AACR 101st ANNUAL MEETING 2010
April 17-21, 2010 • Walter E. Washington Convention Center • Washington, DC

MicroRNA-21 (miR-21) in pancreatic adenocarcinoma: correlation with clinical outcome and pharmacological aspects underlying its role in the modulation of gemcitabine activity

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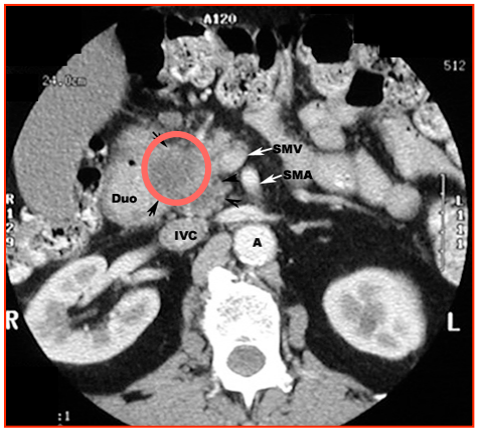
Pancreatic Ductal Adenocarcinoma (PDAC)

PDAC is the most lethal of the common cancers

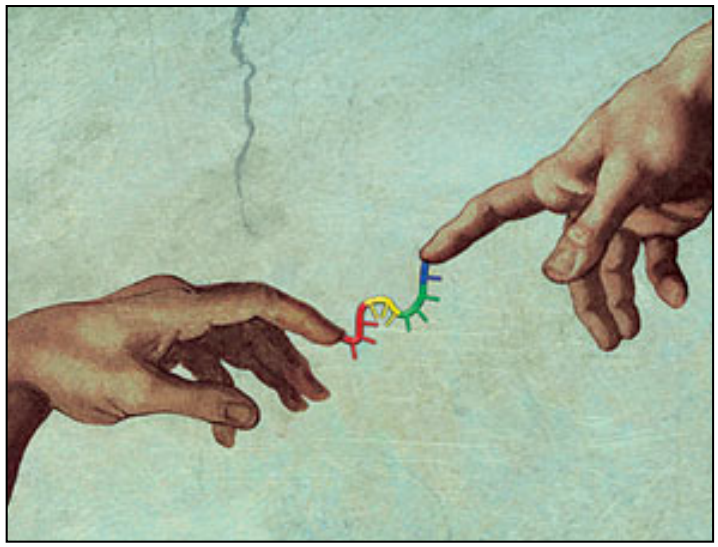


- Early metastatic spread
- Local/metastatic recurrence
- Multifactorial resistance to treatments
- Lack of biomarkers to select "targeted" treaments

New biomarkers/strategies for maximizing therapeutic efficacy and minimizing useless treatment in PDAC patients are urgently warranted

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

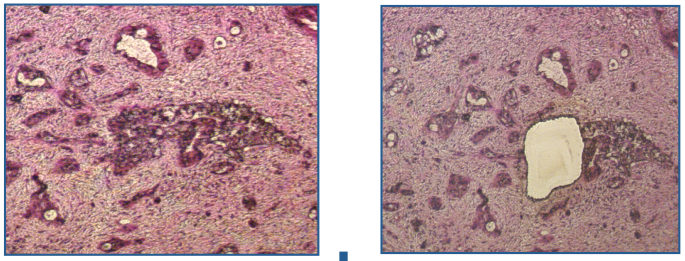
Aim

To characterize miR-21 expression in a wide repository of PDAC tissues and cells, and evaluate the association with clinical outcome and gemcitabine activity

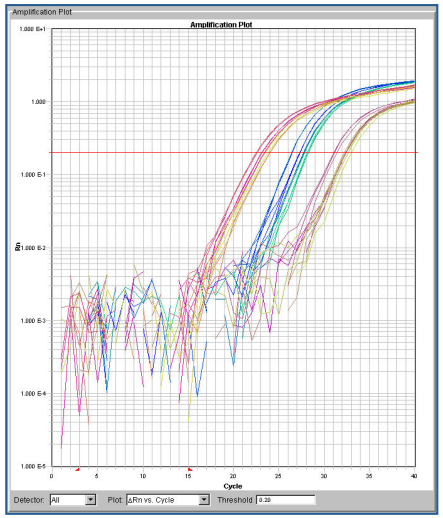
Patients and methods

77 consecutive pancreatic cancer patients underwent surgical procedures consisting of pancreatico-duodenectomy, distal or total pancreatectomy or biopsy

Laser microdissection of frozen tumor tissues with Leica AS/LMD instrument



RNA extraction



Quantitative RT-PCR analysis of miR-21

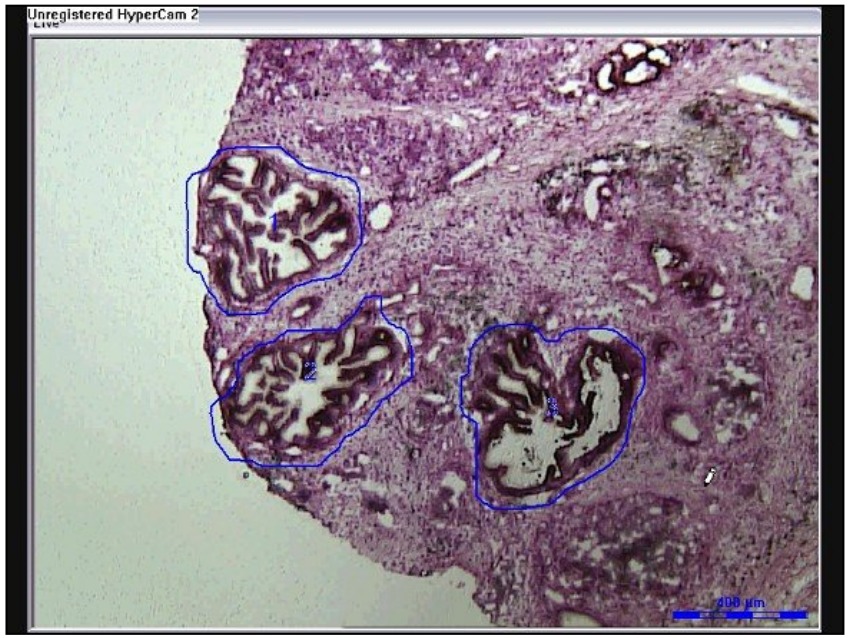
Adjuvant or palliative chemotherapy with gemcitabine 1000 mg/m^2

