



European Cancer Congress 2013

**Comprehensive arrays identify
key microRNAs to distinguish
short and long survivors among
resected pancreatic cancer patients**

Elisa Giovannetti

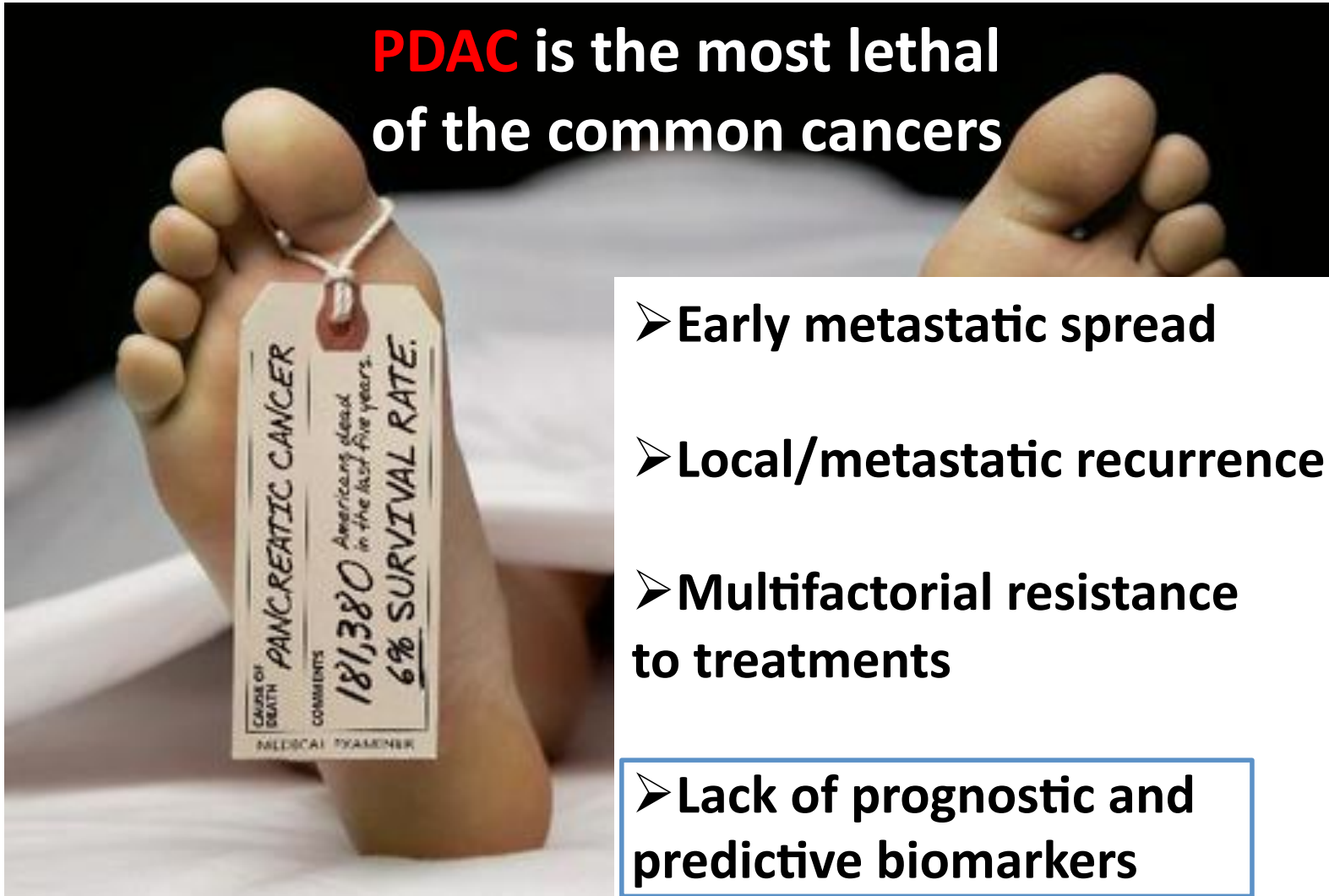
*VU University Medical Center
Amsterdam
The Netherlands*





Pancreatic Ductal Adenocarcinoma (PDAC)

PDAC is the most lethal
of the common cancers

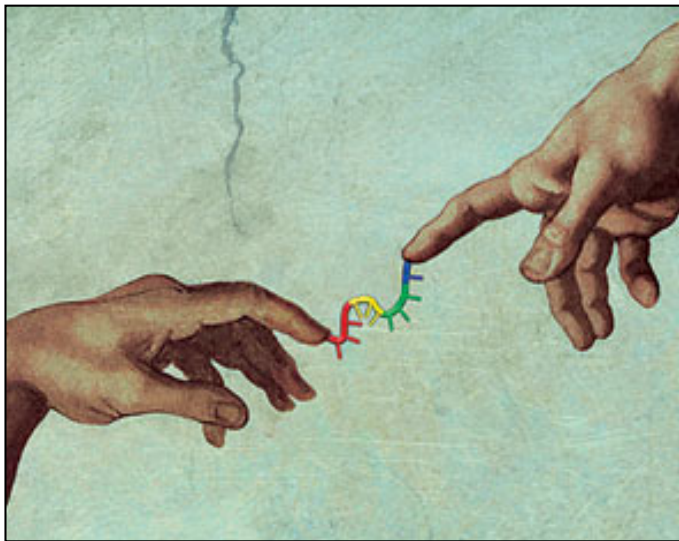


- Early metastatic spread
- Local/metastatic recurrence
- Multifactorial resistance to treatments
- Lack of prognostic and predictive biomarkers



