MicroRNA Gene Expression Deregulation in Human Breast Cancer

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Abstract

MicroRNAs (miRNAs) are a class of small noncoding RNAs that control gene expression by targeting mRNAs and triggering either translation repression or RNA degradation. Their aberrant expression may be involved in human diseases, including cancer. Indeed, miRNA aberrant expression has been previously found in human chronic lymphocytic leukemias, where miRNA signatures were associated with specific clinicobiological features. Here, we show that, compared with normal breast tissue, miRNAs are also aberrantly expressed in human breast cancer. The overall miRNA expression could clearly separate normal versus cancer tissues, with the most significantly deregulated miRNAs being *mir-125b*, *mir-145*, *mir-21*, and *mir-155*. Results were confirmed by microarray and Northern blot analyses. We could identify miRNAs whose expression was correlated with specific breast cancer biopathologic features, such as estrogen and progesterone receptor expression, tumor stage, vascular invasion, or proliferation index. (Cancer Res 2005; 65(16): 7065-70)

Introduction

MicroRNAs (miRNAs) represent a class of naturally occurring small noncoding RNA molecules, distinct from but related to small interfering RNAs. Mature miRNAs are 19- to 25-nucleotide-long molecules cleaved from 70- to 100-nucleotide hairpin pre-miRNA precursors (1). The precursor is cleaved by cytoplasmic RNase III Dicer into ~22-nucleotide miRNA duplex: one strand (miRNA*) of the short-lived duplex is degraded, whereas the other strand serves as mature miRNA. In animals, single-stranded miRNA binds through partial sequence homology to the 3′ untranslated region (3′ UTR) of target mRNAs, and causes either block of translation or, less frequently, mRNA degradation. The discovery of this class of genes has identified a new layer of gene regulation mechanisms, which play an important role in development and in various cellular processes, such as differentiation, cell growth, and cell death (2). Deviations from normal pattern of expression may play a role in diseases, such as in neurologic disorders (3).

Among human diseases, it has been shown that miRNAs are aberrantly expressed or mutated in cancer, suggesting that they may play a role as a novel class of oncogenes or tumor suppressor genes. The first evidence of involvement of miRNAs in human cancer came from molecular studies characterizing the 13q14 deletion in human chronic lymphocytic leukemia (CLL), which revealed that two miRNAs, *mir-15a* and *mir-16-1*, were the only genes within the smallest common region of deletion. The same two genes were affected by a chromosomal translocation in a CLL patient. *mir-16-1* and/or *mir-15a* were then found down-regulated in 50% to 60% of human CLL (4). Following this initial finding, miRNA expression deregulation in human cancer has been proven in other instances. For example, miR143 and miR145 are down-regulated in colon carcinomas (5). Let-7 is down-regulated in human lung carcinomas and restoration of its expression induces cell growth inhibition in lung cancer A549 cells (6). The *BIC* gene, which contains the miR155, is strongly up-regulated in some Burkitt's lymphoma and several other types of lymphomas (7, 8). The findings that miRNAs have a role in human cancer is further supported by the fact that >50% of miRNA genes are located at chromosomal regions, such as fragile sites, and regions of deletion or amplification that are genetically altered in human cancer (9), suggesting that the relevance of miRNAs in human cancer may be presently underestimated.

Only recently, the possibility of analyzing the entire miRNAome has become possible by the development of microarrays containing all known human miRNAs (10–15). The use of miRNA microarrays made possible to confirm miR-16 deregulation in human CLL, but also recognize miRNA expression signatures associated with well-defined clinicopathologic features of human CLL (16). Recognition of miRNAs that are differentially expressed between normal and tumor samples may help to identify those that are involved in human cancer and establish the basis to unravel their pathogenic role. Here, we present results of a genome-wide miRNA expression profiling in a large set of normal and tumor breast tissues demonstrating the existence of a breast cancer–specific miRNA signature.

Materials and Methods

Breast cancer samples and cell lines. RNAs from primary tumors were from 76 samples collected at the University of Ferrara (Italy), Istituto Nazionale dei Tumori, Milano (Italy), and Thomas Jefferson University (Philadelphia, PA). Clinicopathologic information was available for 58 tumor samples. RNAs from normal samples consisted of six pools of five normal breast tissues each and four additional single breast tissues. RNAs of human breast cell lines were from Hs578-T, MCF7, T47D, BT20, SK-BR-3, HBL100, HCC2218, MDA-MB-175, MDA-MB-231, MDA-MB-361, MDA-MB-435, MDA-MB-436, MDA-MB-453, and MDA-MB-468.

