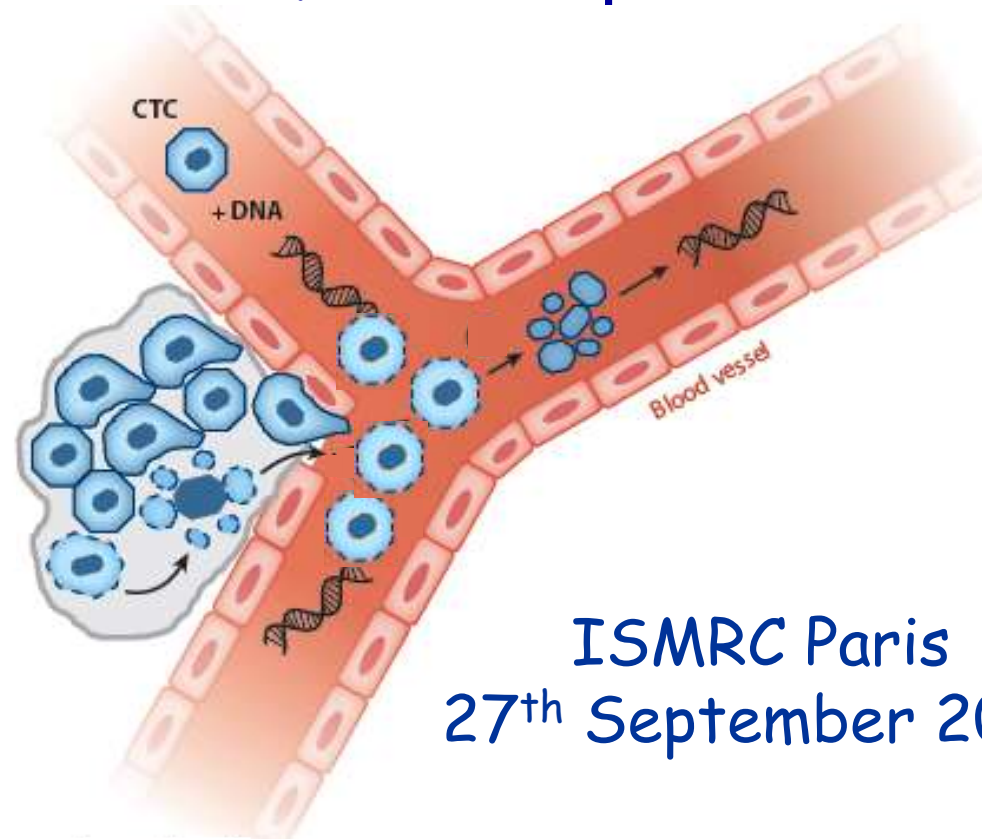
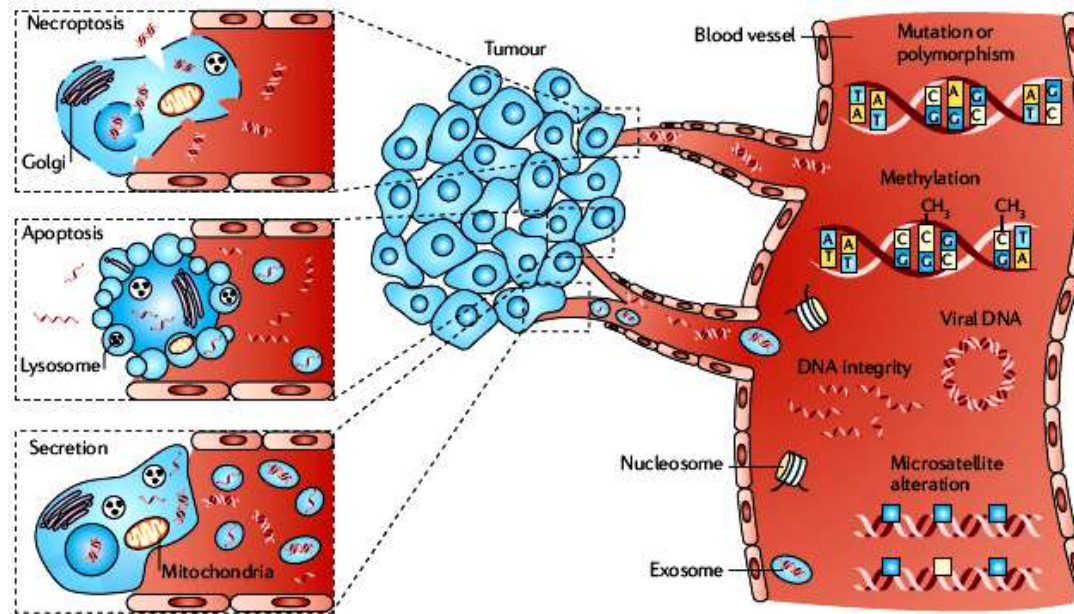


# Circulating tumor DNA and microRNAs in blood of cancer patients



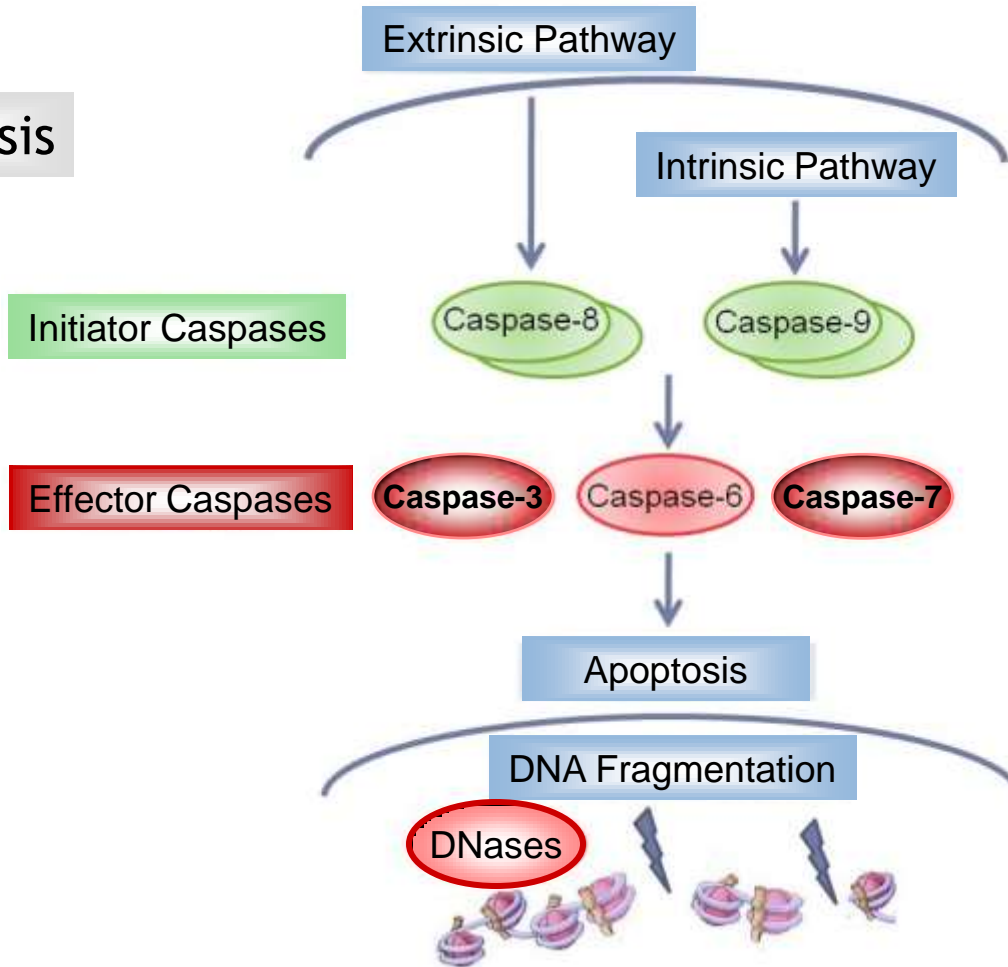
ISMRC Paris  
27<sup>th</sup> September 2013



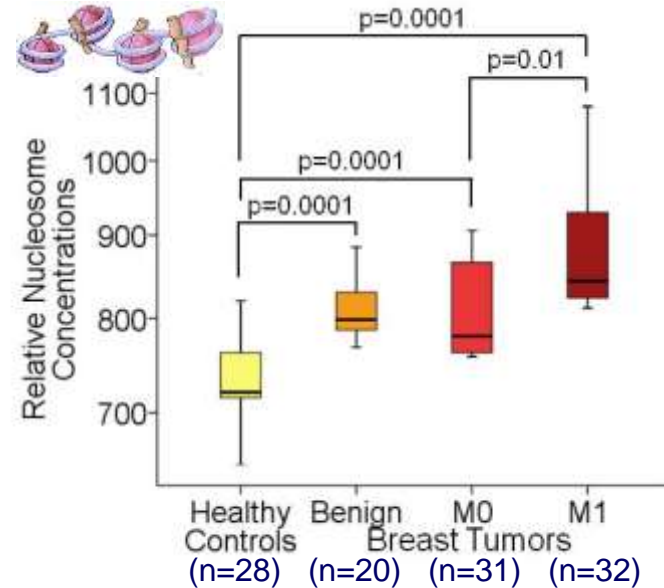
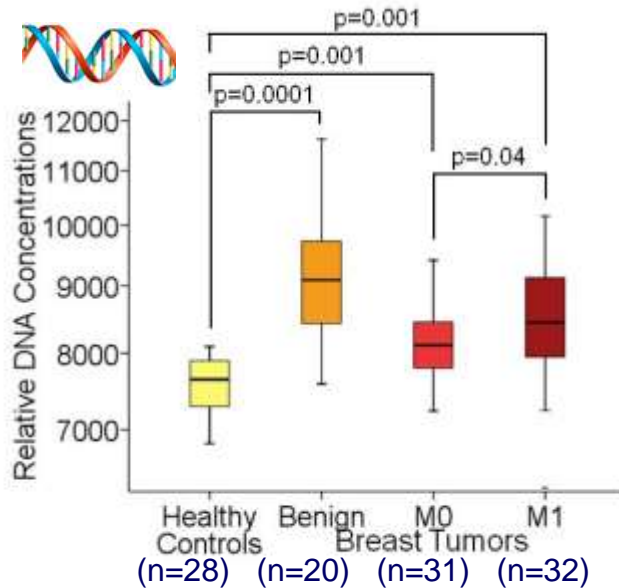
Tumor cells release their nucleic acids into the blood circulation.