Micro-RNA

A class of small non-coding RNAs that interact with the mRNAs of coding genes to direct their post-transcriptional repression



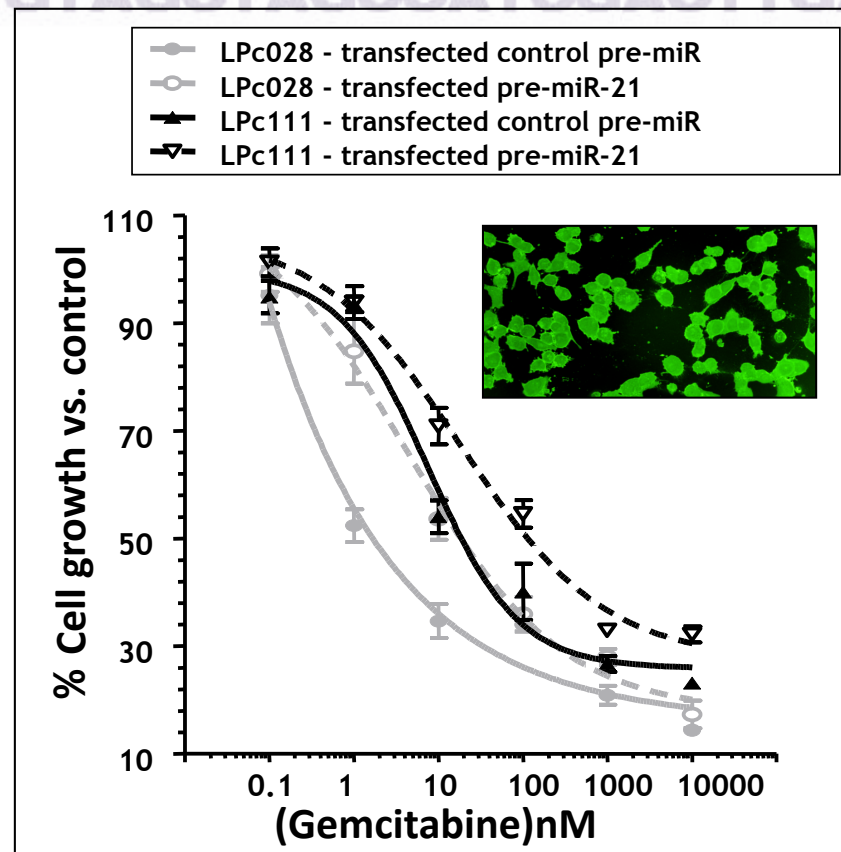
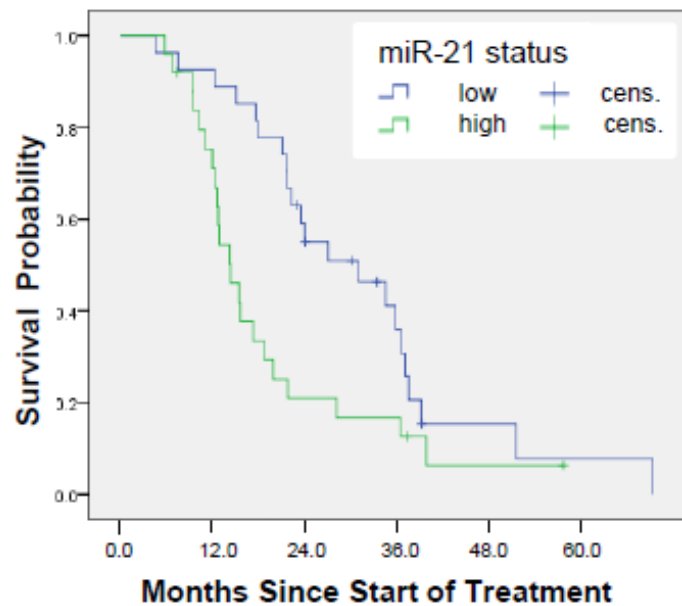
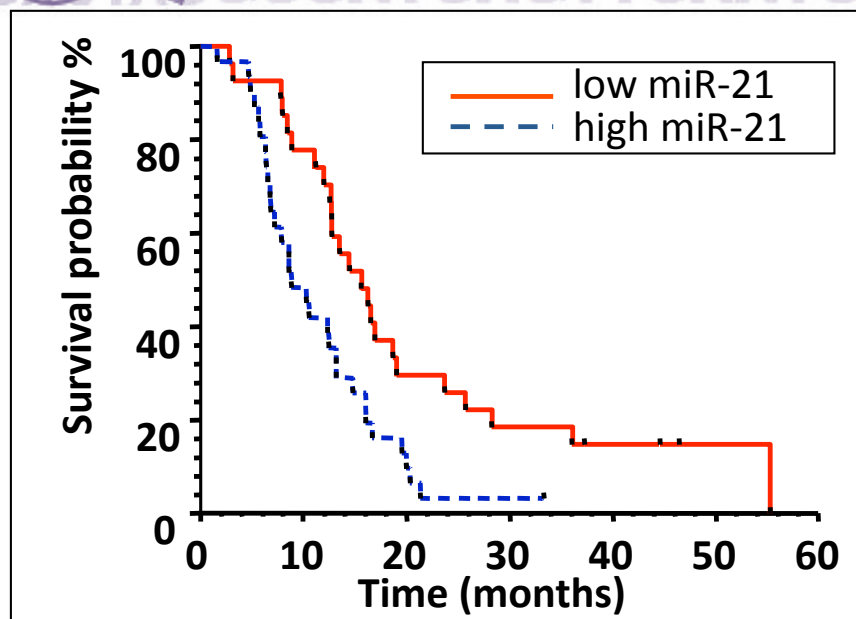
Creation of Adam -
Michelangelo
detail with small variation