Immunohistochemical analysis of breast cancer samples. Hormonal receptors were evaluated with 6F11 antibody for estrogen receptor α and

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Table 1. miRNAs differentially expressed between breast carcinoma and normal breast tissue

	P	Breast cancer			Normal breast		
		Median	Range		Median	Range	
		Normalized	Min	Max	Normalized	Min	Max
let-7a-2	1.94E−02	1.67	0.96	6.21	2.30	1.34	5.00
let-7a-3	4.19E−02	1.26	0.81	3.79	1.58	1.02	2.91
let-7d (=*7d-v1*)	4.61E−03	0.90	0.59	1.54	1.01	0.83	1.25
let-7f-2	6.57E−03	0.84	0.51	1.58	0.92	0.76	1.03
let-7i (= *let-7d-v2*)	3.38E−02	2.05	1.02	7.49	1.53	1.01	3.47
mir-009-1 (*mir-131-1*)	9.12E−03	1.36	0.69	4.16	1.01	0.61	2.44
mir-010b	4.49E−02	1.11	0.69	4.79	1.70	0.96	6.32
mir-021	4.67E−03	1.67	0.66	26.43	1.08	0.80	2.31
mir-034 (=*mir-170*)	1.06E−02	1.67	0.70	6.40	1.09	0.65	3.17
mir-101-1	4.15E−03	0.83	0.52	1.26	0.90	0.77	1.05
mir-122a	3.43E−03	2.21	0.93	8.08	1.48	1.06	3.67
mir-125a	3.28E−03	1.20	0.69	2.36	1.73	1.21	3.34
mir-125b-1	2.65E−02	1.30	0.55	8.85	2.87	1.45	18.38
mir-125b-2	2.33E−02	1.26	0.69	6.29	2.63	1.40	16.78
mir-128b	1.60E−02	1.12	0.68	7.34	1.02	0.89	1.27
mir-136	2.42E−03	1.32	0.74	10.26	1.06	0.76	1.47
mir-143	7.11E−03	0.87	0.68	1.33	0.96	0.81	1.17
mir-145	4.02E−03	1.52	0.92	8.46	3.61	1.65	14.45
mir-149	2.75E−02	1.11	0.53	1.73	1.03	0.83	1.22
mir-155 (*BIC*)	1.24E−03	1.75	0.95	11.45	1.37	1.11	1.88
mir-191	4.26E−02	5.17	1.03	37.81	3.12	1.45	14.56
mir-196-1	1.07E−02	1.20	0.57	3.95	0.95	0.66	1.75
mir-196-2	1.16E−03	1.46	0.57	5.55	1.04	0.79	1.80
mir-202	1.25E−02	1.05	0.71	2.03	0.89	0.65	1.20
mir-203	4.06E−07	1.12	0.50	5.69	0.86	0.71	1.04
mir-204	2.15E−03	0.78	0.48	1.04	0.89	0.72	1.08
mir-206	1.42E−02	2.55	1.22	6.42	1.95	1.34	3.22
mir-210	6.40E−13	1.60	0.98	12.13	1.12	0.97	1.29
mir-213	1.08E−02	3.72	1.42	40.83	2.47	1.35	5.91

PGR-1A6 for progesterone receptor (Ventana, Tucson, AZ). The proliferation index was assessed with MIB1 antibody (DAKO, Copenhagen, Denmark). ERBB2 was detected with CB11 (Ventana) and p53 protein expression was examined with DO7 (Ventana). Staining procedures were done as described (17). Only tumor cells with distinct nuclear immunostaining for estrogen receptor, progesterone receptor, Mib1, and p53 were recorded as positive. Tumor cells were considered positive for ERBB2 when they showed distinct membrane immunoreactivity. To perform a quantitative evaluation of biological markers, the Eureka Menarini computerized image analysis system was used. For each tumor section, at least 20 microscopic fields of invasive carcinoma (40× objective) were measured. The following cutoff values were used: 10% of positive nuclear area for estrogen receptor, progesterone receptor, c-erbB2, and p53; 13% of nuclei expressing Mib1 was introduced to discriminate cases with high and low proliferative activity.

