Poster Discussion:
BASIC SCIENCE AND TRASLATIONAL REASEARCH

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Session Outline

	177PD	Exosomal long non-coding RNA (lncRNA) in Lung Cancer	Goetz Kloecker, US
	1645PD	microRNA-9 and -224 in trastuzumab resistant HER2 positive breast cancer cells.	Karen Howe, IE
15’		**Discussant**	**Erika Martinelli, IT**
15’		**Questions**	
	1646PD	Customized first line chemotherapy according to ERCC1 and RRM1 SNPs in advanced Non-Small-Cell Lung cancer (NSCLC) patients: a phase II study	Francesca Mazzoni, IT
	176PD	Prediction of late breast cancer recurrence by the ROR (PAM50) score in postmenopausal women in the TransATAC cohort	Jack Cuzick, UK
15’		**Discussant**	**Caroline Dive, UK**
15’		**Questions**	

Disclosure slide

- I have no conflict of interest to declare

Focus on the target: classes of human non-coding RNA

Type	Class	Characteristics and function
Small ncRNA (<200 nt)	Small Interfering RNAs (siRNAs)	21-22 nt double-stranded RNAs produced by Dicer and involved in gene silencing and viral defence
	microRNAs (miRNAs)	18–25 nt RNAs that modulate gene expression posttranscriptionally
	Transfer RNAs (tRNA)	An adaptor molecule with an inverted L structure involved in translation of mRNA into protein
	PIWI-interacting RNAs (piRNAs)	Dicer independent 26–31 nt RNAs located in the germline and adjacent somatic cells, involved in germline development and stability through the regulation of transposons
	Small nucleolar RNAs (snoRNAs)	Guide molecules for modification and processing of rRNA, specifically site-specific methylation and pseudouridylation
	microRNA-offset RNAs (moRNAs)	RNAs derived from the ends of pre-miRNAs, predominantly from the 5′ end, independent of the mature miRNA. The function of moRNAs are currently unknown
	Ribosomal 5.8S	Transcribed by pol I as a part of the 45S precursor, 5.8S is a component of the large ribosomal subunit in eukaryotes, and thus involved in protein translation
	Promoter-associated short RNAs (PASRs)	Transcripts within a few hundred bases of protein coding or noncoding transcription start site that may regulate gene expression
Long ncRNA (>200 nt)	Long ncRNA	A broad class of RNAs > 200 nt with functions in epigenetic regulation, splicing, and cellular localization
	Transcribed ultraconserved regions (T-UCR)	Non-coding sequences 100% conserved among humans, mice, and rats, with roles in the regulation of alternative splicing and gene expression, and altered in a number of human cancers
	Pseudogenes	Nonfunctional sequences of genomic DNA originally derived from functional genes but with mutations or premature stop codons that prevent their expression. Known to regulate gene expression and recombination
	Promoter associated long RNAs (PARs)	Transcripts 250–500 nt long within a few hundred bases of protein coding or non coding transcription start sites that may regulate gene expression
	Antisense RNAs	Single stranded RNA complementary to a transcribed mRNA, capable of binding and blocking translation of its complementary mRNA, and promoting target decay.

PD#1645

PD#177

Exosomal long non-coding RNA(lncRNA) in Lung Cancer

(# 177PD)

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University of Louisville School, USA

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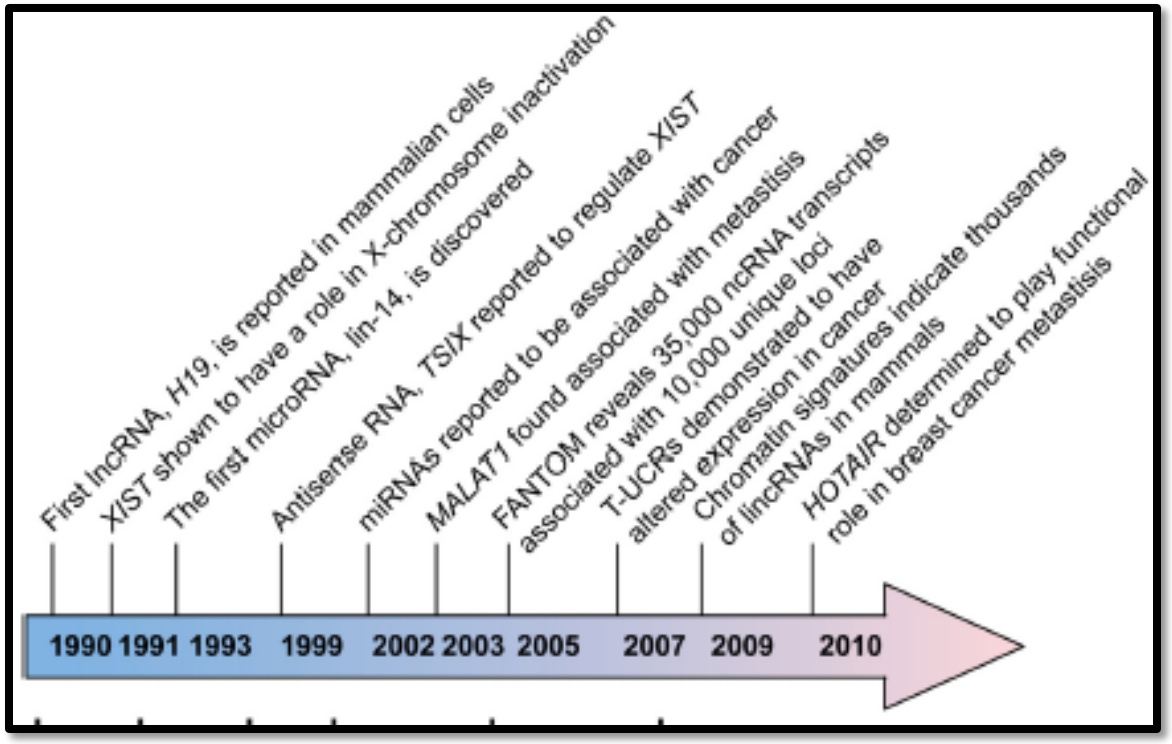
Long non-coding RNA (lncRNA)



Adapted from Gibb Ea et al.Molecular Cancer, 2011

Long ncRNAs, are non-protein coding transcripts longer than 200 nuceotides

Human cancer associated lncRNA

LncRNA	Size	Cytoband	Cancer types	References
HOTAIR	2158 nt	12q13.13	Breast	[18,68]
MALAT1/α/NEAT2	7.5 kb	11q13.1	Breast, lung, uterus, pancreas, colon, prostate, liver, cervix[1], neuroblastoma[1], osteosarcoma	[135,137-139,152,255,256]
HULC	500 nt	6p24.3	Liver, hepatic colorectal metastasis	[170,171]
BC200	200 nt	2p21	Breast, cervix, esophagus, lung, ovary, parotid, tongue	[50,51]
H19	2.3 kb	11p15.5	Bladder, lung, liver, breast, endometrial, cervix, esophagus, ovary, prostate, choricarcinoma, colorectal	[74,92,95,97,102,103,257-264]
BIC/MIRHG155/ MIRHG2	1.6 kb	21q11.2	B-cell lymphoma	[153]
PRNCR1	13 kb	8q24.2	Prostate	[187]
LOC285194	2105 nt	3q13.31	Osteosarcoma	[265]
PCGEM1	1643 nt	2q32.2	Prostate	[188,266,267]
UCA1/CUDR	1.4 kb, 2.2 kb, 2.7 kb	19p13.12	Bladder, colon, cervix, lung, thyroid, liver, breast, esophagus, stomach	[268-270]
DD3/PCA3	0.6 kb, 2 kb, 4 kb	9q21.22	Prostate	[189,190]
anti-NOS2A	~1.9 kb	17q23.2	Brain[1]	[271]
uc.73A	201 nt	2q22.3	Colon	[200]
TUC338 (encodes uc.338)	590 nt	12q13.13	Liver	[203]
ANRIL/p15AS/ CDK2BAS	34.8 kb & splice variants	9p21.3	Prostate, leukemia	[175,176,183,272]
MEG3	1.6 kb & splicing isoforms	14q32.2	Brain (downregulated)	[156-158,162]
GAS5/SNHG2	Multiple isoforms	1q25.1	Breast (downregulated)	[273]
SRA-1/SRA (bifunctional)	1965 nt	5q31.3	Breast, uterus, ovary (hormone responsive tissue)	[274,275]
PTENP1	~3.9 kb	9p13.3	Prostate	[173,174]
ncRAN	2186 nt, 2087 nt	17q25.1	Bladder, neuroblastoma	[276,277]

[1] Cell lines.