A booming field in cancer biology as:

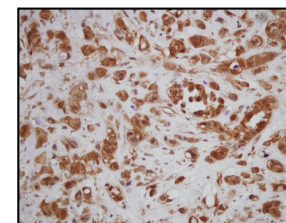
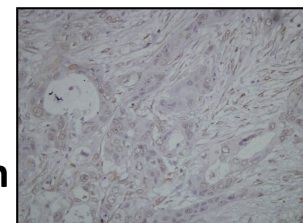
- Oncogenes/tumor suppressor genes
- Diagnostic biomarkers
- Prognostic biomarkers
- Determinants of chemoresistance
- Potential therapeutic targets



From a candidate oncomiR....



14 PDACs
miR-21
low vs high



PTEN

β -actin



Giovannetti et al., Cancer Res 2010

Voortman et al., PLoS ONE 2010



...to a high-throughput screening



The Macro world of MicroRNA
by Maya Pinos

To evaluate
whether
**comprehensive
miRNA expression
profiling** can
distinguish
between PDAC
patients with very
short OS compared
to long-term
survivors



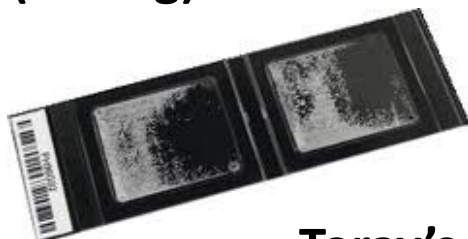
Patients and methods

26 pancreatic cancer patients who underwent PDAC resection selected according to their outcome (short OS < 12 vs long OS > 30 months)

Macrodissection of FFPE tissues



RNA extraction
(500 ng)