MicroRNA microarray. Total RNA isolation was done with Trizol (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. RNA labeling and hybridization on miRNA microarray chips was done as previously described (10). Briefly, 5 μg of RNA from each sample was biotin-labeled during reverse transcription using random examers. Hybridization was carried out on miRNA microarray chip (KCI version 1.0; ref. 10), which contains 368 probes, including 245 human and mouse miRNA genes, in triplicate. Hybridization signals were detected by biotin binding of a Streptavidin–Alexa 647 conjugate using a Perkin-Elmer ScanArray XL5K. Scanner images were quantified by the Quantarray software (Perkin-Elmer, Wellesley, MA).

Statistical and bioinformatic analysis of microarray data. Raw data were normalized and analyzed using the GeneSpring software version 7.2 (Silicon Genetics, Redwood City, CA). Expression data were median centered. Statistical comparisons were done by ANOVA, using the Benjamini and Hochberg correction for false-positive reductions. Prognostic miRNAs for tumor versus normal class prediction were determined by using both the Prediction Analysis of Microarrays software (PAM; ref. 18)[7] and the Support Vector Machine (19) tool. Both algorithms were used for cross-validation and test-set prediction. All data were submitted using MIAMExpress to the Array Express database (accession numbers to be received upon revision).

Northern blotting. Northern blot analysis was done as previously described (4). RNA samples (10 mg each) were electrophoresed on 15% acrylamide, 7 mol/L urea Criterion precasted gels (Bio-Rad, Hercules, CA) and transferred onto Hybond-N+ membrane (Amersham Biosciences, Piscataway, NJ). Hybridization was done at 37°C in 7% SDS/0.2 mol/L Na_2PO_4 (pH 7.0) for 16 hours. Membranes were washed at 42°C, twice with 2× standard saline phosphate [0.18 mol/L NaCl/10 mmol/L phosphate (pH 7.4)], 1 mmol/L EDTA (saline-sodium phosphate-EDTA, SSPE), and 0.1% SDS and twice with 0.5× SSPE/0.1% SDS. The oligonucleotides used as probes are the complementary sequences of the mature miRNA (miR Registry):[8] miR21 5′-TCAACATCAGTCTGATAAGCTA-3′; miR125b1: 5′-TCA-CAAGTTAGGGTCTCAGGGA-3′; miR145: 5′-AAGGGATTCCTGG-GAAAACTGGAC-3′. An oligonucleotide complementary to the U6 RNA (5′-GCAGGGGCCATGCTAATCTTCTCTGTATCG-3′) was used to normalize

[7] http://www-stat.stanford.edu/~tibs/PAM/index.html.

[8] http://www.sanger.ac.uk/Software/Rfam/mirna/.

