Hassan et al. *SECI Oncology* 2015
DOI: 10.18056/seci2015.8

MiRNA-200b as a prognostic factor in invasive duct carcinoma

Nivin A. Hassan[1], Tarek M. Elsaba[2], Samia A. Shouman[3]

[1] Department of Cancer Biology, South Egypt Cancer Institute, Assiut University
[2] Department of Pathology, South Egypt Cancer Institute, Assiut University
[3] Department of Cancer Biology, National Cancer Institute, Cairo University

Correspondence should be addressed to Nivin A. Hassan at Department of Biology, South Egypt Cancer Institute, Assiut University Egypt, nivin_azim@hotmail.com

Published 17 September 2015

Abstract

Background: miR-200b has been reported to be a tumor suppressor and a promising therapeutic target in cancer. miR-200b has been associated with epithelial- mesenchymal transition (EMT) and chemo-resistance in cancer.

Aim of work: To determine the expression of miR-200b in breast cancer, invasive duct carcinoma (IDC) tissues in comparison to normal adjacent breast tissues. Also, the prognostic role of miR-200b expression in breast cancer patients was evaluated.

Material and Methods: Quantitative real time polymerase chain reaction (qRT-PCR) was used to detect the expression level of miR-200 b in 48 tumor tissues and 14 adjacent normal tissues from breast cancer patients with IDC.
Results: The expression of miR-200b in breast cancer tissues was significantly lower than in normal tissues ($p<0.005$). In comparison with the normal tissues, miR-200b was down regulated in 75% (36 /48) of the tumor samples. Low expression of miR - 200b were insignificantly higher in patients with distant metastases (75% vs 25%), vascular invasion (71.1% vs 28.9%), estrogen receptor expression (77.8% vs 22.2%), progesterone receptor expression (84 % vs 16%) and lymph nodes metastasis (72.2% vs 27.8%) in comparison to those with high expression of miR- 200b.

Conclusion: Reduced miR- 200b expression is a frequent event in human breast cancer tissues and could be involved in breast cancer carcinogenesis. miR-200b could be a prognostic factor in breast cancer patients.

Keywords: invasive duct carcinoma, miRNA-200b, qRT-PCR.

Introduction:

Breast cancer is the first ranked female malignancy worldwide, with about 200,000 new incidence and 40,000 deaths per year in US **[1].** Also, the most frequent cancer among females in Egypt estimated using the results of the National- Population based Registry Program of Egypt 2008-2011 was breast cancer (32.04%) **[2]**. Although breast cancers with early stage show excellent outcome after therapy, recurrent and metastatic breast cancer patients remain big problems for cure **[3].**

MicroRNAs, also termed miRNAs, are a class of endogenous, non-protein coding single-stranded RNA molecules with a length of 21–23 nucleotides, which plays a crucial role in the post-transcriptional regulation of gene expression **[4].** miRNAs are highly conserved and specific, and regulate gene expression by binding to the 3′ untranslated region (UTR) of target messenger RNA (mRNAs) and inhibiting translation or inducing degradation of mRNAs. miRNAs has been proved to play vital roles in cancer management, acting as either oncogenes or tumor suppressors **[5].**

Aberrant expression levels of miRNAs have been observed in many solid cancers including breast cancer. Despite significant progress in the last few years on miRNA biology, the exact biological functions and the genetic factors driving their expression have been revealed for only a limited number of miRNAs in breast cancer **[6].**

There are five members of the miR-200 family, which are clustered together at two polycistronic sites: mir-200a, mir-200b, and mir-429 are located on chromosome 1p36 **[7]**; and mir-200c and mir-141 are

located on chromosome 12p13. These microRNAs display marked structural similarities, suggesting that they mainly target the same genes. The wide variety of microRNA targets make them interesting as potential contributors to the plasticity of processes involved in epithelial- mesenchymal transition (EMT) and metastasis **[8].** So, the goal of this study is to identify patterns of miRNA-200b deregulation and its prognostic role in breast cancer.

