

# MicroRNAs and genomics in adrenal tumors

**Dr. Peter Igaz MD MSc PhD DSc**

**Associate professor**

**2<sup>nd</sup> Department of Medicine  
Semmelweis University  
Budapest, Hungary**



# Relevance of genomics (transcriptome and microRNA) studies

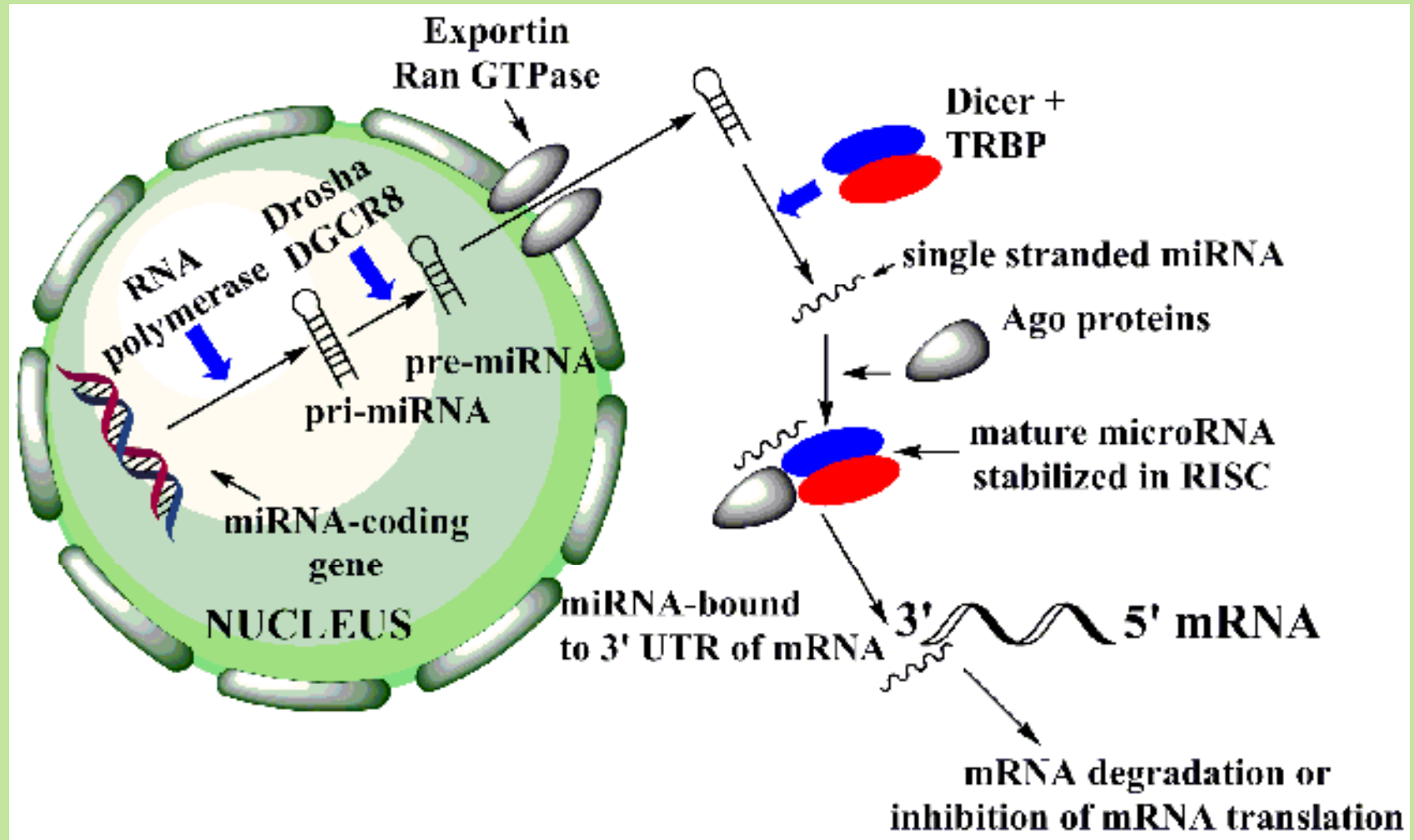
- **Biomarkers**
  - Markers of malignancy
  - Prognostic markers
- **Classification/Subclassification**
- **Pathogenesis**
  - Pathways
- **Novel therapeutic targets**

**Relevant points for adrenocortical-  
adrenomedullary tumour dichotomy**

**Great perspectives for  
transcriptomic and  
microRNA studies for  
biomarkers, pathogenesis  
and tumour classification**

# MicroRNAs

# MicroRNAs (miRNA, miR)




# miRNA function in cancer

## Tumor suppressor miRNAs

  
e.g. miR-15a  
let-7b  
miR-16

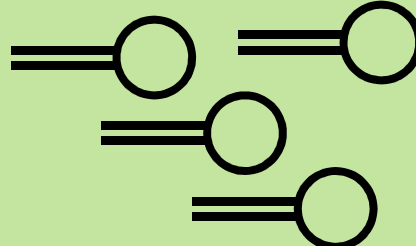


Target mRNA  
  
e.g. BCL2  
CCND1  
FGF2

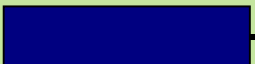


Cell cycle activation  
Cell proliferation  
Invasion  
Decreased apoptosis

## Oncogenic miRNAs

  
e.g. miR-214  
miR-106  
miR-21

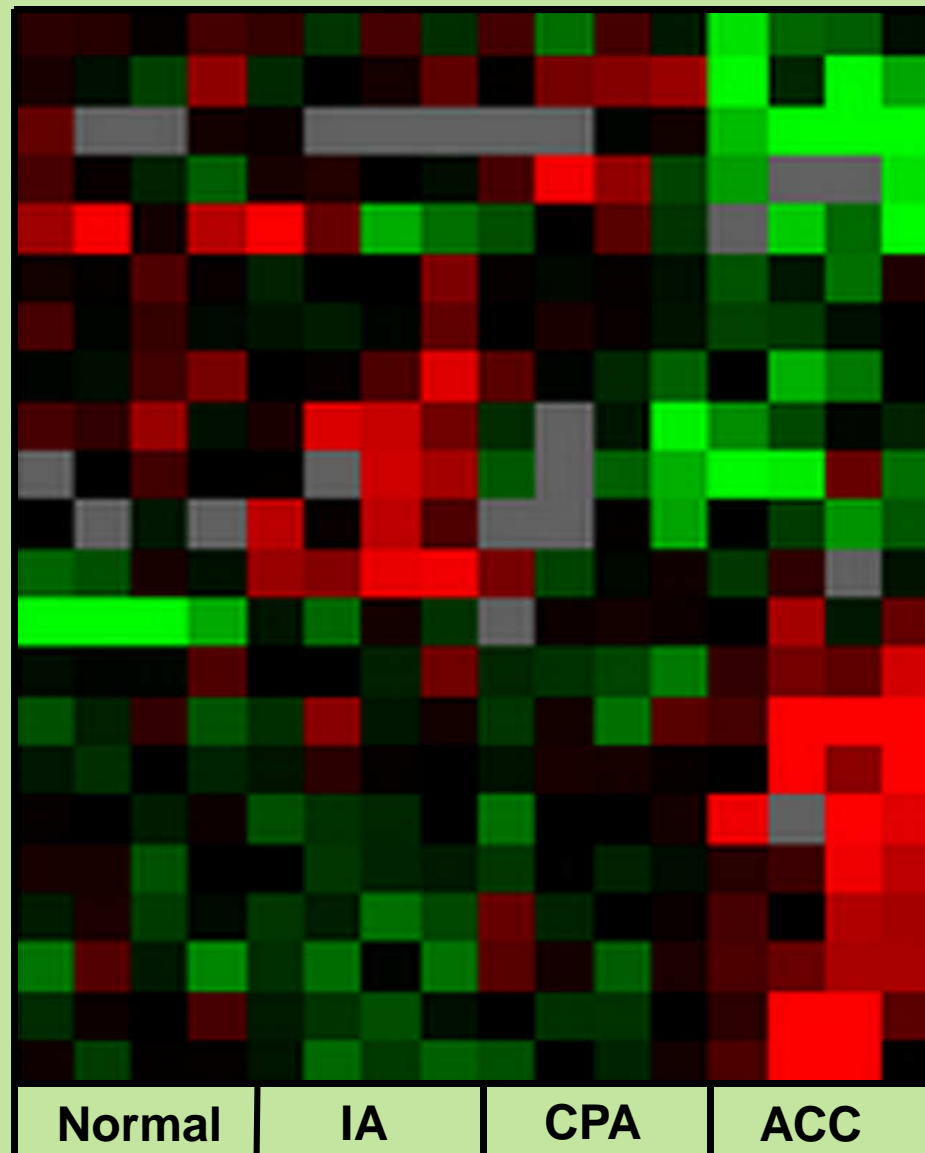


Target mRNA  
  
e.g. PTEN  
RB1  
TPM1



Tumor development

# MicroRNAs in adrenocortical tumors



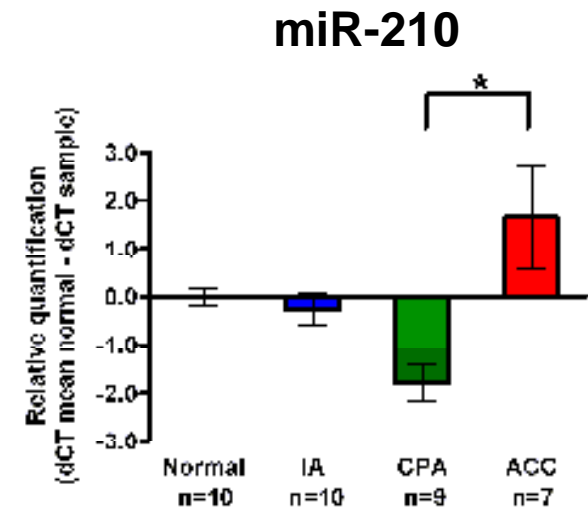
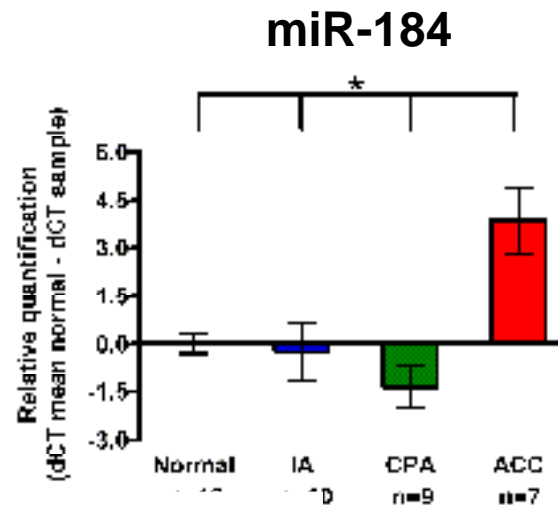
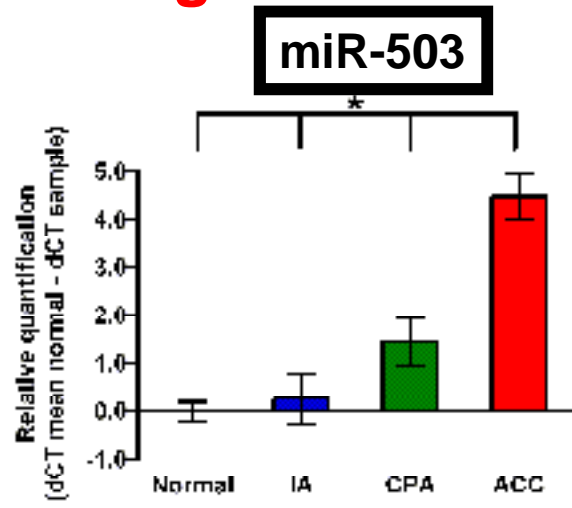
Hsa-miR-196b  
Hsa-miR-210  
Hsa-miR-184  
Hsa-miR-376a  
Hsa-miR-506  
Hsa-miR-181b  
Hsa-miR-181d  
Hsa-miR-192  
Hsa-miR-424  
Hsa-miR-503  
Hsa-miR-215  
Hsa-miR-615  
Hsa-miR-375  
Hsa-miR-222  
Hsa-miR-214  
Hsa-miR-491  
Hsa-miR-511  
Hsa-miR-342  
Hsa-miR-30e-3p  
Hsa-miR-365  
Hsa-miR-299-5p  
Hsa-miR-485-5p