Adapted from Iorio and Croce. JCO, 2009

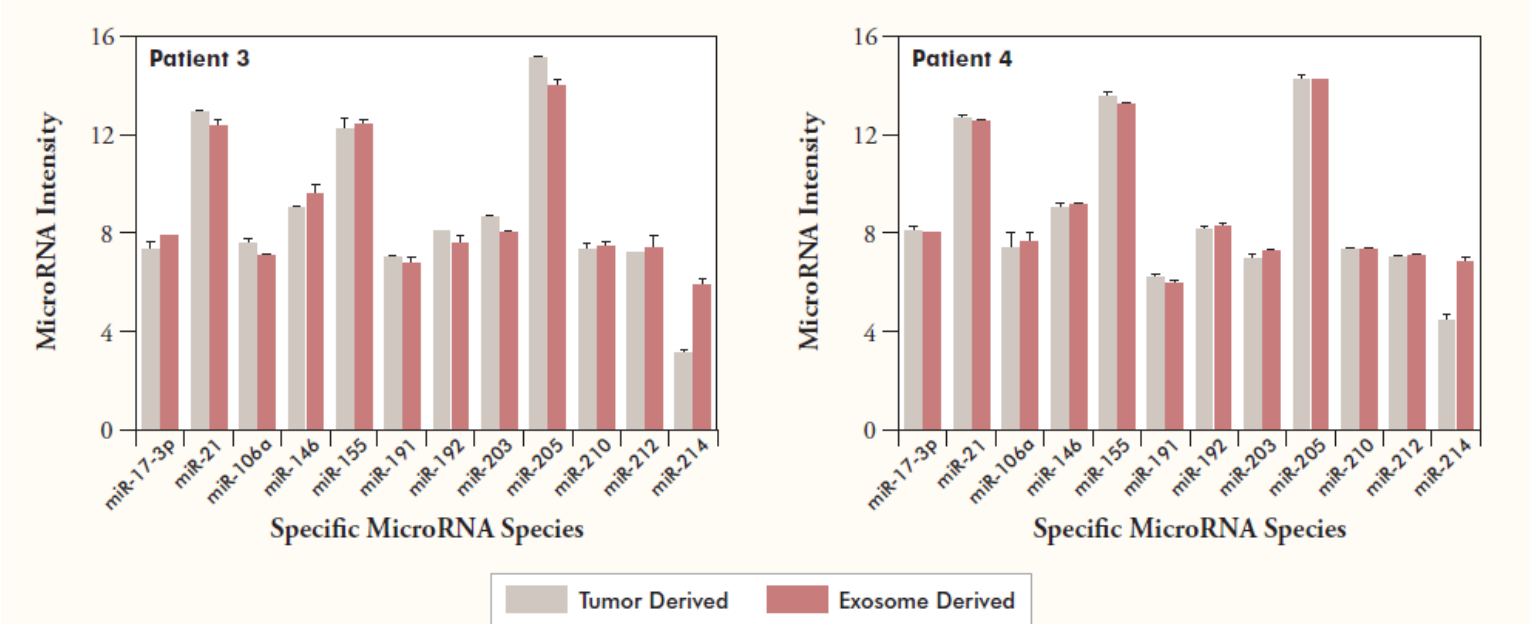
Tumor-derived exosomes

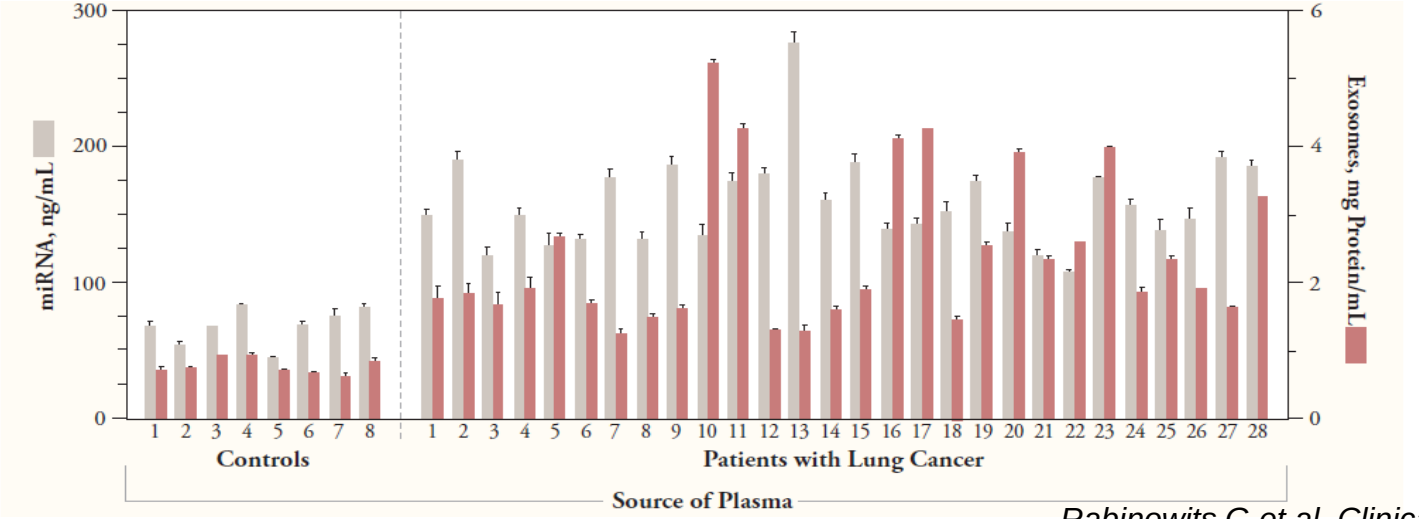
- Small (50-100 nm) membrane vescicles of endocytic origin, initially demontrated in peripheral circulation of cancer patients *(Taylor DD et al. Cancer Res 1980)*

- Role in cell-to-cell comunication by transferring genetics information between cells *(Ratajczak J et al. Leukimia, 2006)*

- Released exosomes contain a subset of both cellular mRNA and miRNA which could be transferred to target cell *(Valadi et al. Nat Cell Biol, 2007)*

Exomal microRNA: a diagnostic marker for lung cancer





Rabinowits G et al. Clinical Lung Cancer, 2009

Research aim

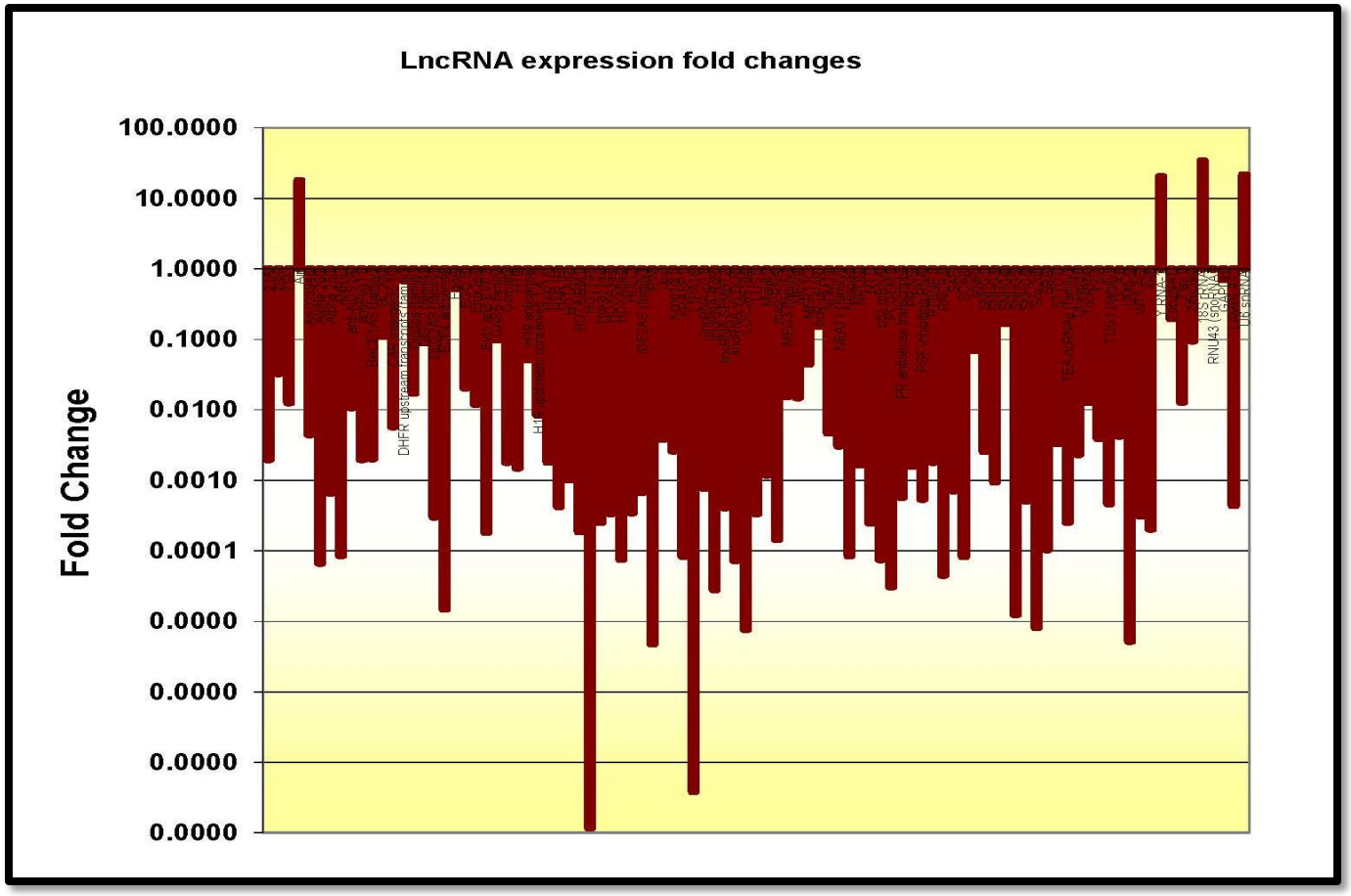
- Examination of the lnRNA content in a human NSCLC cell lines (H838) and compares it to the lnRNA content in the supernatant exosomes.