Material and Methods:

Forty eight cases of female invasive duct carcinoma and fourteen control cases of adjacent normal breast tissue from the same malignant cases were included in this study. Cases were obtained from the registry of the pathology department at South Egypt Cancer Institute. All the specimens were preserved and fixed in 10% buffered formalin, processed as usual then paraffin embedded.

Clinical and pathological data were collected from the registry. These data include patient's age, occurrence of metastasis or recurrence, size of the primary breast mass, vascular invasion, regional lymph nodes status and the status of estrogen and progesterone receptors expression condition in the tumor cells.

All tissue samples were subjected to the measurement of the expression of micro RNA (200b) via quantitative real -time PCR technique: RNA extraction was done using miRNAeasy FFPE kit (catalog no 73504) designed for total RNA extraction including miRNA from formalin fixed, paraffin embedded (FFPE) tissue sections. After that samples were processed by 2 step PCR reaction where cDNA synthesis were done using Taqman®microRNA Reverse transcription kit (Catalog No. 4366596) with a total reaction volume 15 ul (7ul master mix + 3ul RT primers + 5 ul RNA). The primer used is a small RNA specific stem loop RT primer.

qPCR reaction

quantitative PCR reaction was done using Taqman®ssay in 20 ul reaction (10 ul Taqman® universal PCR master mix II (2X) + 3 ul c DNA + 6 ul nuclease free water + 1 ul Taqman® small RNA assay, life technologies (Inventoried Cat # 4427975). Reaction mix loaded to the Applied Biosystem 7500 instrument, life technologies, USA (Catalog No.4366855) .

Cycling program as follow; 50 °c: 2 minutes, 95 °c: 10 minutes and 40 cycles (95 °c: 15 seconds, 60 °c: 60 seconds).

Data Analysis

Relative quantification was done using the comparative CT (ΔΔCT) method. The over or down expression were estimated as folds of rise or decrease in gene expression of the target cases (normalized ratio) in comparison to the calibrator cases (normalized ratio) using the ΔΔCT method calculation by Applied Biosystem detection software.

Statistical Analysis:

Data was entered on a PC using Microsoft Excel 2013 (Microsoft Corporation). Data then was analyzed using SPSS version 21 (IBM Corporation). Data was described as number and percentage for categorical data. For quantitative data, it was described using mean and standard deviation for normally distributed data and median and interquartile range (IQR) for those not normally distributed.

To evaluate difference between groups, the following tests were used; Chi square for categorical data, Student t-test for normally distributed quantitative data and Mann Whitney U test and Kruskal Wallis for not normally distributed quantitative data. Correlation was done for quantitative data using Pearson´s correlation for normally distributed data and Spearman´s correlation for those not normally distributed. P value was considered significant if it is equal to or less than 0.05.

Overall survival and disease free survival were done by using Kaplan-Meier method. The end point for overall survival was death and for disease free survival was relapse. Significance test was done by using Log Rank (Mantel-Cox).

Results:

Our study included 48 female patients, all were diagnosed as breast cancer (IDC). Their mean ages were 53.5± 13.57 years, ranging from 28 to 80 years. 24 patients (50%) were postmenopausal. Concerning tumor characteristics, 28 (58.3%) patients had tumor size ≥2 cm and <5 cm and 18 (37.5%) had tumor ≥ 5 cm. Histologic grade II was reported in 40 patients (88.3%).Estrogen receptors expression in the cells was detected in 27 (56.3%) of the samples while progesterone receptors expression was detected in 25 (52.1%) samples .Lymph node metastasis was detected in 36 (75%) patients while vascular invasion occurred in 38 (79.2%) patients. Distant metastasis was detected in 8 (16.7 %) patients **(Table 1)**.

miR-200b expression in both cancerous and adjacent normal tissue samples are shown in **(Table 2)**. Among cancerous tissue samples, miR-200b over expression was showed in 36 (75%) samples while down regulation was detected in 12 (25%) samples. In contrary, among non cancerous tissue samples, miR-200b over expression was detected in 13 (92.2%) samples while down regulation was detected in 1 (7.1%) sample only. These differences were highly significant (P =<0.001).