Toray's 3D-
Gene[™]
miRNA-chip

Adjuvant chemotherapy
with gemcitabine
1000 mg/m²



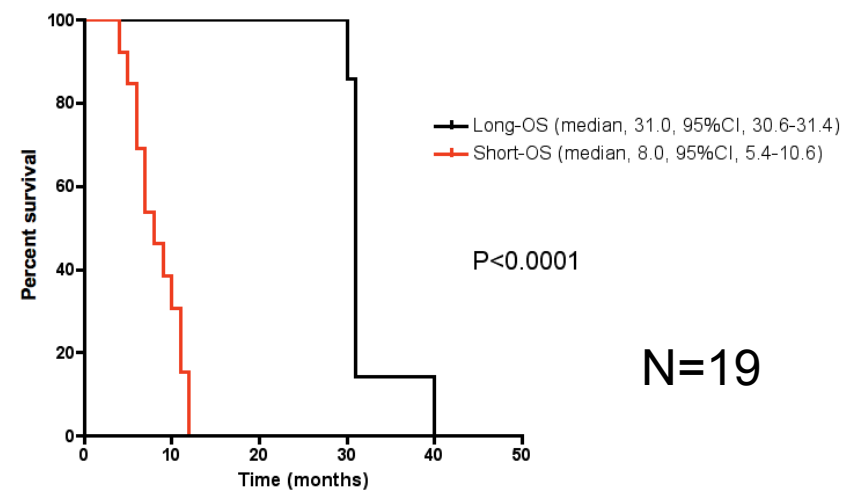
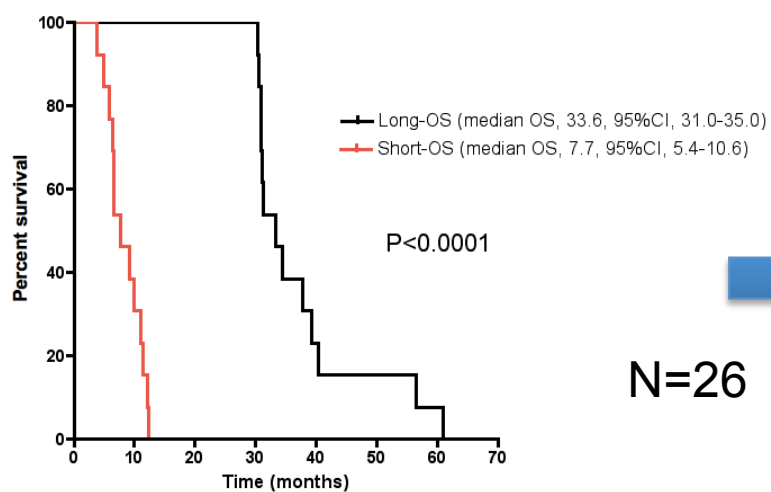
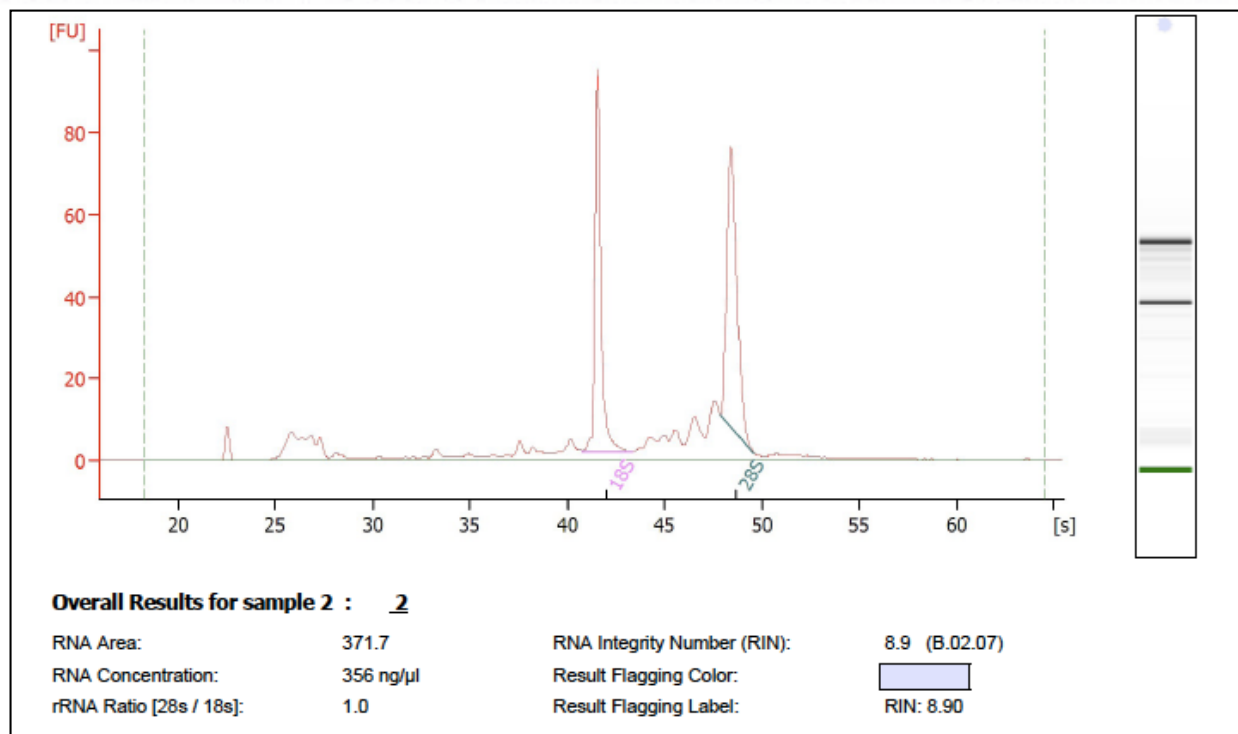
Follow-up

The Kaplan-Meier method was
used to plot DFS and OS

- ✓ Cluster analysis
- ✓ Feature selection algorithm RELIEF to identify the top discriminating miRNAs
- ✓ Validation with PCR in a 2nd cohort
- ✓ *In vitro* studies on possible relevant targets for drug activity