- Comparison beteween lnRNA profiles in blood-borne exosomes from cancer patients and controls.

Methods

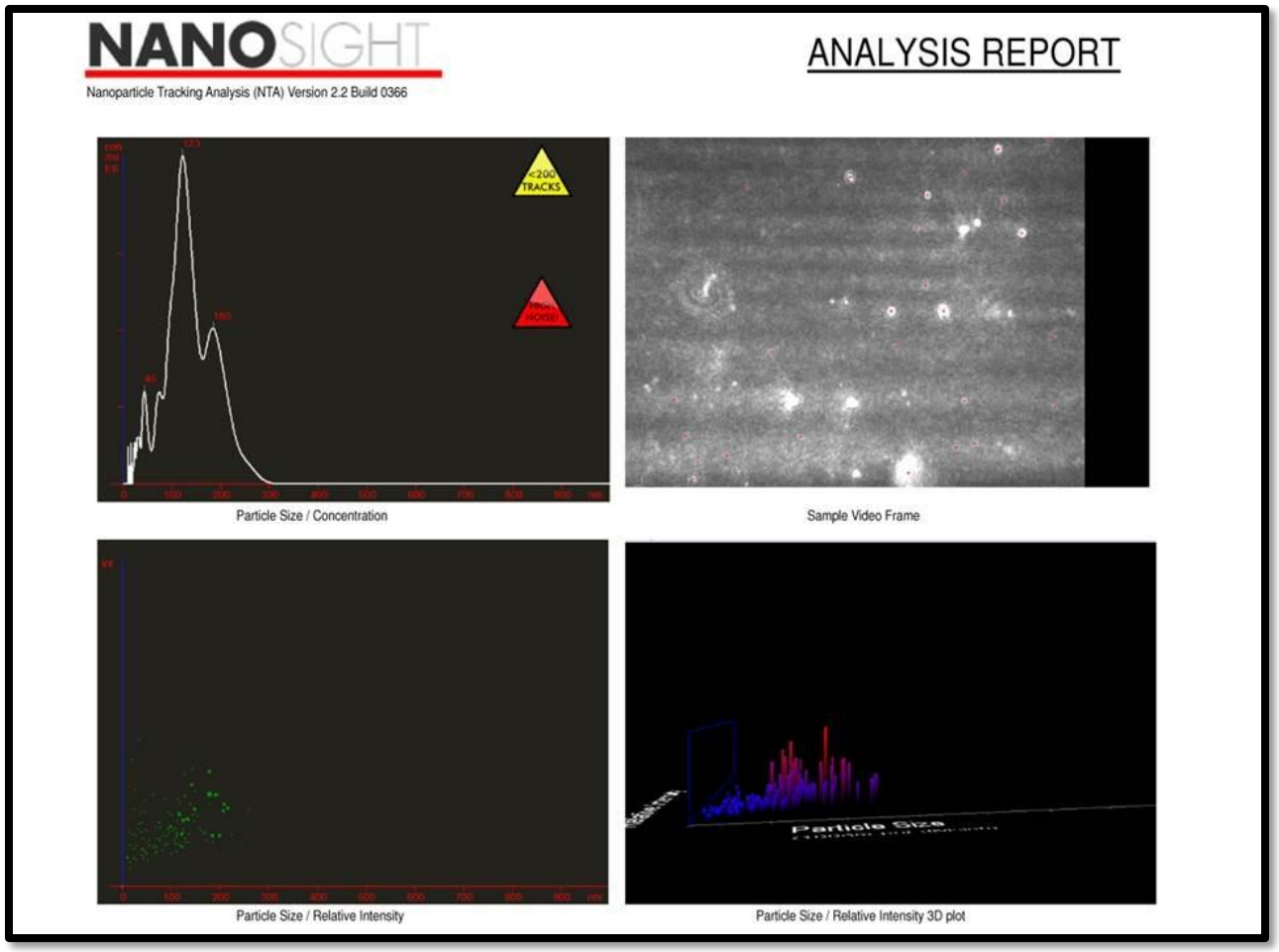
- Microvesicles in the supernatant in cell line culture were isolated by chromatography using Sepharose 2B.
- For serum samples, vesicles were isolated using ExoQuick from 1ml samples.
- Characterization of vesicle populations from the patient serum analyzed with a NanoSight LM10 with Nanoparticle Tracking software
- The total RNA fraction was analyzed for specific lncRNAs using the lncRNA profiler qPCR array (Systems Biosciences)

Ratios of the lncRNAs in exosomes versus lncRNA in H838 cell line (>1, higher concentration in exosomes)



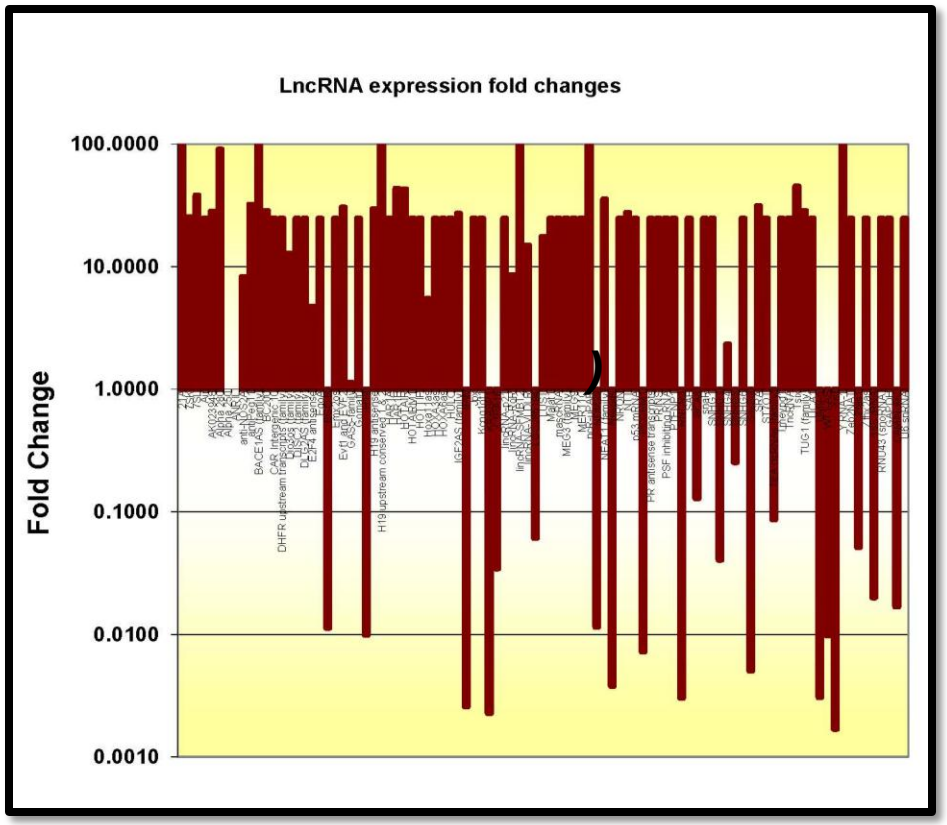
The results showed selective exosomal levels of lnRNA content compared to intracellular lnRNA . CT valuse (ANRIL cells 23.80 vs. 26.44 exosomes. MALAT-1 cells 31.84 vs. 32.85 exosomes. BACE1AS cells 34.14 vs 34.86 exosomes)

Serum samples analysis in patients



Nanoparticle tracking system with vesicles predominately 50-200nm consistent with exosomes, isolated from the serum of lung cancer patients

Ratios of the lncRNA in lung cancer patients' serum versus normal control serum (>1, higher concentration in patients' serum)



In patients serum:

- Increase level (20-fold) in 58 lnc RNA
- Descreae level (10-fold) in 20 lnc RNA

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Study conclusions

- The different profiles of exosomal lnRNA in serum of cancer patients and controls makes lnRNA a potential marker for **screening, diagnosis and monitoring**

- The high lnRNA content of cancer cells, may make lnRNA a **therapeutic target** in the treatment of lung cancer

Circulating miRNA in the serum as diagnostic markers for different tumor entities