As regard to the relative quantity (RQ) of miR-200b, there was highly significant down regulation of miR- 200b in the malignant group in comparison to the control group, median (IQR) = 0.152 (1.208) and 1.000 (0.000), respectively) (P=0.005) as shown in **Table (3)** and **Figure (1).**

Table (1): Clinicopathological data of patients with breast cancer (IDC) (n=48)

Variable	Total No. (%)
Age in years (mean ± SD)	53.5±13.57
Menopausal status	
• Premenopausal	24 (50%)
• Postmenopausal	24 (50%)
Distant metastasis	
• No	40 (83.3%)
• Yes	8 (16.7%)
Tumor size	
• < 2 cm	2 (4.2%)
• 2 – 5 cm	28 (58.3%)
• ≥ 5 cm	18 (37.5%)
Histologic grade	
• Grade I	1 (2.1%)
• Grade II	40 (83.3%)
• Grade III	7 (14.6%)
Lymph-vascular invasion	
• No	10 (20.8%)
• Yes	38 (79.2%)
Lymph node metastasis	
• No	12 (25.0%)
• Yes	36 (75.0%)
Estrogen status	
• ER –ve	21 (43.8%)
• ER +ve	27 (56.3%)
Progesterone status	
• PR –ve	23 (47.9%)
• PR +ve	25 (52.1%)
Her – 2 status	
• Her 2 –ve	9 (18.7%)
• Her 2 +ve	4 (8.3%)
• N/A*	35 (72.9%)

N/A=Not Available
Data was expressed as (Mean ± Standard Deviation), number and percentage

Table (2): Comparison between the expression of miR-200b in cancerous and non cancerous adjacent tissues by PCR

Biological Marker	Group		P value
	Malignant No. = 48	Control No.= 14	
miRNA 200b			
Over expression	12 (25%)	13 (92.9%)	<0.001
Down expression	36 (75%)	1 (7.1%)	

Table (3): Comparison between RQ of miR- 200b in malignant and control groups by PCR

Biological Marker	Group		P value
	Malignant No. = 48	Control No.= 14	
miRNA 200b			
Median (IQR)	0.152 (1.208)	1.000 (0.000)	0.005

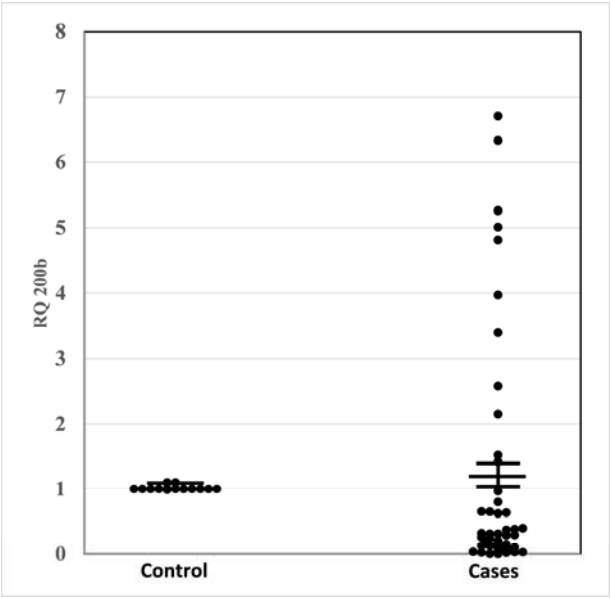

Fig. (1): Comparison between RQ of miR- 200b in malignant and control groups by PCR.

The comparison of various clinico-pathologic characteristics of breast cancer patients and miR-200b expression (high and low expression) in breast tissues in our study are shown in **Table 4**. Among the 48 breast cancer tissues studied, low expression of miR-200b was seen in 75 % of total patients. Low expression of miR-200b was insignificantly higher in patients with distant metastases (75% vs 25%), vascular invasion (71.1% vs 28.9%), estrogen receptor expression (77.8% vs 22.2%), progesterone receptor expression (84 % vs 16%) and lymph nodes metastasis (72.2% vs 27.8%) in comparison to those with high expression of miR- 200b.