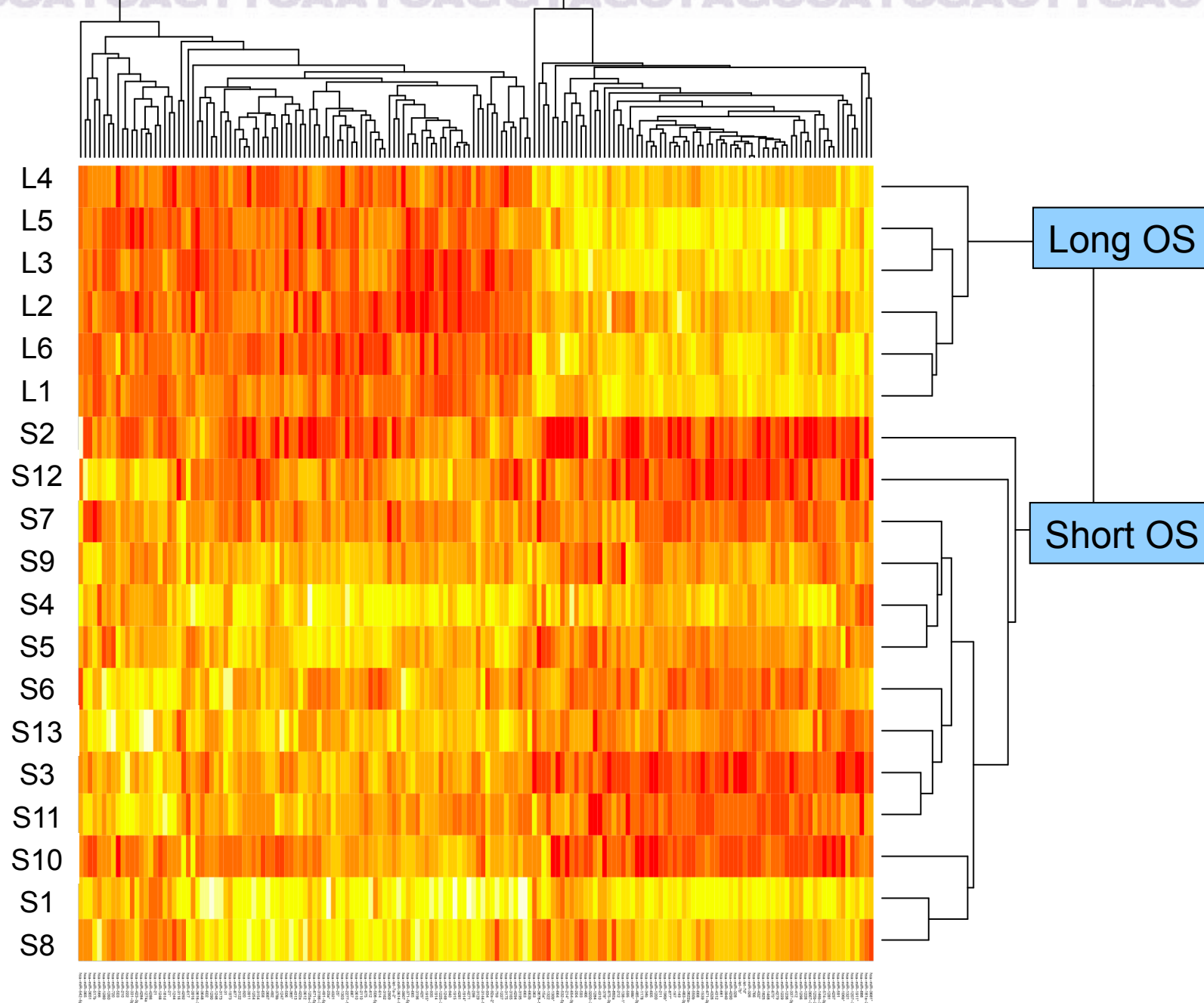


The problem with the RNA





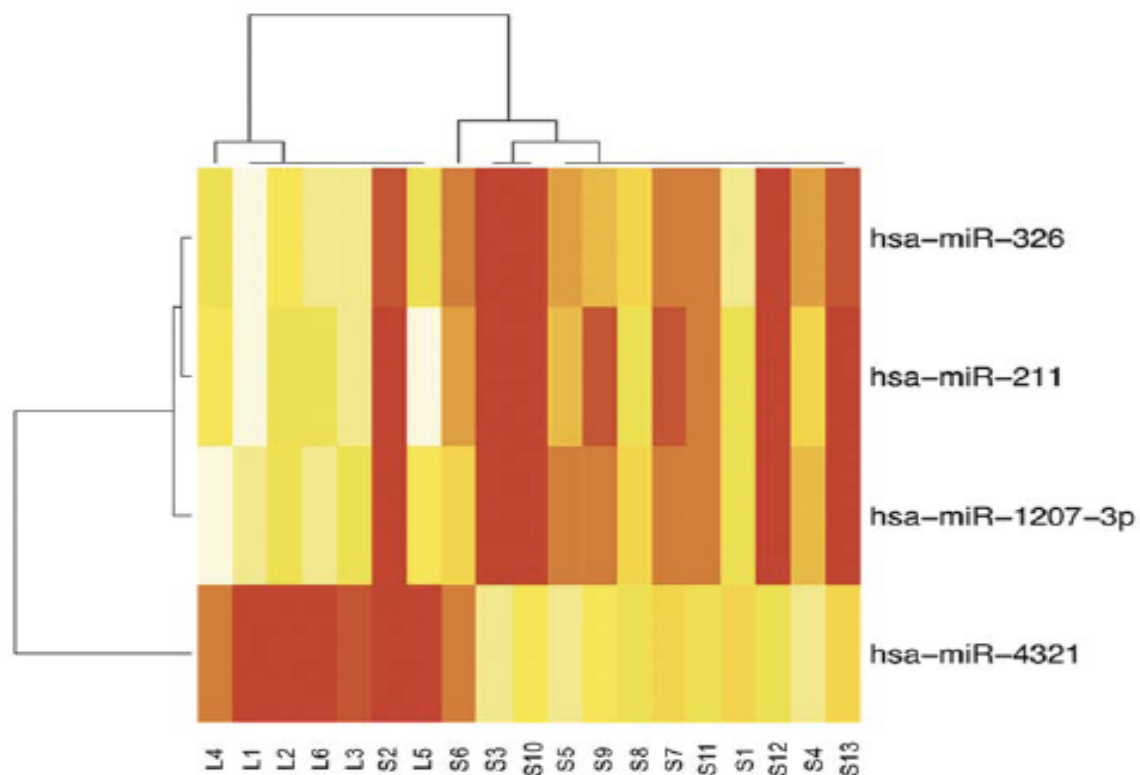
Clustering (with 170 miRNAs)





Top discriminating miRNA

Number	miRNA	RELIEF score	Iterative score
1	miR-211	0.372	86
2	miR-4321	0.332	76
3	miR-1207-3p	0.330	81
4	miR-326	0.321	78