Tumor entity	References	Study Design	Sample Size	Circulating miRNAs examined	Technology	Normalization	Promising circulating miRNAs
B-Cell Lymphoma	Lawrie et al. [23]	Tumor vs. normal, retrospective study on prognosis	60 patients vs. 43 healthy controls	3	Quantitative RT-PCR	*miRNA-16*	*miRNA-155*, *miRNA-210*, and *miRNA-21*
Breast Cancer	Heneghan et al. [73]	Tumor vs. normal	83 patients vs. 44 healthy controls	7	Quantitative RT-PCR	*miRNA-16*	*miRNA-195* and *let7a*
	Zhu et al. [83]	Tumor vs. normal	13 patients vs. 8 healthy controls	3	Quantitative RT-PCR	18 s rRNA	*miRNA-155*
Colon Cancer	Huang et al. [60]	Tumor vs. normal	Screening: 20 patients vs. 20 healthy controls Validation: 80 patients, 37 adenomas and 39 healthy controls	12	Quantitative RT-PCR	*miRNA-16*	*miRNA-29* and *miRNA92a*
	Ng et al. [57]	Tumor vs. normal, tissue and serum	Screening: 5 plasma samples, associated tumor/ normal tissue 1.validation: 25 patients vs. 20 healthy controls 2. validation 180 samples	95	Quantitative RT-PCR Array	*RNU6B*	*miR-17-3p* and *miR-92*
Gastric Cancer	Tsujiura et al. [85]	Tumor vs. Normal	Screening: 8 samples and associated tissue Validation: 69 patients vs. 30 healthy controls	5	Quantitative RT-PCR	*RNU6B*	*miR-17-5p*, *miR-21*, *miR-106a*, *miR-106b* and *let-7a*
Leukemia	Tanaka et al. [56]	Tumor vs. Normal	Screening: 2 patients vs. 7 healthy controls Validation: 61 patients vs. 16 healthy controls	723	microRNA Microarray (Agilent Technologies)	*miRNA-638*	*miRNA-92a*
Lung Cancer	Chen et al. [24]	Tumor vs. normal	Screening: Pool analysis Validation: 152 patients vs. 75 healthy controls	Genome-wide profiling by Solexa sequencing	Solexa sequencing, Quantitative RT-PCR	Directly normalized to total RNA	*miRNA-25* and *miRNA-223*
	Hu et al. [74]	Study on prognosis (Overall survival)	Screening: 60 patients Validation: 243 patients	Genome-wide profiling by Solexa sequencing	Solexa sequencing, Quantitative RT-PCR	Referenced to control healthy serum sample	*miR-486*, *miR-30 d*, *miR-1* and *miR- 499*
Oral Cancer	Liu et al. [80]	Tumor vs. normal	43 patients vs. 21 healthy controls	1	Quantitative RT-PCR arrays	*miRNA-16*	*miR-31*
Ovarian Cancer	Resnick et al. [67]	Tumor vs. normal	Screening: 9 patients vs. 4 healthy controls Validation: 19 patients vs. 11 healthy controls	365	Quantitative RT-PCR arrays	*U44/U48 and miRNA-142-3p*	*miRNA-21*, *miRNA-92*, *miRNA-93*, *miRNA-126*, *miRNA-29a*, *miRNA-155*, *miRNA-127* and *miRNA-99b*
Pancreatic Cancer	Ho et al. [28]	Tumor vs. normal	Screening: 11 patients vs. 14 healthy controls, Validation: 11 patients vs. 11 healthy controls	1	Quantitative RT-PCR arrays	c. elegans spike-in *miRNA-54*	*miRNA-210*
	Wang et al. [61]	Tumor vs. normal	49 patients vs. 36 healthy controls	4	Quantitative RT-PCR arrays	*miRNA-16*	*miR-21*, *miR-210*, *miR-155*, and *miR-196a*
Prostate Cancer	Mitchell et al. [25]	Tumor vs. normal	Screening: Pool analysis Validation: 25 patients vs. 25 healthy controls	6	Quantitative RT-PCR	c. elegans spike-in *cel-miR-39*, *celmiR-54*, and *cel-miR-238*	*miRNA-141*
	Brase et al. [72]	Low grade vs. high grade	Screening: 7 high grade vs. 14 low grade Validation: 116 patients	667	Quantitative RT-PCR arrays	c. elegans spike-in *cel-miR-39*, *celmiR-54*, and *cel-miR-238*	*miRNA-141*, *miRNA-375*
Squamous Cell Carcinoma	Wong et al. [81]	Tumor vs. Normal tissue screening, Validation in serum	30 patients vs. 38 healthy controls	1	Quantitative RT-PCR arrays	*miRNA-16*	*miRNA-184*

Putting the data in the context as biomarkes

lncRNA	Significance	Technology	Source tissue	Reference
MALAT1	**P**: predict metastasis survival in early-stage NSCLC	Subtractive hybridization method, sequencing and quantitative RT-PCR	Shock frozen primary NSCL tumors	*Ji P, et al. Oncogene (22) 2003*
BC200	**D**: Detectable at significant levels in tumors (lung)	In situ hybridization	Tumor and normal tissue frozen in liquid nitrogen	*Chen W, et al. Journal of Pathology (183) 1997*
H19	**D:** Loss of imprinting in lung adenocarcinoma	RT-PCR	Lung cancer and normal tissue	*Kodha M, et al. Molecular Carcinogenesis (31) 2001*
DD3PCA3	**D**: high level in prostate cancer tumor vs benign tumor	RT-PCR	Urine sediment	*Tinzl M et al. European Urology (46) 2004*
HULC	**D:** detected in the blood of HCC patients and in corresponding tissue samples	RT-PCR	Blood sample and tumor tissue (cryo-preserved, and paraffin-embedded)	*Panzitt K, et al. Gastrenterology (342) 2007*

P: prognostic
D: diagnostic

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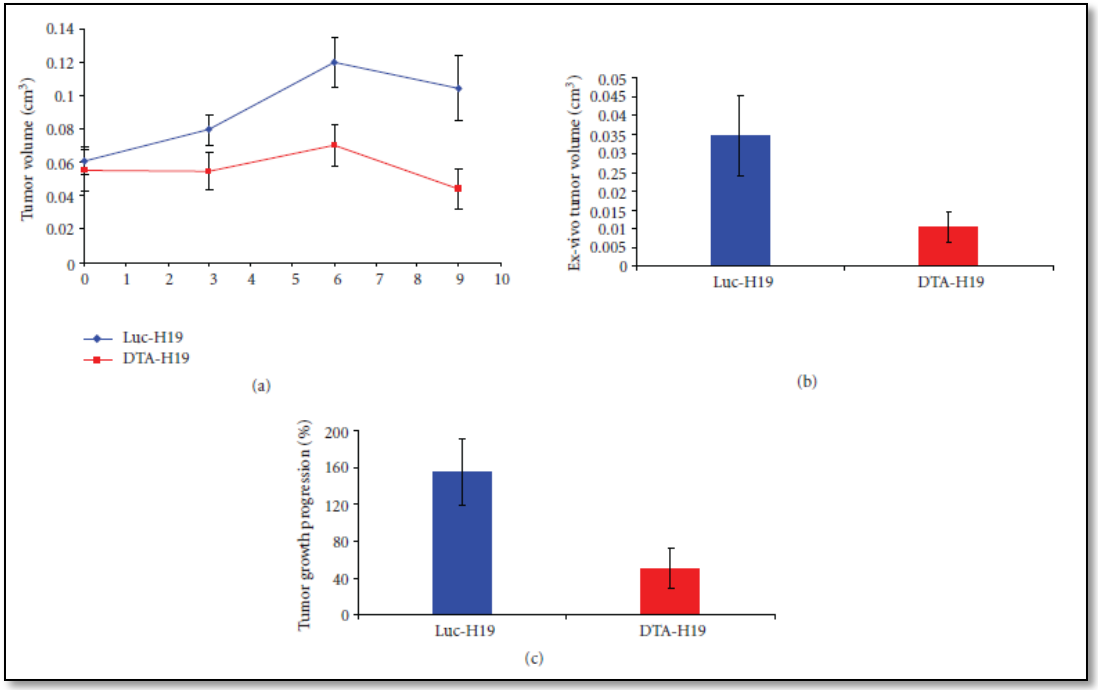
Study conclusions

- The different profiles of exosomal lnRNA in serum of cancer patients and controls makes lnRNA a potential marker for **screening, diagnosis and monitoring**

- The high lnRNA content of cancer cells, may make lnRNA a **therapeutic target** in the treatment of lung cancer

Putting the data in the context: utility as cancer therapies

- Ohana P, et al. Gene *Therapy and Molecular Biology* (8) 2004
- Smaldone MC, et al. *Curr Opin Mol Ther* (12) 2010
- Midoux P, et al. *Curr Gene Ther* (8) 2008
- Amit D, et al. *J Transl Med* (8) 2010
- Mizrahi A, et al. *J Transl Med* (7) 2009



- *Scaiewicz V,et al. J Oncol 2010*

MICRORNA-9 AND -224 IN TRASTUZUMAB RESISTANT HER2 POSITIVE BREAST CANCER CELLS

(# 1645PD)