Overall survival (OS) in high and low miR-200b expression cases was 32.22 months and 49.55 months ,respectively while disease free survival (DFS) in high and low miR-200b expression cases was 24.75 months and 39.50 months ,respectively (**Table 5 and 6).** No significance difference could be detected for both overall and disease free survival among malignant cases showed up or down regulation of miR-200b (P=0.988 and P=0.726, respectively).

Table (4): Cinico-pathological variables and the expression of miR-200b in breast cancer (IDC)

Variables	miR- 200b		P value
	Low expression No. = 36	High expression No.= 12	
Age (mean ± SD) year	5.50±10.37	52.83±12.62	0.513
Menopausal State			
• Pre (≤51 yrs)	20 (83.3%)	4 (16.7%)	0.182
• Post(>51yrs)	16 (66.7%)	8 (33.3%)	
Distant Metastasis			
• Yes	6 (75%)	2 (25%)	1.000
• No	30 (75%)	10 (25%)	
Tumor Size (cm)			
• < 2	2 (100%)	0 (0%)	
• ≥ 2 < 5	22 (78.6%)	6 (21.4%)	0.467
• ≥ 5	12 (66.7%)	6 (33.3%)	
Tumor Grade			
• Grade I	1 (100%)	0 (0%)	
• Grade II	29 (72.5%)	11 (27.5%)	0.639
• Grade III	6 (85.7%)	1 (14.3%)	
Vascular Invasion			
• Yes	27 (71.1%)	11 (28.9%)	0 218
• No	9 (90%)	1 (10%)	
ER			
• + ve	21 (77.8%)	6 (22.2%)	0.614
• - ve	15 (71.4%)	6 (28.6%)	
PR			
• + ve	21 (84%)	4 (16%)	0.133
• - ve	15 (65.2%)	8 (34.8%)	
Her-2 neu (n=13)			
• + ve	3 (75%)	1 (25%)	0.764
• - ve	6 (66.7%)	3 (33.3%)	
LN Metastasis			
• Yes	26 (72.2%)	10 (27.8%)	0.441
• No	10 (83.3%)	2 (16.7%)	

Table (5): Comparison of overall survival of malignant cases and the expression of miR 200b

Biological Marker	Mean	Confidence Interval		P value
		Lower	Upper	
miRNA 200b				
Over expression	32.22	27.08	37.35	0.988
Down expression	49.55	36.40	62.71	

Table (6): Comparison of disease free survival of malignant cases and the expression of miR 200b

Biological Marker	Mean	Confidence Interval		P value
		Lower	Upper	
miRNA 200b				
Over expression	27.75	16.80	32.71	0.726
Down expression	39.50	26.35	52.65	

Discussion:

The miR-200 family has been reported to be a fundamental regulator of epithelial-mesenchymal transition (EMT), thus highlighting their roles in cancer progression. As a founding member in miR-200 family, miR-200b attracts much focus both in carcinogenesis and cancer therapy in recent years **[9].** miR-200b as tumor suppressor regulating EMT has been reported in several malignancies, such as prostate cancer **[10],** colon cancer **[11],** non-small cell lung cancer **[12**], and so on. The dysregulation of miR- 200b in cancer could be transcriptional inhibition or epigenetic modifications, such like DNA methylation and histone modifications **[13,14].** Moreover, miR-200b is thought to be related to cell differentiation by targeting GATA-4 **[15].** Loss of miR-200b contributes to the breast cancer stem cell status maintaining **[16].** Besides, miR-200b has also been associated with cancer chemo-sensitivity by modulating PTEN, PTPN12 and thus their downstream oncogenes like src and ras **[17, 18].**

Breast cancer is the most frequent carcinoma and the second most common cause of cancer-related mortality in women **[19].** Also, Iorio and colleagues **[20]** identified a global pattern of miRNA deregulation in breast cancer tissue when compared with normal breast tissue, hinting at the importance of miRNA deregulation in the development of breast cancer in general.

