhai HS, Yan CL, et al. The value of serum CA15-3, CEA in the early diagnosis of breast cancer. Qiqihar Yi Xue Yuan Xue Bao (in Chinese) 2010;31:1849-50.

16- Hayes J, Peruzzi P, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy . Trends in Molecular Medicine, vol. 20, no. 8, pp. 460–469, 2014.

17- Wang S, Li H , Wang J, et al . Prognostic and biological significance of microRNA-127 expression in human breast cancer. Disease Markers, vol. 2014, Article ID 401986, 12 pages, 2014.

18- Shao Y, Geng Y, Gu W, et al . Prognostic significance of microRNA-375 downregulation in solid tumors: a meta-analysis. Disease Markers, vol. 2014, Article ID 626185, 10 pages, 2014.

19- Wang S, Yang M , Wang X, et al . Increased expression of miRNA-182 in colorectal carcinoma: an independent and tissue-specific prognostic factor. International Journal of Clinical and Experimental Pathology, vol. 7, no. 6, pp. 3498–3503, 2014.

20- Stenvold H , Donnem T , Andersen S, et al . Stage and tissue-specific prognostic impact of miR-182 in NSCLC. BMC Cancer, vol. 14,no. 1, article 138, 2014.

21- Casanova-Salas I , Rubio-Briones J , Calatrava A ,et al . Identification of miR-187 and miR-182 as biomarkers of early diagnosis and prognosis in patients with prostate cancer treated with radical prostatectomy .The Journal of Urology, vol. 192, no. 1, pp. 252–259, 2014.

22-Zhu W, Qin W, Atasoy U , Sauter ER: Circulating microRNAs in breast cancer and healthy subjects. BMC Res Notes 2: 89, 2009.

23-ping‑yu wang , hai‑tao gong , bao‑feng li , et al. Higher expression of circulating miR‑182 as a

novel biomarker for breast cancer. ONCOLOGY LETTERS 6: 1681-1686, 2013.
24-Chiang CH , Hou M , Hung W. Up-regulation of miR-182 by β-catenin in breast cancer increases tumorigenicity and invasiveness by targeting the matrix metalloproteinase inhibitor RECK . Biochim Biophys Acta 1830: 3067-3076, 2013.
25-Krishnan K, Steptoe A , Martin H , et al . MicroRNA-182-5p targets a network of genes involved in DNA repair. RNA 19: 230-242, 2013.
26-Moskwa P, Buffa F, Pan Y, et al . miR-182-mediated downregulation of BRCA1 impacts DNA repair and sensitivity to PARP inhibitors. Mol Cell 41: 210-220, 2011.
27-Cui X, Schiff R, Arpino G, Osborne CK and Lee AV: Biology of progesterone receptor loss in breast cancer and its implications for endocrine therapy. J Clin Oncol 23: 7721-7735, 2005.
28-Osborne CK, Schiff R, Arpino G, Lee AS and Hilsenbeck VG: Endocrine responsiveness: understanding how progesterone receptor can be used to select endocrine therapy. Breast 14: 458-465, 2005.
29-Faheem M, Mahmood H, Khurram M, Qasim U and Irfan J: Estrogen receptor, progesterone receptor, and Her 2 Neu positivity and its association with tumour characteristics and menopausal status in a breast cancer cohort from northern Pakistan. E cancer medical science 6: 283, 2012.