K. Howe, A. Eustace, S. Souahli, B.C. Browne, S. Aherne, N. Barron, N. Walsh, J.P. Crown, N. O'Donovan

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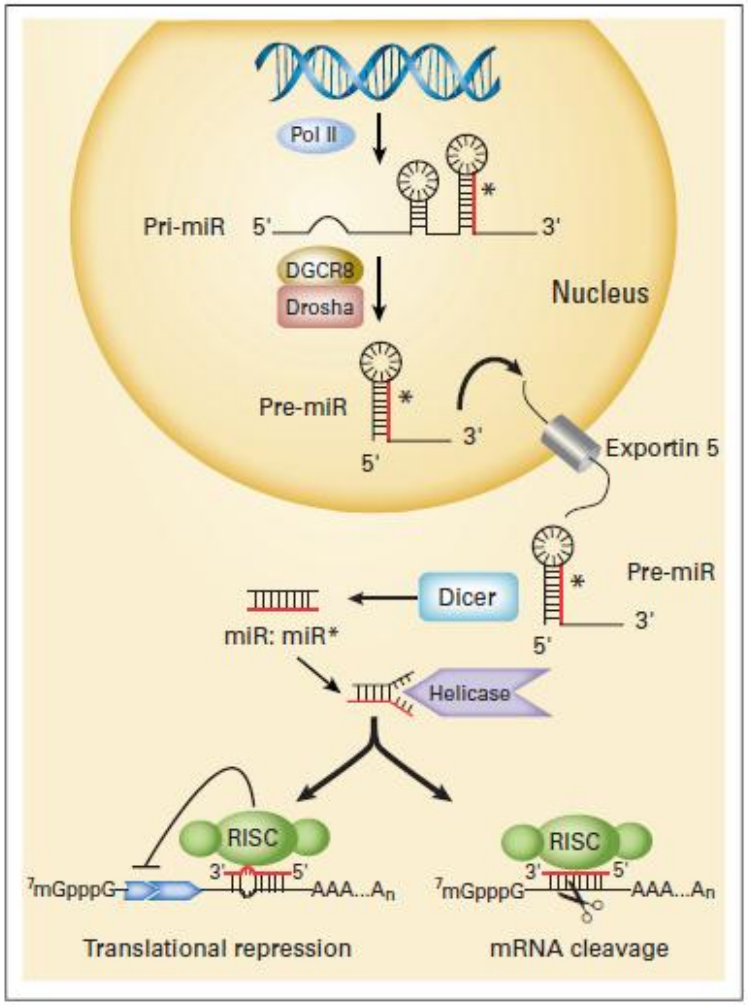
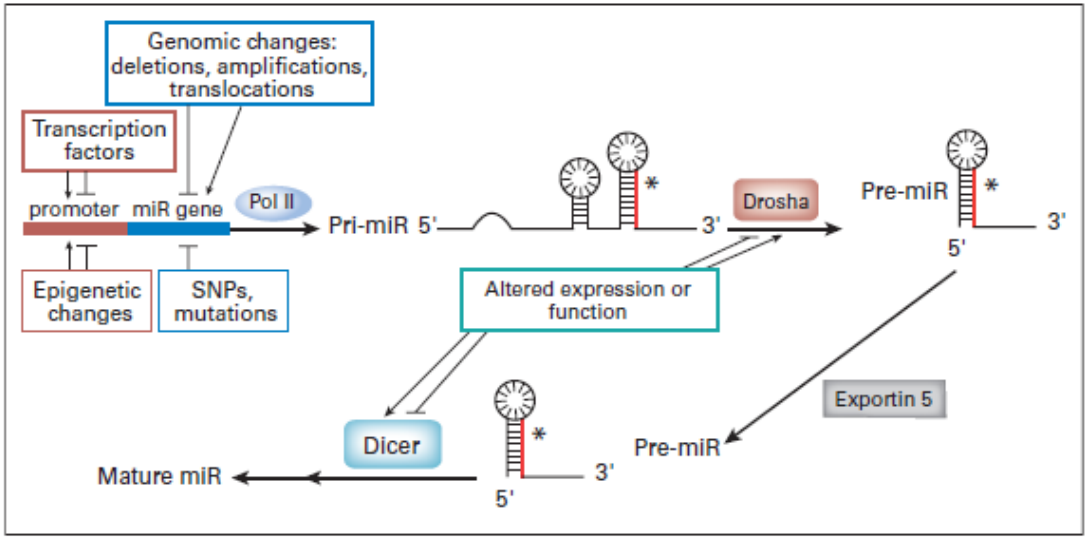
Focus on a target: MicroRNA (miRNA)





Adapted from Iorio and Croce. JCO, 2009

miRNA in cancer

microRNA	Expression	Cancer	Targets
miR-21	Upreguleted	Breast	PTEN, PDCD4, TPM1
miR-125b	Downregulated	Breast	HER2, HER3
miR-205	Downregulated	Breast	HER3
miR10b	Downregulated	Breast	HOXD10
Mir-155 Let-7	Upreguleted Downregulated	Lung cancer	RAS, HMGA2, c-MYC
mir-221 miR-122a miR- 34a	Upregulated Downregulated	HCC	P27 Cyclin G1 MET
miR-141/200	Upregulated Down	Ovarian, Prostate Kidney	ZEB, ZEB2

Research aim

- To identify novel microRNAs that play a role in trastuzumab resistance
- To investigate the function of microRNA targets in cell growth and/or trastuzumab sensitivity

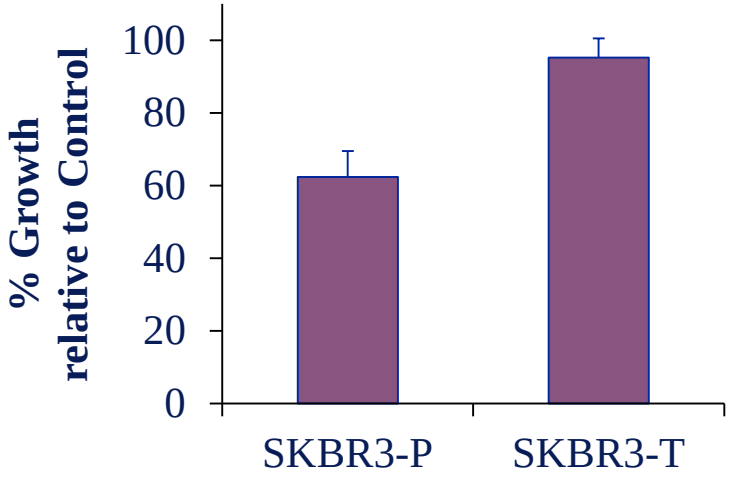
Methods

- Acquired trastuzumab resistant cell line, SKBR3-T, was developed previously, by treatment with trastuzumab (SKBR3-T) for 6 months and a media control cell line, SBR3-Parental was developed in parallel
- microRNA was isolated using the miRVANA microRNA isolation kit (Ambion)
- cDNA was synthesised using the TaqMan® MicroRNA RT Kit (Applied Biosystems) and qRT-PCR was performed TaqMan® Universal PCR Master mix
- miRNAs were with quantified using TaqMan MicroRNA Assays (Applied Biosystems), normalised with RNU48 on an An ABI Prism 7900HT

- Functional studies were carried out using Ambion® Pre-miR™ miRNA Precursors and Anti-miR™ miRNA Inhibitors. Cell counts were preformed using Guava Viacount reagent on the Guava EasyCyte.

miRNA profiling of trastuzumab resistant cells (SKBR3-T)

A



B

	Fold Change	P-value
miR-205	3.4	0.02
miR-221	3.2	0.01
miR-222	2.4	0.02
miR-9	2.4	3.93634E-06
miR-550	-2.2	0.03
miR-192	-2.8	0.01
miR-31	-3.1	0.05
miR-30d	-4.0	0.04
miR-148a	-4.4	0.03
miR-30a-5p	- 4.7	0.04
miR-224	-5.3	0.05
miR-194	-22.3	0.02

Fold change ≥ 2 and p value ≤ 0.05 were used as cut-offs.

miRNA qRT-PCR validation

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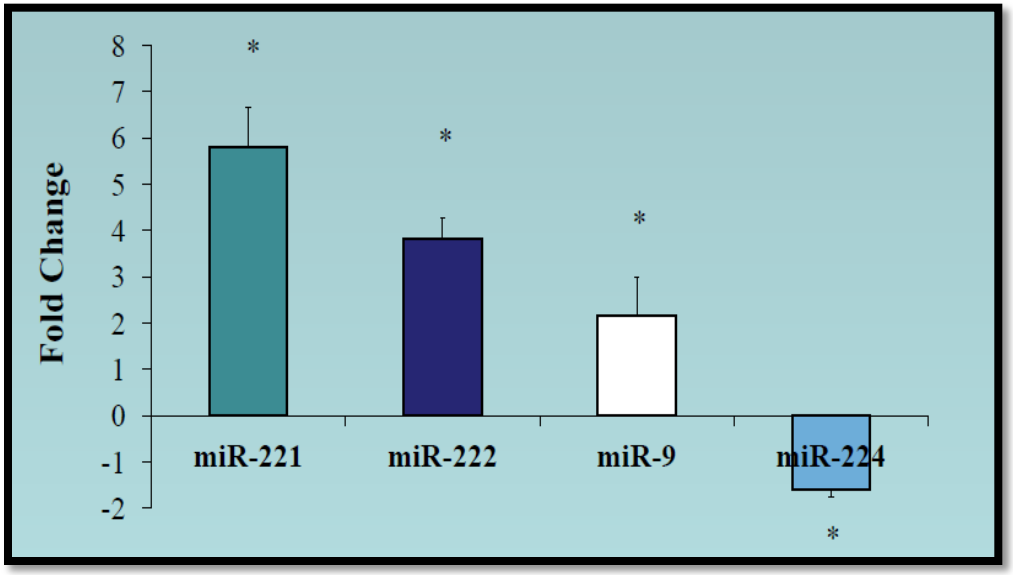
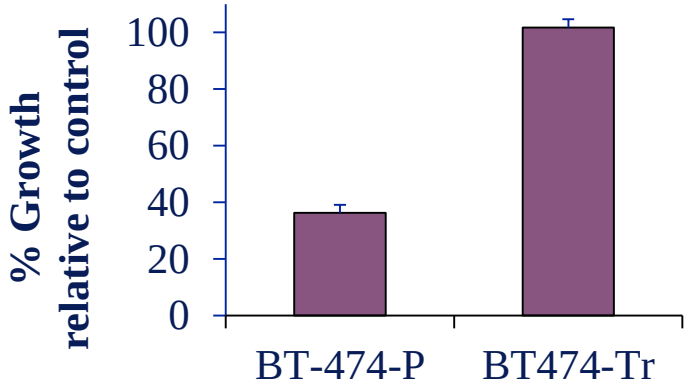
A