Validation with PCR in a second cohort

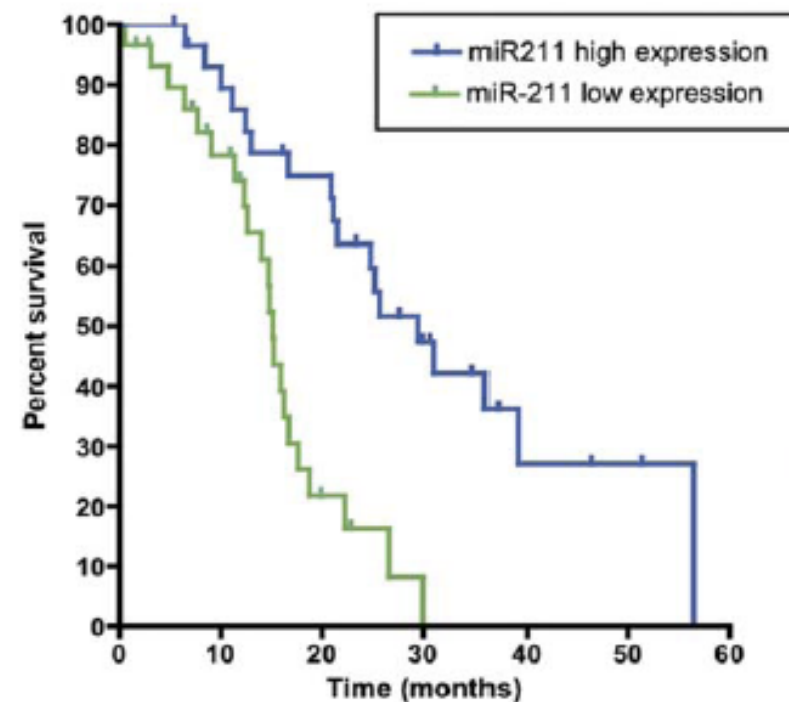
Clinical characteristics of the PDAC patients

Characteristic	Patients for the miRNA profiling n (%)		Patients of the validation cohort n (%)
	Short-OS	Long-OS	
<i>No. Patients</i>	13	13	60
<i>Age. median [range]</i>	64 (37-71)	63 (56-79)	
≤65	8 (61.5)	6 (46.2)	36 (60.0)
>65	5 (38.5)	7 (53.8)	24 (40.0)
<i>Sex</i>			
Male	7 (53.8)	3 (23.1)	28 (46.7)
Female	6 (46.2)	10 (76.9)	32 (53.3)
<i>Operation procedure</i>			
pancreatico-duodenectomy	11 (84.6)	9 (69.2)	46 (76.7)
total pancreatectomy	0 (0.0)	0 (0.0)	3 (5.0)
distal pancreatectomy	2 (15.4)	4 (30.8)	11 (18.3)
<i>TNM Stage</i>			
pT3 N0 Mx	0 (0.0)	1 (7.7)	0 (0.0)
pT3 N1 Mx	13 (100.0)	12 (92.3)	60 (100.0)
<i>Nodal status</i>			
N0	0 (0.0)	1 (7.7)	0 (0.0)
N1	13 (100.0)	12 (92.3)	60 (100.0)
<i>Grading</i>			
G1	0 (0.0)	0 (0.0)	3 (5.00)
G2	8 (61.5)	9 (69.2)	27 (45.0)
G3	5 (38.5)	4 (30.8)	30 (50.0)
Unknown	0 (0.0)	0 (0.0)	0 (0.0)
<i>Resection margins</i>			
R0	12 (92.2)	12 (92.2)	54 (90.0)
R1	1 (7.8)	1 (7.8)	6 (10.0)
<i>Vascular invasion</i>			
Yes	4 (30.8)	3 (23.1)	23 (38.3)
No	9 (69.2)	10 (76.9)	37 (61.7)
<i>Perineural invasion</i>			
Yes	7 (53.8)	9 (69.2)	31 (51.7)
No	6 (46.2)	4 (30.8)	29 (48.3)