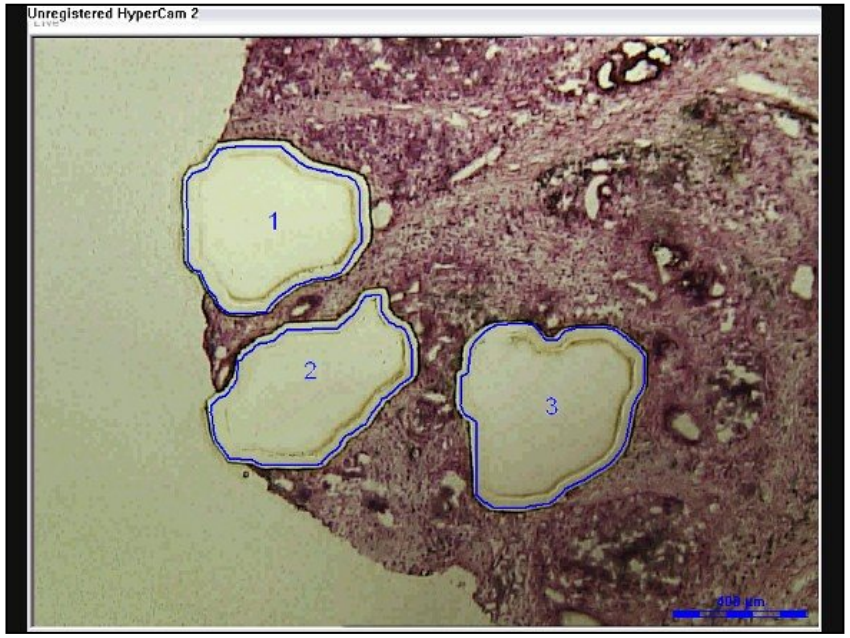
Follow-up

Response to treatment was evaluated using the RECIST criteria, while the Kaplan-Meier method was used to plot DFS, TTP and OS

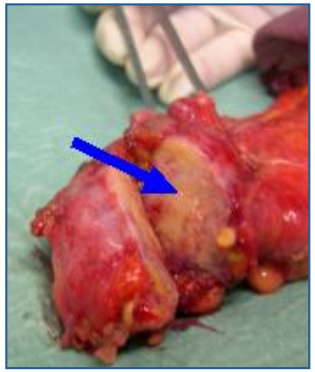
Analysis of the association of clinical and pathological factors and miR-21 by
✓Log-rank test (OS, PFS and DFS curves)
✓Cox proportional hazard multivariate

Laser microdissection

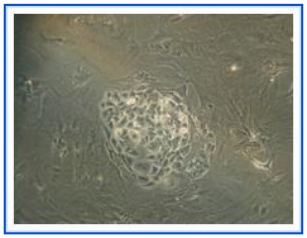


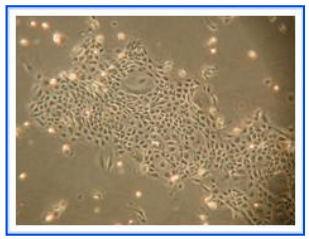


… not only PDAC tissues

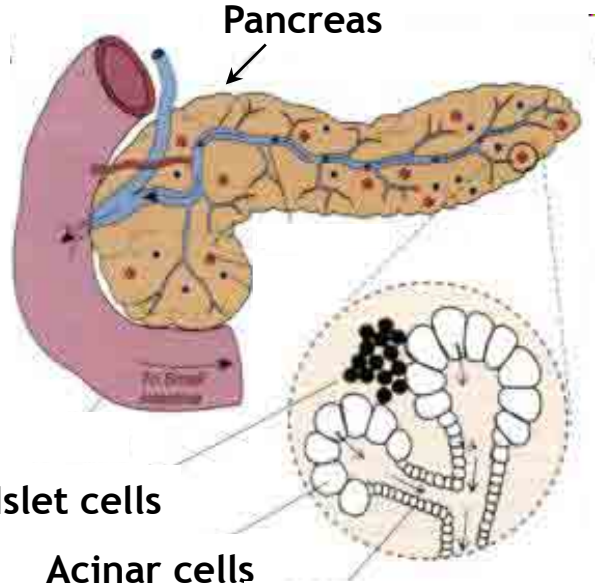


Micro- and non-microdissected PDAC specimens

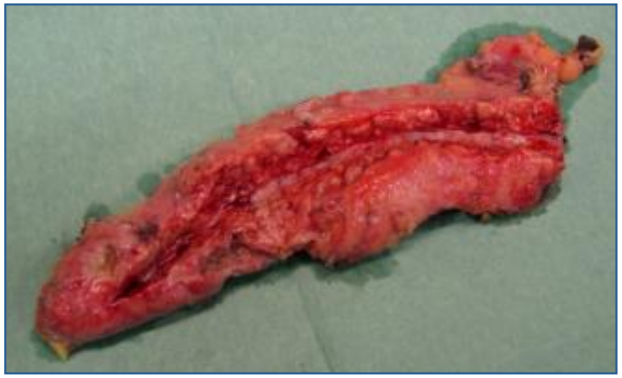




8 primary cultures (+ 7 ATCC cell lines)