# Apoptotic cascade

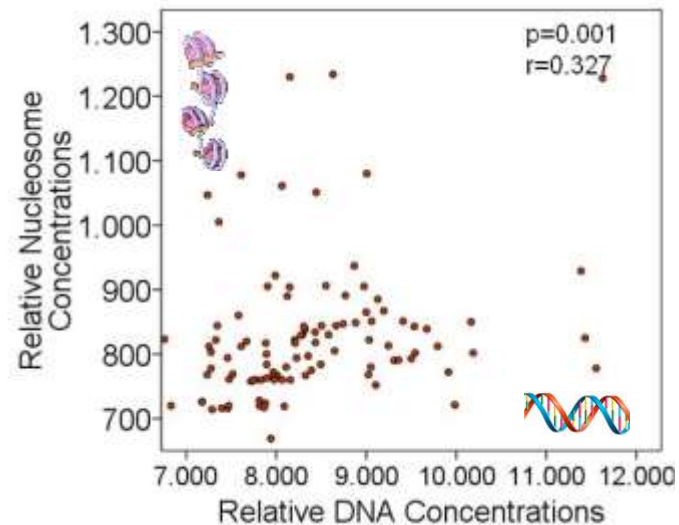
Apoptosis



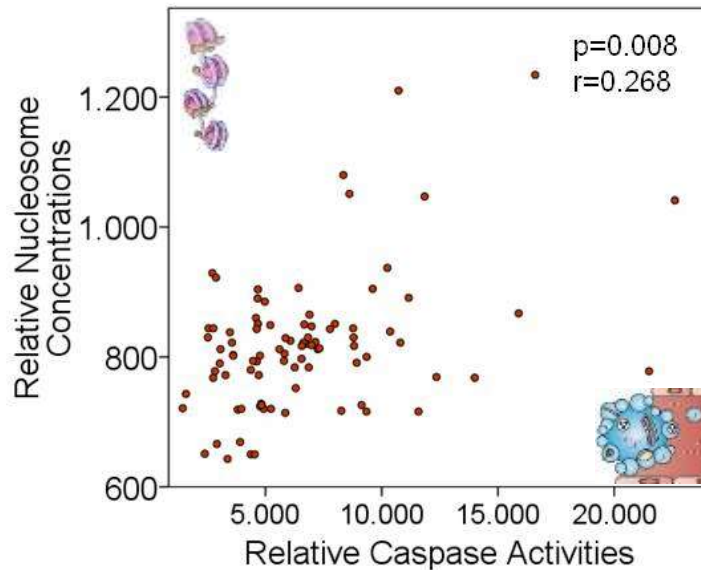
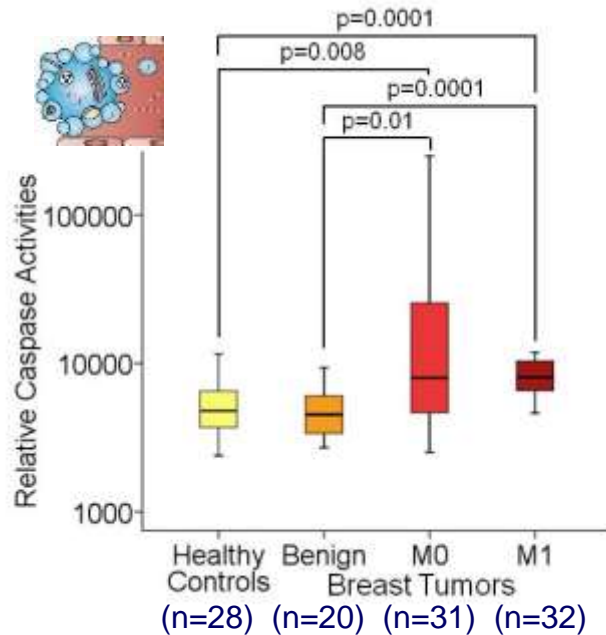
# Serum levels of circulating DNA and nucleosomes in breast cancer patients



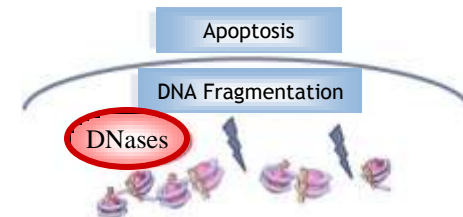
Cell-free DNA circulates as nucleosomes in human blood.



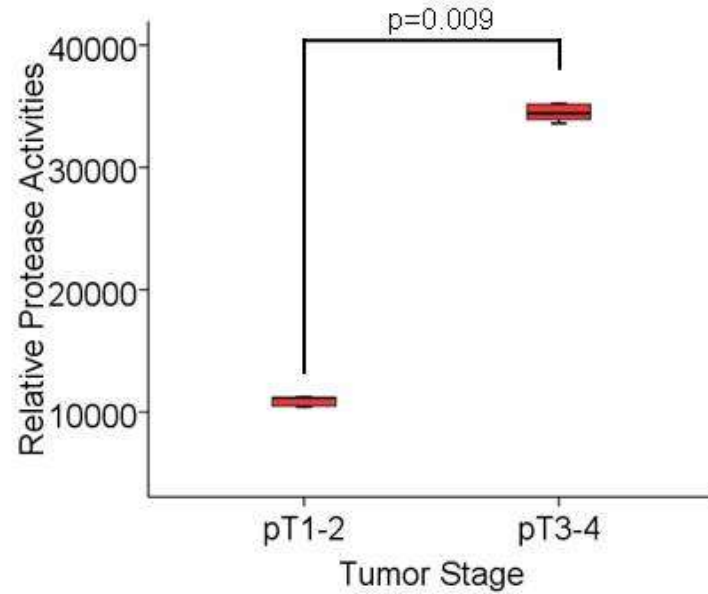
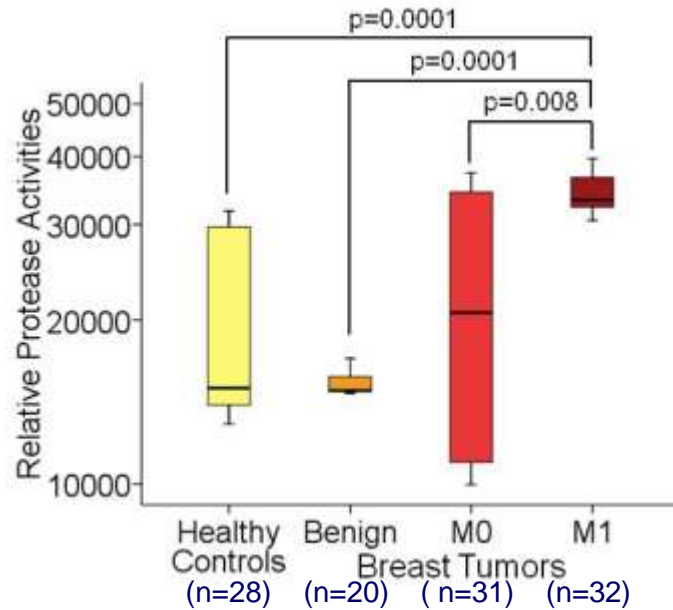
# Serum levels of circulating caspase activities in breast cancer patients



Elevated serum levels of circulating nucleosomes in breast cancer patients are caused by caspases 3 and 7 which are involved in the apoptosis of tumor cells.

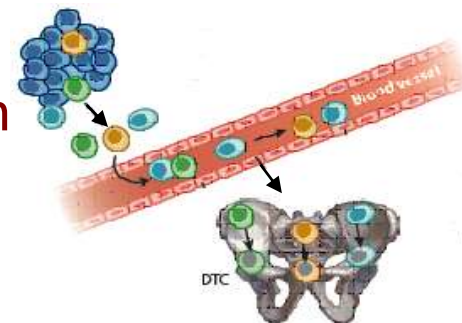


# Serum levels of circulating protease activities in breast cancer patients



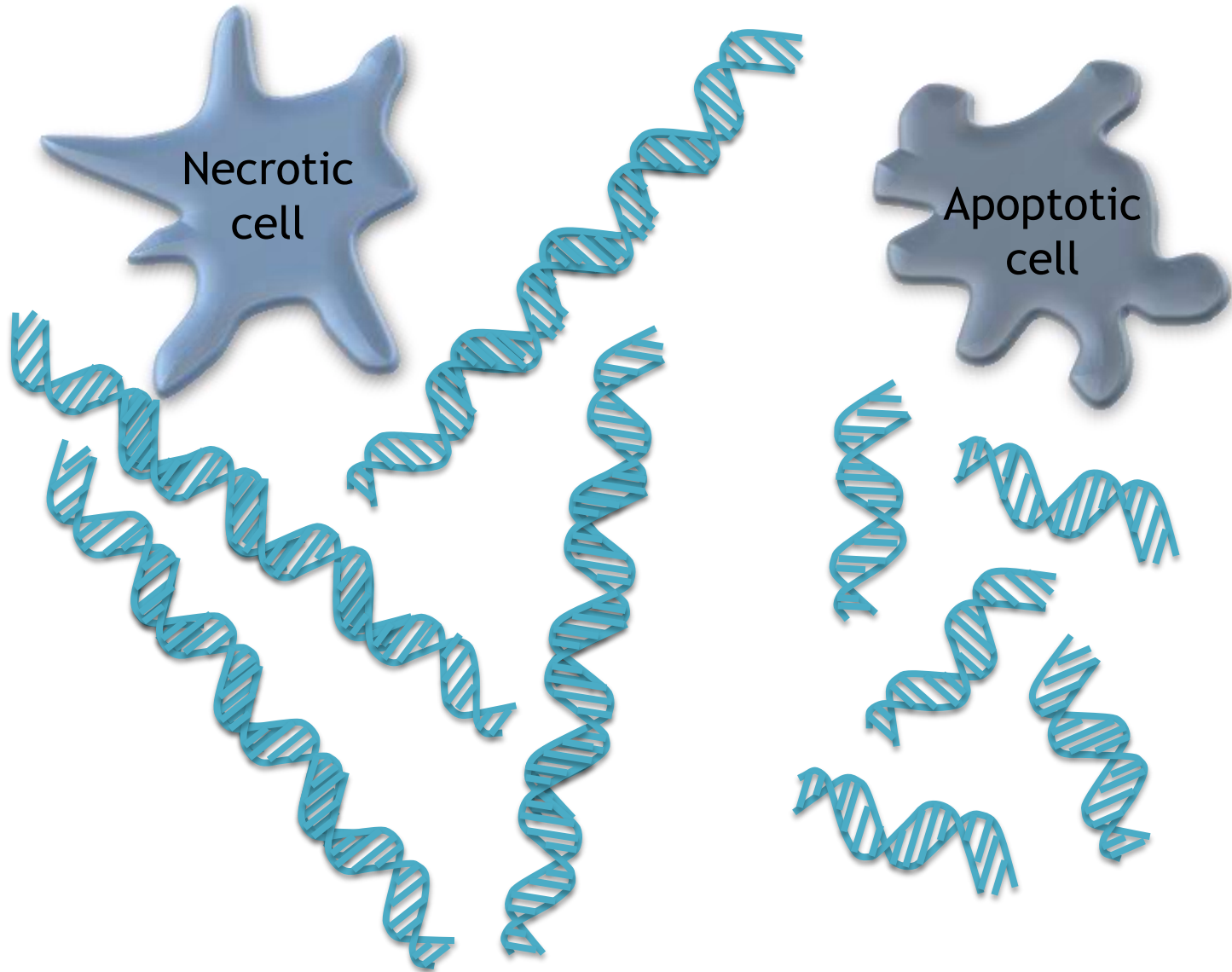
Degradation of the extracellular matrix by proteases supports the dissemination of tumor cells.

Increased levels of circulating protease activities in advanced tumor stages and metastatic disease.





# DNA release by apoptotic and necrotic tumor cells





Extraction of  
DNA from blood plasma and leukocytes

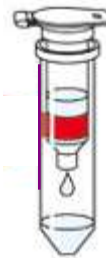


Fractionation of the circulating cell-free plasma DNA

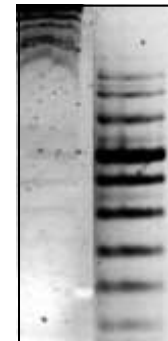
in long DNA fragments and short DNA fragments



by a two column system.



