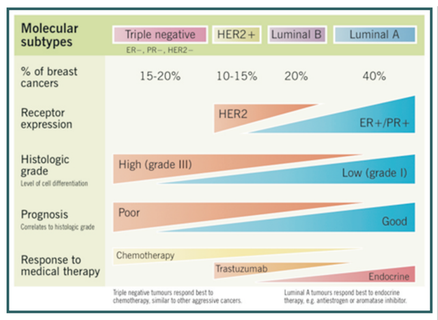
miRNome profile reveals shared features in breast cancer subtypes and points to a novel miRNAs signature involved in disease

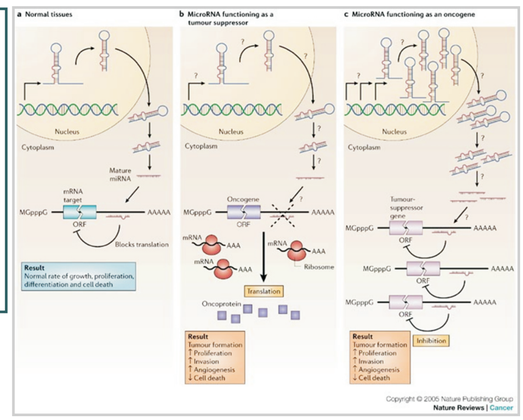
Stephany Corrêa[1]; Thais Basili [1]; Renata Binato[1], Eliana Abdelhay[1,].

[1]Laboratório Célula-Tronco, CEMO, Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brasil

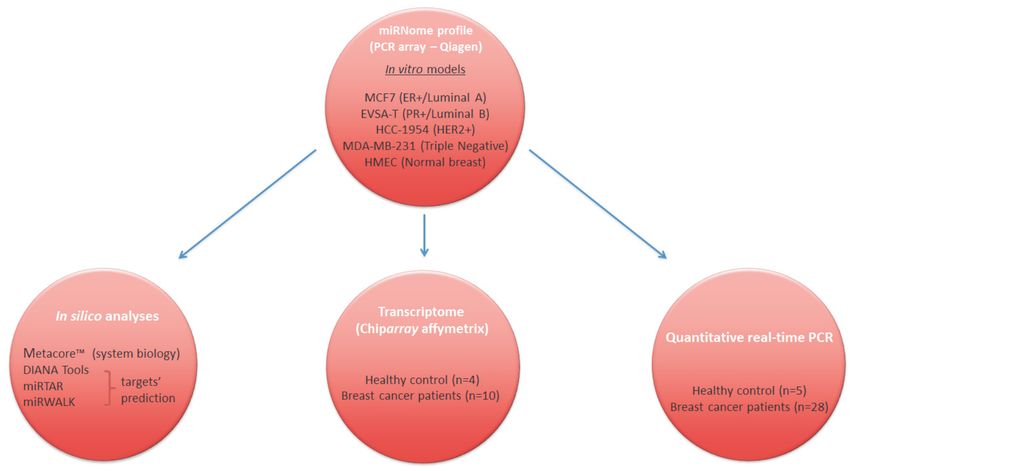
INTRODUCTION AND OBJECTIVE

MicroRNAs (miRNAs) have been investigated in Breast Cancer (BC) mostly with focused perspective due to their high complexity role in gene expression regulation; however, it is still pertinent to perform a large-scale evaluation in order to uncover novel miRNAs related to disease initiation and progression that may be potentially applied as BC markers.



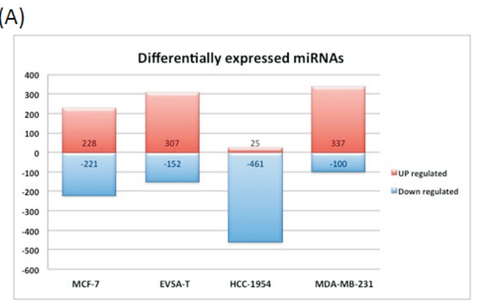


MATERIAL AND METHODS



RESULTS

Comparison among BC cell lines miRNomes



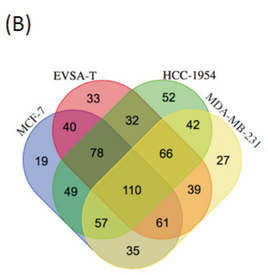


Figure 1: Differentially expressed miRNAs in human breast cancer cell lines. (A) RT-qPCR analysis of 1008 miRNA levels from miRNome PCR array. snoRNAs/snRNAs were used as housekeeping genes for normalization by arithmetic mean. Quantification was performed by the ΔΔCT method. Results were normalized relative to the HMEC cell line. (B) Venn diagram of differentially expressed miRNAs in breast cancer cell lines. Venn diagram drafted from http://bioinformatics.psb.ugent.be /webtools/Venn, showing the total number and overlapping miRNAs identified as differentially expressed by quantitative PCR array from the following breast cancer cell lines