SKBR3-T versus SKBR3-P



B

BT-474-Tr versus BT474-P



	Fold Change	P-values
miR-9	1.14	0.68
miR-224	**On - Off**	**0.0004**

*On - Off fold change denotes Ct values < 36 to Ct values of > 36 in the trastuzumab resistant cell lines.

miR-9 and miR-224 in “innate” trastuzumab resistance cells lines

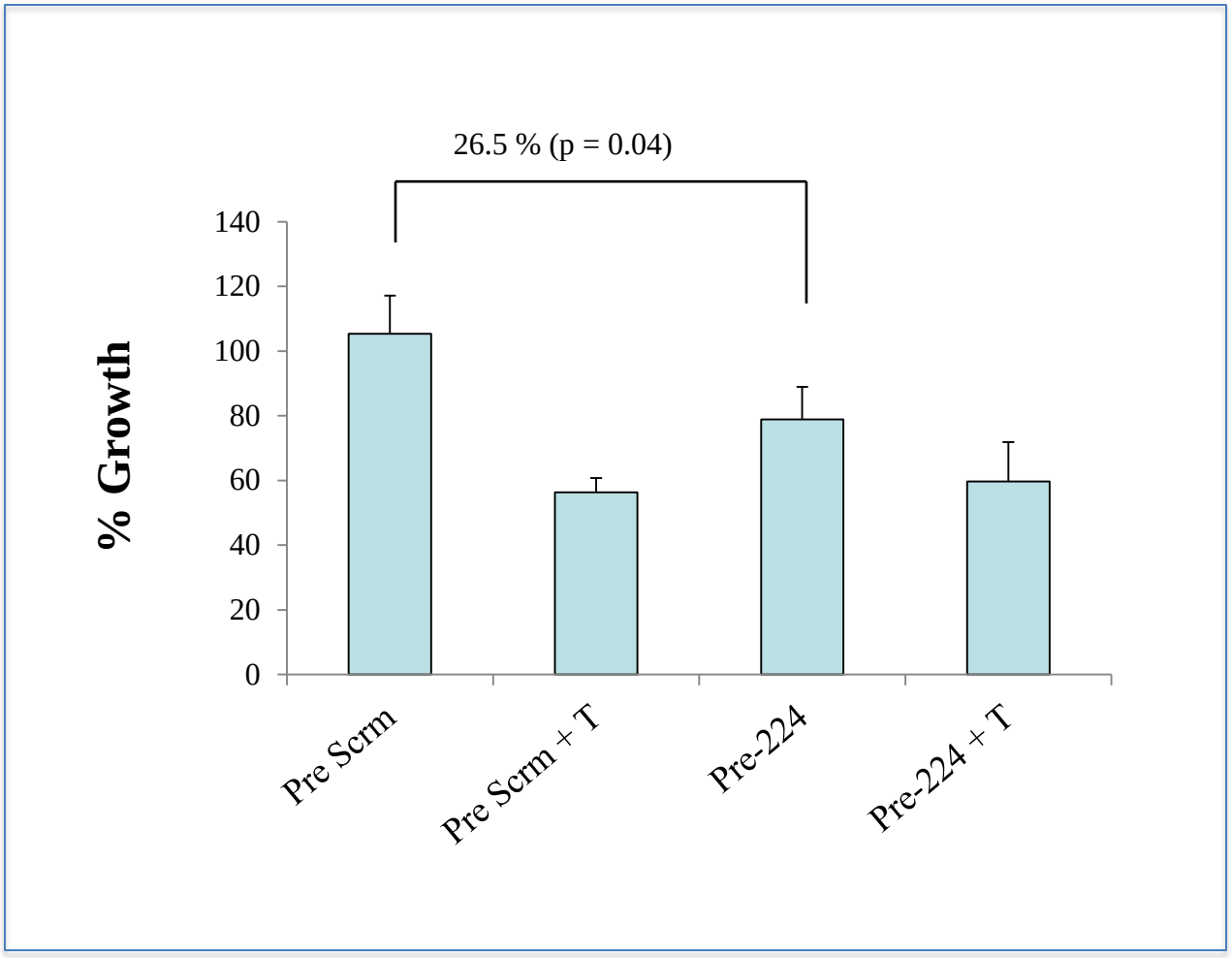
miR-9

	Fold Change	P-Value
UACC-732	4.0	0.14
JIMT-1	4.9	0.06
HCC-202	1.3	0.45
HCC-1954	2.9	0.20
HCC-1569	**587.1**	**0.00004**
MDA-MB-453	1.2	0.77

miR-224

	Fold Change	P-Value
UACC-732	**On - Off**	**0.001**
JIMT-1	**On - Off**	**0.03**
HCC-202	**277.5**	**0.0001**
HCC-1954	**1490.4**	**0.00003**
HCC-1569	On - Off	0.20
MDA-MB-453	On - Off	0.06

Functional study on SKBR3



Study conclusions

(P# 1645PD)

- First report of involment of miRNA-9 and miRNA-224 in trastuzumab resitance in HER2 positive breast cancer

- Preliminary functional studies suggest that miRNA-224 may play a role in regulating cell growth in HER2 positive breast cancer cells

Study conclusions

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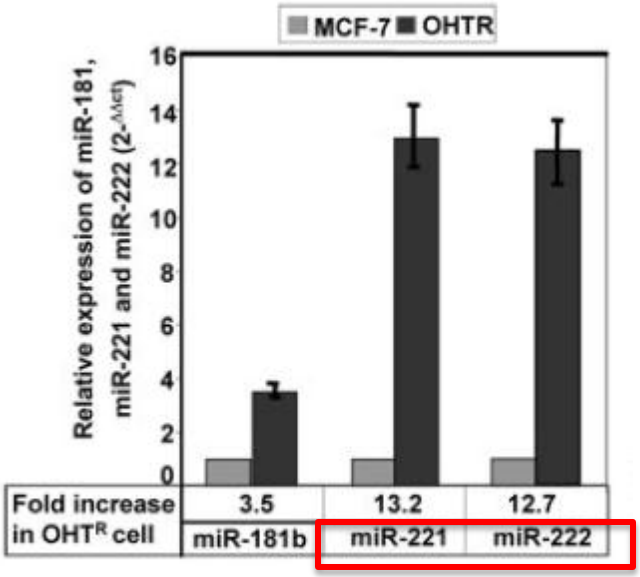
miRNAs modulate multidrug resistance in breast cancer

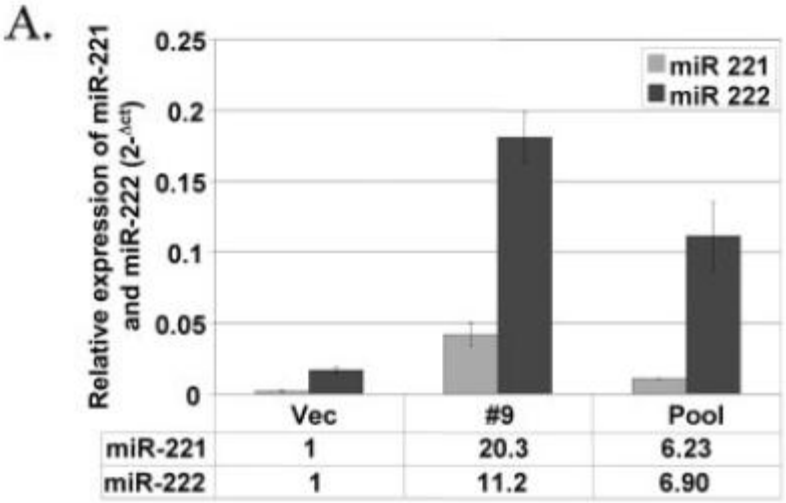
Mechanism	miRNA	Alteration	Targets	Resistant to	References
ABC transporters	miR-200c	Down-regulated	Pgp/MDR1	Doxorubicin	Chen et al. [13]
	miR-451	Down-regulated	Pgp/MDR1	Doxorubicin	Kovalchuk et al. [14]
	miR-345,-7	Down-regulated	MRP-1/ABCC1	Cisplatin	Pogribny et al. [11]
	miR-326	Down-regulated	MRP-1/ABCC1	VP-16	Liang et al. [15]
	miR-328,-519c	Down-regulated	BCRP/ABCG2	Mitoxantrone	Pan et al. [16]; Li et al. [18]
	miR-19	Up-regulated	MDR-1	Taxol	Liang et al. [19]
			MRP-1	VP-16	
			BCRP	Mitoxantrone	
Anti-apoptotic proteins	miR-125b	Up-regulated	Bak1	Taxol	Zhou et al. [12]
	miR-19	Up-regulated	PTEN	Taxol	Liang et al. [19]
				VP-16	
				Mitoxantrone	
	miR-21	Up-regulated	PTEN	Doxorubicin	Wang et al. [21]
	miR-21	Up-regulated	PTEN	Trastuzumab	Gong et al. [22]
EMT	miR-200	Down-regulated	E-cadherin	Doxorubicin	Tryndyak et al. [26]; Howe et al. [27]; Jurmeister et al. [28]
			ZEB1/ZEB2		
	miR-221/222				Stinson et al. [29, 30]
	miR-203				Moes et al. [31]
CSCs	miR-128	Down-regulated	Bmi-1		Zhu et al. [34]
			ABCC5		