miR-211

P<0.001

HR=2.3

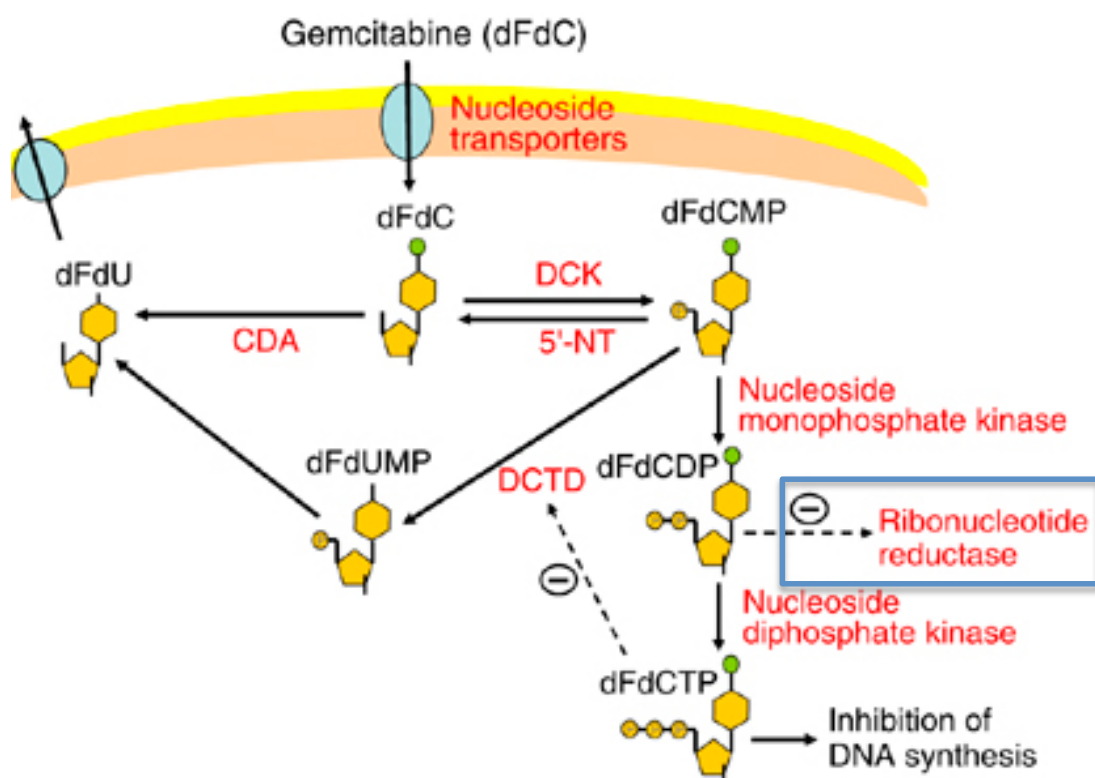


.. but no correlation for **miR-4321**
(only a trend towards significantly longer OS, P=0.194)

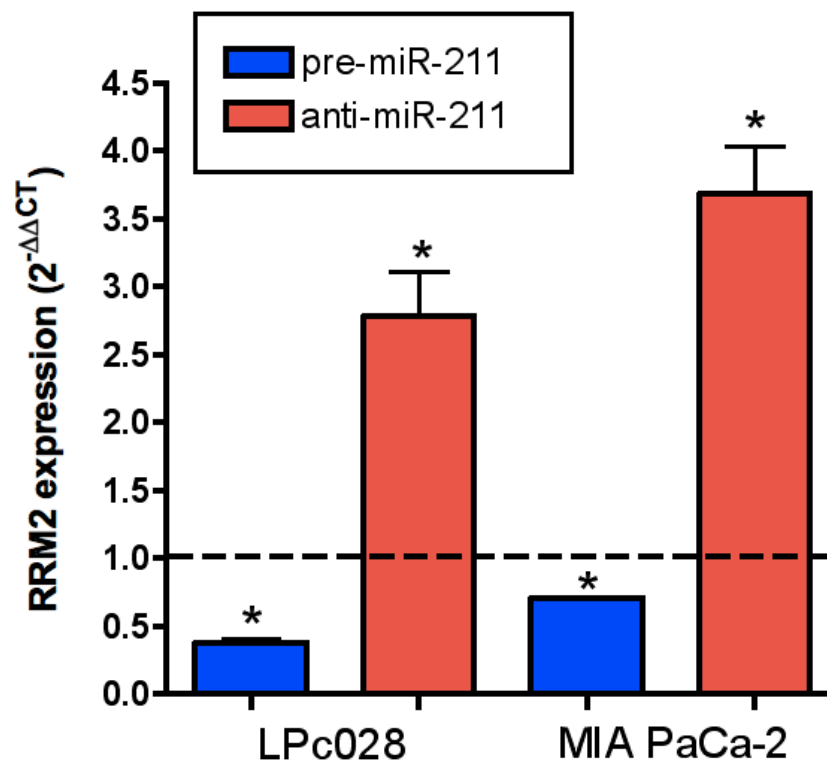


miRNA targets

Number	miRNA	Chromosome	miRDB targets*	TargetScan targets**
1	miR-211	15q13.3	702	669
2	miR-1207-3p	8q24.21	217	488
3	miR-326	11q13.4	309	441
4	miR-4321	chr19:2250638-2250717 ^o	14	161