Table 1: miRNAs that presented as up-regulated or down-regulated among BC subtypes from the 110 common miRNAs presented by Venn Diagram.

miRBase ID	MCF7	EVSA-T	HCC-1954	MDA-MB-231
up-regulated				
hsa-miR-1203	3.78	2.64	3.42	2.28
hsa-miR-1238-3p	36.55	363.30	27.22	189.36
hsa-miR-1266	38.59	4.21	2.87	34.46
hsa-miR-146b-3p	4.05	9.64	2.92	8.16
hsa-miR-326	19.77	46.10	4.05	23.72
hsa-miR-3909	3.52	4.88	3.14	2.83
hsa-miR-497-3p	27.67	54.00	2.26	101.59
hsa-miR-95	30.00	3.75	13.45	176.48
down-regulated				
hsa-miR-127-5p	-12.25	-5.82	-1324.90	-45.83
hsa-miR-129-5p	-4.24	-2.03	-11.17	-3.53
hsa-miR-1305	-9.16	-54.32	-513.18	-21.73
hsa-miR-134	-6.69	-27.00	-470.59	-10772.01
hsa-miR-149-5p	-15.91	-6.89	-3.41	-6316.90
hsa-miR-205-5p	-659.40	-30.48	-6.37	-58183.94
hsa-miR-2355-3p	-190.02	-66.87	-42.62	-19.54
hsa-miR-34c-5p	-10897.18	-2967.43	-16028.29	-4787.31
hsa-miR-376c-3p	-81.10	-9.72	-1178.99	-328.18
hsa-miR-382-5p	-4.39	-5.05	-1686.71	-19.65
hsa-miR-423-3p	-4.44	-4.01	-2.17	-4225.77
hsa-miR-431-3p	-4.74	-11.74	-56.62	-3.37
hsa-miR-431-5p	-3.67	-5.17	-990.26	-52.65
hsa-miR-455-3p	-19.49	-269.35	-49.29	-2.02
hsa-miR-487a	-4.35	-4.33	-1259.24	-14512.42
hsa-miR-487b	-7.52	-4.91	-1359.00	-162.58
hsa-miR-582-5p	-34.58	-2.14	-53.38	-435.04
hsa-miR-708-5p	-13.47	-553.20	-3136.63	-151.34
hsa-miR-874	-14.22	-17.43	-228.07	-3.50

Table 2: miRNAs wiht shifted expression among the BC subtypes from the 110 common miRNAs presented by Venn Diagram.

miRBase ID	MCF7	EVSA-T	HCC-1954	MDA-MB-231
shift in expression				
hsa-let-7e-3p	-2.14	-5.96	-6.18	8.28
hsa-miR-1270	-14.32	-13.41	-66.56	7.24
hsa-miR-1271-5p	-5.62	-3.80	-46.42	2.07
hsa-miR-1283	-6.20	-3.19	-8.49	51.21
hsa-miR-130a-5p	-12.42	-5.48	-30.06	4.12
hsa-miR-138-5p	-151.69	-101.83	-1597.57	18.53
hsa-miR-139-5p	-11.35	-11.96	-10.07	4.66
hsa-miR-144-3p	2.86	-2.99	-15.71	-2.10
hsa-miR-193a-3p	2.90	43.92	4.78	-2.19
hsa-miR-200a-5p	6.14	18.96	-124.36	-58.69
hsa-miR-28-3p	-4.87	-2.25	-2.53	5.86
hsa-miR-29a-3p	-4.20	-8.70	-5.44	2.47
hsa-miR-29a-5p	-28.54	-5.75	-5.91	4.14
hsa-miR-29b-1-5p	-14.67	-5.79	-7.38	3.41
hsa-miR-29c-3p	-2.99	-2.64	-3.98	2.72
hsa-miR-3065-5p	-4.23	-2.63	-8.10	4.82
hsa-miR-34b-3p	-215.52	-10.42	-258.68	2.16
hsa-miR-410	-3.64	-51.45	-560.28	1377225.47
hsa-miR-429	11.46	63.34	-679.50	-33.71
hsa-miR-4326	-58.49	-5.43	-2.23	5.23
hsa-miR-455-5p	-16.49	-51.80	-86.22	5.87
hsa-miR-516a-5p	-10.27	-2.51	-12.44	29.38
hsa-miR-615-3p	2.14	33.24	-2.35	-3.26
hsa-miR-639	8.81	7.54	-2.11	-24.34
hsa-miR-643	-4.36	-4.64	-6.59	17.31
hsa-miR-651	-10.65	-3.75	-28.44	15.60
hsa-miR-99a-5p	2.60	4.72	-28.31	-2.69