As miRNAs appear to be critical regulators of tumor biology, their potential as prognostic and predictive biomarkers has recently been given attention. In addition, their great stability when compared with mRNA molecules, both in blood samples and in formalin-fixed, paraffin-embedded tissue samples, offers a great advantage **[21, 22].**

In this study, we investigated the expression of miR- 200b and its prognostic role in breast cancer in 48 female patients, all were diagnosed with breast cancer (IDC). The expression of miR- 200b in both cancerous and adjacent normal tissue samples showed over expression of miRNA in 13 (92 .2%) of normal tissue samples in comparison to 12 (25 %) of cancerous samples. Down expression of miR- 200b was showed in 36 (75%) cancerous samples while it was reported in 1 (7.1%) normal sample only. These differences were highly significant (P=<0.001).

As regard to the RQ of miR- 200b, there was highly significant down regulation of miR- 200b in the malignant group in comparison to the control group, (median (IQR) = 0.152 (1.208) and 1.000 (0.000), respectively) (P=0.005).

The difference in miR- 200b expression between normal and tumor samples underlines the important role of miRNA deregulation in the development of breast cancer. We observed attenuated expression levels of miR- 200b in the tumor samples relative to normal tissues . The global repression of miRNAs in cancerous tissue relative to normal tissue has been reported previously and suggests that most miRNAs have a tumor suppressive function **[23].**

Ye et al **[24]** study showed that expression of miR-200b was significantly lower in breast cancer tissues than normal tissues, in 40 samples of tumor and para-carcinoma (normal) tissues, by using qRT -PCR to detect the expression level. In comparison with the normal tissues, miR-200b was down regulated in 77.5% (31/40) of the tumor samples. .Also, they reported similar results in breast cancer cell lines. They concluded that reduced miR- 200b expression was a frequent event in human breast cancer tissues and could be involved in breast cancer carcinogenesis.

Comparison of various clinico –pathological variables as well as both overall and disease free survival in malignant cases among those showing over and down expression of miRNA showed no significant differences among the two groups . Low expression of miR- 200b correlate with higher distant metastases (75% vs 25%) ,vascular invasion (71.1% vs 28.9%) and lymph nodes metastasis (72.2% and 27.8%) in comparison to those with high expression of miR- 200b. These results indicated that miR-200b could be a prognostic factor in breast cancer patients.

The prognostic role of the expression of miR- 200b in breast cancer patients was analyzed .Ye et al **[24]** found that low expression of miR-200b correlated with advanced clinical stage and more distant metastasis in breast cancer. Since miR-200b was reported as a regulator of EMT, which was thought to be the initiation of metastasis, the results were reasonable. Further we showed that the patients with low miR-200b expression correlated with worse outcome, indicating miR-200b as a tumor suppressor in breast cancer.

miRNA families, including members of the miR-200 family, members of the let-7 family, and NF_B-regulating miRNAs **[25],** are known to inhibit stem cell specific pathways, epithelial-to-mesenchymal transition, cell proliferation, and other global oncogenic processes **[26 ,27, 28, 29, 30].** Hence, their over expression would induce a more-differentiated, less-proliferative, less-mesenchymal, and less-migratory/invasive cell phenotype. The huge difference in miRNA expression of theses members between normal and tumor samples underlines the important role of miRNA deregulation in the development of breast cancer **[31].** The global repression of miRNAs in cancerous tissue relative to normal tissue has been reported previously and suggests that most miRNAs have a tumor suppressive function **[23].**

Conclusion:

In conclusion, our study demonstrated that miR-200b could be a tumor suppressor and a potential biomarker in breast cancer patients. We hypothesize that such profiles of miR- 200b expression can be informative for breast cancer detection and prognosis and might assist in defining specific targets for future therapy.

List of Abbreviations:

DFS		Disease free survival
EMT	:	Epithelial- mesenchymal transition
FFPE	:	Formalin fixed-paraffin embedded
IDC	:	Invasive duct carcinoma
IQR	:	Interquartile range
OS		Overall survival
q RT-PCR	:	Quantitative real time – polymerase chain reaction
RQ	:	Relative quantity

Acknowledgements:

The authors thank Dr. Nabiel NH Mikhail for his contribution in the statistics of this study.