Tömböl ... Igaz, 16, 895-906  
Endocrine-Related Cancer, 2009

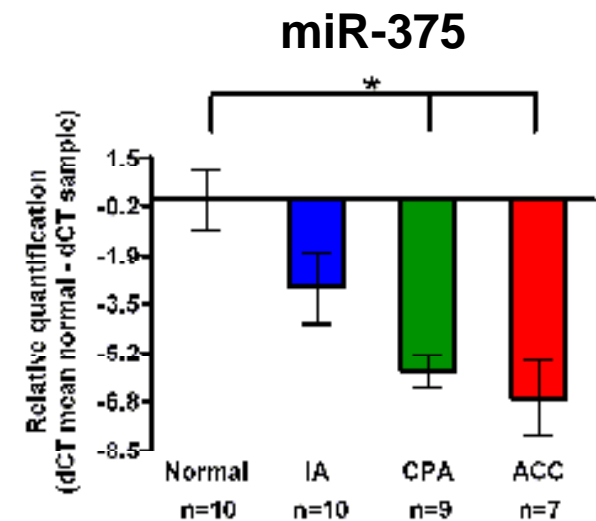
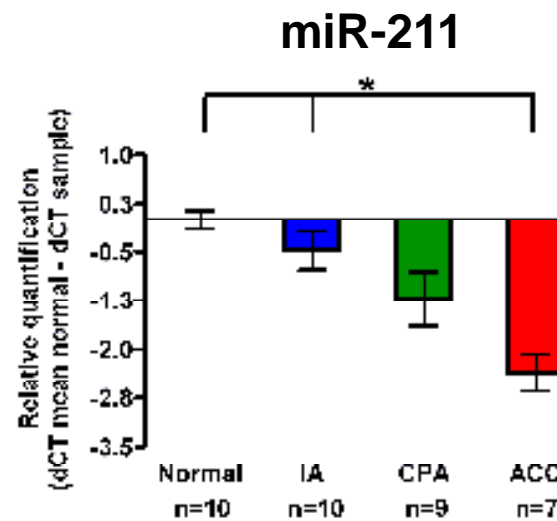
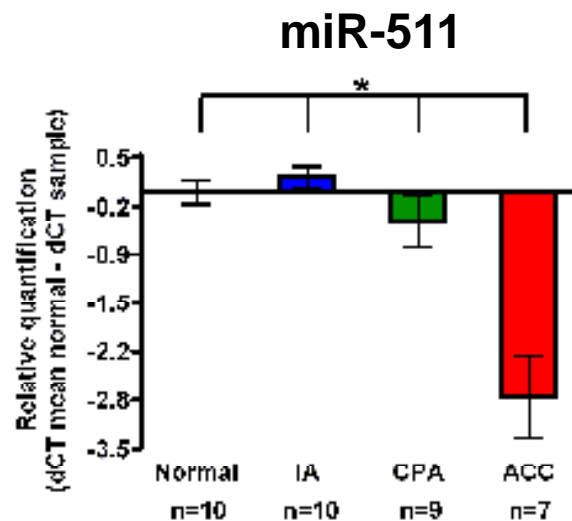
One-way ANOVA,  $p < 0.05$ ; post hoc test: Scheffe

# qRT-PCR validated microRNAs

## Oncogenic microRNAs

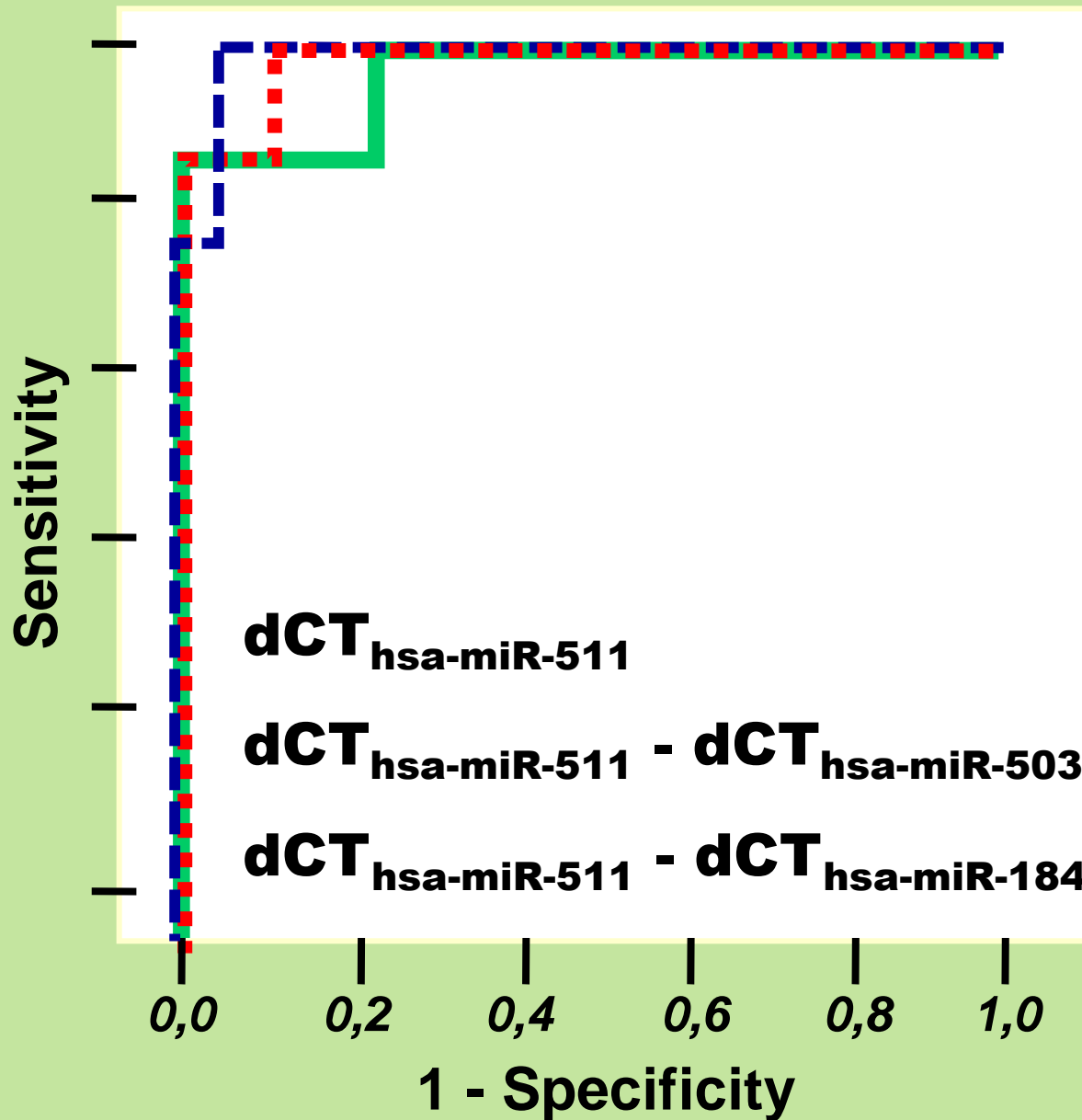


## Tumor suppressor microRNAs



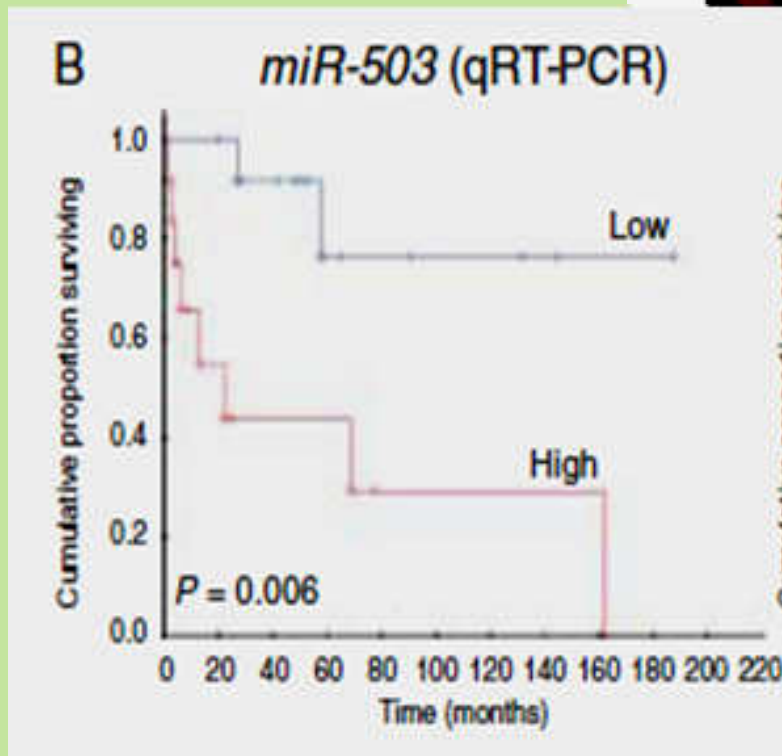
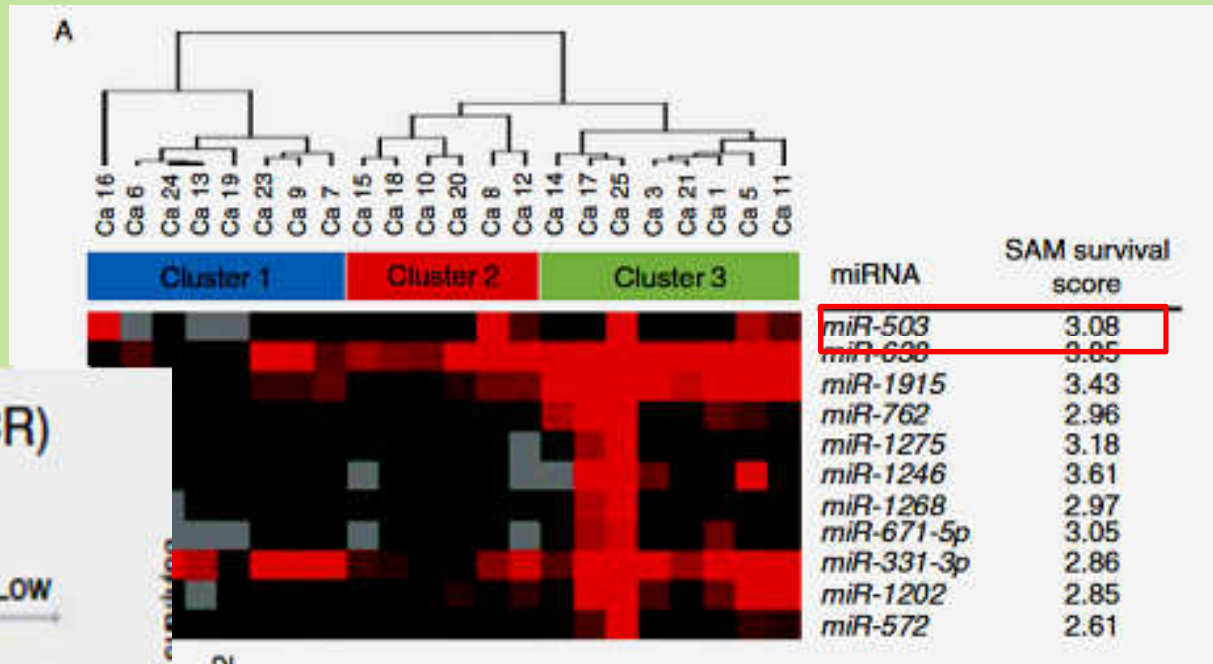