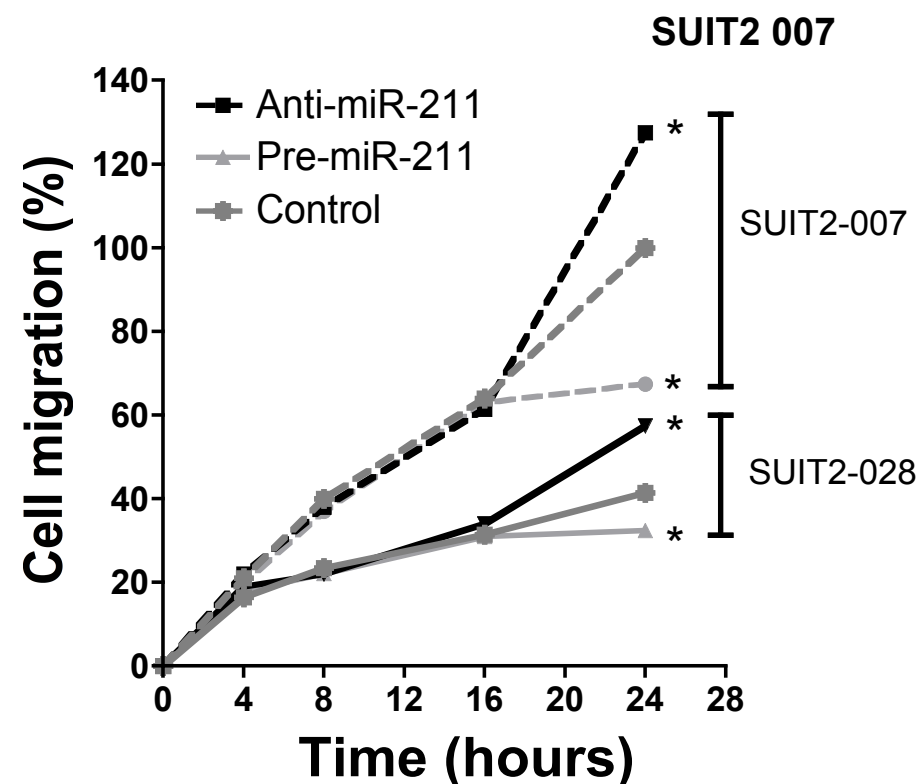
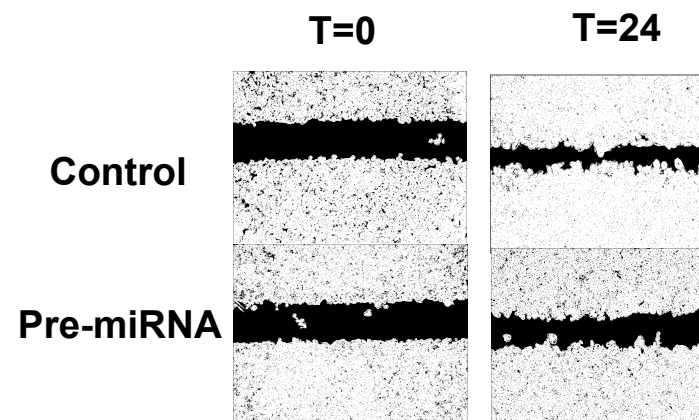
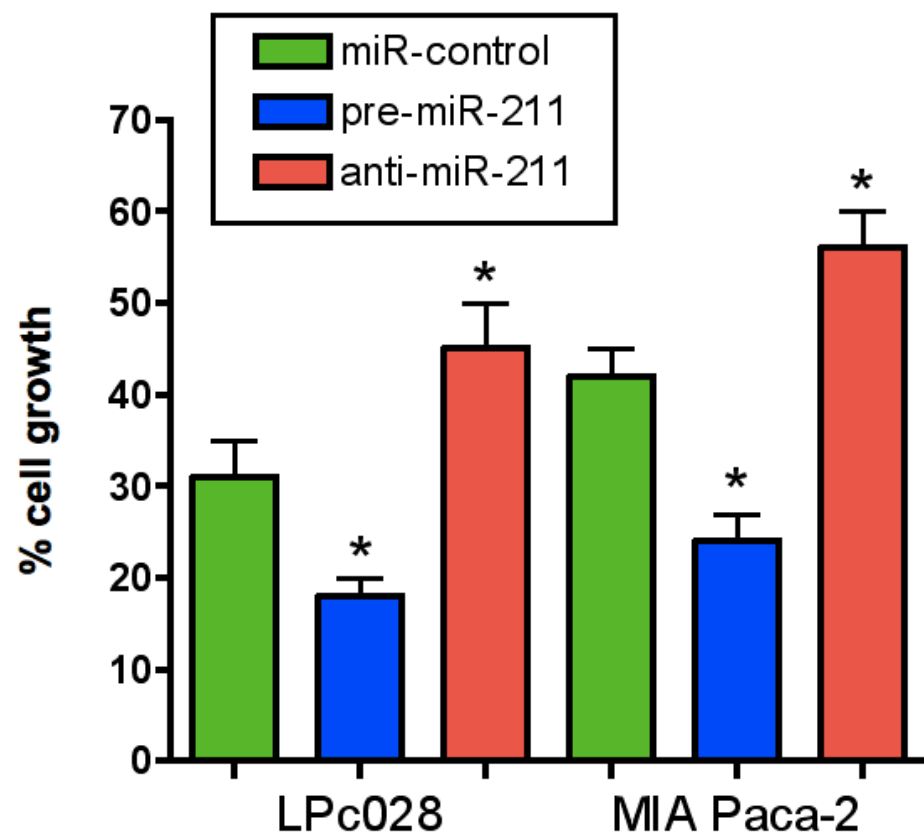
Modulation of RRM2 by miR-211





Potential role of RRM2

Chemosensitivity to gemcitabine
1 μ M, 48 hours





Conclusions

- ✓ Analysis of the array data revealed that PDAC specimens clustered according to their short/long-OS classification
- ✓ The feature selection algorithm *RELIEF* identified the top discriminating miRNAs between the two groups, with miR-211 emerging as the best
- ✓ The prognostic value of miR-211 expression was validated by quantitative-PCR in an independent cohort of PDAC patients
- ✓ miR-211 targets RRM2 and affects gemcitabine sensitivity
- ✓ miR-211 has also a potential role in PDAC cells invasion

MiR-211 represents a promising target for prognostic (and therapeutic) approaches, and our results prompt further (prospective) studies as well as research on the biological role of miR-211 in PDAC





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