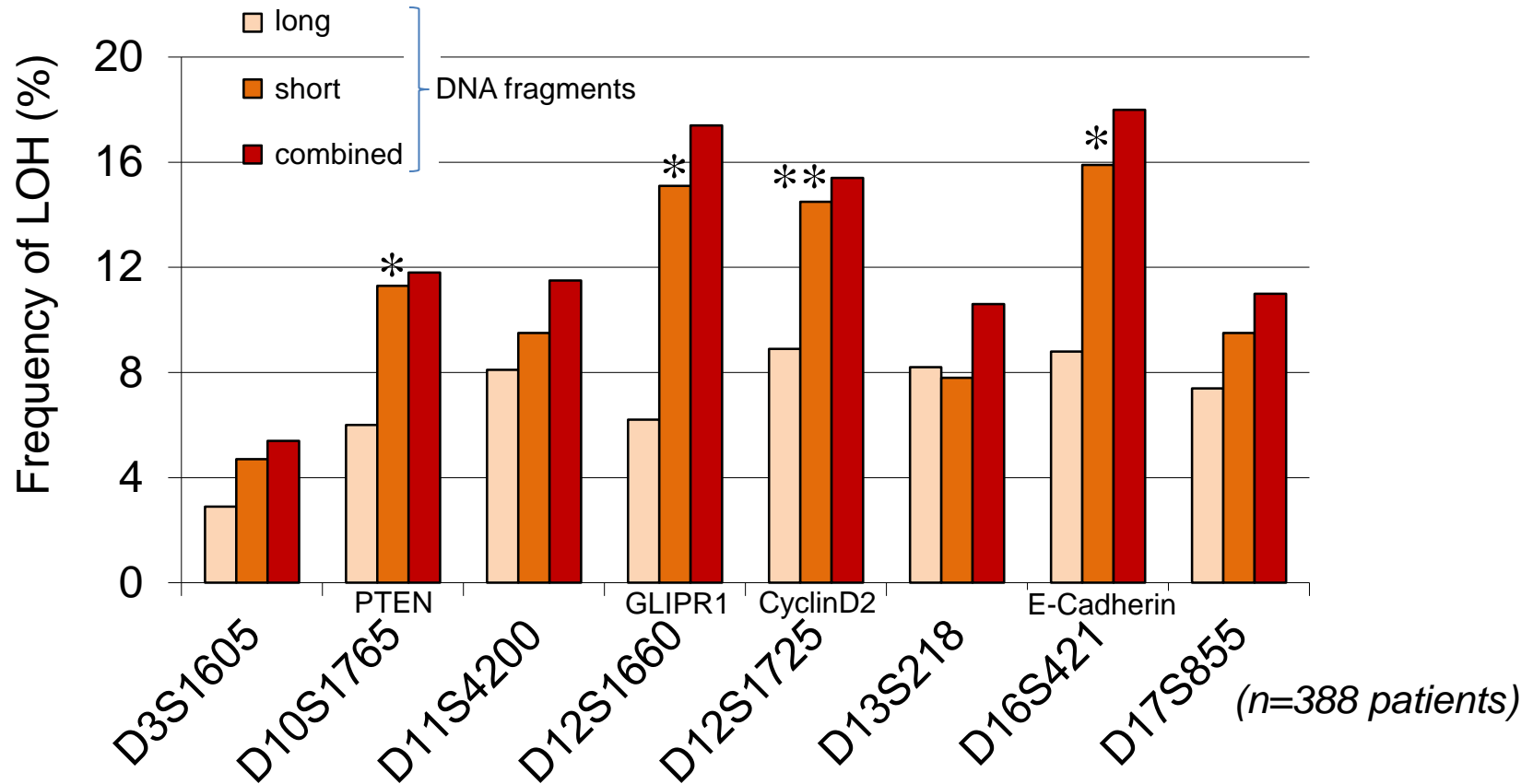
Fractions  
1 2



Gel Electrophoresis



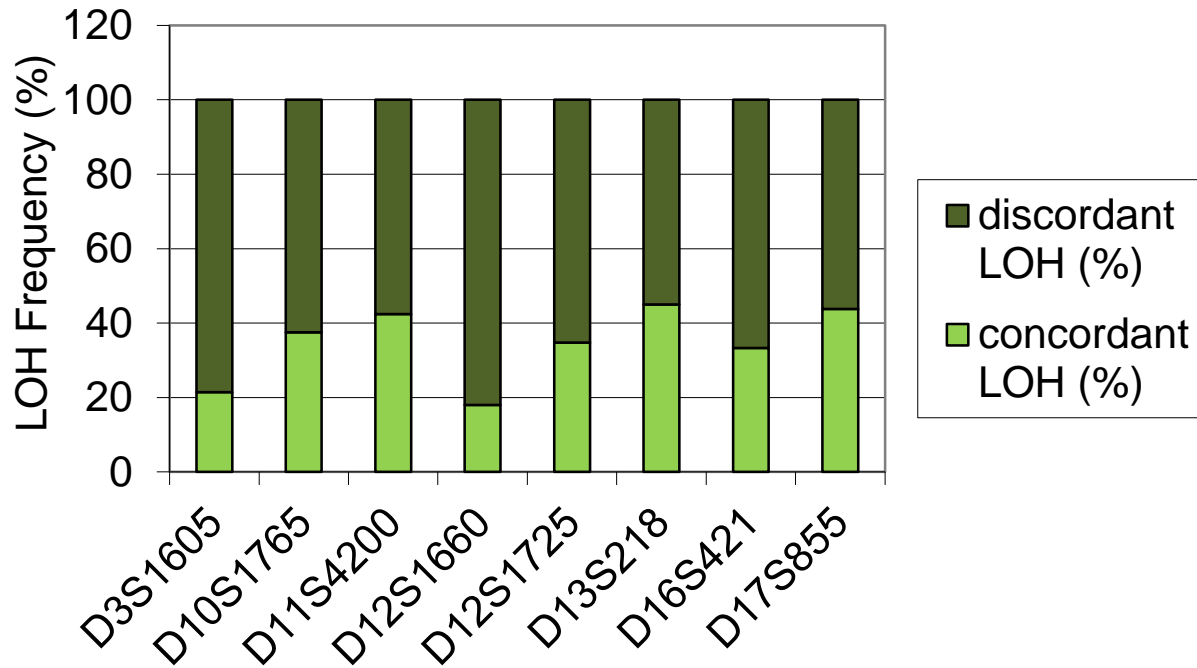
# LOH frequencies in the plasma DNA fractions of breast cancer patients



Significantly more breast cancer patients (38%) had LOH at one or more microsatellite markers in the fraction containing short DNA fragments than in the fraction containing long molecules (28%,  $p=0.0001$ ).



# Concordance of LOH patterns in both plasma DNA fractions of **breast** cancer patients

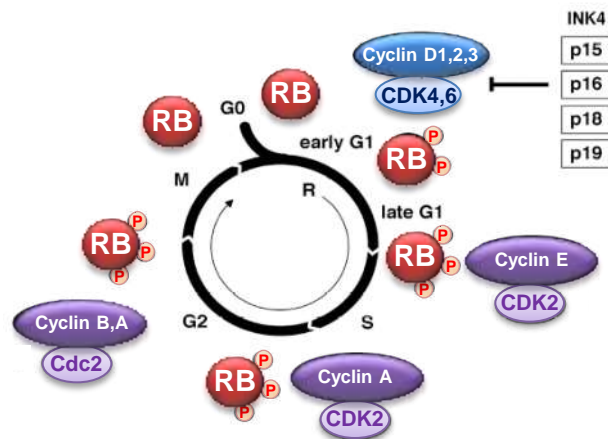


Concordance of 32.85%



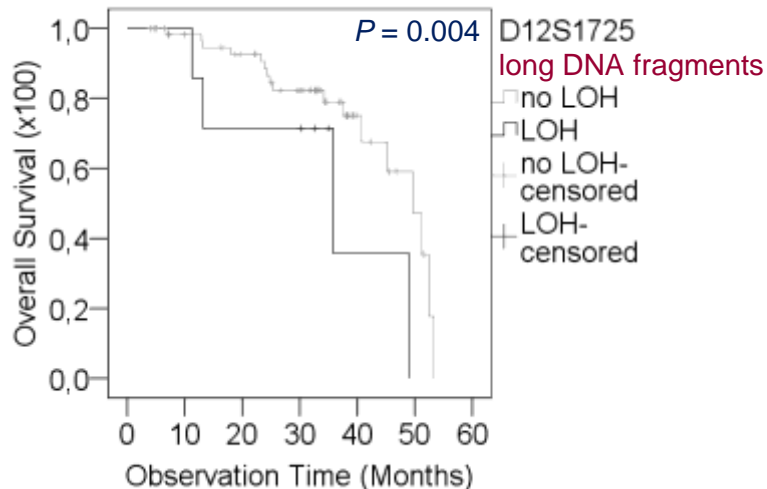
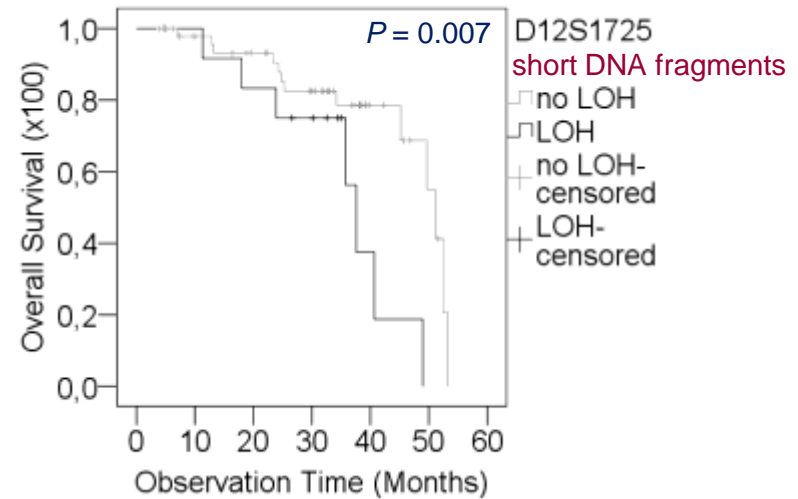
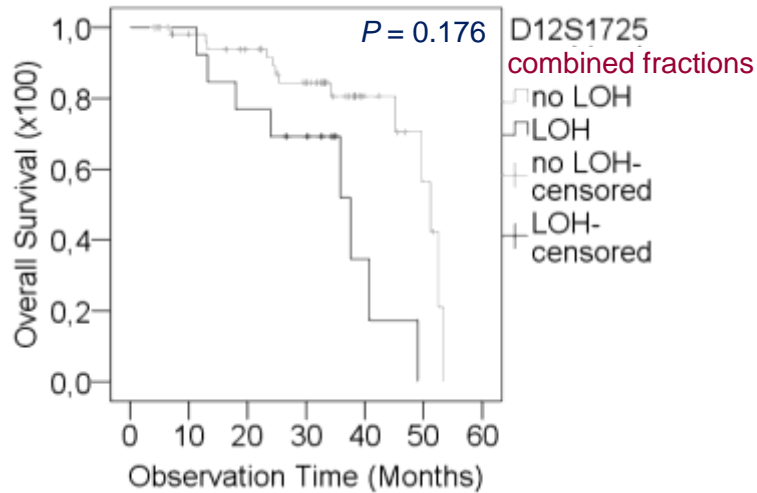
# Associations of LOH at Cyclin D2, RB1 with clinical data of breast cancer patients