# ROC-analysis



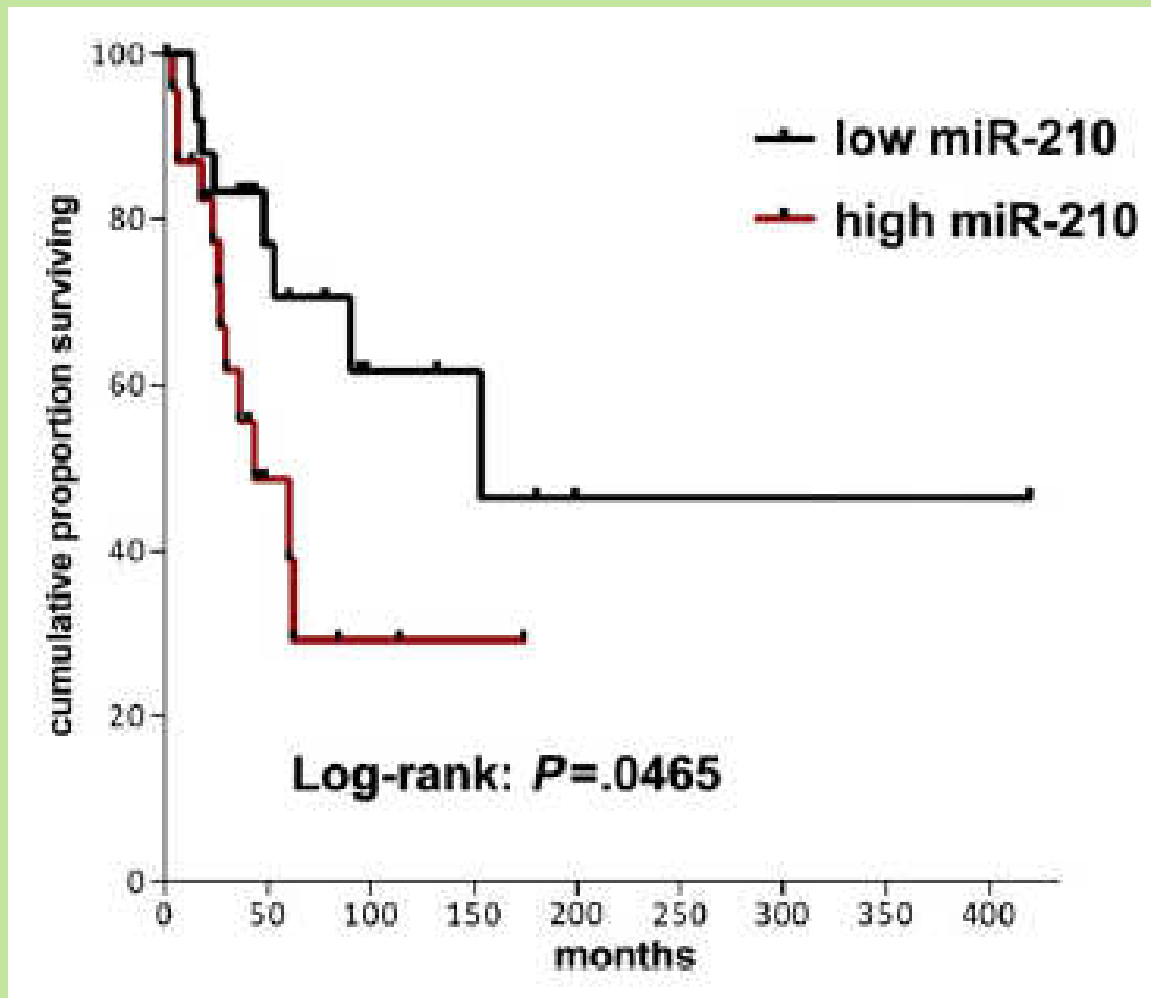
100 % sensitivity  
97% specificity  
for ACC-ACA  
differentiation

# microRNAs associated with poor ACC survival



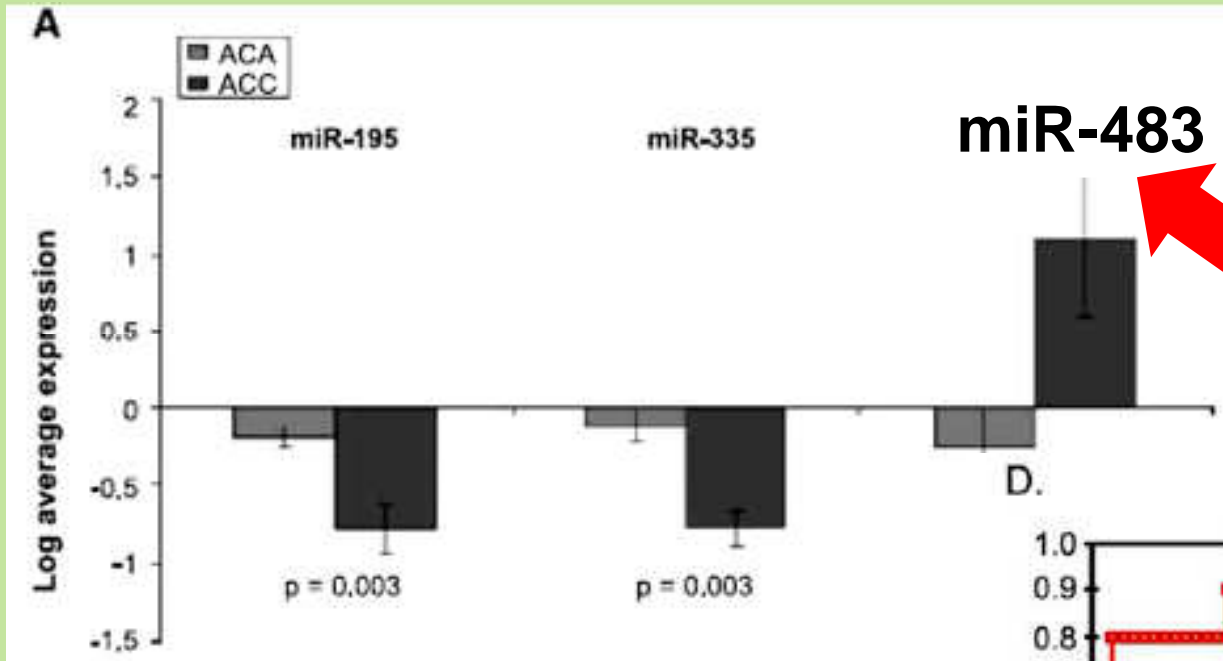
Özata et al., Endocrine-Related Cancer, 2011

# Overexpressed miR-210 is associated with poor survival of ACC

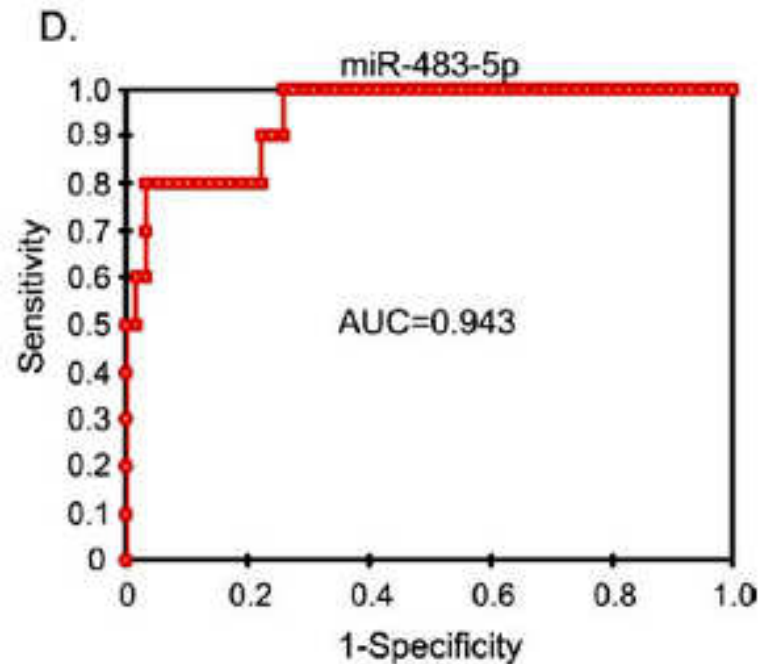


Duregon et al., Hum Pathol, 2014

# qRT-PCR validated miRNAs in Soon's study (Clin Cancer Res, 2009)



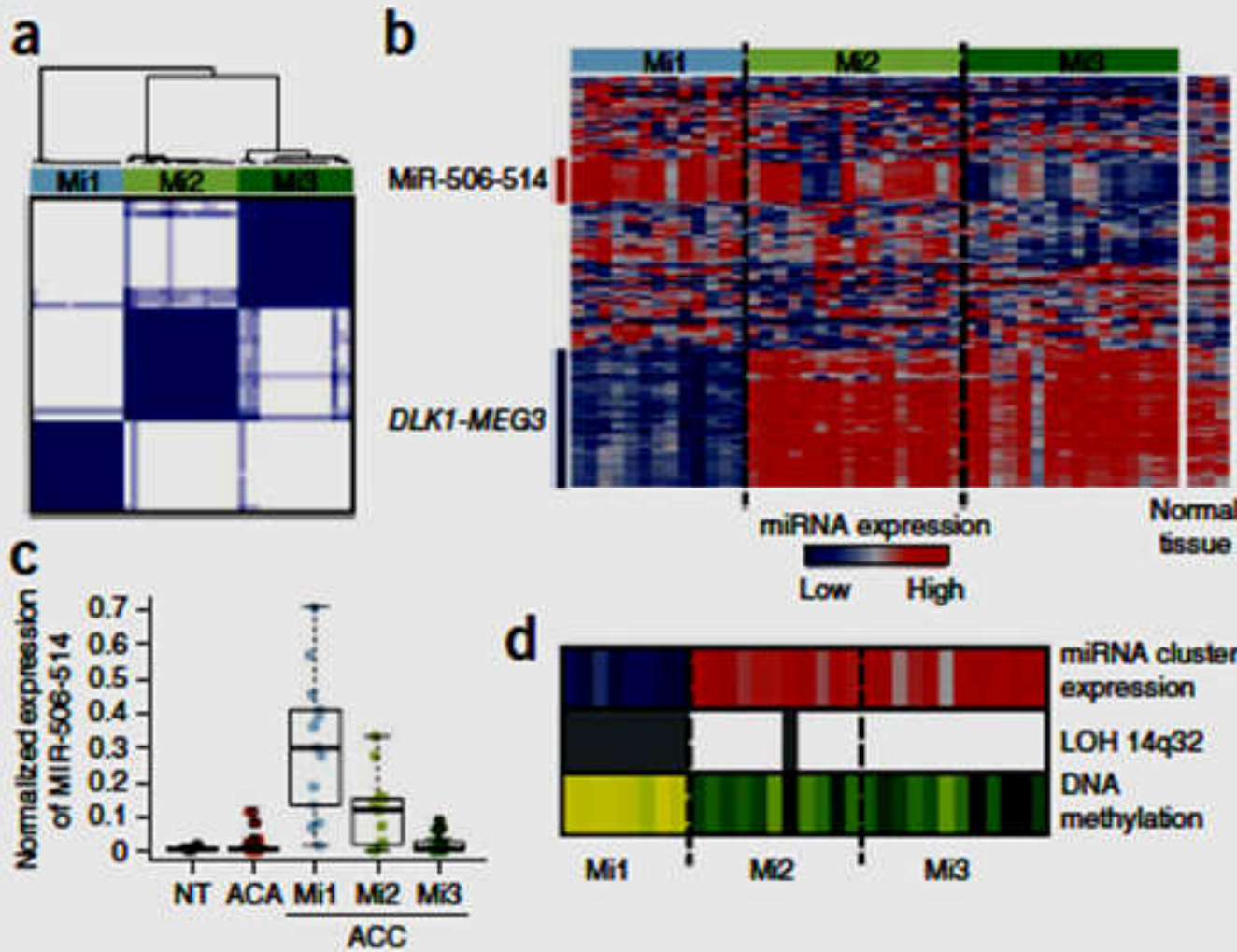
its gene is located  
in an intron of  
the IGF-2 gene