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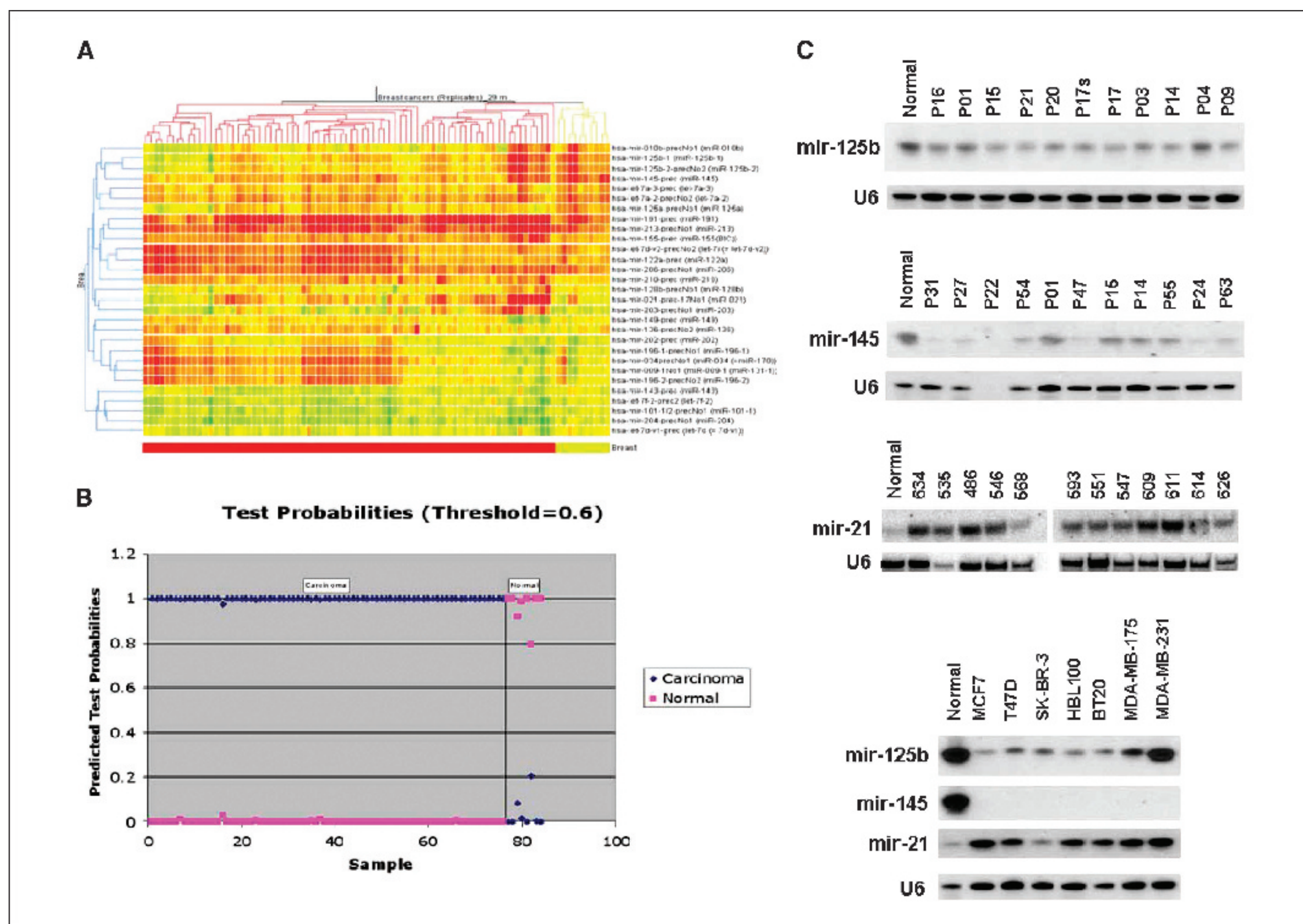

Figure 1. Cluster analysis and PAM prediction in breast cancer and normal breast tissues. *A,* tree generated by a cluster analysis showing the separation of breast cancer from normal tissues on the basis of miRNA differentially expressed ($P < 0.05$) between breast cancer and normal tissue (see Supplementary Table S1). The bar at the bottom indicates the group of cancer samples (*red*) or the group of normal breast tissues (*yellow*). *B,* PAM analysis displaying the graphical representation of the probabilities (0.0-1.0) of each sample for being a cancer or a normal tissue. All breast cancer and normal tissues were correctly predicted by the miR signature shown in Table 1. *C,* Northern blot analysis of human breast carcinomas and breast cancer cell lines with probes *mir-125b*, *mir-145*, and *mir-21*. The U6 probe was used for normalization of expression levels in the different lanes.

expression levels. Two hundred nanograms of each probe was end labeled with 100 mCi [γ-^{32}P]ATP using the polynucleotide kinase (Roche, Basel, Switzerland). Blots were stripped in boiling 0.1% SDS for 10 minutes before rehybridization.

Results

A microRNA expression signature discriminates between normal and cancer breast tissues. We used a miRNA microarray (10) to evaluate miRNA expression profiles of 10 normal and 76 neoplastic breast tissues. Each tumor sample was derived from a single specimen; 6 of the 10 normal samples consisted of pools made of five different normal breast tissue RNAs; hence, 34 normal breast samples were actually examined in the study.

To identify miRNA whose expression was significantly different between normal and tumor samples and could identify the different nature of these breast tissues, we made use of ANOVA and class prediction statistical tools.

To identify differentially expressed miRNAs among all the human miRNAs spotted on the chip, the ANOVA analysis on normalized data generated a list of differentially expressed miRNAs (at $P <$ 0.05) between normal breasts and breast cancers (Table 1). Cluster analysis, based on differentially expressed miRNA, generated a tree with clear distinction between normal and cancer tissues (Fig. 1*A*).