Circulating Microsatellite Markers			p Values					
LOH at	Chromosomal Loci	Gene products	pT	pN	Tumor Size	PR <sup>+</sup>	HER2 <sup>+</sup>	OS
<b>D12S1725 L</b>	<b>12p13.32</b>	<b>Cyclin D2</b>	<b>0.003</b>	0.375	0.077	/	0.219	0.176
<b>D12S1725 S</b>			<b>0.0001</b>	<b>0.014</b>	<b>0.010</b>	/	<b>0.028</b>	<b>0.007</b>
<b>D12S1725</b>			<b>0.0001</b>	<b>0.037</b>	<b>0.0001</b>	/	<b>0.012</b>	<b>0.004</b>
<b>D13S218 L</b>	<b>13q12-14</b>	<b>RB1</b>	<b>0.002</b>	/	/	/	/	/
<b>D13S218 S</b>			<b>0.039</b>	/	/	/	/	/
<b>D13S218</b>			<b>0.013</b>	/	/	/	/	/





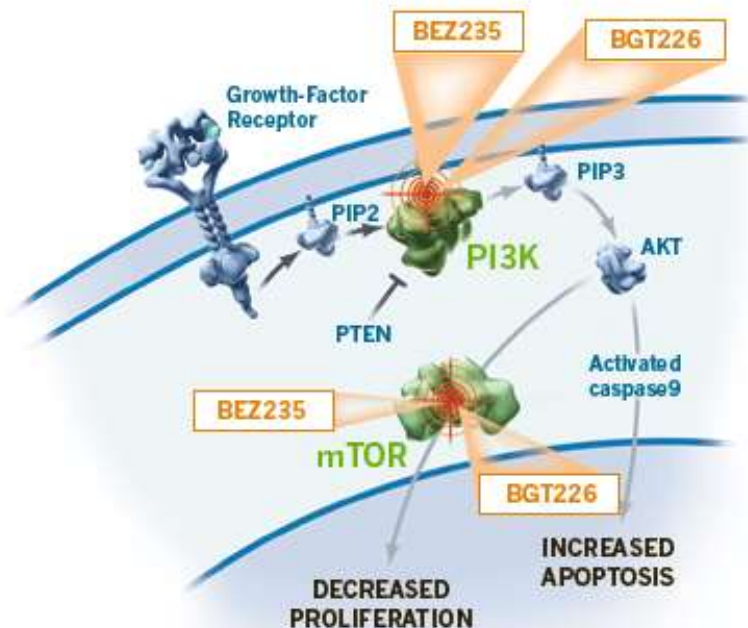
# Prognostic value of LOH at circulating Cyclin D2 in **breast** cancer patients



LOH at D12S1725 mapping to  
Cyclin D2 correlates with  
shorter overall survival.

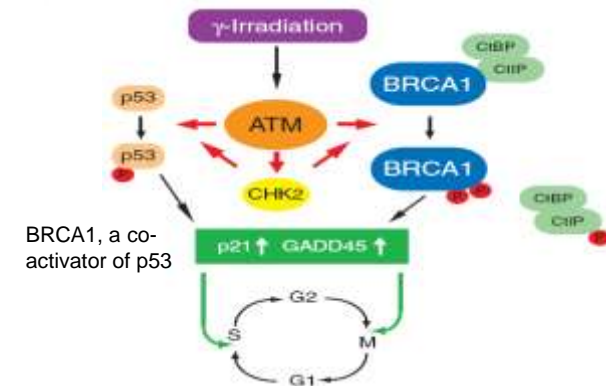
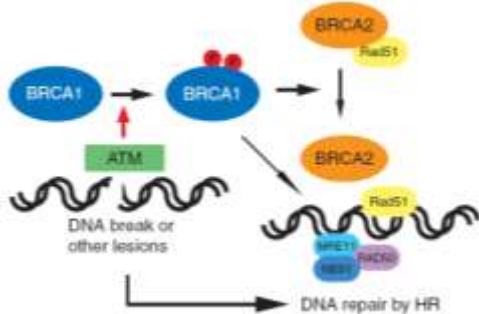
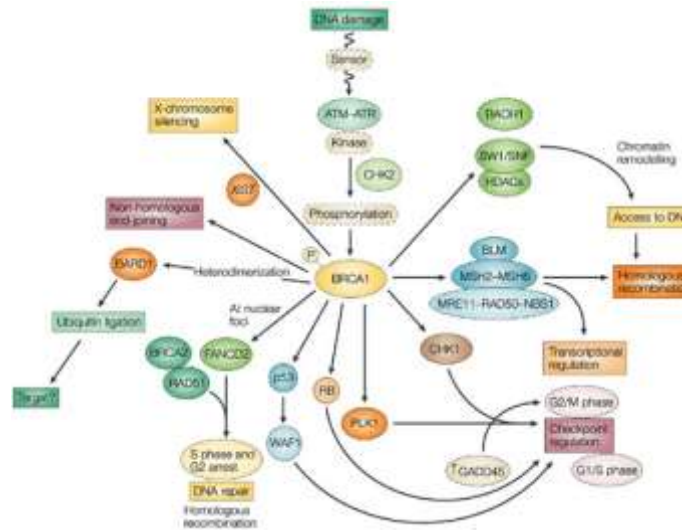
# Associations of LOH at PTEN with clinical data of breast cancer patients

Circulating Microsatellite Markers			p Values					
LOH at	Chromosomal Loci	Gene products	pT	pN	Tumor Size	PR <sup>+</sup>	HER2 <sup>+</sup>	OS
D10S1765 L	10q23.3	PTEN	0.0001	/	0.009	/	/	/
D10S1765 S			0.004	/	0.025	/	/	/
D10S1765			0.0001	/	0.006	/	/	/



# Associations of LOH at BRCA1 with clinical data of breast cancer patients

Circulating Microsatellite Markers			p Values					
LOH at	Chromosomal Loci	Gene products	pT	pN	Tumor Size	PR <sup>+</sup>	HER2 <sup>+</sup>	OS
D17S855 L	17q21	BRCA1	/	/	/	/	0.170	/
D17S855 S			/	/	/	/	<b>0.003</b>	/
D17S855			/	/	/	/	<b>0.002</b>	/



BRCA1, a co-activator of p53

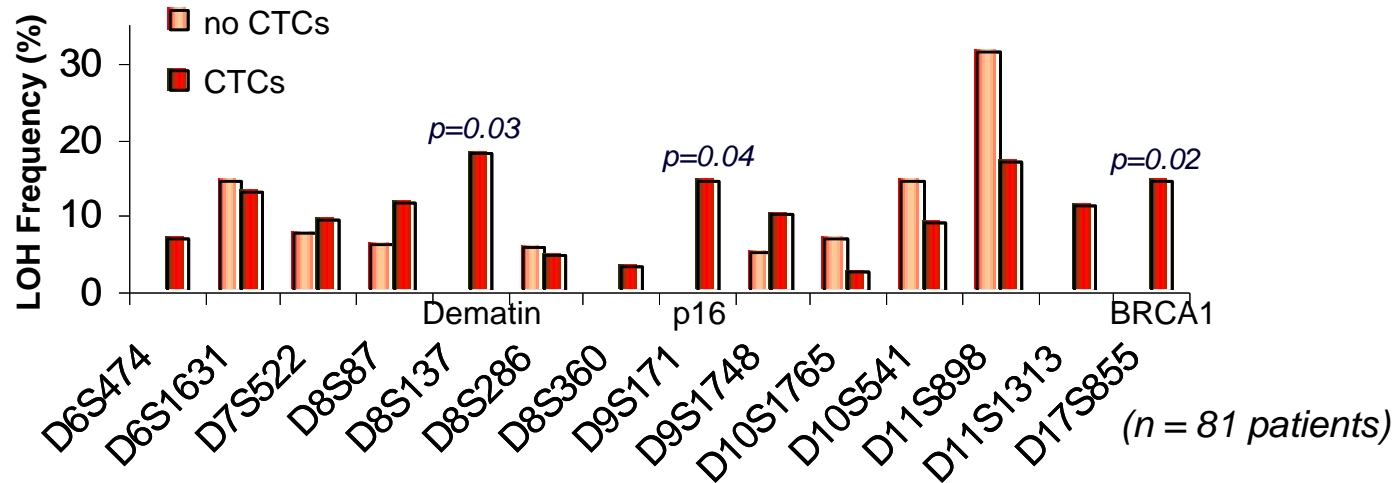


# Associations of LOH at TIG1 with clinical data of breast cancer patients

Circulating Microsatellite Markers			p Values					
LOH at	Chromosomal Loci	Gene products	pT	pN	Tumor Size	PR <sup>+</sup>	HER2 <sup>+</sup>	OS
<b>D3S1705 L</b>	<b>3q25.31-32</b>	<b>TIG1</b>	<b>0.008</b>	0.066	/	0.129	/	/
<b>D3S1705 S</b>			0.082	0.056	/	<b>0.010</b>	/	/
<b>D3S1705</b>			<b>0.004</b>	<b>0.011</b>	/	<b>0.010</b>	/	/

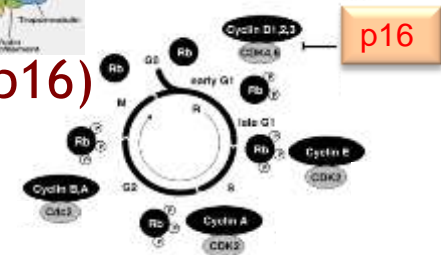
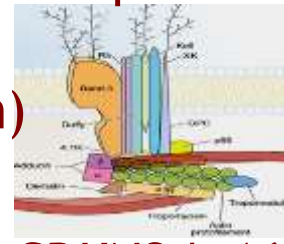
LOH at Tazarotene-induced gene 1 (TIG1), a cell adhesion molecule, leads to a reduced cell to cell contact and an increased cell proliferation.

# Associations of CTCs with marker-specific LOH in prostate cancer patients



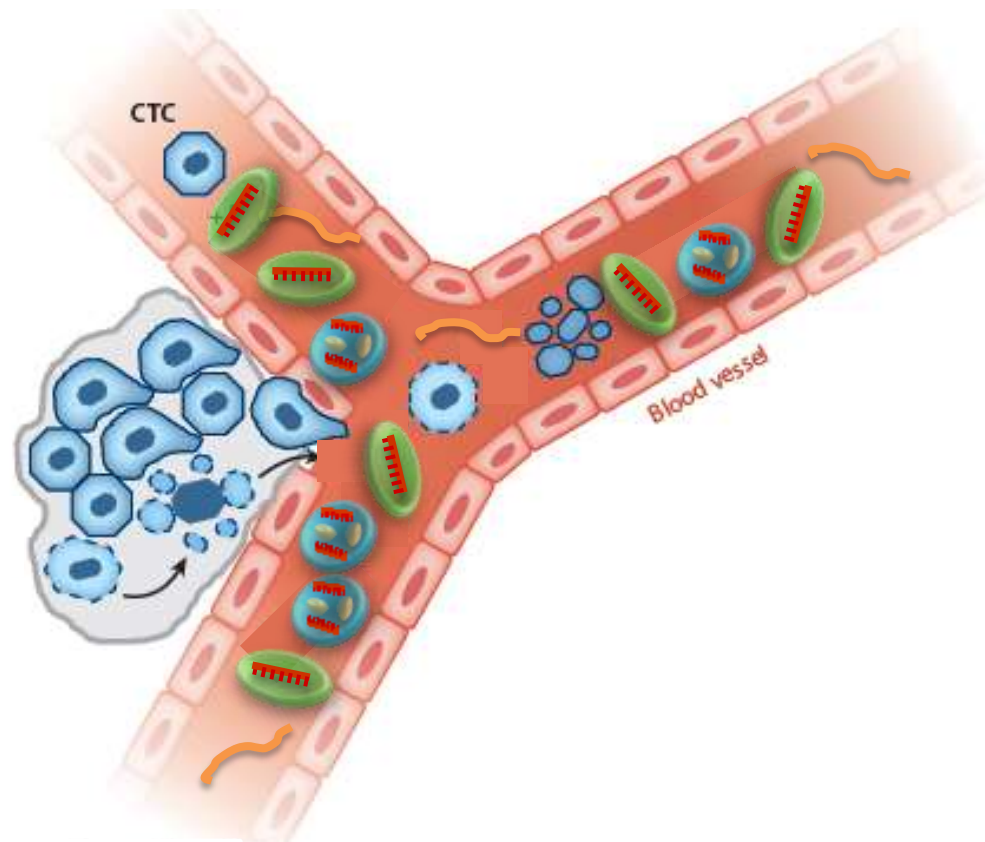
Significant associations between the presence of CTCs and LOH frequencies at the markers in prostate cancer patients:

- ❖ D8S137 (Cytoskeleton protein Dematin)
- ❖ D9S171 (Inhibitor of dependent kinase CDKN2/p16)
- ❖ D17S855 (BRCA1)



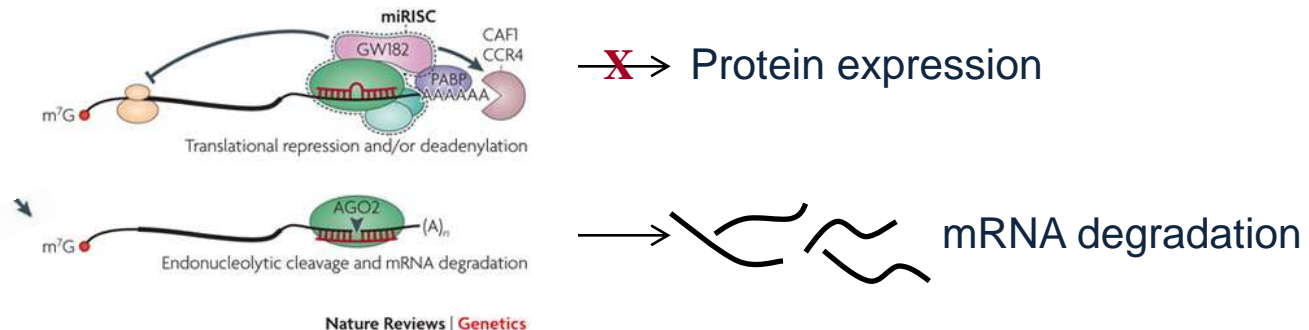


# Circulating microRNAs in the blood of cancer patients



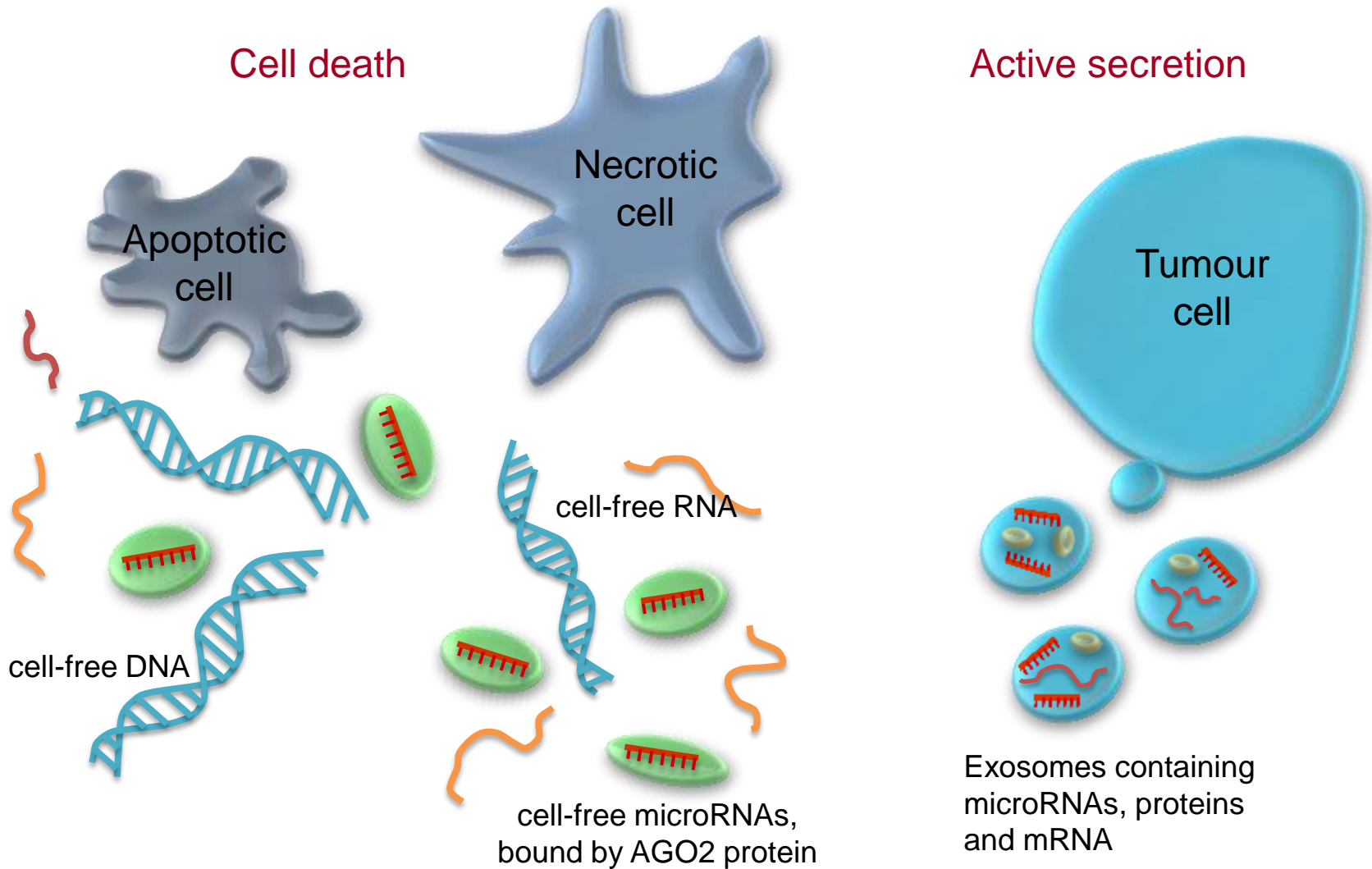
# MicroRNA expression, processing and function

- ❖ Small regulatory, non-coding RNA molecules of ~22 nucleotides.
- ❖ Binding affinity to hundreds of different mRNAs and regulation of several signaling pathways.
- ❖ Apoptosis, hematopoietic cell differentiation, metabolism, neural development, metastasis.
- ❖ Frequent location in fragile chromosomal regions exhibiting amplifications, deletions or translocations.