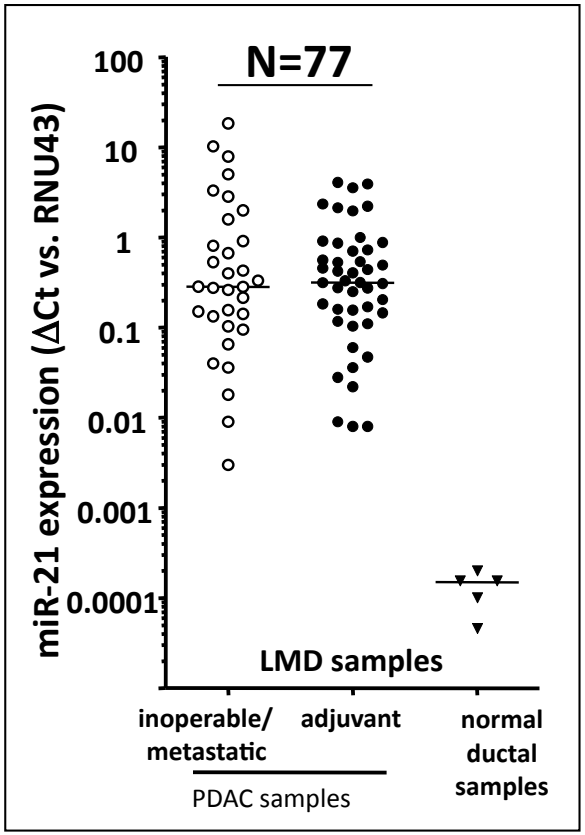


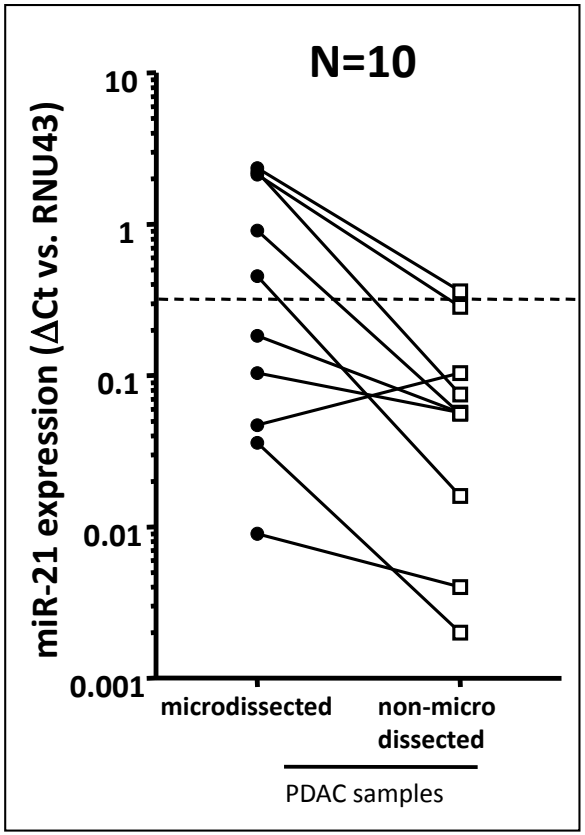
Normal pancreatic tissues



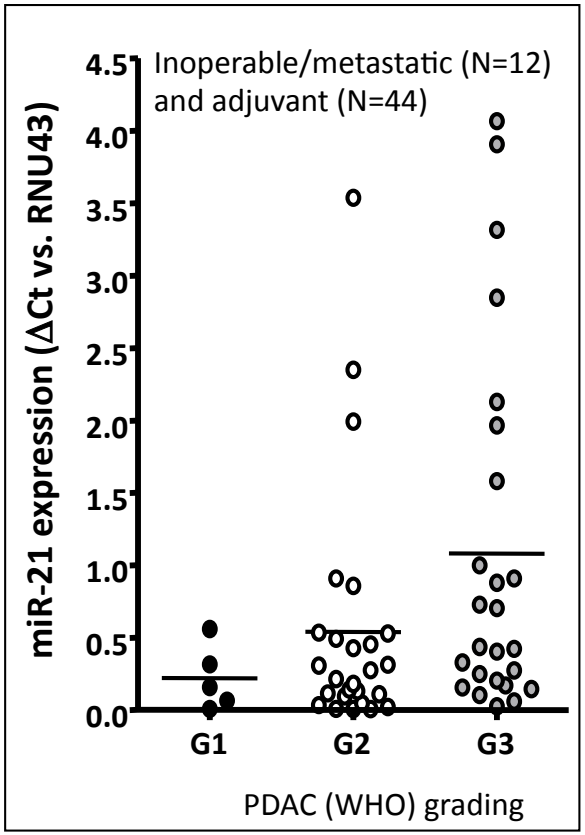
5 normal LMD-ductal tissues (+ hTERT-HPNE ductal pancreatic immortalized cells and Hs27 fibroblasts)