**miR-483-5p:**  
**Positive predictive value: 100%**  
**Negative predictive value: 92%**

**Patterson et al., Cancer, 2011**

# NGS of 45 ACC samples – Assié et al., Nature Genetics, 2014



3 clusters

-mRNA

-methylome

-microRNA

miR-483-5p –  
overexpression

overexpressed  
cluster miR 506-514

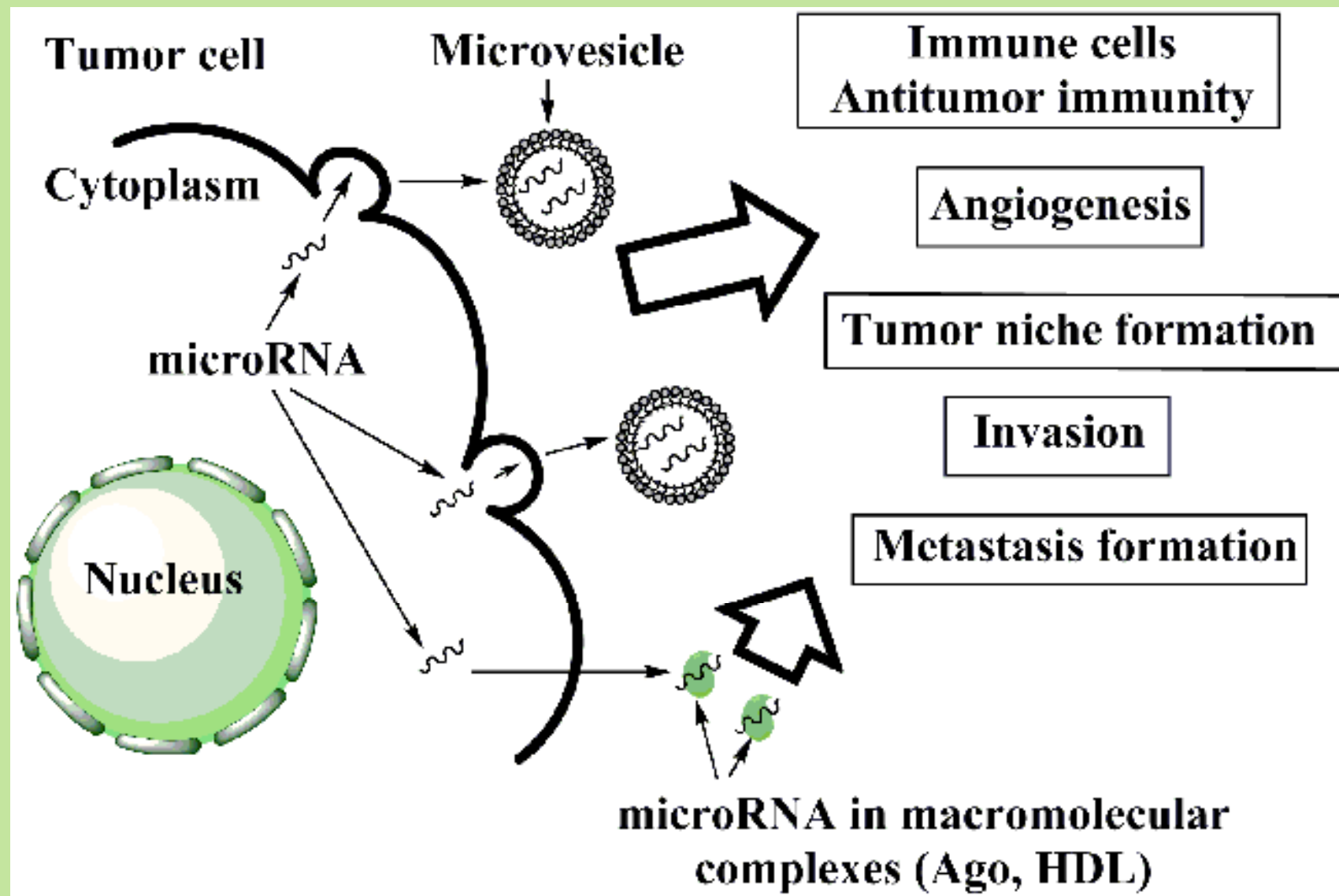
**Blood-borne circulating  
microRNAs  
(plasma/serum)**

# Circulating microRNA

- **Origin of circulating microRNA:**
  - i. passive release (necrosis, inflammation)

**Active release**

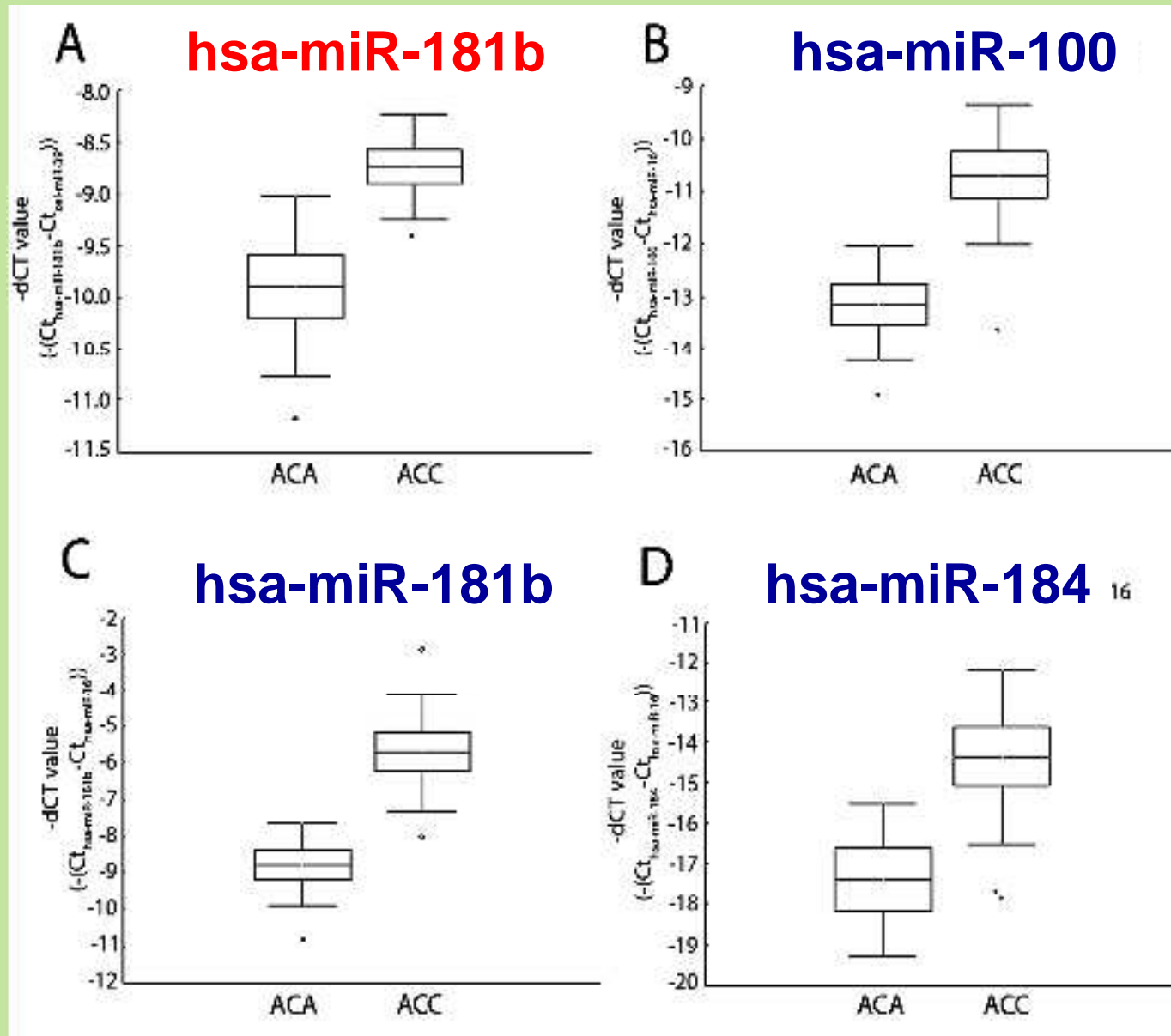
  - ii. membrane vesicles (exosomes, microvesicles)
  - iii. macromolecular complexes (Ago, HDL)
- **Circulating microRNA – hormones?**
- **Conveying epigenetic gene expression information to other tissues?**





# Circulating microRNAs in ACC – RT-qPCR 1.

Reference gene: **cel-miR-39**, **hsa-miR-16**

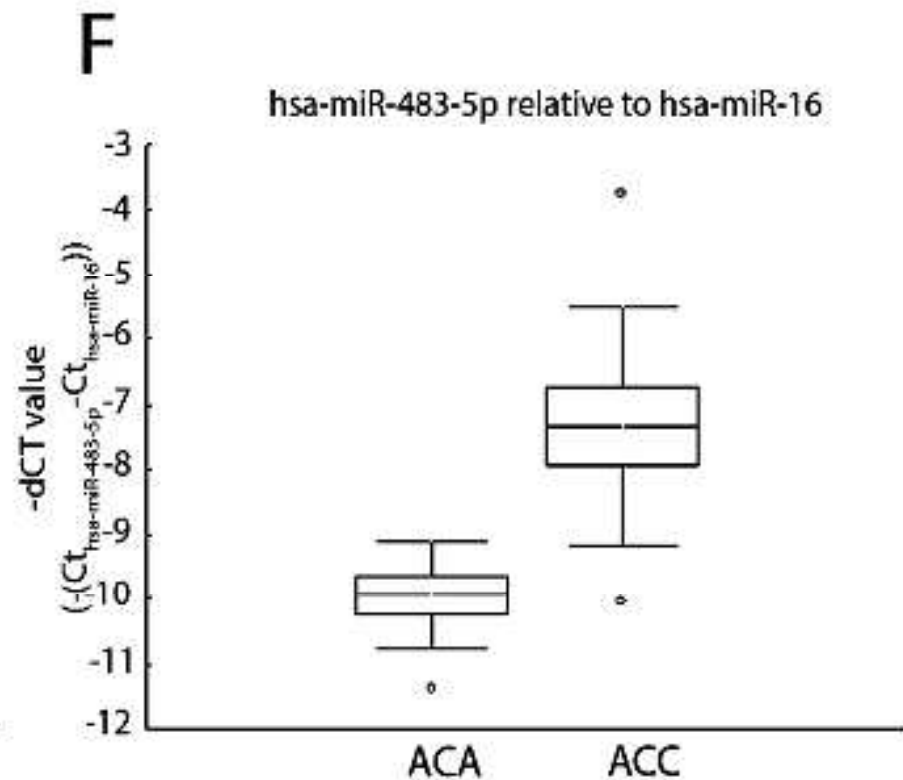
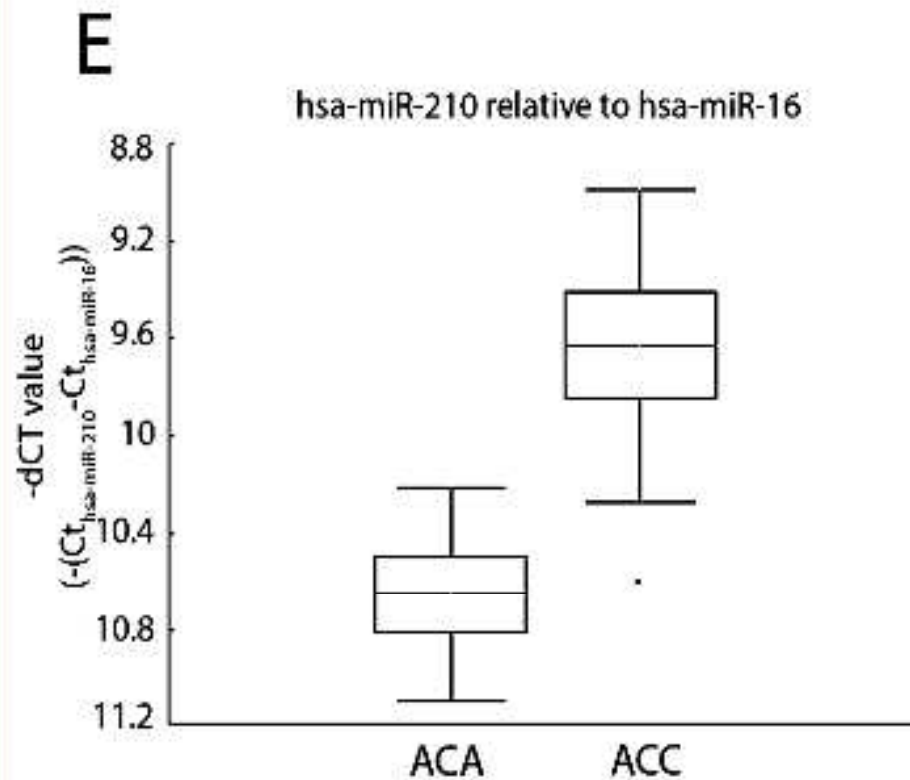


