



MicroRNA Involvement in Breast Cancer Multidrug Resistance

Zhongxing Liang¹, Hui Wu¹, James Xia², Yuhua Li¹, Nick Wagar¹, Aizhi Zhu¹, Sijia Wang¹, Dean Blevins¹, Younghyun Yoon¹, Santosh Reddy¹, Jeanine Southerland¹, Stefania Scalò³, Susan E. Bates¹, Hyunuk Shim¹.

1. Emory Univ., Atlanta, GA; 2. GenoSensor Corp., Tempe, AZ; 3. National Cancer Institute, Naples, Italy; 4. NCI/NIH, Frederick, MD



ABSTRACT and INTRODUCTION

Multidrug resistance (MDR) has been frequently associated with elevated expression of one or more ATP binding cassette (ABC) transporters such as three well-known drug efflux proteins: P-glycoprotein (MDR-1), multidrug resistance associated protein (MRP-1), and breast cancer resistance protein (BCRP). However, the regulation of these transporters remains controversial. A recently discovered class of small, functional, noncoding RNAs, named microRNAs (miRNAs), has been shown to function as regulatory molecules by inhibiting protein translation and to play an important role in development, differentiation, cell proliferation, and apoptosis. Very little is currently known about how changes in certain miRNA expression levels among drug resistant tumor cells affect breast cancer multidrug resistance.

In order to determine whether expression of certain miRNAs is altered in various breast cancer MDR cell lines, we performed analyses of miRNA expression in MDR cell lines (MCF7/Adr200, MCF7/TX200, MCF7/MX100) compared to their parent cell line (MCF7) using a microarray containing 463 human mature and precursor miRNA probes. Specific miRNA expression profiles in MDR cells were observed. The alteration of expression of 115 miRNAs were found at least in one MDR subtype, fifteen of which were differentially expressed in all three MDR sublines compared to their parental cells. We found that there are two different profiles of microRNAs between MCF-7/Adr200 overexpressed MDR-1 and MCF-7/TX200 overexpressed MRP-1 as well as MCF-7/MX100 overexpressed BCRP and they may be regulated by different microRNAs. miR-326 and miR-26C have demonstrated the involvement in multidrug resistance. We will further modulate the levels of more selected miRNAs in MDR cells or in MCF7 cells to determine whether these altered expression of miRNAs is involved in breast cancer MDR. Our findings will be beneficial for the prediction of multidrug resistance in patients as well as the design of personalized therapy for breast cancer patients.

MATERIALS AND METHODS

Cell Lines. MCF-7 and its multidrug resistant sublines: MCF-7/Adr200, which was selected by adriamycin, and MCF-7/TX200, which was selected by etoposide. MCF-7/MX100, which was selected by mitoxantrone, were used in this study.

microRNA microArray Hybridization. The miRNAs, purified and labeled by using the related kits from GenoSensor. Hybridizations, image scans, and data analysis were performed by our microarray core facility. In brief, the hybridized slides were subjected to scanning with ScanArray Express HT scanner and to image analysis with GE software.

Data Analysis. For data analysis, we used GeneSpring software. Hierarchical clustering was performed by Average Linkage.

Real-time PCR. Expression levels of a cluster of the decreased expressive miRNAs and a cluster of the elevated expressive miRNAs were confirmed by real time reverse transcription polymerase chain reaction.

siRNA Transfection. The selected decreased expressive miRNAs were upregulated by transfection of synthetic RNA encoding the miRNAs, and conversely, the selected elevated expressive miRNAs were downregulated by transfection of synthetic siRNAs against the precursors of these miRNAs.

MTT Cell Proliferation Assay. Drug resistance was tested by increasing the concentration of the tested drugs and measuring the surviving cells with the MTT cell proliferation assay.

Western Blot Analyses. The proteins were resolved by SDS/PAGE and subjected to immunoblot analysis with MDR-1, MRP-1 and BCRP.

Statistical Analysis. All quantitative data from MTT, and real-time RT-PCR were repeated at least 3 times and statistically analyzed with a t-test.