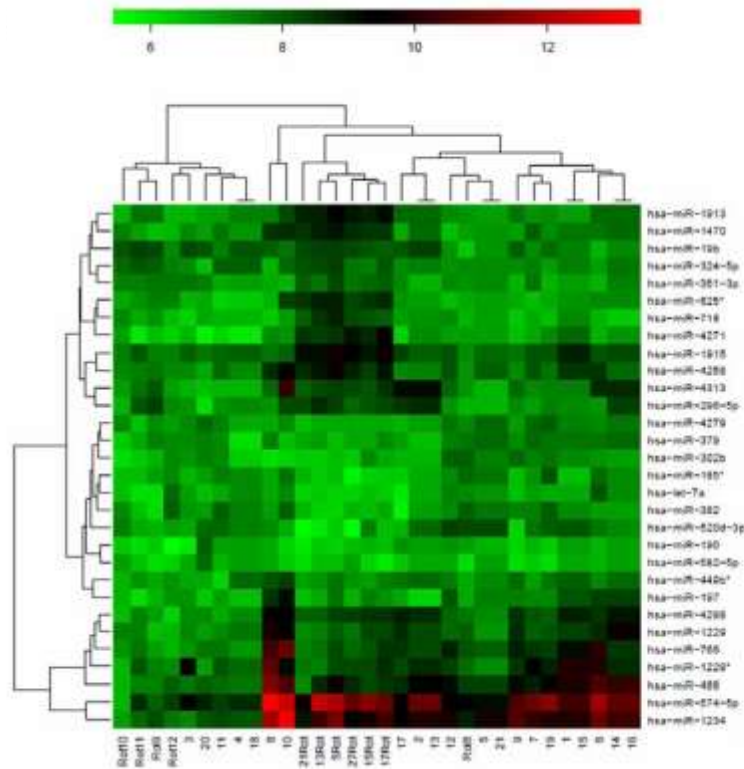


# Release of microRNAs into the blood circulation

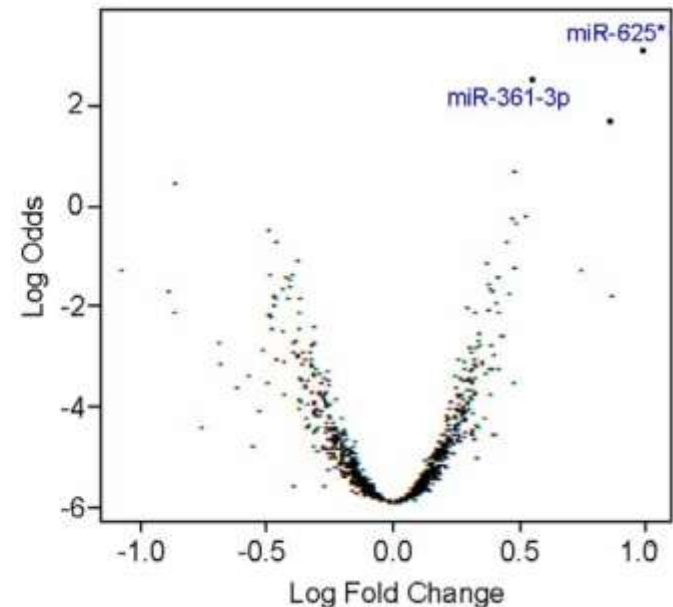


# Serum microRNA profiling in lung cancer patients by a blood-based microarray

Hierarchical cluster heat map



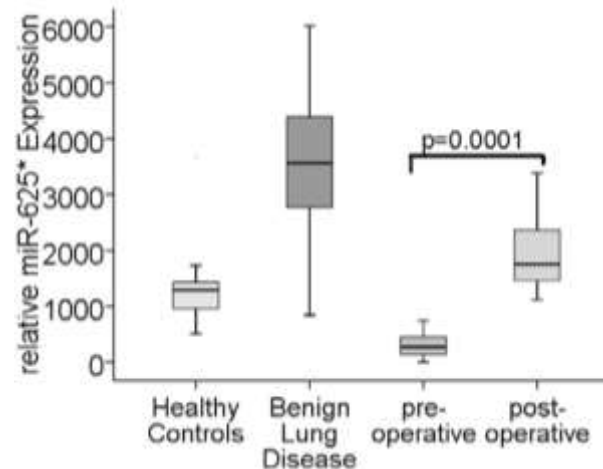
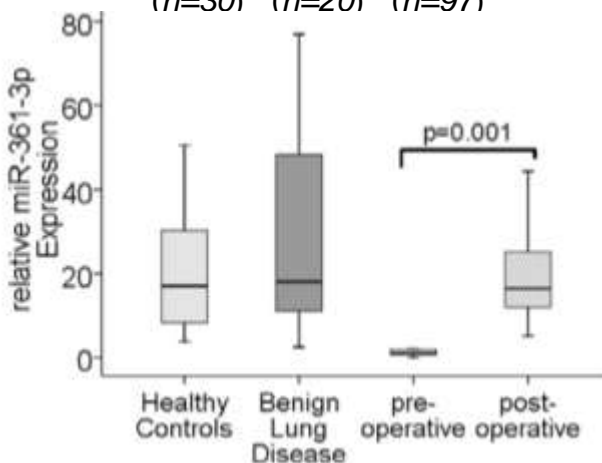
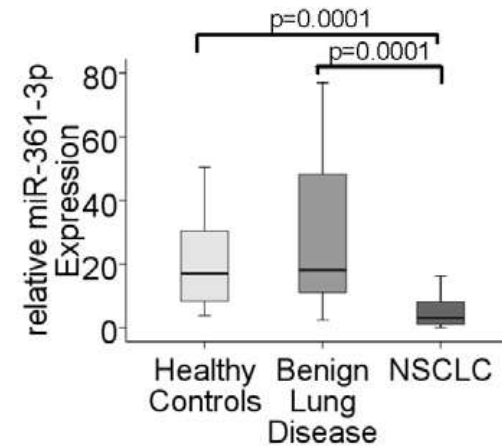
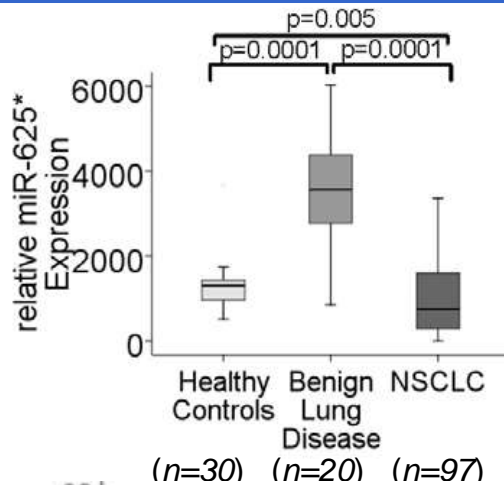
Volcano plot



Microfluidic biochips containing 1158 different microRNAs using serum of 21 NSCLC patients and 11 healthy individuals.

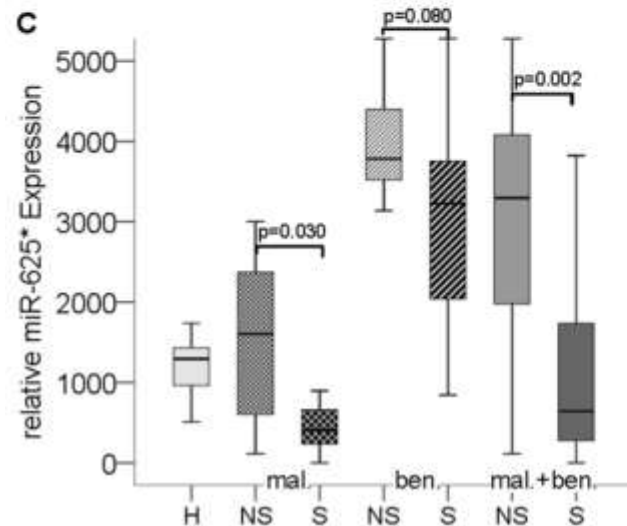
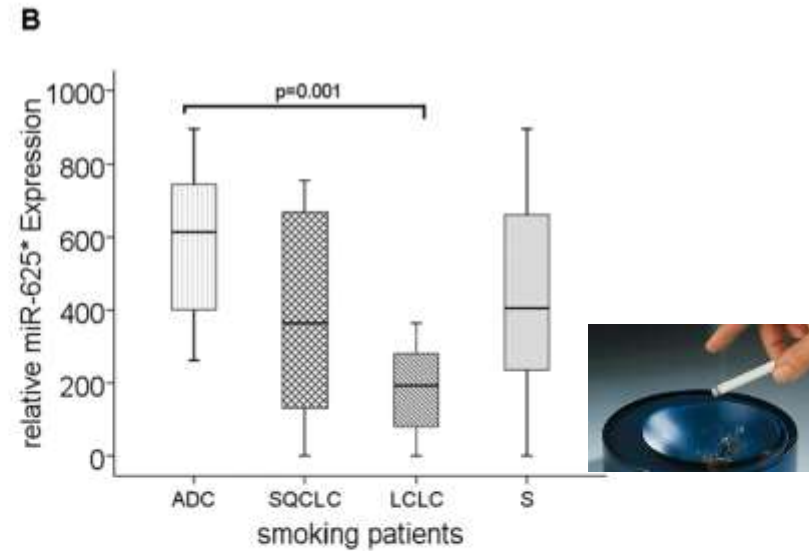
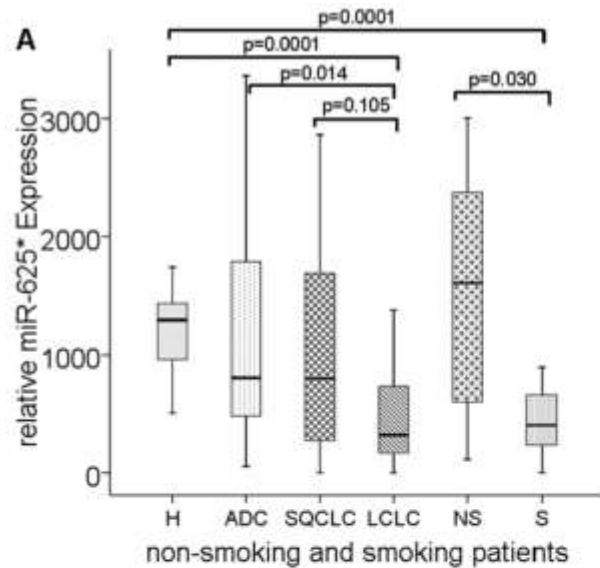
Comparison of microRNA levels between NSCLC patients and healthy individuals.

# Serum miR-625\* and miR-361-3p in lung cancer patients



- ❖ Serum levels of miR-625\* and miR-361-3p discriminate malignant from benign tumors.
- ❖ Serum levels of miR-625\* and miR-361-3p levels increase in postoperative serum.

# Correlation of serum miR-625\* with histological type of lung cancer and smoking behavior

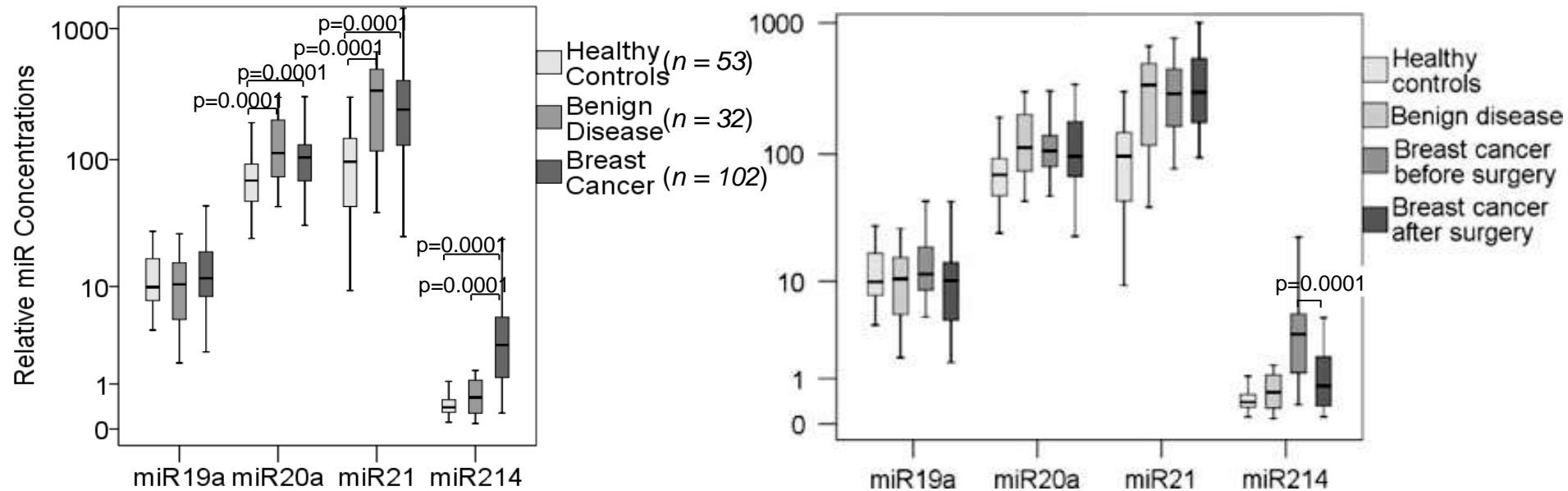


4 major histological types:

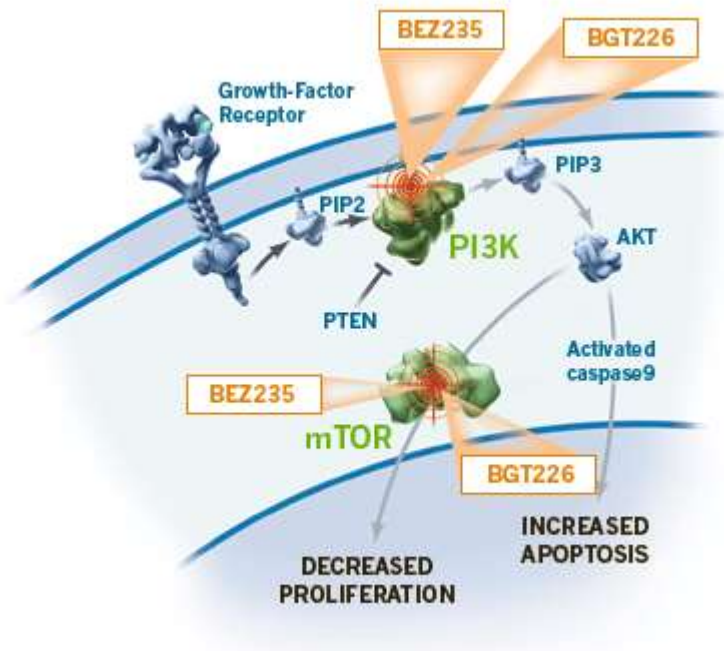
- ❖ non-small cell lung cancer (NSCLC):  
squamous cell lung cancer (SQCLC)  
adenocarcinoma (ADC)
- ❖ large cell lung cancer (LCLC)
- ❖ small cell lung cancer (SCLC)