In silico analysis of miRNAs disclose shared pathways in BC subtypes

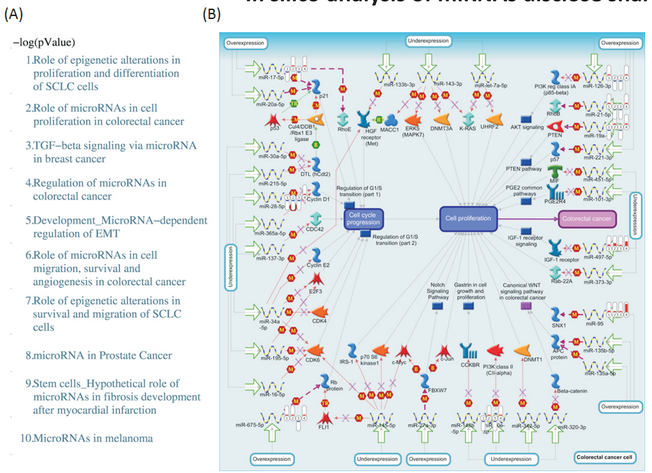


Figure 2: System biology analysis of the BC miRNOme profiles. (A) Enrichment analysis of the 10 most representative pathways maps from the obtained miRNome profiles. Pathway maps of (B) Role of microRNAs in cell proli**feration in colorectal** cancer and (C) Regulation of epithelial-to mesenchymal transtition. (D) Representative interaction map of miR-205-5p.Pathway map were generated by Metacore™ software. The lists of DE miRNAs were uploaded into software and compared to correlate the fold change obtained in PCRarray with disease aggressiveness. 1: MCF7; 2: EVSA-T; 3: HCC-1954; 4:MDA-MB-231.