To identify the smallest set of predictive miRNAs differentiating normal versus cancer tissues, we used the Support Vector Machine (GeneSpring software; ref. 19) and PAM (18).[9] Results from the two types of class prediction analysis were largely overlapping (Table 2; Fig. 1*B*). Among miRNAs listed in Table 2, 11 of 15 have an ANOVA P value of <0.05.

To confirm results obtained by microarray analysis, we carried out Northern blot analysis on some of the differentially expressed miRNAs. We analyzed the expression of *mir-125b*, *mir-145*, and *mir-21* in human breast cancers and in breast cancer cell lines. All Northern blots confirmed results obtained by microarray analysis, and in many cases differences seemed even stronger than that anticipated from microarray studies (Fig. 1*C*).

Given that biological significance of miRNA deregulation relies on their protein-coding gene targets, we analyzed the predicted targets of the most significantly down-regulated and up-regulated

[9] http://www-stat.stanford.edu/~tibs/.

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miRNAs: *miR-10b*, *miR125b*, *miR-145*, *miR-21*, and *miR-155*. The analysis was done using the three algorithms, miRanda, TargetScan, and PicTar, commonly used to predict human miRNA gene targets (20–22). Because any of the three approaches generates an unpredictable number of false positives, results were intersected to identify the genes commonly predicted by at least two of the methods. Results are shown in Supplementary Table S1.

Biopathologic features and microRNA expression. We analyzed results from miRNA expression profiles in breast cancer to evaluate whether a correlation existed with various biopathologic features associated with tumor specimens. We analyzed lobular versus ductal histotypes, breast cancers with differential estrogen receptor α or progesterone receptor expression, lymph nodes metastasis, vascular invasion, proliferation index, expression of ERBB2, and immunohistochemical detection of p53. Lobular versus ductal and ERBB2 expression classes did not reveal any differentially expressed miRNA, whereas all other comparisons revealed a small number of differentially expressed miRNAs ($P < 0.05$). Tumor grade was not analyzed because the only two grade 1 samples were a size too small to be compared with a large number of grade 2 or 3 samples. Complete results are shown in Table 3.

Discussion

We have analyzed 76 breast cancer and 10 normal breast samples to identify miRNAs whose expression is significantly deregulated in cancer versus normal breast tissues. We have indeed identified 29 miRNAs whose expression is significantly deregulated (at $P < 0.05$) and a smaller set of 15 miRNAs that were able to correctly predict the nature of the sample analyzed (i.e., tumor or normal breast tissue) with 100% accuracy. These results leave few doubts that aberrant expression of miRNA is indeed involved in human breast cancer.

Among the differentially expressed miRNAs, *miR-10b*, *miR-125b*, *miR145*, *miR-21*, and *miR-155* emerged as the most consistently deregulated in breast cancer. Three of them, *miR-10b*, *miR-125b*, and *miR-145*, were down-regulated and the remaining two, *miR-21* and *miR-155*, were up-regulated, suggesting that they may potentially act as tumor suppressor genes or oncogenes, respectively.

It has been reported that the *miR-125b*, a putative homologue of *lin-4* in *Caenorhabditis elegans*, and the *let-7* miRNAs are induced during *in vitro* retinoic acid–induced differentiation of Tera-2 or embryonic stem cells. Furthermore, high expression of human *miR-125b* seems to be present in differentiated cells or tissues (23). Here, we show that breast cancer primary tumors and cell lines show evidence of a decreased level of *miR-125b* expression, suggesting that lack of *miR-125* may impair differentiation capabilities of cancer cells.

At present, the lack of knowledge about bona fide miRNA gene targets hampers a full understanding on the biological functions deregulated by miRNA aberrant expression. To partially overcome this limitation, we made use of presently available computational approaches to predict gene targets (21, 22, 24). Supplementary Table S1 shows targets that were predicted by at least two of the methods, and shows that various cancer-associated genes are potentially regulated by miRNAs aberrantly expressed in breast cancer.