Results: miR-21 expression





P=0.014

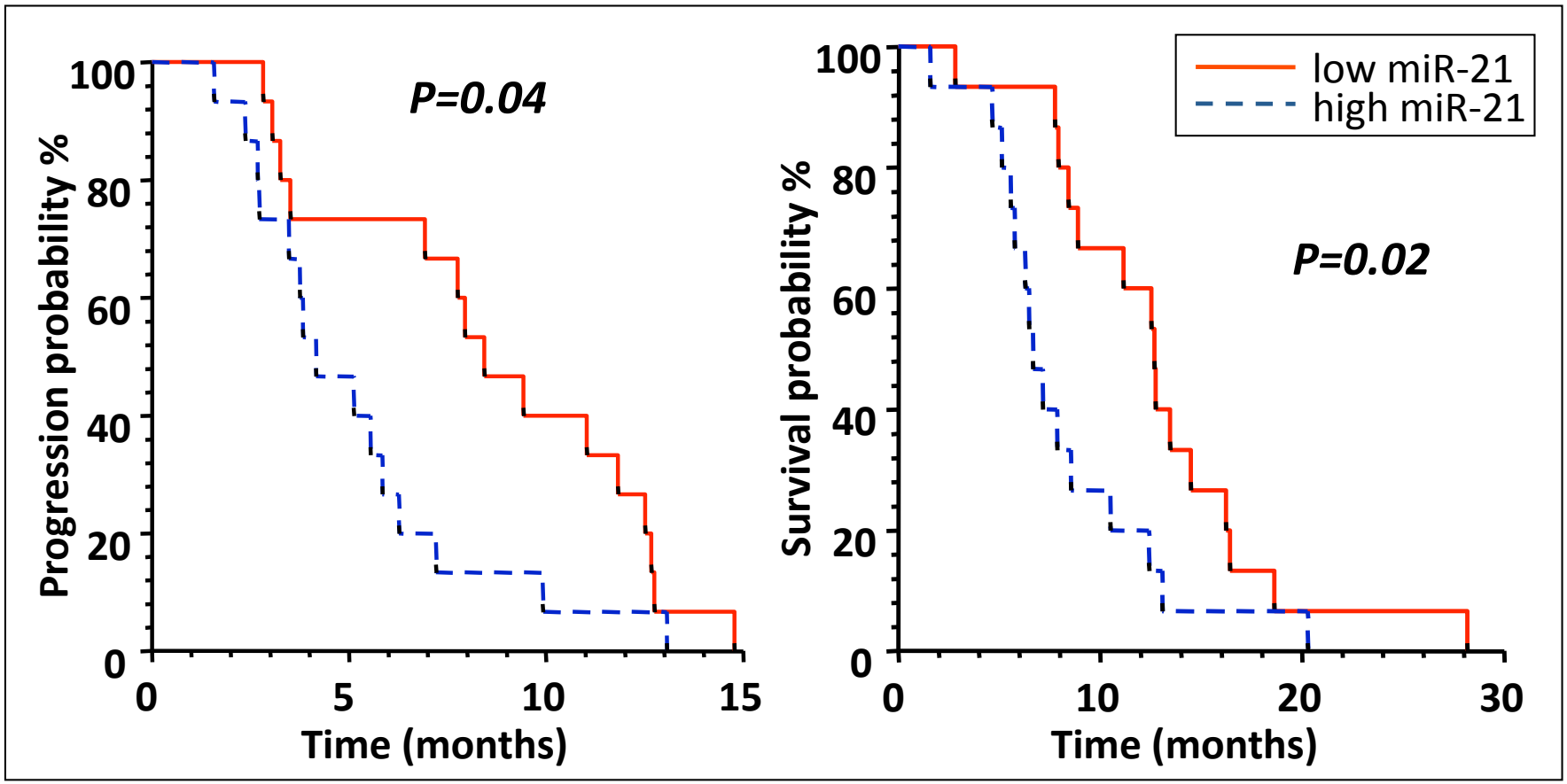


P=0.034

Results: correlation with outcome

In the inoperable/metastatic setting (N=32)

- Trend toward a significant association with clinical benefit = PR+SD (P=*0.07*)
- Significant association with PFS and OS



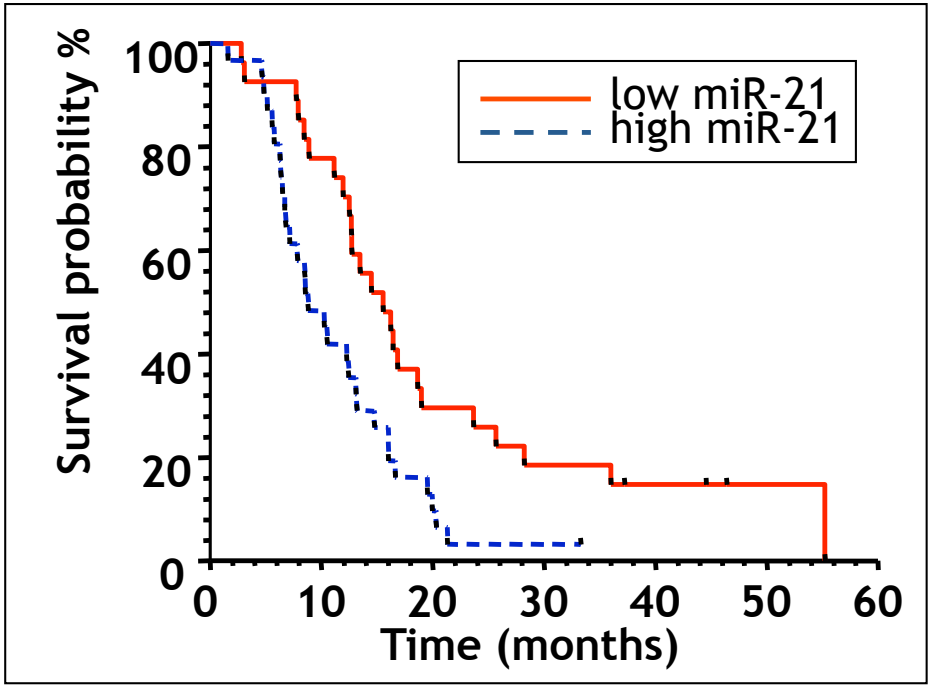
Results: correlation with outcome

In the adjuvant setting

➢Significant association with DFS (*P=0.004*) and OS (*P=0.009*)