BC patients present differential expression of *in silico* miRNAs targets

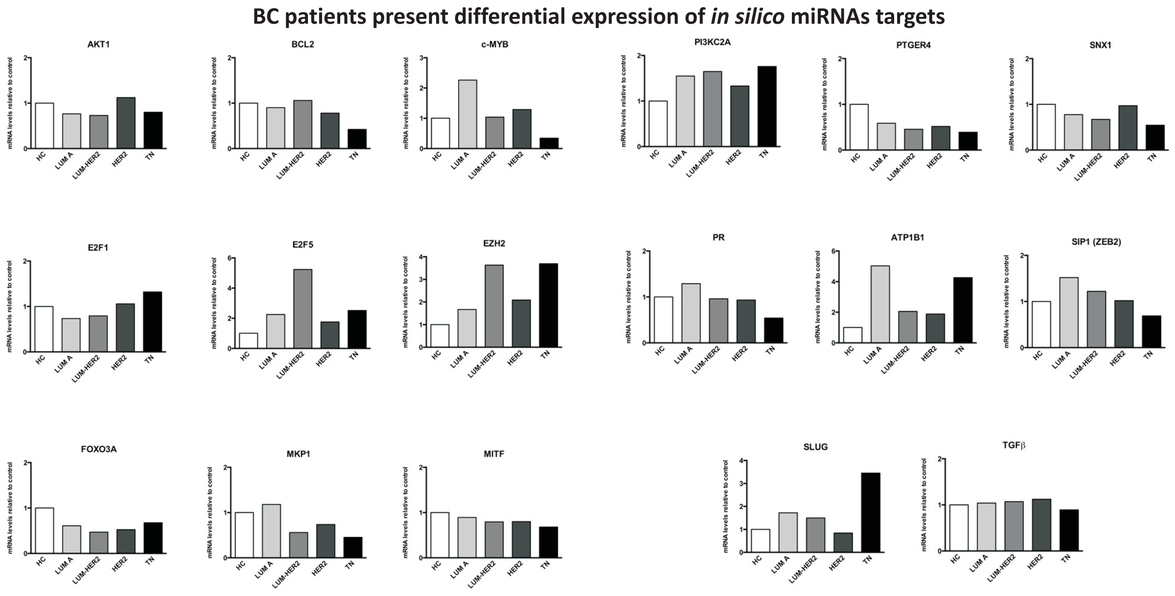


Figure 3: Validation of putative miRNA targets related to proliferation, survival, epigenetic regulation, progesterone-mediated and the TGF signaling pathways in BC. Transcriptome analysis of miRNAs targets exposed by Metacore™ software. mRNA levels of each gene was assessed by raw expression values normalized to GAPDH expression. HMEC mRNAs levels were used as a control. Quantification was performed by the ΔΔCT method.

miRNAs Targets' prediction points to novel miRNAs involved in disease

Table 3: Prediction analysis of miRNA-target interaction from *CMYB*, *EZH2* and *MITF*

	miRTAR	miRwalk	Targetscan	miRnet	miRor
CMYB					
hsa-miR-130a-5p					x
hsa-miR-2355-3p		CDS			
hsa-miR-28-3p					
hsa-miR-29a-5p					
hsa-miR-29b-1-5p		CDS			
hsa-miR-34b-3p					x
hsa-miR-4326		CDS			
hsa-miR-144					x
hsa-miR-429					x
hsa-miR-138					x
hsa-miR-874	CDS				
hsa-miR-127-5p	CDS				
hsa-miR-34c-5p	3UTR				
EZH2					
hsa-miR-129-5p	3UTR				
hsa-miR-149-5p		5UTR, CDS			
hsa-miR-205-5p		CDS			
hsa-miR-2355-3p					
hsa-miR-34c-5p		5UTR, CDS			x
hsa-miR-423-3p		CDS			
hsa-miR-431-3p		CDS			
hsa-miR-431-5p		5UTR, CDS			
hsa-miR-455-3p	3UTR	CDS			
hsa-miR-487a	3UTR	5UTR, CDS			
hsa-miR-487b	3UTR	CDS			
hsa-miR-582-5p	3UTR				x
hsa-miR-708-5p		CDS *		x	
hsa-miR-874		5UTR, CDS			
hsa-miR-127-5p	3UTR	3UTR			
hsa-miR-1305	3UTR				
hsa-miR-144					x
hsa-miR-138		5UTR, CDS*			x
hsa-miR-34b		CDS			x
hsa-miR-429	3UTR				
MITF					
hsa-miR-2355-3p		3UTR, 5UTR, CDS			
hsa-miR-34c-5p	3UTR	CDS			x
hsa-miR-376c-3p					
hsa-miR-429	3UTR				x
hsa-miR-129-5p	3UTR	3UTR, CDS			
hsa-miR-582-5p	3UTR				x
hsa-miR-874	3UTR, CDS	3UTR, CDS			
hsa-miR-1305	3UTR	3UTR, 5UTR, CDS			
hsa-miR-144		3UTR			x
hsa-miR-34b		3UTR			x