Szabó DR...lgaz, Lab Invest 2014

# Circulating microRNAs in ACC – RT-qPCR 2.

## hsa-miR-210

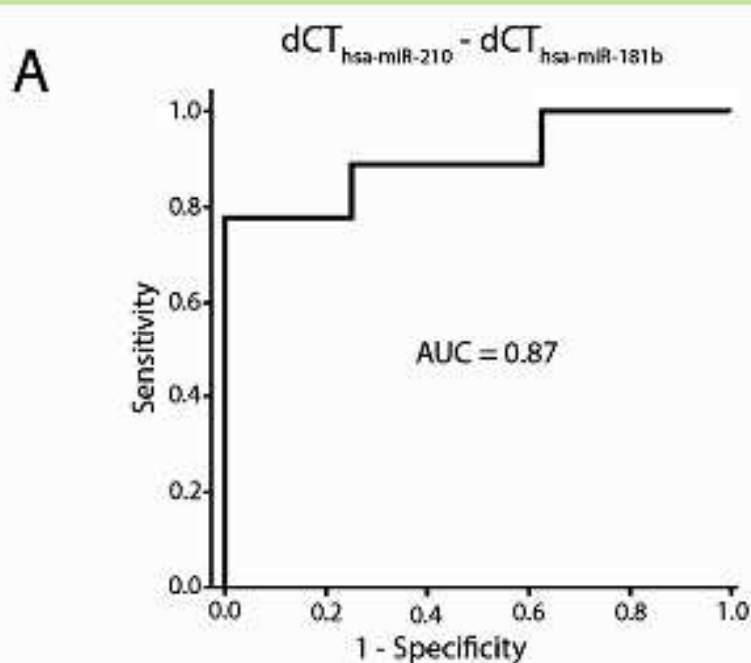
## hsa-miR-483-5p



Szabó DR...Igaz, Lab Invest 2014

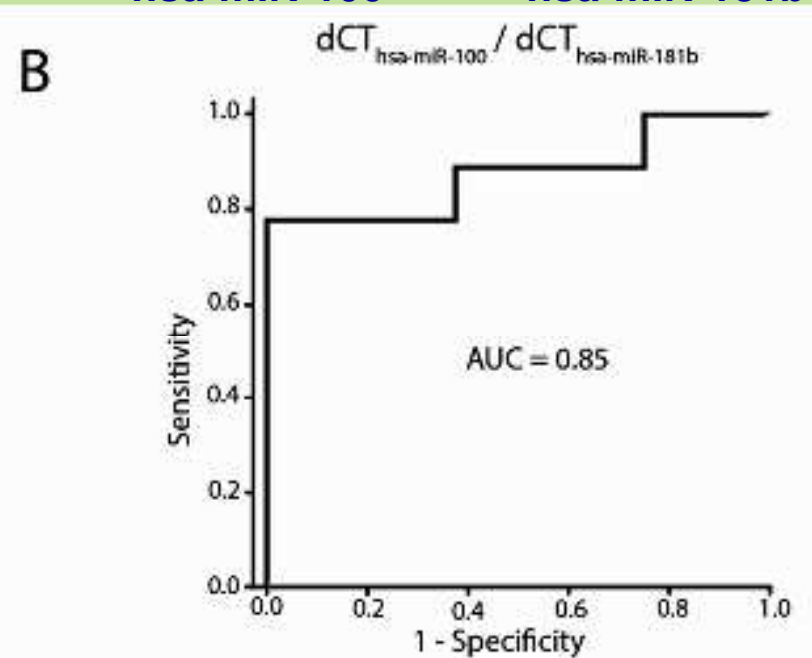
# ROC analysis

$dCT_{\text{hsa-miR-210}} - dCT_{\text{hsa-miR-181b}}$



**Sensitivity: 88.9 %**  
**Specificity: 75.0 %**

$dCT_{\text{hsa-miR-100}} / dCT_{\text{hsa-miR-181b}}$



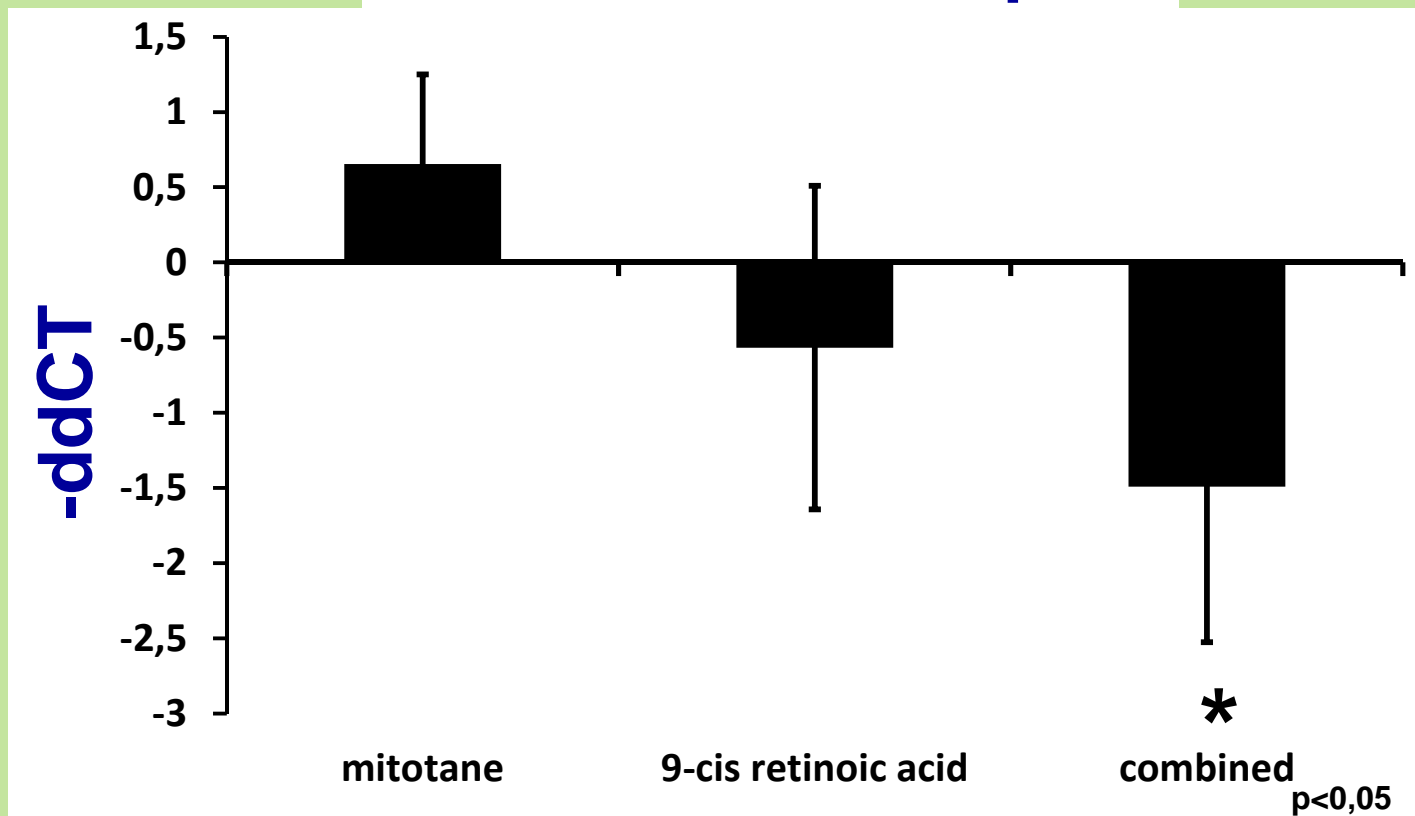
**Sensitivity: 77.8 %**  
**Specificity: 100 %**

## Problems with circulating microRNA studies

- **Very low quantity of circulating microRNA**
- **Choice of analysis – real-time PCR vs. Microarray**
- **Reference gene?**
  - Cel-miR-39 – artificial control
  - miR-16
  - (RNU6B – unsuitable)
- **Plasma vs. serum**

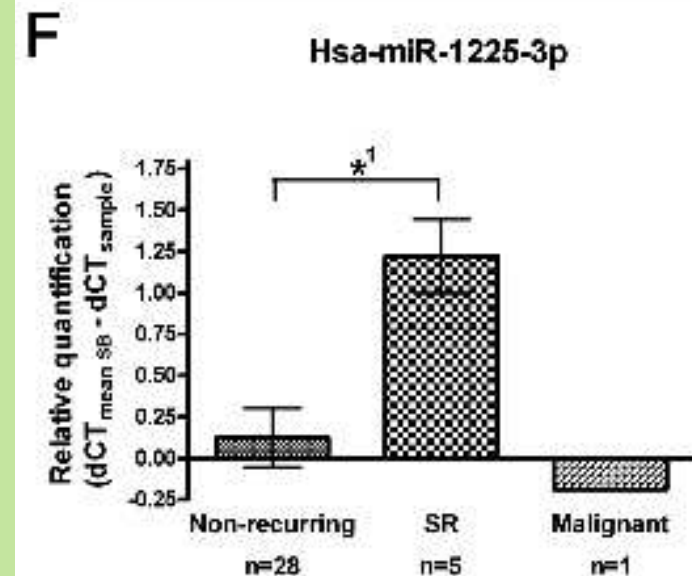
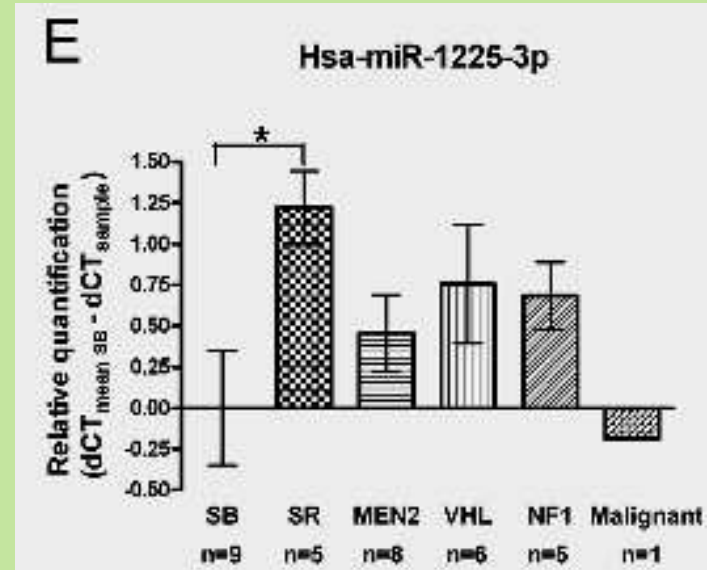
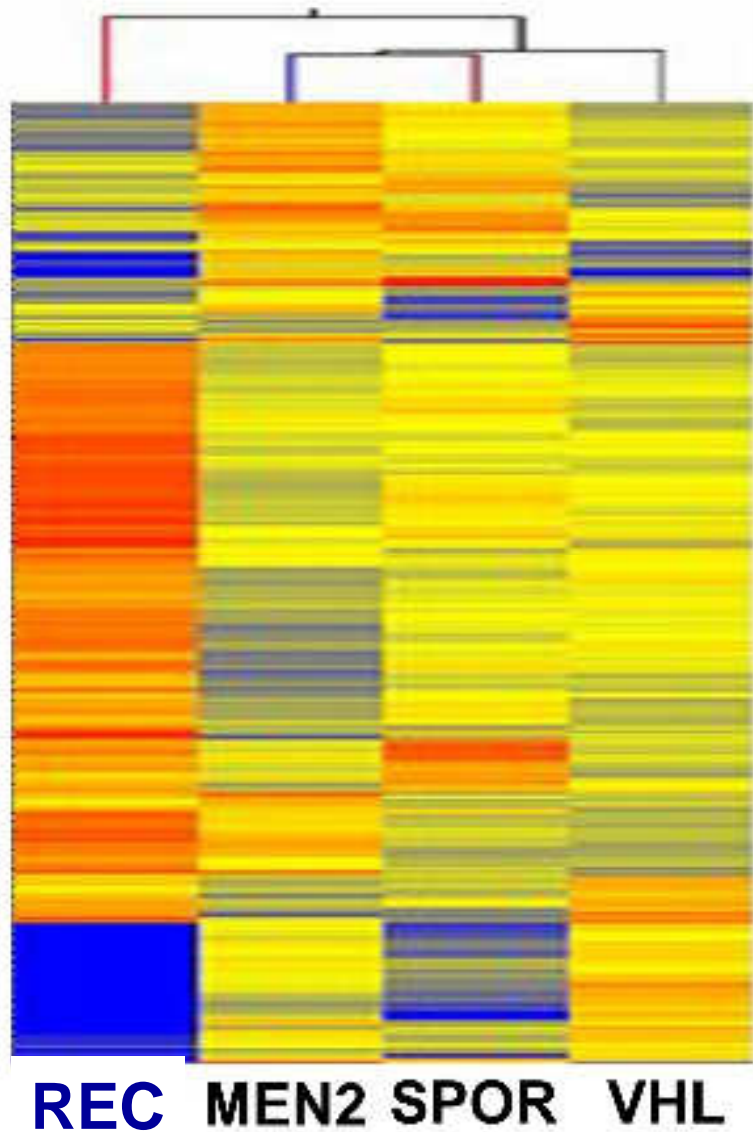
# Circulating microRNA for monitoring treatment efficacy