I. Expression of MDR-related genes in multidrug resistant sublines

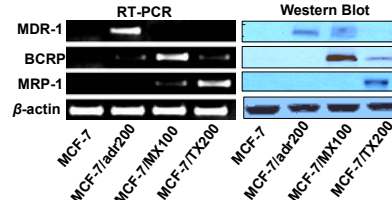


Fig. 1. Expression of MDR-related genes of MCF-7 and its MDR sublines. MCF-7/Adr200, which was selected by adriamycin (doxorubicin), overexpresses MDR-1 but not BCRP and MRP-1; MCF-7/MX100, which was selected by mitoxantrone, overexpresses BCRP but not MDR-1 and MRP-1; MCF-7/TX200, which was selected by etoposide, overexpresses MRP-1 but not MDR-1 and BCRP.

II. Altered expression of microRNAs in multidrug resistant cells

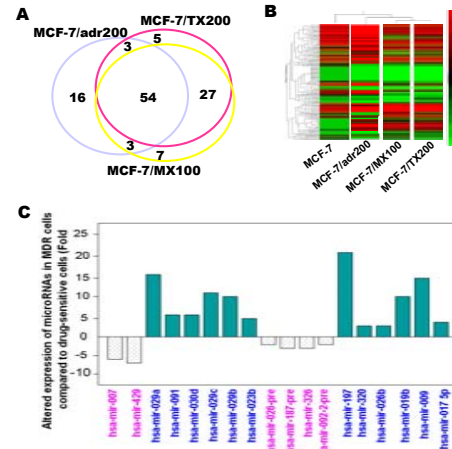


Fig. 2. miRNAs with changed expression in multidrug resistant cells compared to their parental cells. Panel A, the Venn diagram shows relationship of number of miRNAs with very significant change of expression between three MDR sublines. Fifty-four miRNAs are constitutively differentially expressed in all three MDR sublines compared to their parental cells. Sixteen miRNAs are differentially expressed only in MCF-7/Adr200 cells compared to their parental cell line MCF-7. Five miRNAs and 7 miRNAs are differentially expressed respectively only in MCF-7/TX200 and in MCF-7/MX100. Panel B, Dendrogram of clustering analysis of significant miRNA expression in three MDR cell sublines and their parental cell line. The names of cell lines are listed on the bottom of the figure. The dendrogram of clustering cell lines with microRNA files is placed on the top of this figure. Colors represent normalized median value of miRNA expression according to their color legend on the right side of the figure the range of the normalized median value was designated from 0 to 6 depending on their colors from green to red. The dendrogram on the left shows clustering analysis of miRNA expression. Panel C, The graph shows the fold number of expressive difference of fifteen miRNAs with very significant change in MDR cells compared to their parental cells. The y-axis shows a different fold number of the normalized median intensity of microRNA expression between MDR cells and their parental cells

III. microRNAs as potentially specific modulators of MDR-related genes:MDR-1, MRP-1 and BCRP

Table 2. Altered expression of microRNAs in various type of MDR sublines

	hsa-miR-021	hsa-let-7f	hsa-let-7e	hsa-miR-098	hsa-miR-203	hsa-miR-181a	hsa-miR-181b	hsa-miR-100	hsa-miR-130a	hsa-miR-221	hsa-miR-030e 5p	hsa-miR-424	hsa-miR-200a	hsa-miR-099a	hsa-miR-222	hsa-miR-342	hsa-miR-032	hsa-miR-092	hsa-miR-030b	hsa-miR-030e 3p	hsa-miR-106a	hsa-miR-148b	hsa-miR-484	hsa-miR-15a
Normalized	6.91	1.1	2.2	1.6	0.12	0.5	0.3	0.01	0.01	0.019	0.16	0.018	0.37	0.018	0.01	1.5	0.8	0.6	0.4	0.3	0.2	0.13	0.05	0.11
Median	45.5	2.6	4.4	3.2	0.42	1.4	1	0.01	0.01	0.01	0.20	0.01	0.61	0.01	1.1	1.5	0.6	0.7	0.5	0.1	0.12	0.08	0.14	
MCF-7/Adr200	5.89	2.1	4	2.7	0.21	1	0.6	0.70	2.28	1.85	1.38	0.52	0.01	0.71	1.39	0.5	1.6	2.1	1.7	2.2	0.9	0.04	0.27	0.16
MCF-7/MX100	7.69	2	3.1	2.2	0.07	0.5	0.4	0.74	3.05	2.36	2.16	0.86	0.03	0.73	1.37	0.3	2.3	2.5	2	2.8	2.2	0.17	0.06	0.38
MCF-7/TX200	7	2.4	2	2	3.5	2.8	3.3	1	-1.9	1.3	-1.8	1.7	-1.7	1	-1.4	1.9	1	1.8	-1.7	-1.9	1	1.6	1.3	
MCF-7/Adr200	-1	1.8	1.4	1.4	-1.7	1	1.3	74	305	124	139	471	-42	408	137	-6	2.9	4.2	5	9.3	11	1.3	1.2	3.5