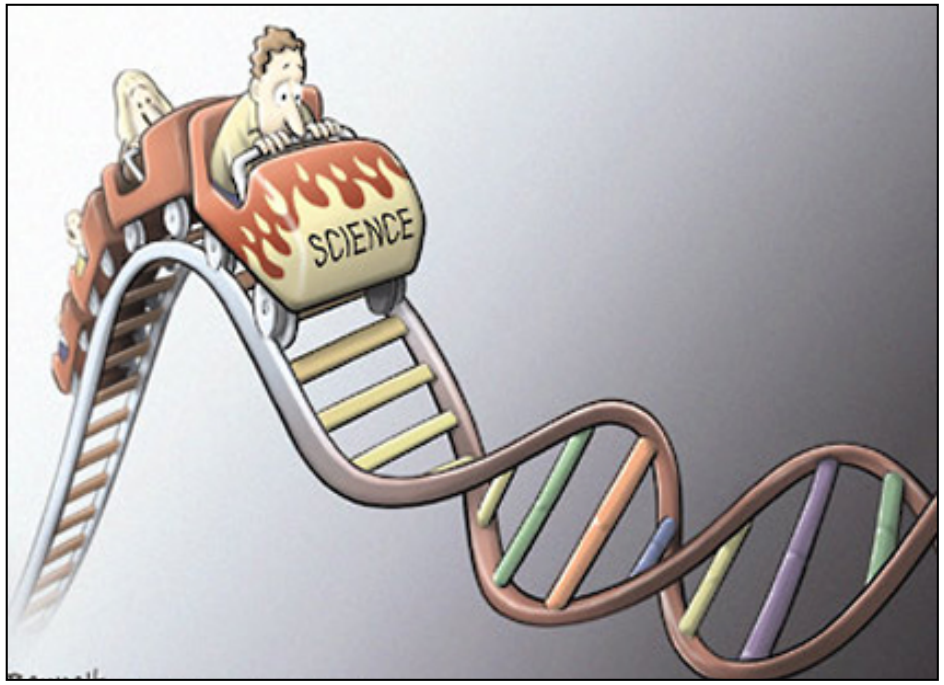
In the inoperable/metastatic + adjuvant setting (N=58)

➢Significant association with OS



➢<u>Multivariate analysis</u> indicated that adjuvant setting of therapy and high miR-21 expression were independent predictors of prognosis (HR=0.3, with *P<0.001* for adjuvant setting, and HR=2.6, with *P=0.001* for high miR-21 expression, respectively)

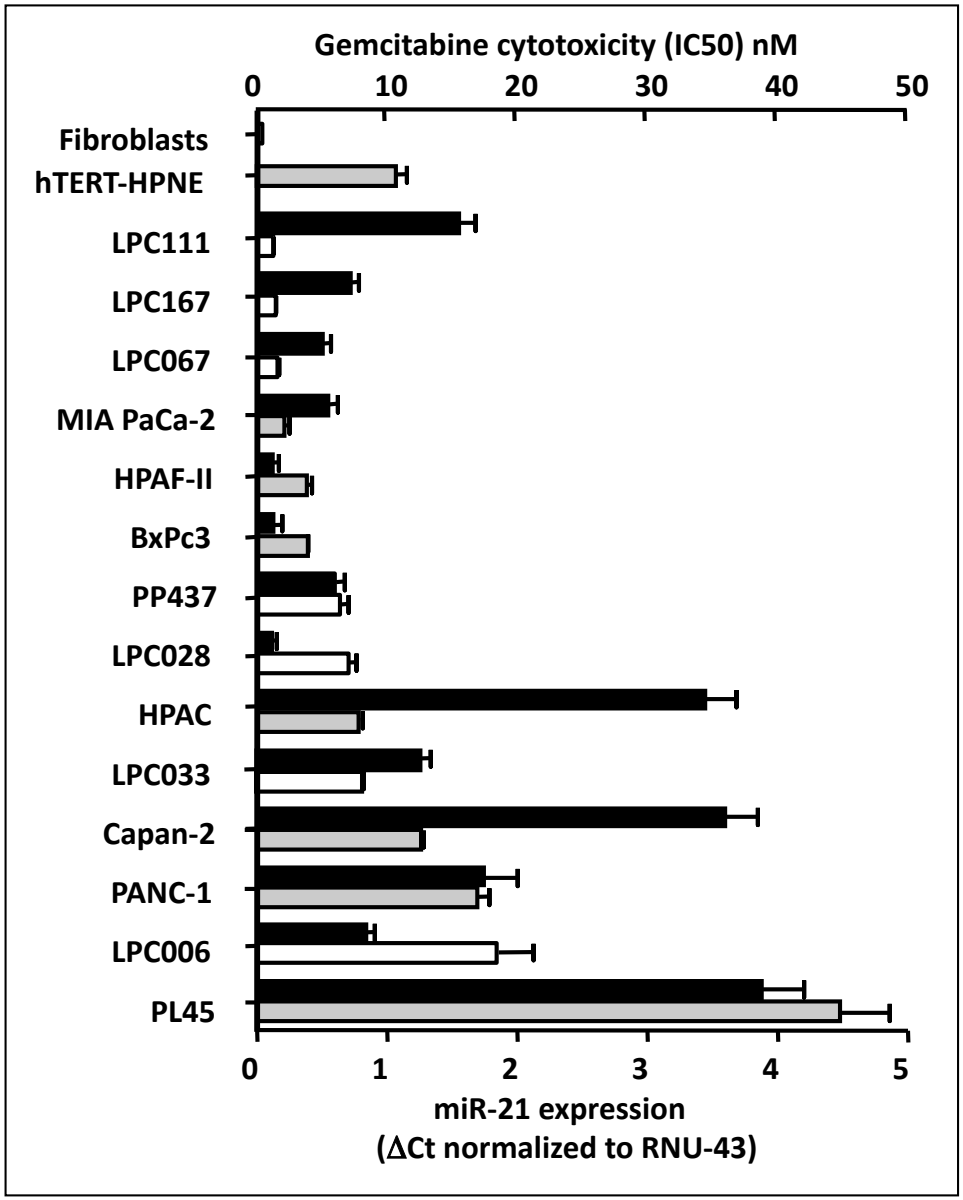
“... one more ride”