It may be expected that targets of down-regulated miRNAs include oncogenes or genes encoding proteins with potential oncogenic functions. Indeed, among putative targets, several genes with potential oncogenic functions could be found, such as *FLT1* and the v-crk homologue, the growth factor *BDNF*, and the transducing factor *SHC1* predicted as *miR-10b* targets. Among putative targets of *miR-125b*, potential oncogenic functions

Table 2. Normal and tumor breast tissue class predictor miRNAs

miRNA name	Median expression		ANOVA* *P*	SVM prediction strength†	PAM score‡		Chromosome map
	Cancer	Normal			Cancer	Normal	
mir-009-1	1.36	1.01	0.0091	8.05	0.011	−0.102	1q22
mir-010b	1.11	1.70	0.0449	8.70	−0.032	0.299	2q31
mir-021	1.67	1.08	0.0047	10.20	0.025	−0.235	17q23.2
mir-034	1.67	1.09	0.0106	8.05	0.011	−0.106	1p36.22
mir-102 (*mir-29b*)	1.36	1.14	>0.10	8.92	0.000	−0.004	1q32.2-32.3
mir-123 (*mir-126*)	0.92	1.13	0.0940	9.13	−0.015	0.138	9q34
mir-125a	1.20	1.73	0.0033	8.99	−0.040	0.381	19q13.4
mir-125b-1	1.30	2.87	0.0265	14.78	−0.096	0.915	11q24.1
mir-125b-2	1.26	2.63	0.0233	17.62	−0.106	1.006	21q11.2
mir-140-as	0.93	1.10	0.0695	11.01	−0.005	0.050	16q22.1
mir-145	1.52	3.61	0.0040	12.93	−0.158	1.502	5q32-33
mir-155 (*BIC*)	1.75	1.37	0.0012	10.92	0.003	−0.030	21q21
mir-194	0.96	1.09	>0.10	11.12	−0.025	0.234	1q41
mir-204	0.78	0.89	0.0022	8.10	−0.015	0.144	9q21.1
mir-213	3.72	2.47	0.0108	9.44	0.023	−0.220	1q31.3-q32.1

*ANOVA (Welch *t* test in the Genespring software package) as calculated in Table 1.
†Support Vector Machine prediction analysis tool (from Genespring 7.2 software package). Prediction strengths are calculated as negative natural log of the probability to predict the observed number of samples, in one of the two classes, by chance. The higher is the score, the best is the prediction strength.
‡Centroid scores for the two classes of the PAM (18).

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Table 3. Differentially expressed miRNAs associated with invasive breast cancer biopathologic features

	Median expression		
No. samples	20	13	*P*
Feature	ER+	ER−	
mir-26a	2.473	1.483	0.0273
mir-26b	3.751	1.932	0.0273
mir-29b	1.280	0.935	0.0188
mir-30a-5p	1.779	1.202	0.0191
mir-30b	1.810	1.184	0.0250
mir-30c	1.587	1.040	0.0191
mir-30d	2.986	1.736	0.0273
mir-185	1.568	2.296	0.0399
mir-191	6.354	2.908	0.0273
mir-206	1.811	2.373	0.0273
mir-212	2.811	3.905	0.0403
No. samples	18	14	*P*
Feature	PR+	PR−	
let-7c	1.445	1.129	0.0130
mir-26a	2.451	1.673	0.0474
mir-29b	1.283	0.997	0.0194
mir-30a-5p	1.879	1.219	0.0012
mir-30b	1.898	1.220	0.0044
mir-30c	1.643	1.089	0.0047
mir-30d	3.211	1.777	0.0055
No. samples	9	22	*P*
Feature	pT1	pT2-3	
mir-9-2	0.894	0.840	0.0078
mir-15a	0.905	0.830	0.0024
mir-21	1.080	1.348	0.0040
mir-30a-s	0.944	0.875	0.0065
mir-133a-1	0.928	0.843	0.0025
mir-137	0.894	0.818	0.0100
mir-153-2	0.896	0.833	0.0096
mir-154	0.924	0.852	0.0062
mir-181a	1.024	1.225	0.0045
mir-203	0.905	1.102	0.0011
mir-213	1.915	3.197	0.0003
No. samples	16	6	*P*
Feature	pN_0	pN_{10+}	
let-7f-1	1.195	1.053	0.0378
let-7a-3	1.191	1.039	0.0303
let-7a-2	1.470	1.213	0.0300
mir-9-3	1.634	1.344	0.0152
No. samples	21	11	*P*
Feature	Vascular invasion absent	Vascular invasion present	
mir-9-3	1.059	0.988	0.0451
mir-10b	1.048	0.972	0.0210
mir-27a	1.104	0.992	0.0317
mir-29a	1.101	0.970	0.0346
mir-123	1.125	0.852	0.0161
mir-205	1.299	0.762	0.0451
No. samples	26	23	*P*
Feature	Low PI	High PI	
let-7c	1.817	1.361	0.0071
let-7d	1.594	1.310	0.0073
mir-26a	2.602	1.928	0.0492
mir-26b	4.039	2.695	0.0297
mir-30a-5p	1.783	1.394	0.0257
mir-102	1.389	1.037	0.0017
mir-145	1.557	1.281	0.0136
No. samples	39	14	*P*
Feature	p53+	p53−	
mir-16a	0.895	1.030	0.0026
mir-128b	0.964	1.059	0.0096