References:

1. Siegel R, Naishadham D, Jemal A: **Cancer statistics**, 2013. CA Cancer J Clin 2013, **63**:11–30.

2. Ibrahim AS, Mikhail NNH: **Review Article: The Evolution of Cancer Registration in Egypt: From Proportions to Population-based Incidence Rates.** SECI Oncology, Volume:15, Publication ID:4. DOI:10.18056/seci2015.4.

3. Carlson RW, Allred DC, Anderson BO, Burstein HJ, Edge SB, Farrar WB, Forero A, Giordano SH, Goldstein LJ, Gradishar WJ: **Metastatic Breast Cancer**, Version 1.2012 Featured Updates to the NCCN Guidelines. J Natl Compr Canc Netw 2012, **10**:821–829.

4. Liu H: **MicroRNAs in breast cancer initiation and progression**. Cell Mol Life Sci 2012, **69**:3587–3599.

5. Calin GA, Croce CM: **MicroRNA signatures in human cancers**. Nat Rev Cancer 2006, **6**:857–866.

6. Riaz M, van Jaarsveld MTM , Hollestelle A,Prager-van der Smissen WJC , HeineAAJ , Antonius WM Boersma AWM, Liu J, Helmijr J, Ozturk B, Smid M, Wiemer EA, Foekens JA and Martens JWM : **Human breast cancer cell lines reveals subtype and driver mutation-specific miRNAs** . World Journal of Surgical Oncology 2008, **6**:56 DOI:10.1186/1477-7819-6-56

7. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ: **The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1**. Nat Cell Biol 2008, **10**:593–601.DOI: 10.1038/ncb 1722.

8. Bojmar L, Karlsson E, Ellegård S, Olsson H, Björnsson B, Hallböök O, Larsson M, Stål O, Sandström P . **The Role of MicroRNA-200 in Progression of Human Colorectal and Breast Cancer** PLos One: December 20, 2013:8 (12):e84815. DOI: 10.1371/journal.pone.0084815.

9. Feng B, Wang R, Chen L-B: **Review of miR-200b and cancer chemosensitivity**. Biomed Pharmacother 2012, **66**:397–402.

10. He M, Liu Y, Deng X, Qi S, Sun X, Liu G, Zhao M: **Down-regulation of miR-200b-3p by low p73 contributes to the androgen-independence of prostate cancer cells**. Prostate 2013, **73**:1048–1056.DOI: 10.1002/pros.22652.

11. Cai ZG, Zhang SM, Zhang H, Zhou YY, Wu HB, Xu XP: **Aberrant expressionof microRNAs involved in epithelial-mesenchymal transition of HT-29 cell line**. Cell Biol Int 2013, **37**:669–674.DOI: 10.1002/cbin.10087.

12. Pacurari M, Addison JB, Bondalapati N, Wan YW, Luo D, Qian Y, CastranovaV, Ivanov AV, Guo NL: **The microRNA-200 family targets multiple non-small cell lung cancer prognostic markers in H1299 cells and BEAS-2B cells**. Int J Oncol 2013, **43**:548–560.DOI: 10.3892/ijo.2013.1963.

13. Castilla MA, Diaz-Martin J, Sarrio D, Romero-Perez L, Lopez-Garcia MA, Vieites B, Biscuola M, Ramiro-Fuentes S, Isacke CM, Palacios J: **MicroRNA-200 family modulation in distinct breast cancer phenotypes**. PLoS One 2012,**7**:e47709.DOI: 10.1371/journal.pone.0047709.

14. Davalos V, Moutinho C, Villanueva A, Boque R, Silva P, Carneiro F, Esteller M: **Dynamic epigenetic regulation of the microRNA-200 family mediates epithelial and mesenchymal transitions in human tumorigenesis**. Oncogene 2011, **31**:2062–2074.DOI: 10.1038/onc.2011.383.

15. Yao C-X, Wei Q-X, Zhang Y-Y, Wang W-P, Xue L-X, Yang F, Zhang S-F, Xiong C-J, Li W-Y, Wei Z-R: **miR-200b targets GATA-4 during cell growth and differentiation**. RNA Biol 2013, **10**:0–1.DOI: 10.4161/rna.24370.