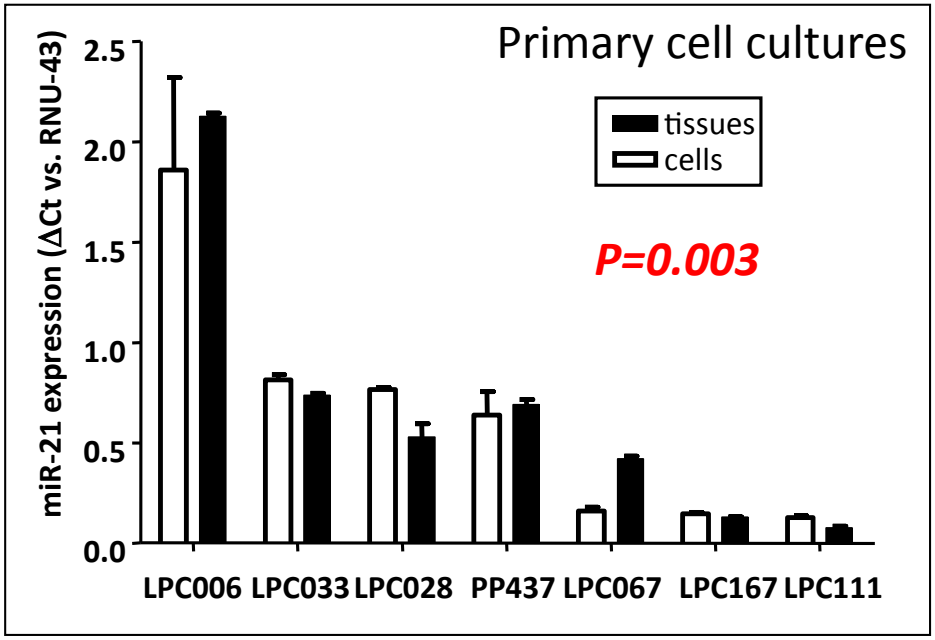


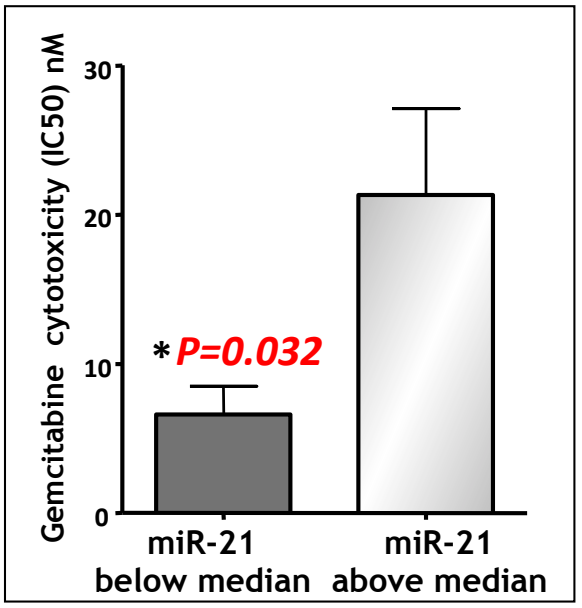
Preclinical studies on the role of miR-21:

- **Cytotoxicity / apoptosis**
- **PTEN/Akt expression/phosphorylation**
- **Expression of MMP-2/-9**

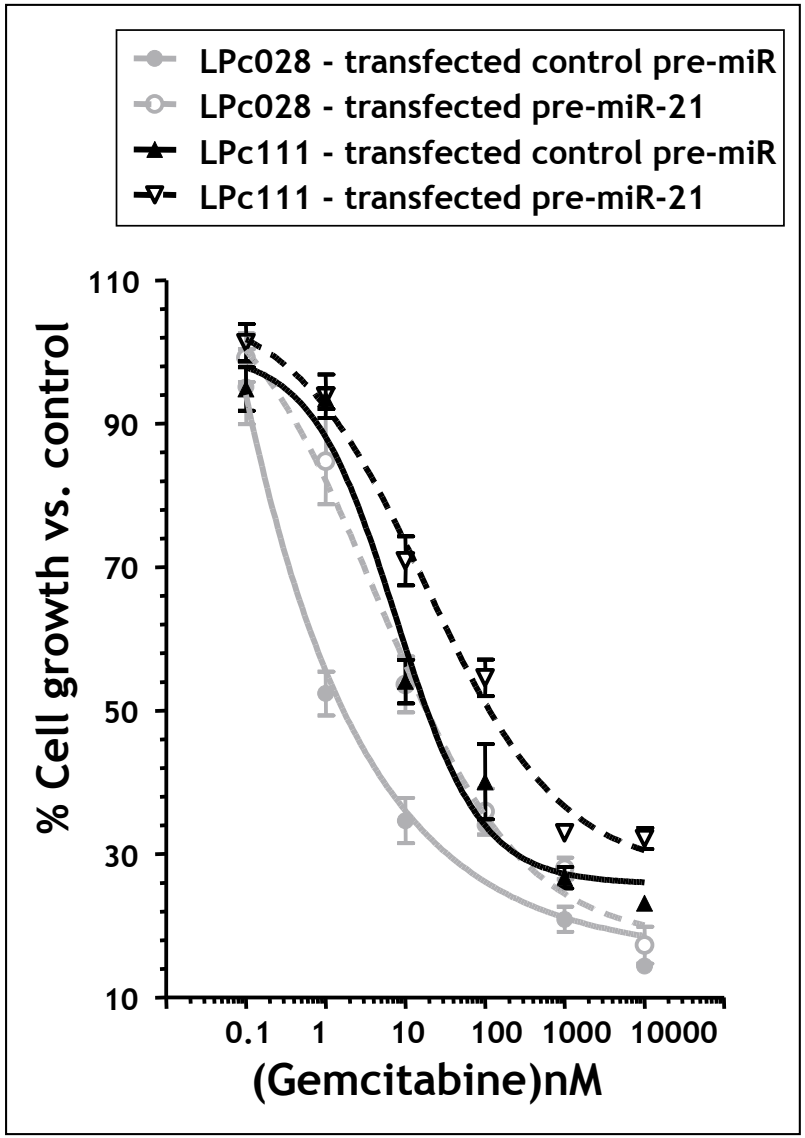
Gemcitabine cytotoxicity and basal miR-21 expression