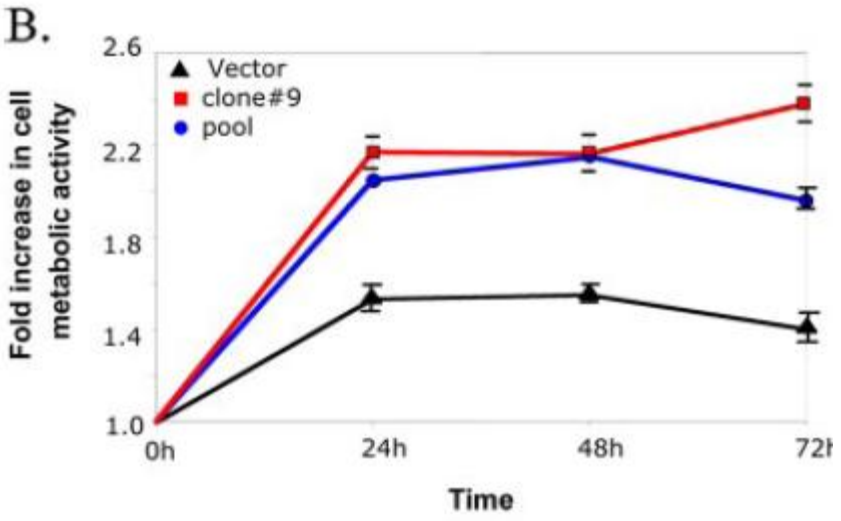
Tian W, et al. Clin Transl Oncol 2012

miRNA in drugs resistance



Distinct miRNA expression profilein tamoxifen resistance breast caner cell lines (OHTR)





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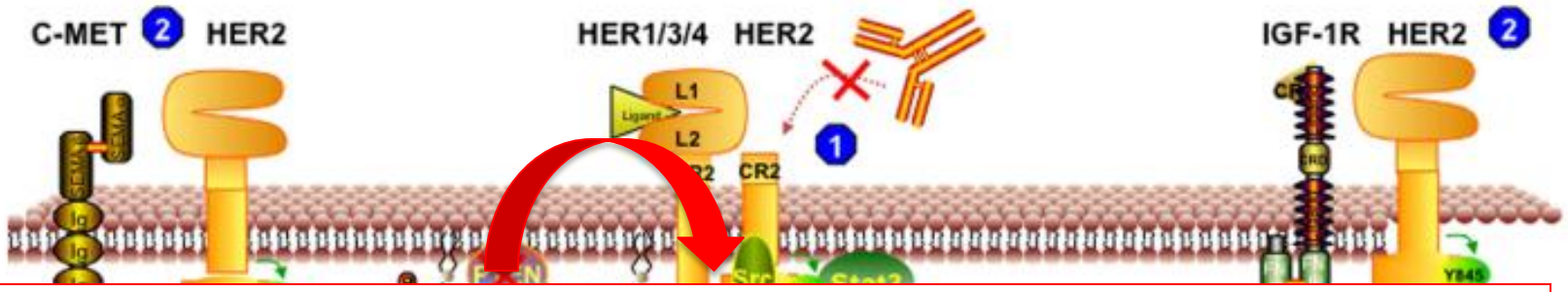
Miller TE et al. J Biol Chem 2008

Resistant mechanism to Trastuzumab



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Up-regulation of *miR-21* Mediates Resistance to Trastuzumab Therapy for Breast Cancer*[S]

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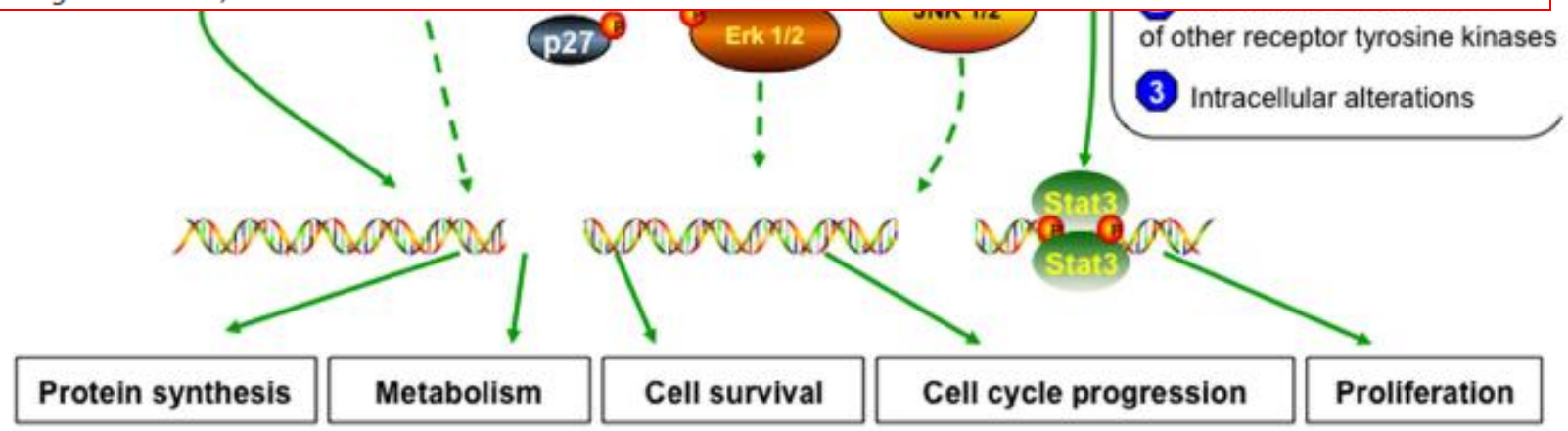
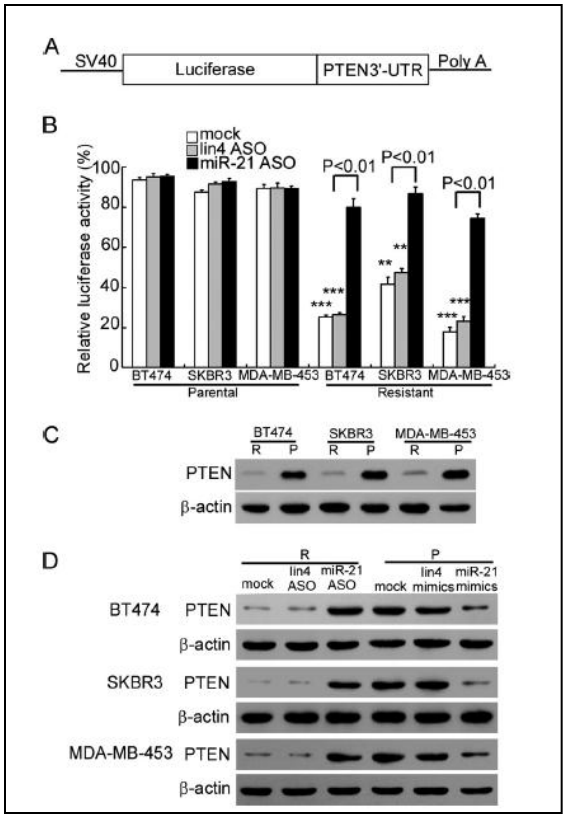
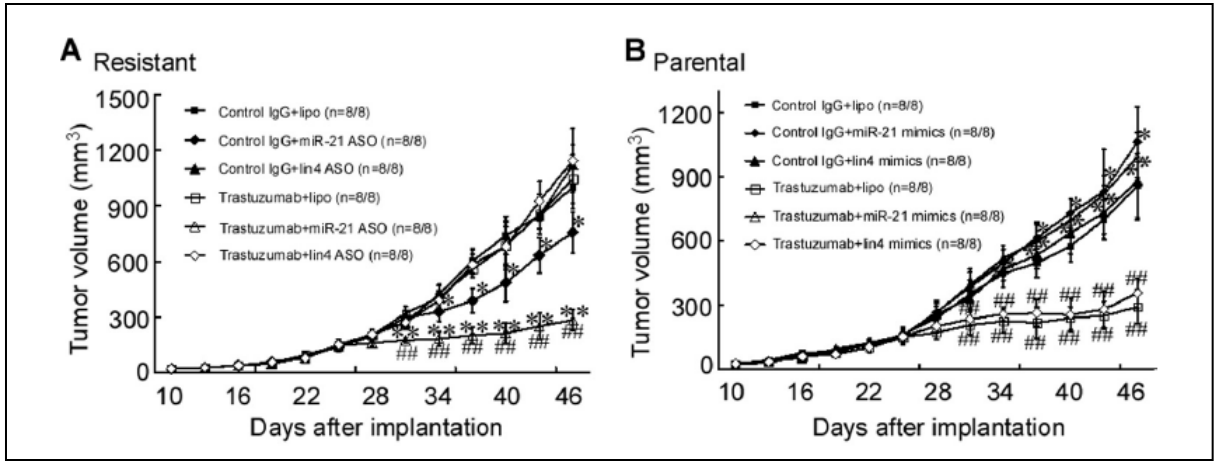
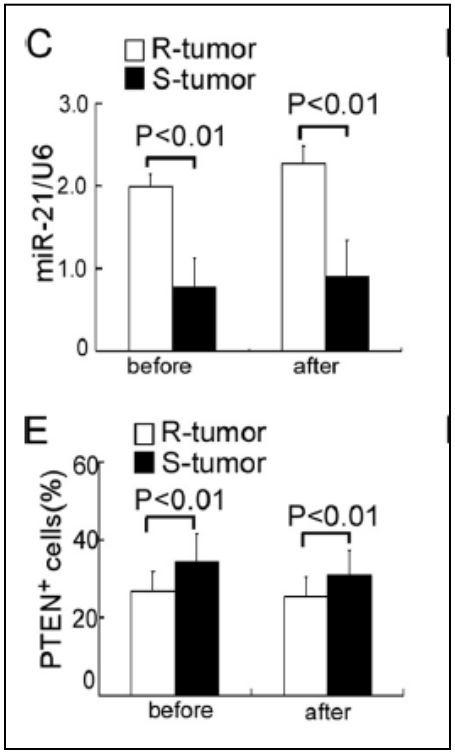