## hsa-miR-483-5p



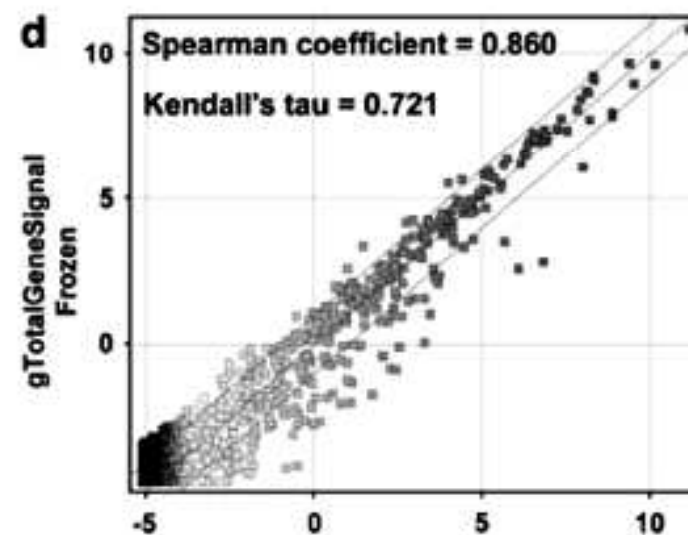
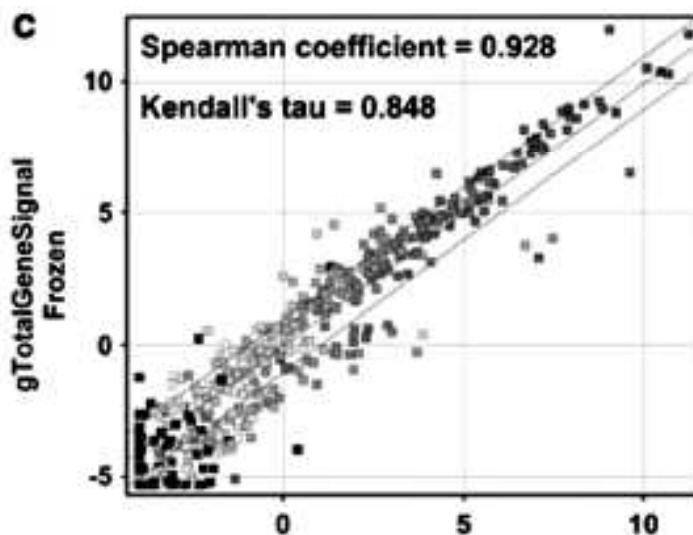
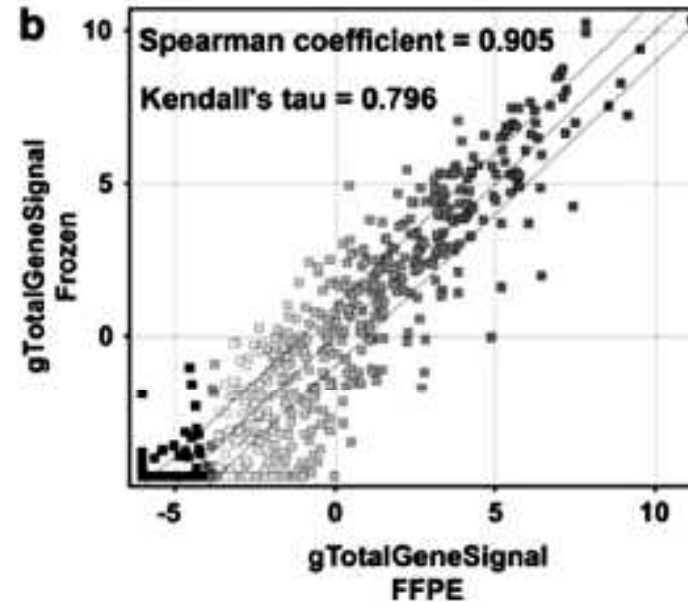
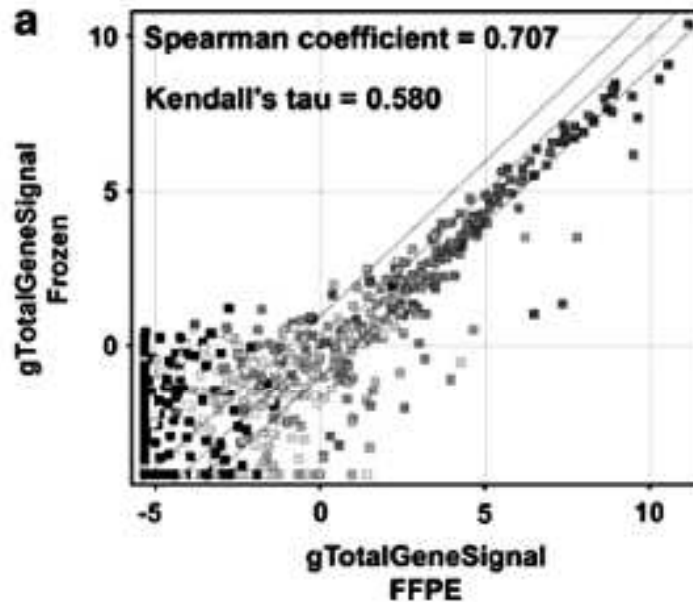
Nagy Z,..., Igaz P, submitted

# MicroRNA expression in recurring pheo



Tömböl ... Igaz, Mod Pathol ,  
2010, 23, 1583-1595

# FFPE samples can be well used for the analysis of microRNAs



Tömböl ... Igaz, Mod Pathol ,  
2010, 23, 1583-1595



# Notch-signaling may be involved in pheochromocytoma recurrence

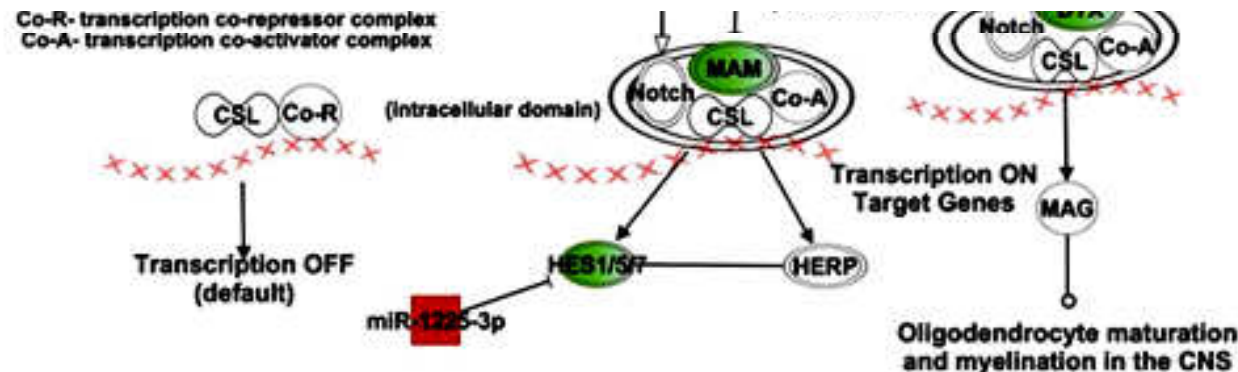
miR-1225-3p miR-1225-3p miR-1225-3p

*Surgery*. 2008 December ; 144(6): 956–962. doi:10.1016/j.surg.2008.08.027.

## Histone deacetylase inhibitors upregulate Notch1 and inhibit growth in pheochromocytoma cells

Joel T. Adler, BA, Daniel G. Hottinger, Muthusamy Kunnimalaiyaan, PhD, and Herbert Chen, MD, FACS

*Endocrine Surgery Research Laboratories, Department of Surgery, University of Wisconsin, Madison, WI*