* Validated miRNA-target interaction

Table 4: Prediction analysis of miRNA-target interaction from *SIP1* and *SNX1*

	miRTAR	miRwalk	Targetscan	miRnet	miRor
SIP1					
hsa-miR-205-5p		3UTR*	x	x	
hsa-miR-429	3UTR			x	x
hsa-miR-708-5p		3UTR*, CDS*		x	
hsa-miR-127-5p	3UTR, CDS	3UTR, CDS			
hsa-miR-129-5p	3UTR, CDS	5UTR, CDS	x		
hsa-miR-1305	3UTR	3UTR, 5UTR, CDS			
hsa-miR-34c-5p		5UTR, CDS			x
hsa-miR-376c-3p					
hsa-miR-382-5p		5UTR, CDS			
hsa-miR-431-3p		3UTR, 5UTR, CDS			
hsa-miR-431-5p		5UTR, CDS			
hsa-miR-455-3p	3UTR, CDS	3UTR			
hsa-miR-487b	3UTR	3UTR, CDS			
hsa-miR-582-5p	3UTR	3UTR			x
hsa-miR-874	3UTR	3UTR, 5UTR, CDS			
hsa-miR-487a	3UTR	CDS			
hsa-miR-4326	3UTR	CDS			
hsa-miR-423-3p	3UTR, CDS	CDS			
hsa-miR-144		CDS*			x
hsa-miR-34b		CDS			x
hsa-miR-130a		3UTR, 5UTR			x
SNX1					
hsa-miR-1305		3UTR, CDS			
hsa-miR-205-5p		3UTR		x	
hsa-miR-2355-3p		3UTR, CDS			
hsa-miR-34c-5p	3UTR	3UTR			x
hsa-miR-431-3p		3UTR			
hsa-miR-455-3p	3UTR	3UTR			
hsa-miR-708-5p		3UTR, CDS			
hsa-miR-874	3UTR	3UTR			
hsa-miR-4326	3UTR	3UTR			
hsa-miR-423-3p	3UTR	3UTR			
hsa-miR-429	3UTR				x
hsa-miR-129-5p	3UTR	3UTR, CDS			
hsa-miR-582-5p	3UTR	3UTR			
hsa-miR-144		3UTR			x
hsa-miR-138		3UTR, CDS			x
hsa-miR-34b		3UTR			x

* Validated miRNA-target interaction

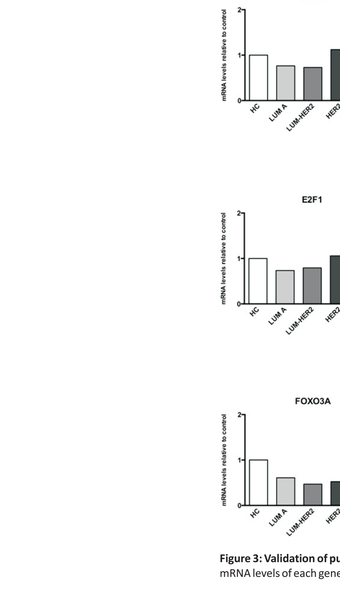


Figura 3: Representative miRNA target prediction analysis from miRTAR online tool

Potential miRNAs signature in Breast Cancer

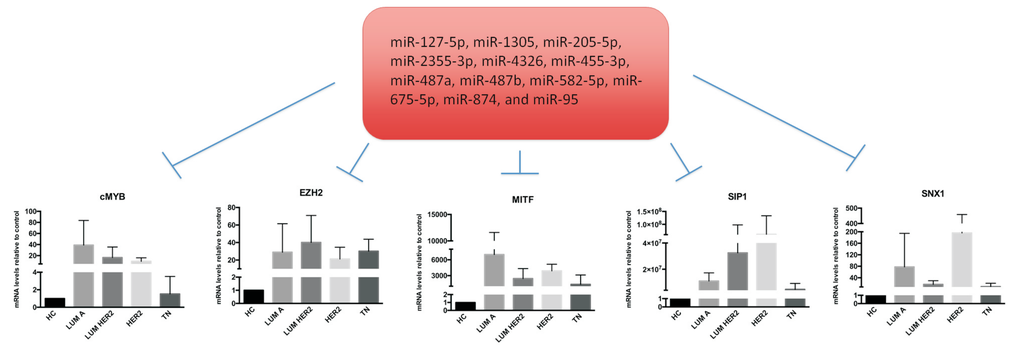


Figure 4: Validation of putative miRNA targets. RT-qPCR analysis of *CMYB*, *EZH2*, *MITF*, *SIP1* and *SNX1*, pointed as the miRNAs targets. mRNA levels of each gene was assessed by raw expression values normalized to GAPDH expression. 5 healthy controls (HC) samples were evaluated, together with n= 28 BC patients' samples from major subtypes. mRNAs levels were used as a control. Quantification was performed by the ΔΔCT method.

CONCLUSION

The approach applied in this study presented signaling molecules and pathways involved in BC tumorigenesis and pointed to novel interactions and potential regulation regarding predicted targets differentialy expressed *in vivo* with identified miRNAs, some of which are still unexplored and may be related to BC aggressiveness.

Auxílio Financeiro: CNPq, FAPERJ, MS

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

SUS MINISTÉRIO DA SAÚDE