# Circulating PTEN-targeting miR-214 in breast cancer patients



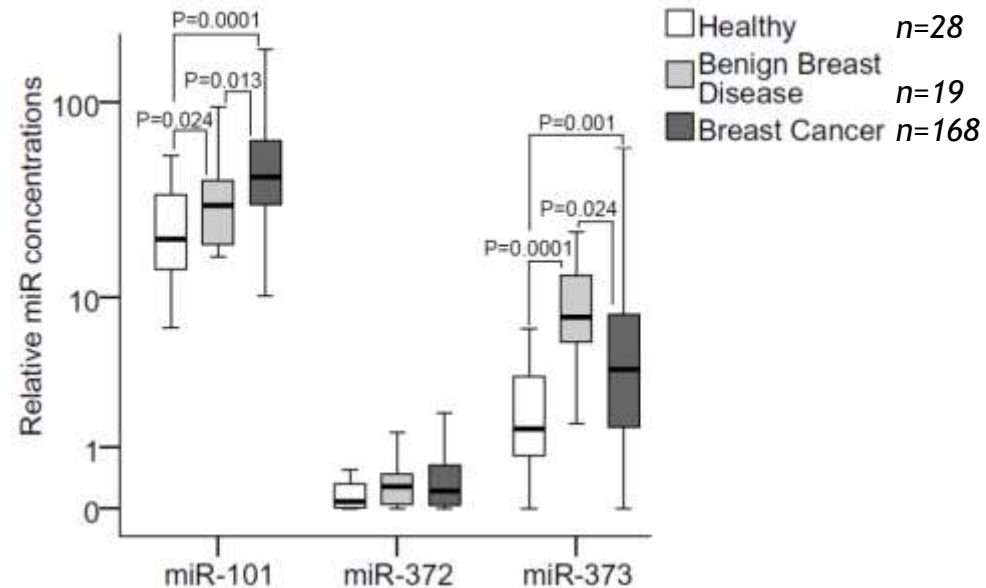
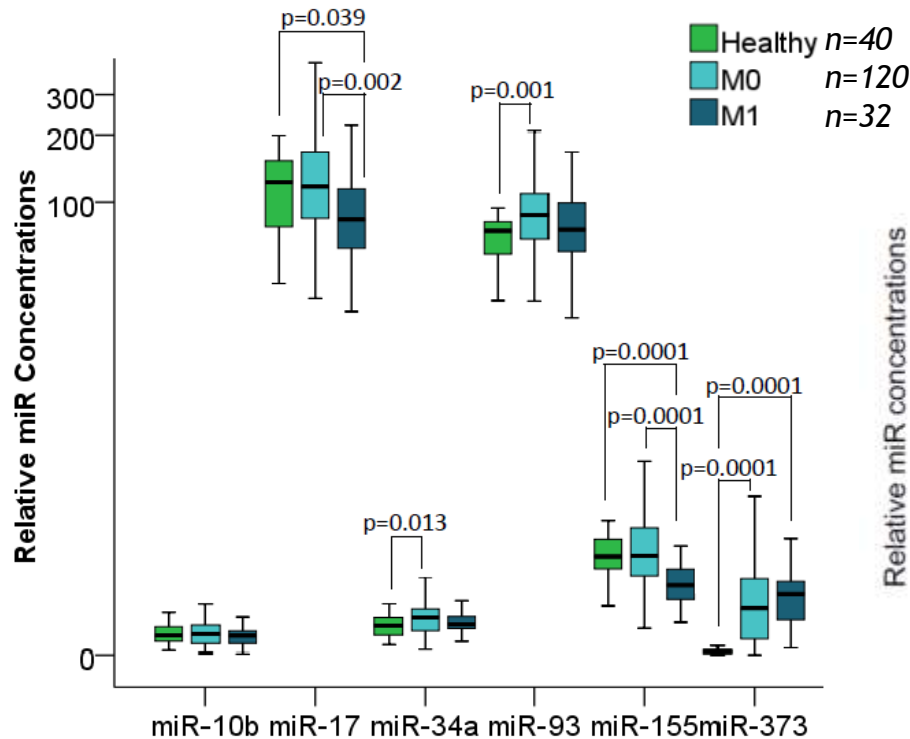
- ❖ Serum levels of miR-214 discriminate malignant from benign tumors.
- ❖ Serum levels of miR-214 significantly decrease in postoperative serum.



Increased serum levels of miR-214 are associated with a positive lymph node status



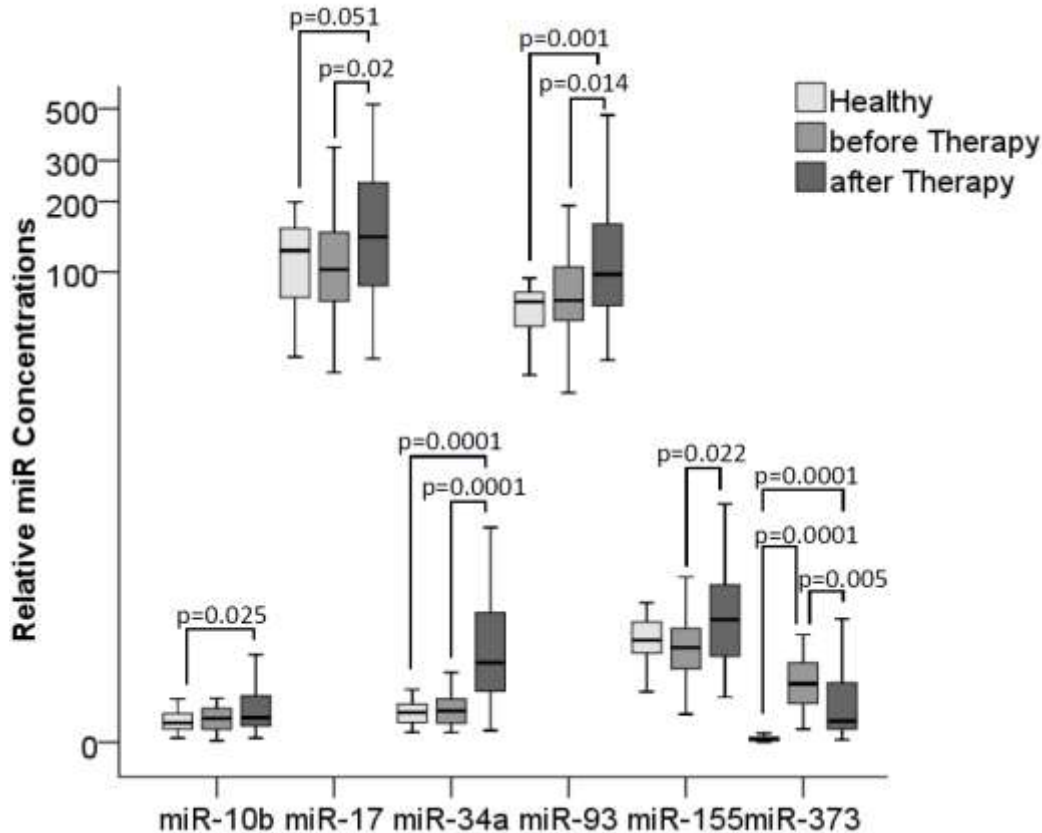
# Serum miR-373 in breast cancer patients



Serum levels of miR-373 significantly increase in the cohorts of patients with primary breast cancer, metastases and benign breast disease.

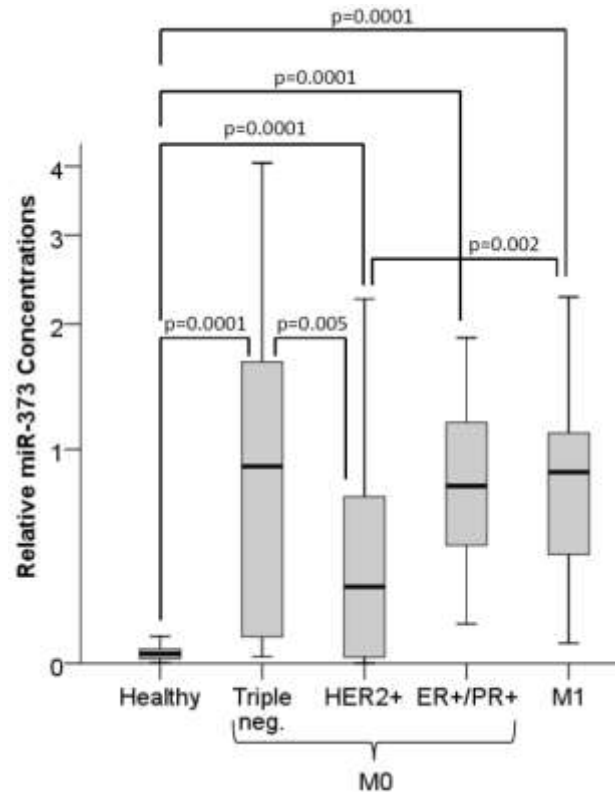


# Changes of serum miR-373 in ER<sup>+</sup>/PR<sup>+</sup> breast cancer patients after therapy



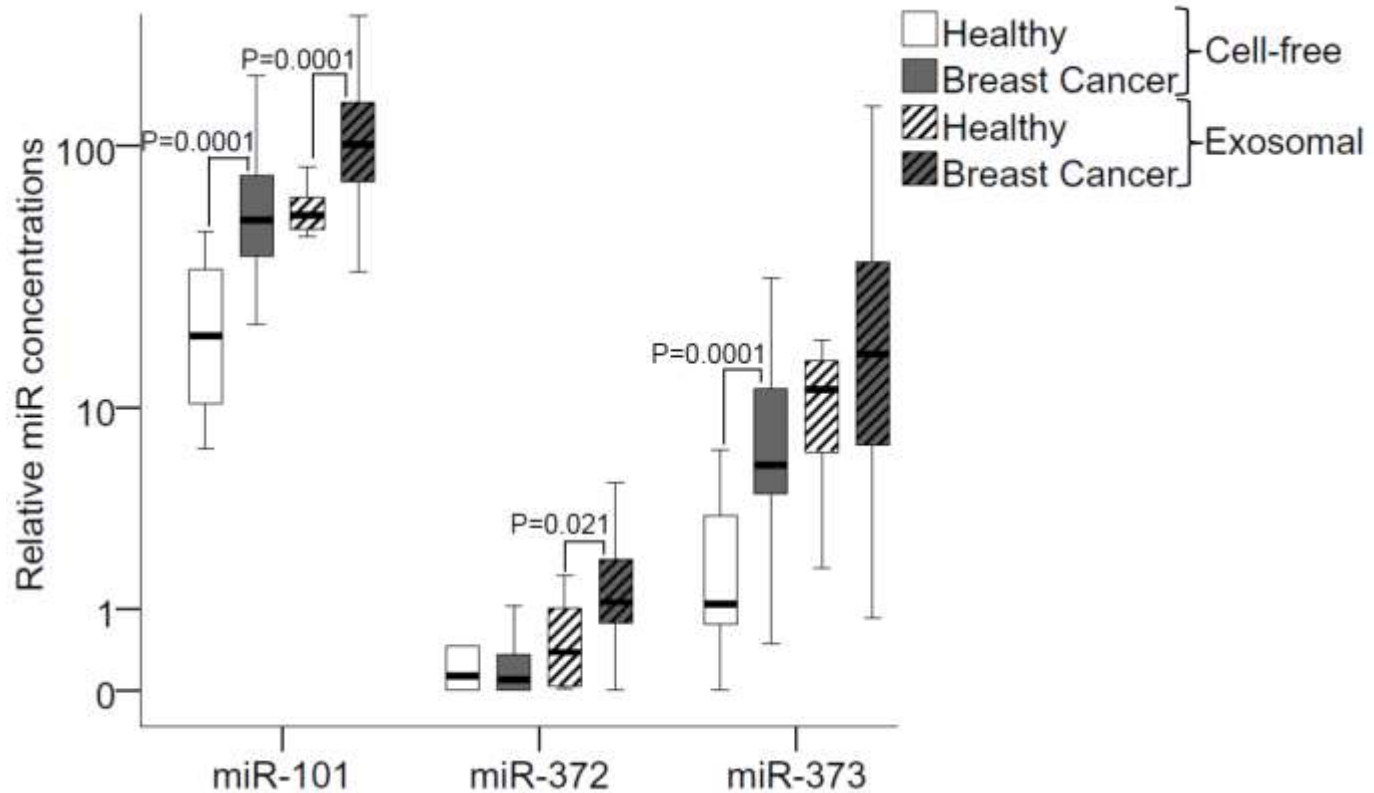
- ❖ Serum levels of miR-17, miR-93 and miR-155 significantly increase after chemotherapy.
- ❖ Serum levels of miR-373 decrease after chemotherapy.