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; pT, tumor stage; pN, positive lymph nodes; low PI, low proliferation index, MIB-1 < 20; high PI, high proliferation index, MIB-1 > 30.

included the oncogenes *YES*, *ETS1*, *TEL*, and *AKT3*; the growth factor receptor *FGFR2*; or members of the mitogen-activated signal transduction pathway *VTS58635*, *MAP3K10*, *MAP3K11*, and *MAPK14*. The oncogenes *MYCN*, *FOS*, *YES*, and *FLI1*; integration site of Friend leukemia virus; cell cycle promoters such as *cyclins D2* and *L1*; and MAPK transduction proteins such as MAP3K3 and MAP4K4 were predicted targets for *miR-145*. Interestingly, the proto-oncogene *YES* and the core-binding transcription factor *CBFB* were potential targets of both *miR-125* and *miR-145*.

For the up-regulated miRNAs *miR-21* and *miR-155*, it may be expected that gene targets belong to the class of tumor suppressor genes. For *miR-21*, the *TGFB* gene was predicted as target of *miR-21* by all three methods. For *miR-155*, potential targets included the tumor suppressor genes *SOCS1* and *APC*, and the kinase *WEE1*, which blocks the activity of Cdc2 and prevents entry into mitosis. The hypoxia-inducible factor *HIF1A* was also a predicted target. Interestingly, among predicted genes, the tripartite motif-containing protein *TRIM2*, the proto-oncogene *SKI*, and the RAS homologues *RAB6A* and *RAB6C* were found as potential targets of both *miR-21* and *miR-155*.

miRNAs were found differentially expressed in various biopathologic features distinctive of human breast cancer. Some of

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these findings are worth noticing. For example, *mir-30*s are all down-regulated in both estrogen receptor– and progesterone receptor–negative tumors, suggesting that expression of these miRNAs is regulated by these hormones. Another interesting observation is the finding that the expression of various *let-7* miRNAs was down-regulated in breast cancer samples with either lymph node metastasis or higher proliferation index, suggesting that a reduced *let-7* expression could be associated with a poor prognosis. An association between *let-7* down-regulation and poor prognosis was previously reported in human lung cancer (6). The finding that the *let-7* family of miRNAs regulates the expression of the *RAS* oncogene family provides a potential explanation for the role of the *let-7* miRNAs in human cancer (25). Two miRNA, *miR-145* and *miR-21*, whose expression could differentiate cancer versus normal tissues, were also differentially expressed in cancers with different proliferation indexes or different tumor stage. In particular, *miR-145* is progressively down-regulated from normal breast to cancer with high proliferation index. Similarly, but in opposite direction, *miR-21* is progressively up-regulated from normal breast to cancers with high tumor stage. These findings suggest that deregulation of these two miRNAs may affect critical molecular events involved in tumor progression. Another miRNA potentially involved in cancer progression is *miR-9-3*. *miR-9-3* was down-regulated in breast cancers with either high vascular invasion or presence of lymph node metastasis, suggesting that its down-regulation was acquired in the course of tumor progression and, in particular, during the acquisition of cancer metastatic potential.

It has been reported that miRNA genes are frequently located in chromosomal regions characterized by nonrandom aberrations in human cancer, suggesting that resident miRNA expression might be affected by these genetic abnormalities (9). *miR-125b*, which is down-modulated in breast cancer, is located at chromosome 11q23-24, one of the regions most frequently deleted in breast, ovarian, and lung tumors (26, 27). The recognition of a bona fide tumor suppressor gene located at 11q23-24 involved in the pathogenesis of human breast cancer is still lacking. The *miR-125b* gene establishes itself as an important candidate for this role.

Results reported here increase our understanding of the molecular basis of human breast cancer and suggest that aberrant expression of miRNA genes may be important for the pathogenesis of this human neoplasm.

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