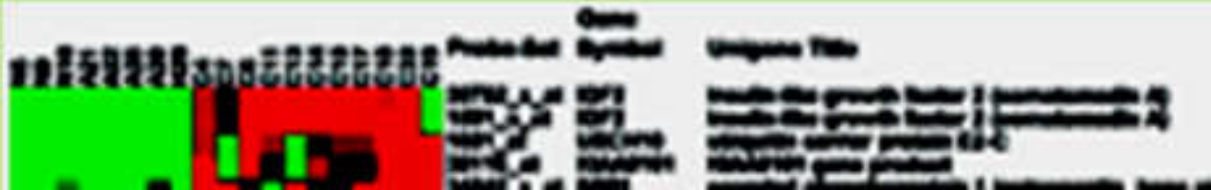


Tömböl ... Igaz  
2010, 23, 1583-

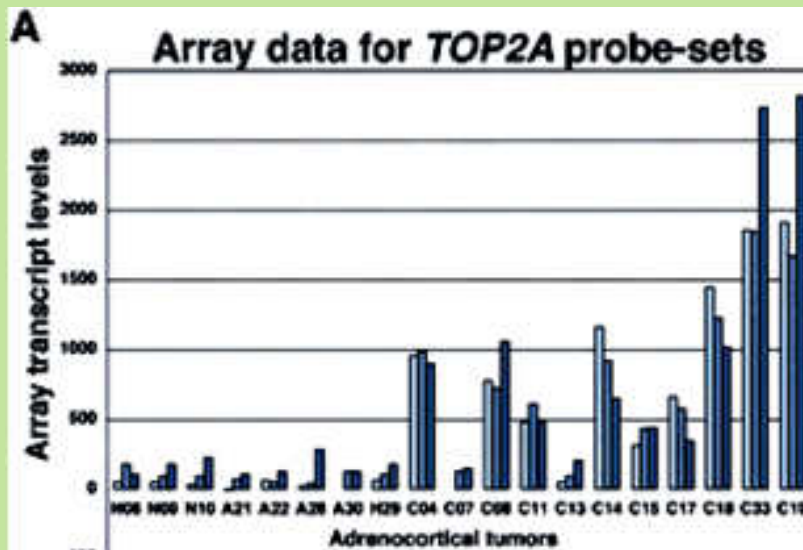


**Transcriptomics studies in  
adrenal tumours – functional  
genomics**

# The malignancy signature in ACC

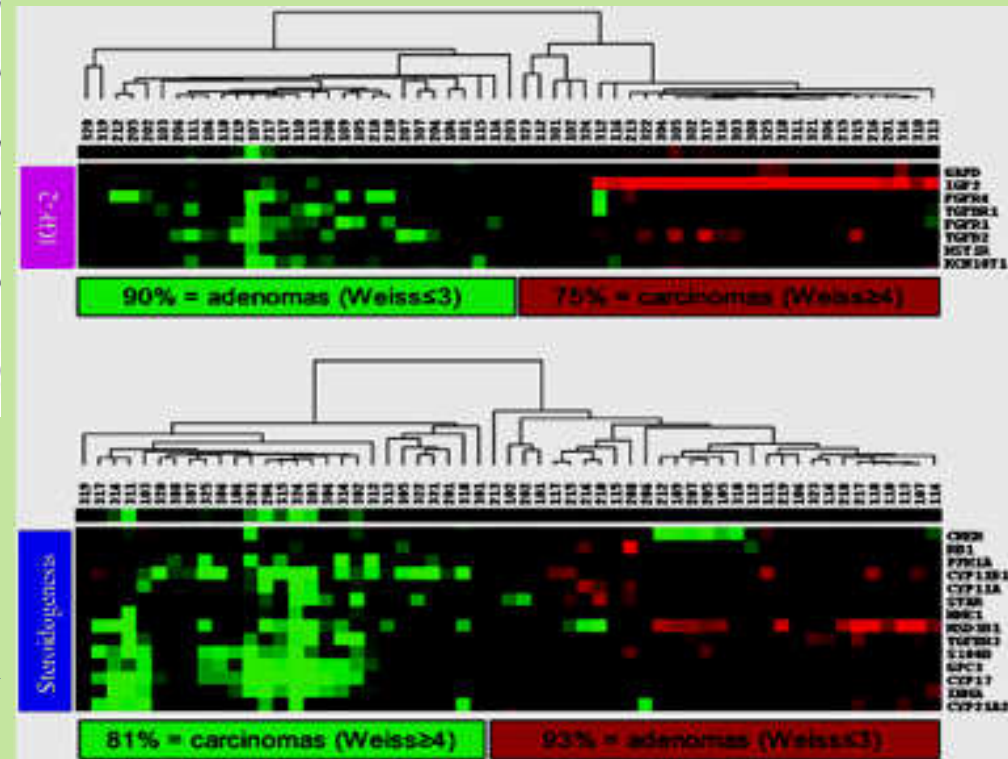


Overexpression of IGF-2 in ACC samples (106x) – Giordano et al., 2003

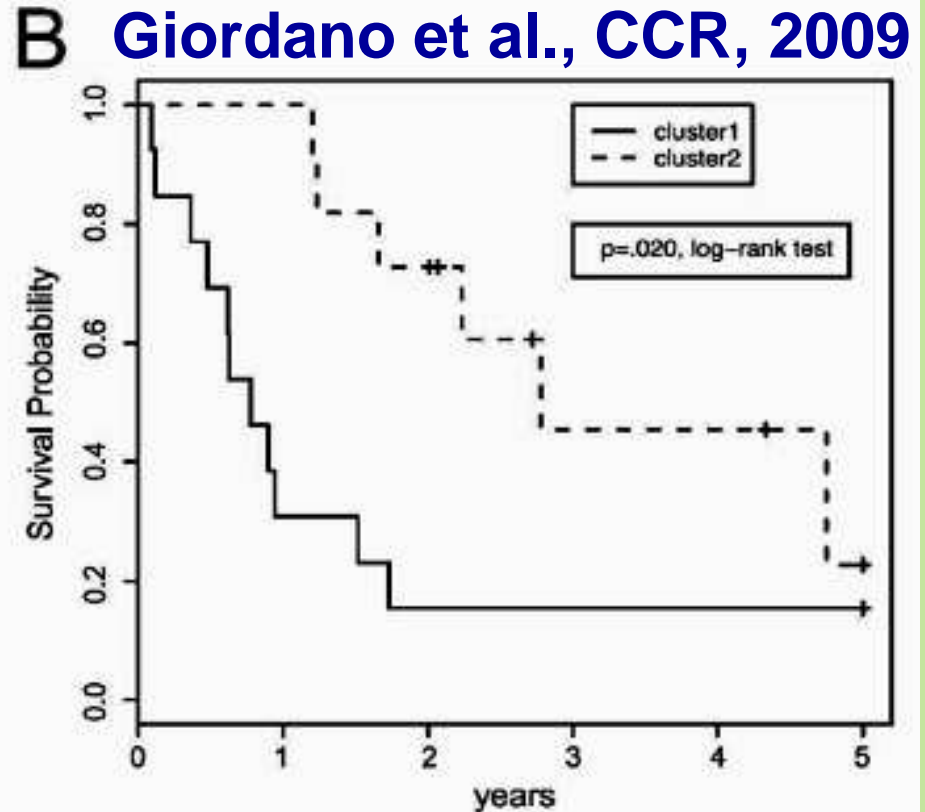
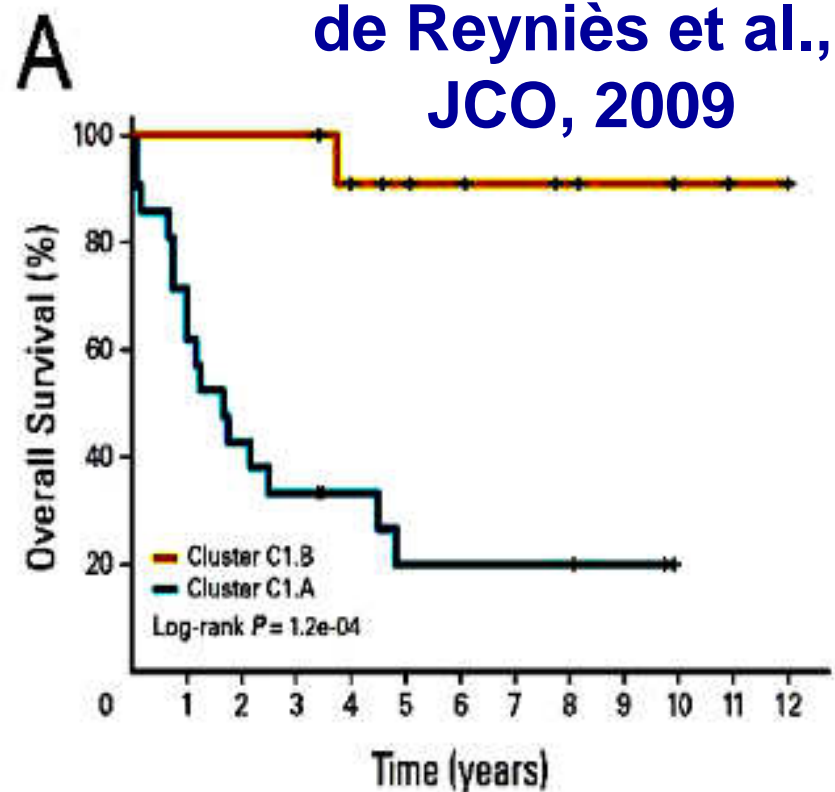


Overexpression of topoisomerase 2A in ACC (Giordano et al., 2003)

IGF-2 and steroidogenesis gene clusters can be used for the differentiation of benign and malignant tumors (de Fraipont et al., 2004)



# Microarray-based subclassification of ACC



**Genes upregulated in the poor outcome group:**

regulators of cell cycle and transcription, Wnt/ $\beta$ -catenin

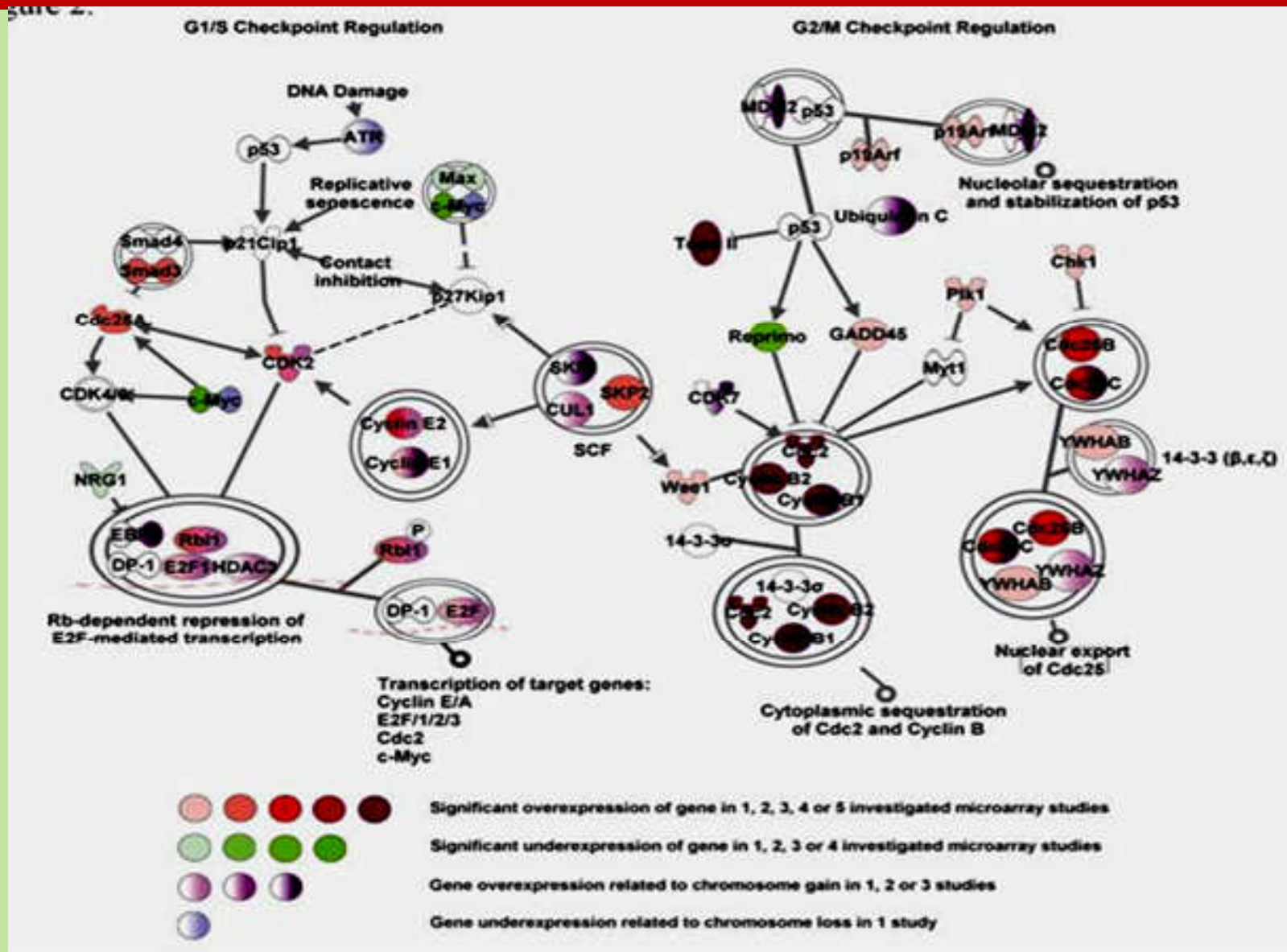
**Genes upregulated in the good outcome group:**