# Correlation of serum miR-373 with receptor status of breast cancer



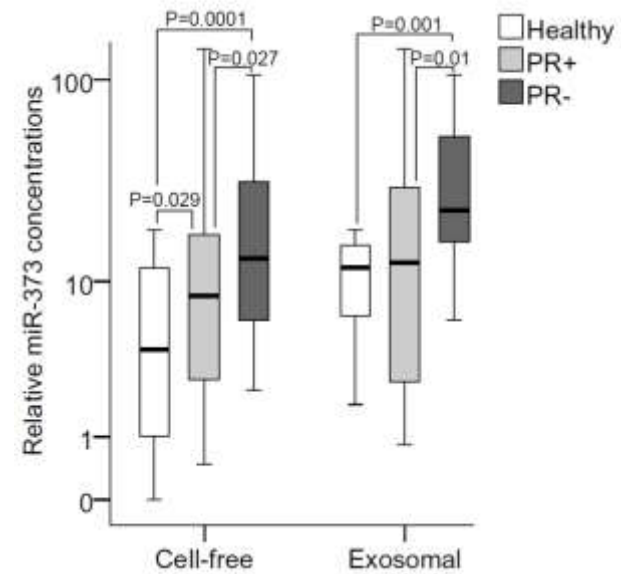
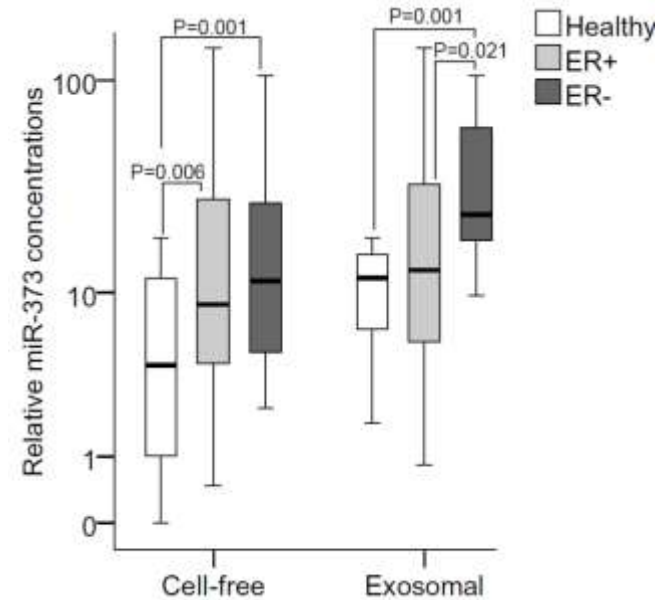
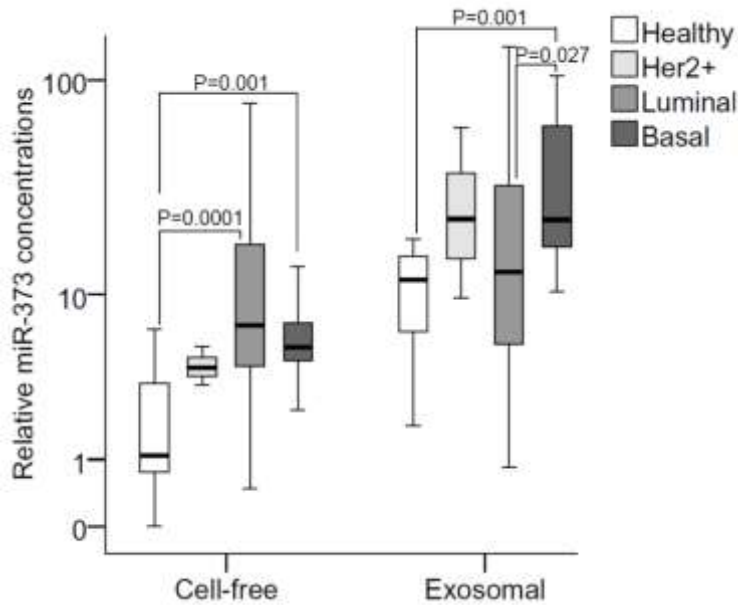
Significantly higher serum levels of miR-373 in patients with triple-negative cancer or metastatic disease than in HER2-positive patients.

# Cell-free and exosomal serum microRNAs in **breast** cancer patients



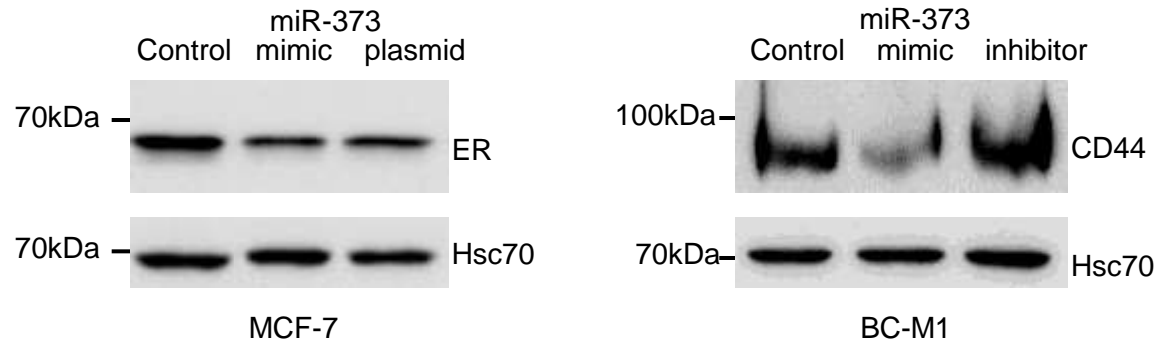
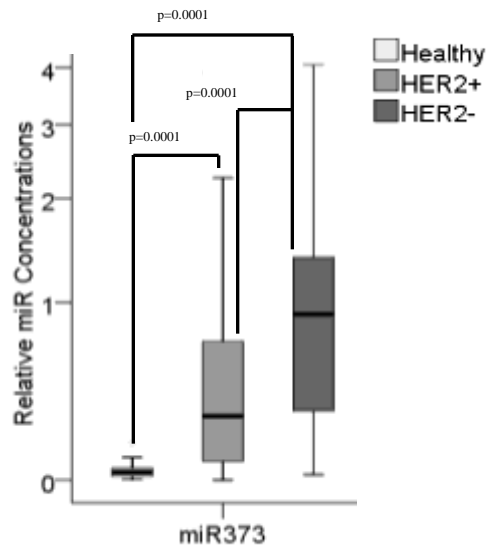
A prevalence of serum miR-101, miR-372 and miR-373 are found in exosomes.

# Correlation of exosomal miR-373 with receptor status of breast cancer



- ❖ Higher levels of exosomal miR-373 in basal-like than in luminal carcinomas.
- ❖ Higher levels of exosomal miR-373 in estrogen- and progesterone-receptor negative tumors than in patients with hormone-receptor positive tumors.

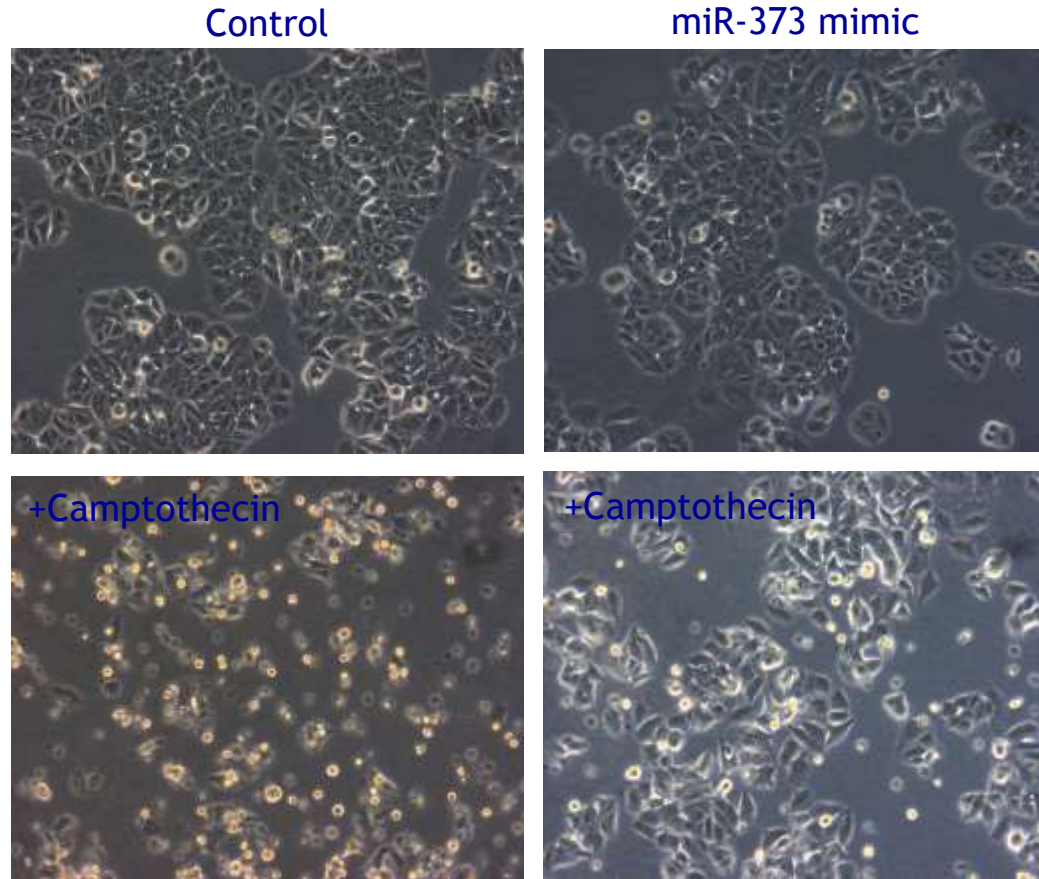
# CD44 and estrogen receptor are targets of miR-373



- ❖ Association of elevated serum levels of miR-373 with HER2 status of the primary tumor.
- ❖ Downregulation of the cell surface marker CD44 and estrogen receptor (ER) by miR-373 in MDA-231, MDA-468 and BC-M1 cells.



# miR-373 inhibits the apoptosis of MCF-7 cells mediated by camptothecin



MCF-7 cells transfected with miR-373 and treated with the topoisomerase I inhibitor camptothecin. Camptothecin, which is used in cancer chemotherapy, induces apoptosis.



# Conclusion of the deregulation of microRNAs in blood of cancer patients

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- ❖ In lung cancer serum levels of miR-625\* correlate with histological type and smoking behavior.
- ❖ In breast cancer serum levels of PTEN-targeting miR-214 discriminate malignant from benign tumors and are associated with lymph node status.
- ❖ Tumor-specific increase in serum levels of miR-373 and its association with triple-negative, hormone receptor and HER2 status.
- ❖ MiR-373 can inhibit translation of the cell surface marker CD44 and estrogen receptor.
- ❖ MiR-373 has an anti-apoptotic effect on MCF-7 cells.
- ❖ Prevalence of circulating microRNAs in exosomes.





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Corinna Eichelser  
Dr. Carina Roth  
(University Medical Center Hamburg-Eppendorf, Germany)
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Thanks for your attention



A long way to cure cancer