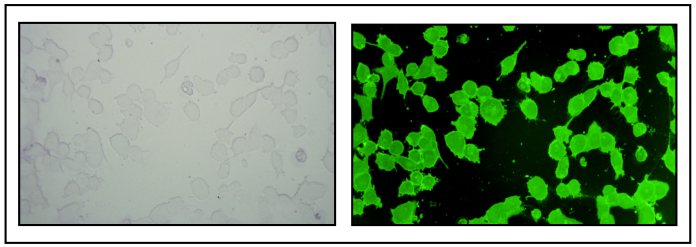


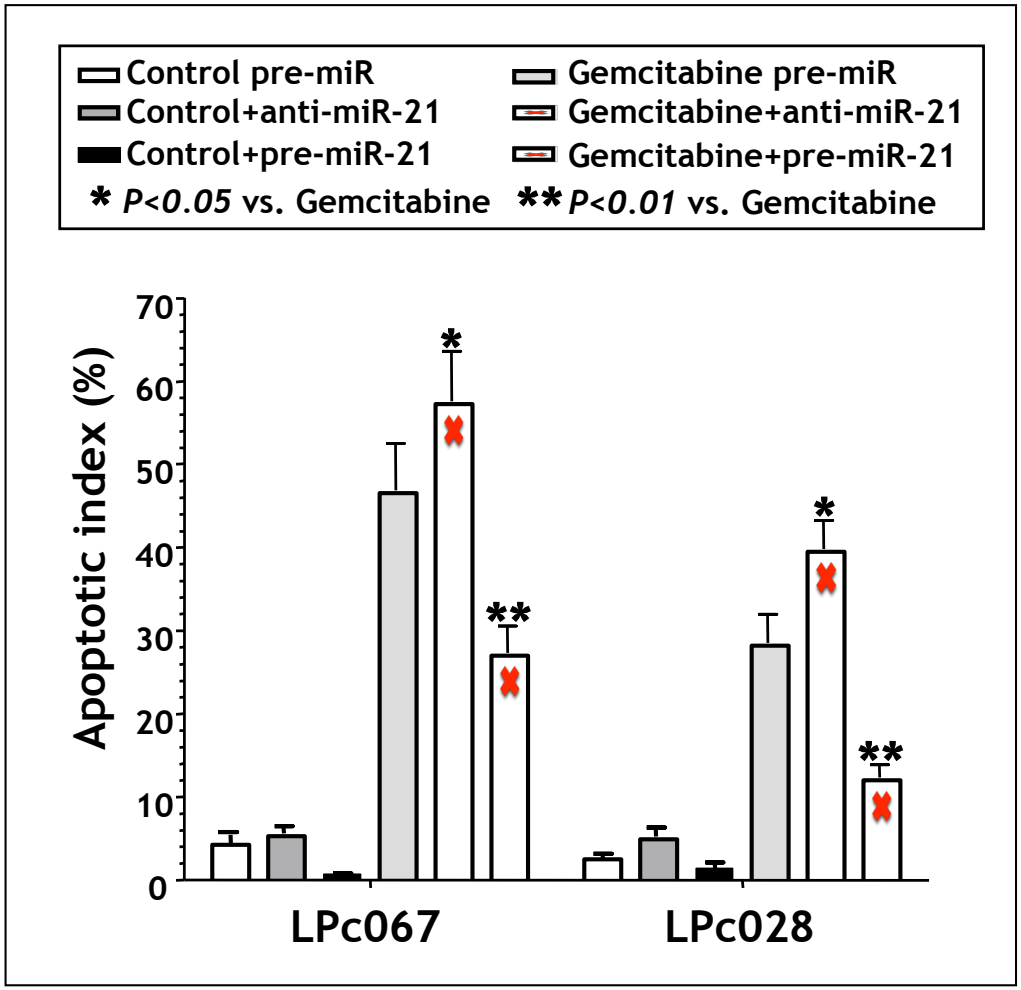


Modulation of gemcitabine cytotoxicity and apoptosis

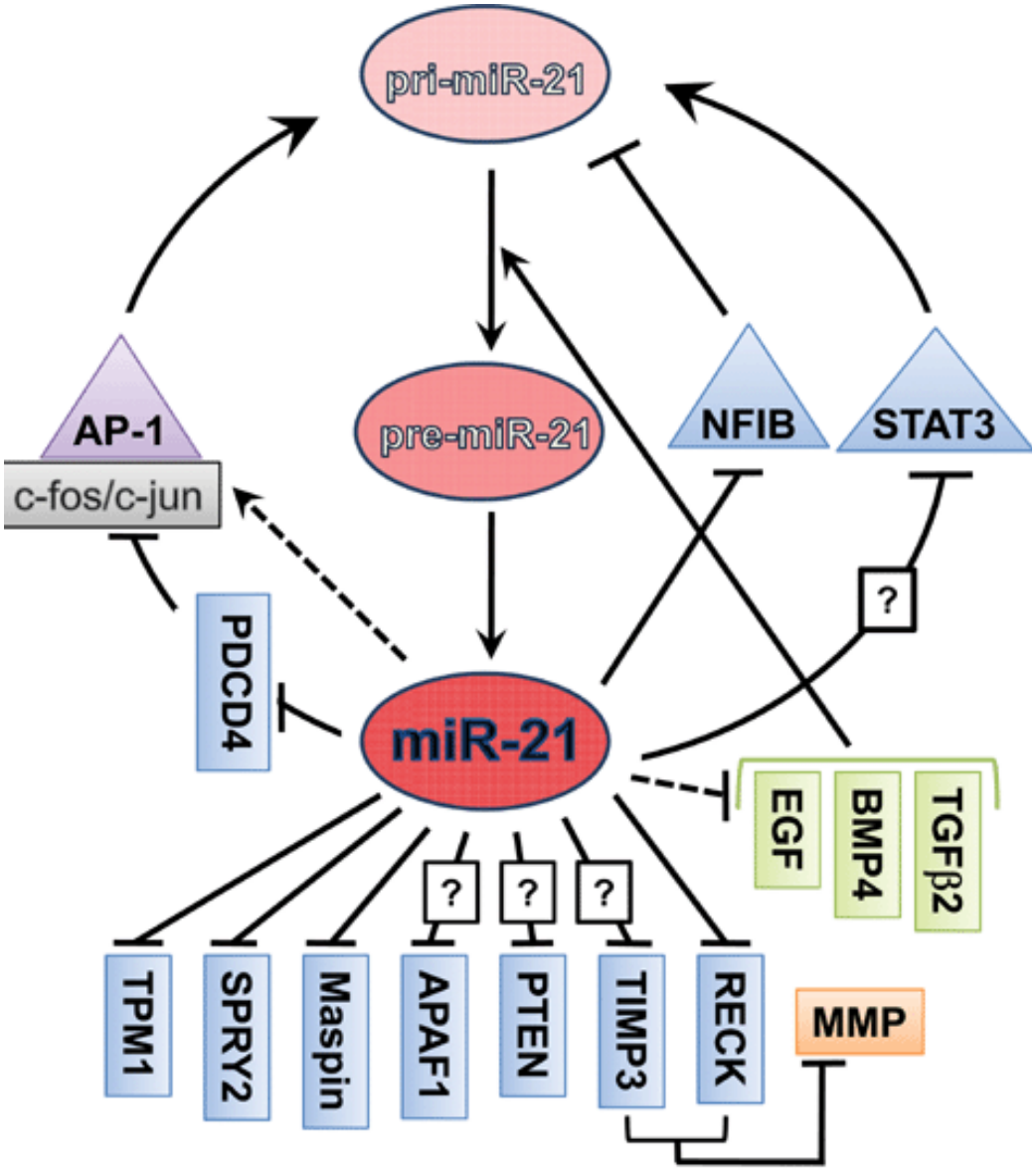


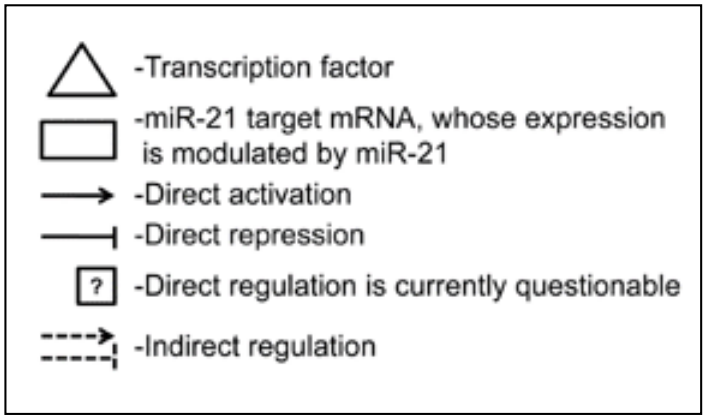