cell metabolism and intracellular transport

Ragazzon et al., ERC, 2011

**PATHOGENIC PATHWAYS IN  
ACC BASED ON OUR  
META-ANALYSIS**

# Pathways with altered gene expression patterns in the regulation G1/S and G2/M checkpoint in ACCs



Szabó PM ... Igaz P,  
Oncogene (2010), 29, 3163–3172

# Scale-independent networks

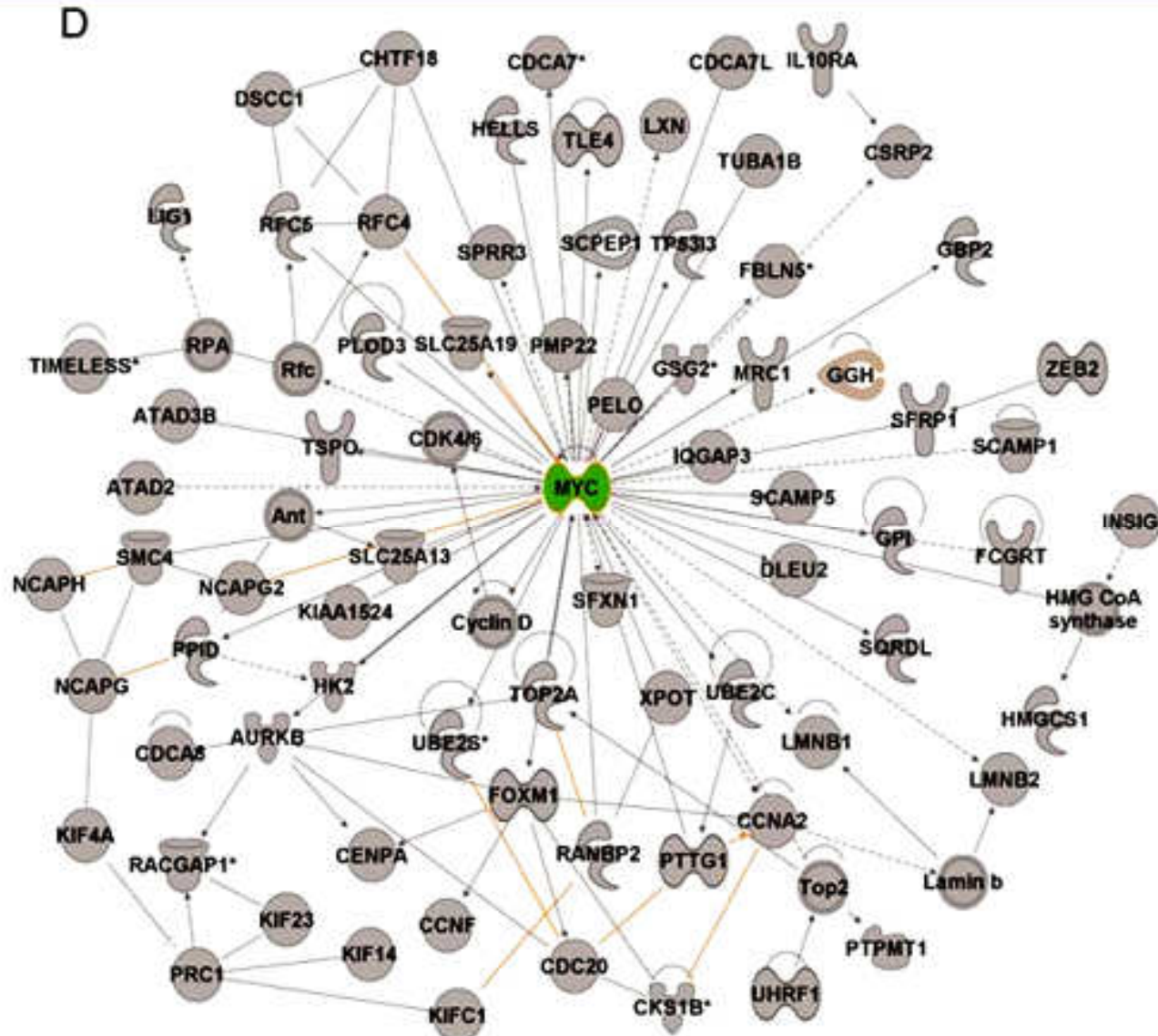
Google  
Magyarország

Internet hub



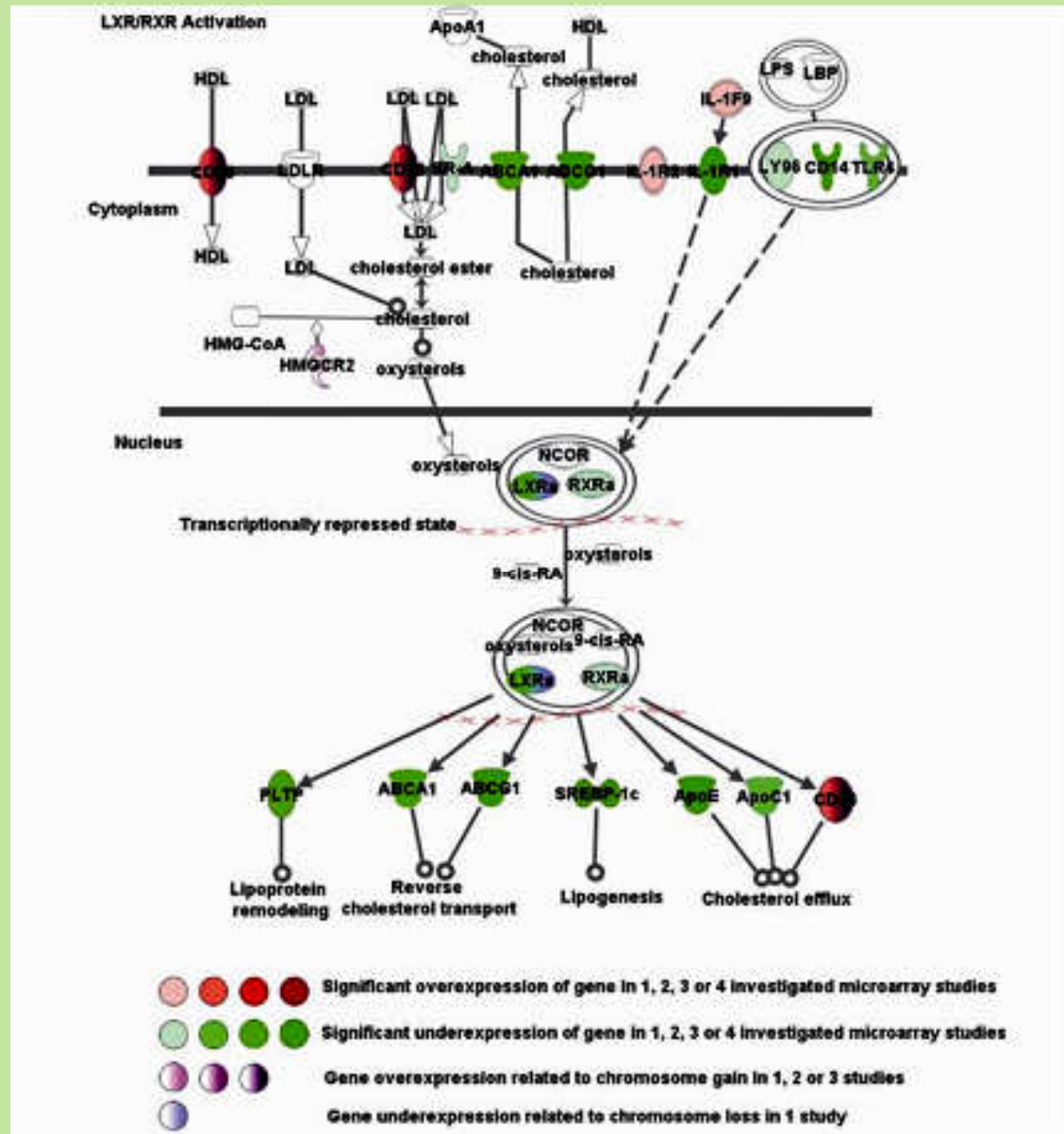


# Underexpressed c-myc is a central player in the ACC gene expression network



Szabó, RÁCz, Igaz, Horm Metab Res 2011

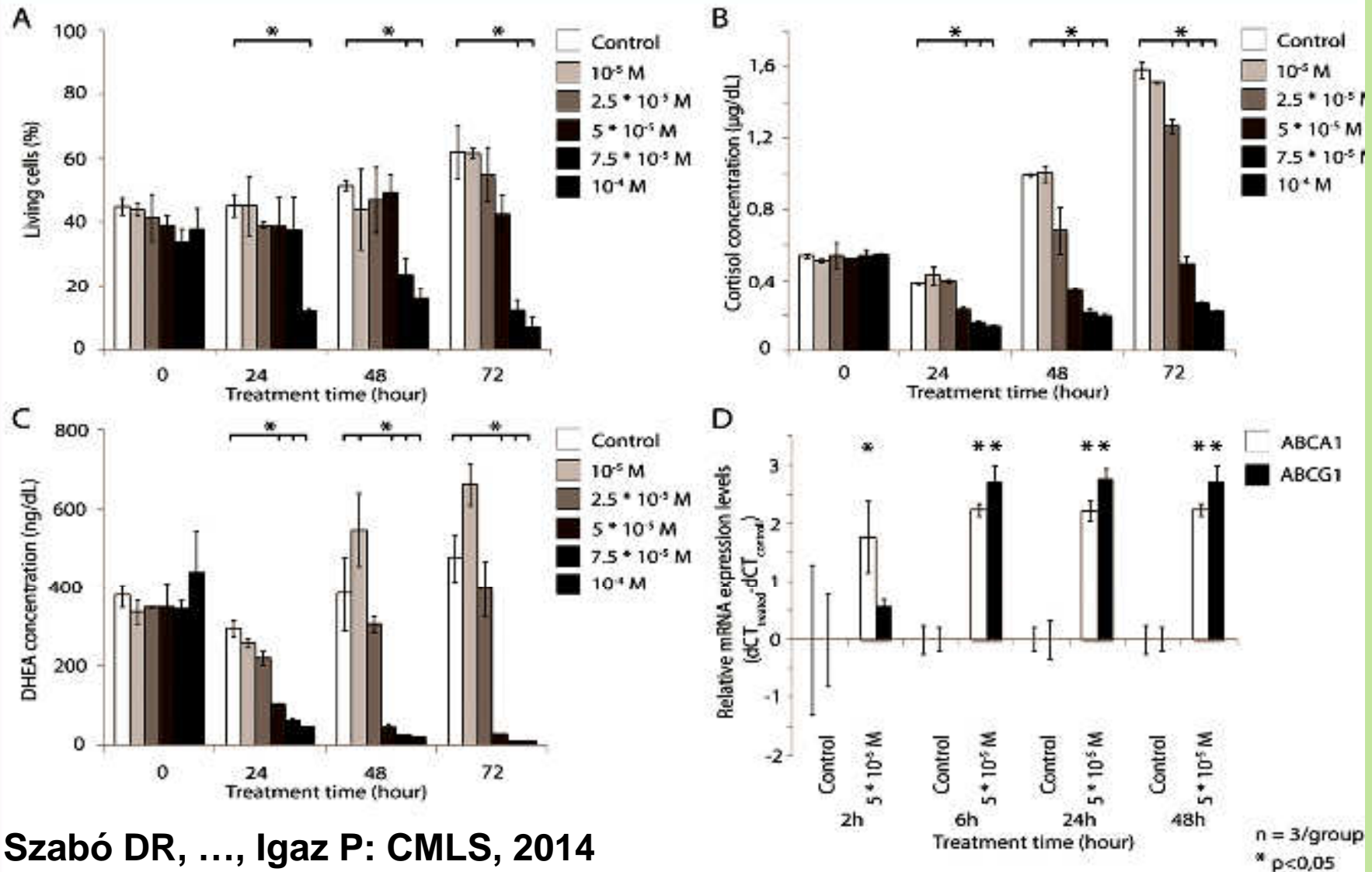
# Pathways with altered gene expression patterns involved in LXR/RXR signalling in ACCs



Szabó PM ... Igaz P,  
Oncogene (2010), 29, 3163–3172

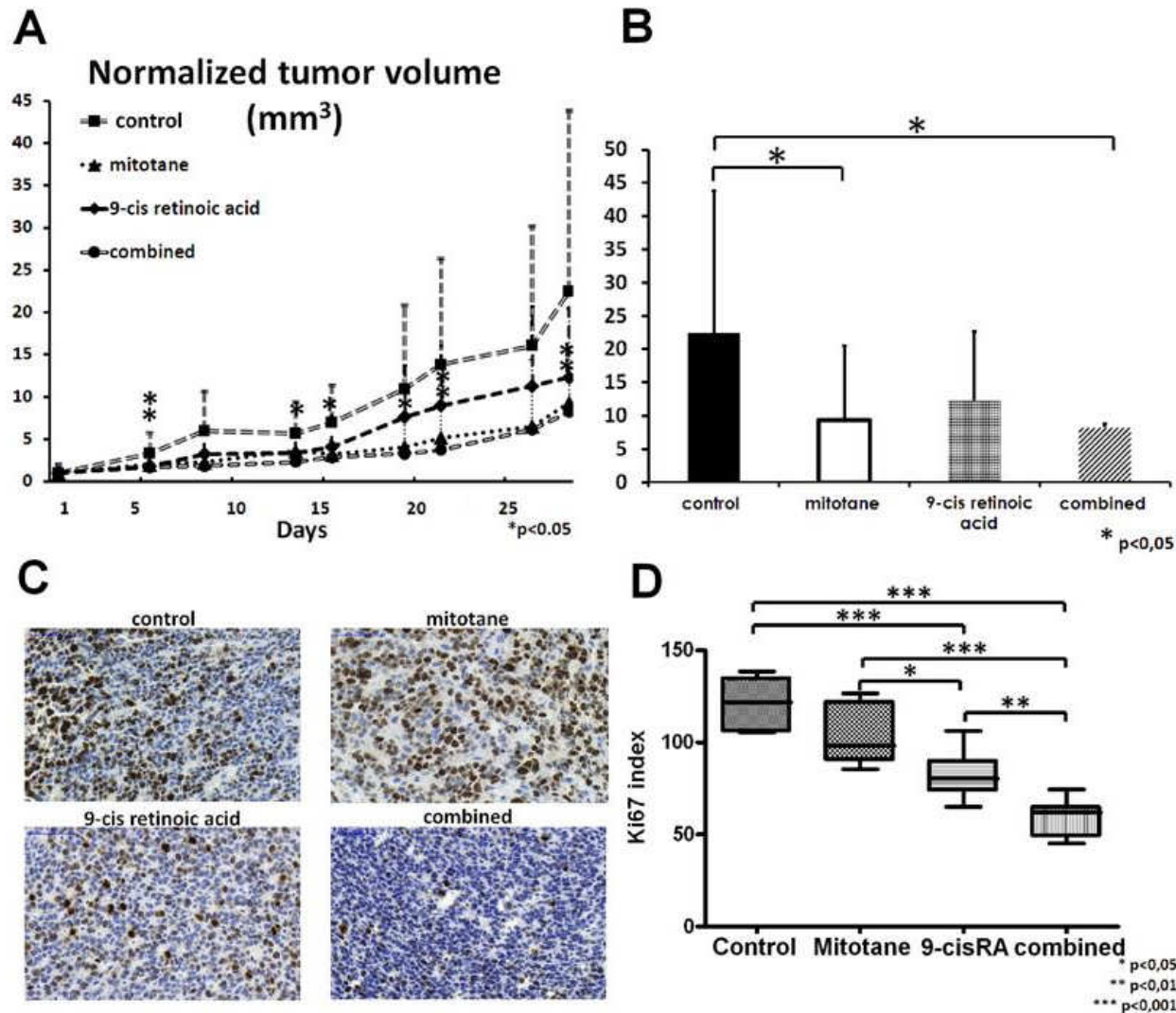


# 9-cisRA inhibits H295R proliferation and hormone secretion



Szabó DR, ..., Igaz P: CMLS, 2014

# Large scale xenograft study



- 43 male SCID mice
- 5 mg/kg 9-cisRA or mitotane or their combination

Nagy Z, ..., Igaz P:  
submitted

# Future trends

- Large-scale transcriptomic and microRNA studies – novel biomarkers, classification
- Circulating microRNA – **liquid biopsy**
- Potential for next generation sequencing
- Integrative view of molecular alterations (mRNA, CGH, microRNA, methylation, SNP-s, transcription factors, proteomics, metabolomics etc.)
- Genomics and epigenomics (microRNA, methylation, histone modification)
- Need for confirmation in *in vitro* and *in vivo* models.
- Novel therapeutic targets
- Molecular intervention?

2nd  
Me  
Pr  
Dr



Ph  
Dr  
Dr  
Dia  
Ad  
Dr  
Dr. Pal Perge