16. Lim Y-Y, Wright JA, Attema JL, Gregory PA, Bert AG, Smith E, Thomas D,Lopez AF, Drew PA, Khew-Goodall Y: **Epigenetic modulation of the miR-200 family is associated with transition to a breast cancer stem-cell-like state**. J Cell Sci 2013, **126**:2256–2266.DOI: 10.1242/jcs.122275.

17. Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, Jiang J, Schmittgen TD, Patel T: **Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines**. Gastroenterology 2006, **130**:2113–2129.

18. Rossi L, Bonmassar E, Faraoni I: **Modification of miR gene expression pattern in human colon cancer cells following exposure to 5-fluorouracil in vitro**. Pharmacol Res 2007, **56**:248–253.

19. Xu P, Guo M, Hay BA: **MicroRNAs and the regulation of cell death**. Trends Genet 2004, **20**:617-624.

20. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E,Pedriali M, Fabbri M, Campiglio M, Ménard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce C: **MicroRNA gene expression deregulation in human breast cancer**. Cancer Res 2005, **65**:7065-7070

21. Xi Y, Nakajima G, Gavin E, Morris CG, Kudo K, Hayashi K, Ju J: **Systematic analysis of microRNA expression of RNA extracted from fresh frozen and formalin-fixed paraffin-embedded samples**. RNA 2007, **13**:1668-1674.

22. Li J, Smyth P, Flavin R, Cahill S, Denning K, Aherne S, Guenther SM, O'Leary JJ, Sheils O: **Comparison of miRNA expression patterns using total RNA extracted from matched samples formalin-fixed paraffin embedded(FFPE) cells and snap frozen cells**. BMC Biotechnol 2007, **7**:1-6.

23. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet- Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR: **MicroRNA expression profiles classify human cancers.** Nature 2005, **435**:834-838.

24. Ye, F, Tang , H, Liu , Q , Xie , X , Wu ,M , Liu , X, Chen ,B and Xie ,X : **miR-200b as a prognostic factor in breast cancer targets multiple members of RAB family**. Journal of Translational Medicine 2014, **12**:17.DOI: 10.1186/1479-5876-12-17.

25. Ma X, Becker Buscaglia LE, Barker JR, Li Y: **MicroRNAs in NF-kappaB signaling**. J Mol Cell Biol 2011, **3**:159-166. DOI: 10.1093/jmcb/mjr007

26. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R,Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM: **A microRNA expression signature of human solid tumors defines cancer gene targets.** Proc Natl Acad Sci USA 2006, **103**:2257-2261.

27. Herschkowitz JI, Zhao W, Zhang M, Usary J, Murrow G, Edwards D, Knezevic J, Greene SB, Darr D, Troester MA, Hilsenbeck SG, Medina D, Perou CM, Rosen JM: **Breast Cancer Special Feature: Comparative oncogenomics identifies breast tumors enriched in functional tumor initiating cells**. Proc Natl Acad Sci USA 2011 Feb 21; **109**(8):2778-83.DOI: 10.1073/pnas.1018862108.

28. Gregory PA, Bracken CP, Smith E, Bert AG, Wright JA, Roslan S, Morris M,Wyatt L, Farshid G, Lim YY, Lindeman GJ, Shannon MF, Drew PA, Khew- Goodall Y, Goodall GJ: **An autocrine TGF-beta/ZEB/miR-200 signaling network regulates establishment and maintenance of epithelial mesenchymal transition**. Mol Biol Cell 2011, **22**:1686-1698. DOI: 10.1091/mbc.E11-02-0103

29. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ: **The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1**. Nature 2008, **10**:593-601 DOI: 10.1038/ncb 1722.

30. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E: **let-7 regulates self renewal and tumorigenicity of breast cancer cells**. Cell 2007, **131**:1109- 1123.

31. van Schooneveld E, Wouters MCA, Van der Auwera I, Peeters DJ, Wildiers H, Van Dam PA, Vergote I, Vermeulen PB, DirixLY and Van Laere1 SJ : **Expression profiling of cancerous and normal breast tissues identifies microRNAs that are differentially expressed in serum from patients with (metastatic) breast cancer and healthy volunteers**. Breast Cancer Research 2012, **14**:R34.