IV. microRNAs are involved in multidrug resistance

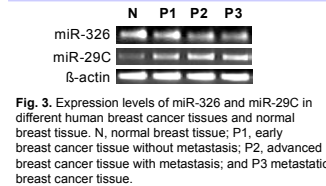


Fig. 3. Expression levels of miR-326 and miR-29C in different human breast cancer tissues and normal breast tissue. N, normal breast tissue; P1, early breast cancer tissue without metastasis; P2, advanced breast cancer tissue with metastasis; and P3 metastatic breast cancer tissue.

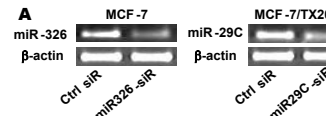


Fig. 4. Expression alteration of miR-326 in MCF-7 cells and miR-26C in MCF-7/TX200 cells modulated multidrug resistance of tumor cells. Panel A, expression levels miR-326 gene was downregulated in MCF-7 cells with miR-326 siRNA and expression levels of miR-29C was deregulated with siRNA against miR-29C. Panel B, Effect of downregulation of miR-326 in MCF-7 (on the right) or miR-29C in MCF-7/TX200 (on the left) on multidrug resistance. Each point is an average of triplicate determinations. Effect of miRNA siRNAs (closed symbols) on the drug sensitivity of cells compared to control siRNA (open symbols).

RESULTS

Table 1. miRNAs with changed expression in all three MDR sublines

	Normalized median value	MCF-7/Adr200	MCF-7/TX200	MCF-7/MX100	P value
hsa-miR-007	0.13	0.01	0.042	0.048	7.60E 06
hsa-miR-429	0.097	0.039	0.01	0.01	4.90E 05
hsa-miR-029a	0.618	1.268	15.22	12.32	0.0204
hsa-miR-091	0.005	1.837	5.976	7.893	0.0092
hsa-miR-030d	0.239	0.086	2.159	2.383	0.0101
hsa-miR-029c	0.598	1.665	9.671	6.887	0.0118
hsa-miR-029b	0.473	1.522	3.523	8.622	0.0274
hsa-miR-023b	1.373	10.47	5.523	5.522	0.0023
hsa-miR-026 pre	0.02	0.01	0.01	0.01	0.0029
hsa-miR-187 pre	0.03	0.01	0.008	0.01	0.0004
hsa-miR-326	0.026	0.01	0.01	0.01	6.60E 06
hsa-miR-092 2-pr	0.021	0.01	0.01	0.01	0.0001
hsa-miR-197	0.006	0.358	0.206	0.089	0.0215
hsa-miR-320	0.2	0.5	1	1	0.0111
hsa-miR-026b	0.4	2	0.9	1	0.0245
hsa-miR-019b	0.06	0.12	0.62	1.08	0.0428
hsa-miR-009	0.01	0.02	0.21	0.22	0.0426
hsa-miR-017 5p	0.7	1.5	3.4	3.7	0.0123

CONCLUSION and PLANS

Specific profile of miRNAs and significant miRNAs with altered expression in MDR cells were found. These findings will contribute further study of mechanism of multidrug resistance in cancer therapy. In addition, these findings will be beneficial for the prediction of multidrug resistance in patients as well as the design of personalized therapy for breast cancer Patients.

We will further modulate the levels of more selected miRNAs in MDR cells or in MCF7 cells to determine whether these altered expressions of miRNAs are involved in breast cancer MDR. The targeting genes of significant miRNAs will be searched by bioinformatics and microarray.

ACKNOWLEDGEMENTS

This study was supported by DOD Breast Cancer Program Concept Award and a research grant from Georgian Cancer Coalition (to ZL) as well as a Distinguished Cancer Scientist Development Award from Georgian Cancer Coalition (to HS).