Adapted from Thuy Vu et al. Frontiers in Oncology, 2012

miR21/PTEN and acquired resistance to Trastuzumab







Gong C, et al. J Biol Chem, 2011

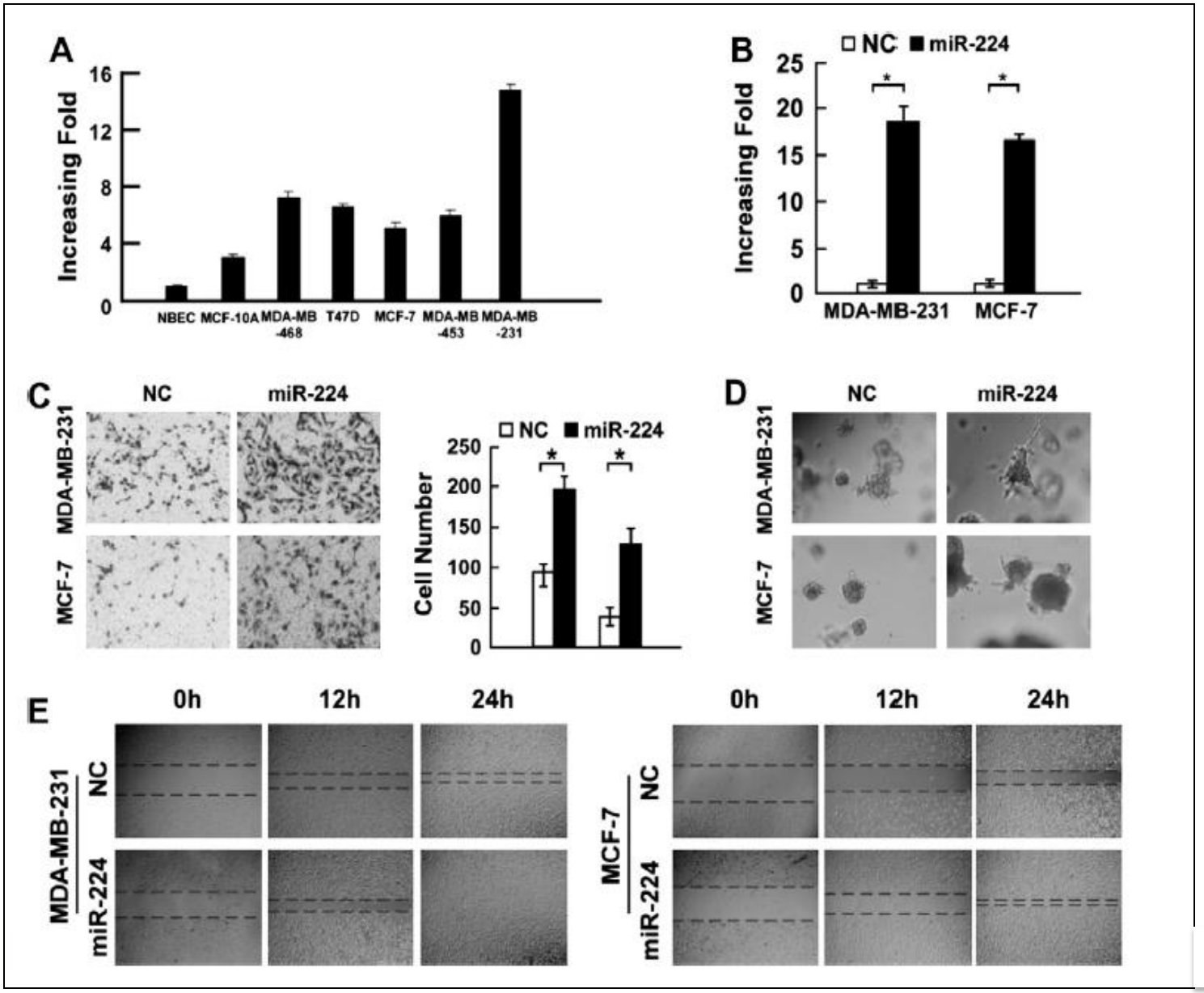
Study conclusions

(P# 1645PD)

- First report of involment of miRNA-9 and miRNA-224 in trastuzumab resitance in HER2 positive breast cancer

- Preliminary functional studies suggest that miRNA-224 may play a role in regulating cell growth in HER2 positive breast cancer cells

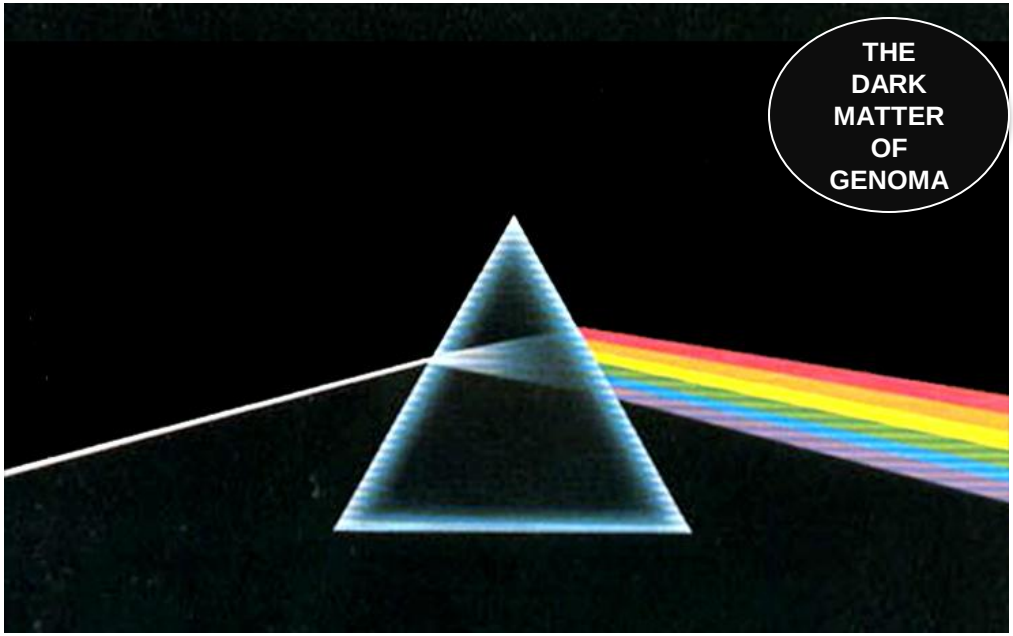
miRNA-224 in human breast cancer cells



Huang L, et al. Biochem Biophys Res Commun, 2012

CONCLUSIONS

New insight to understand more about the non coding RNA (ncRNA)



ncRNA will be probably be a major therapeutic modality in the near future !