Cells transfected with Pre-miR





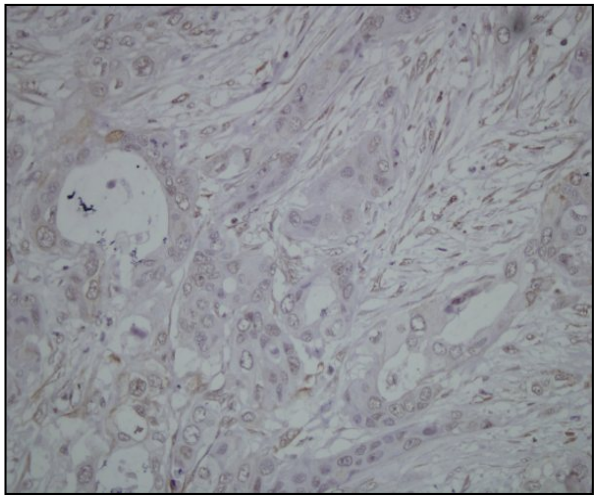
miR-21 targets and its regulatory network



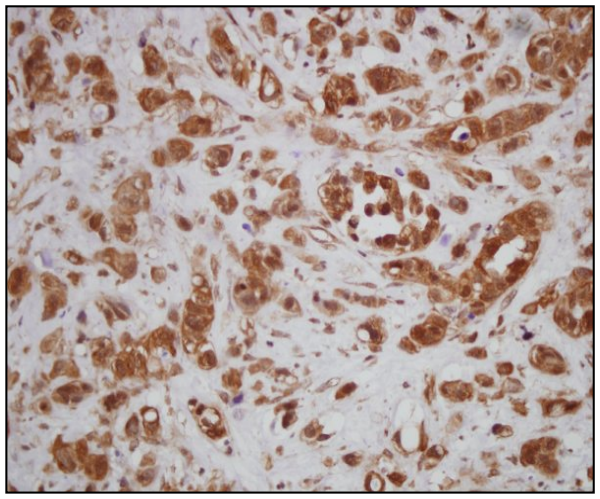


Krichevsky and Gabriely, 2009

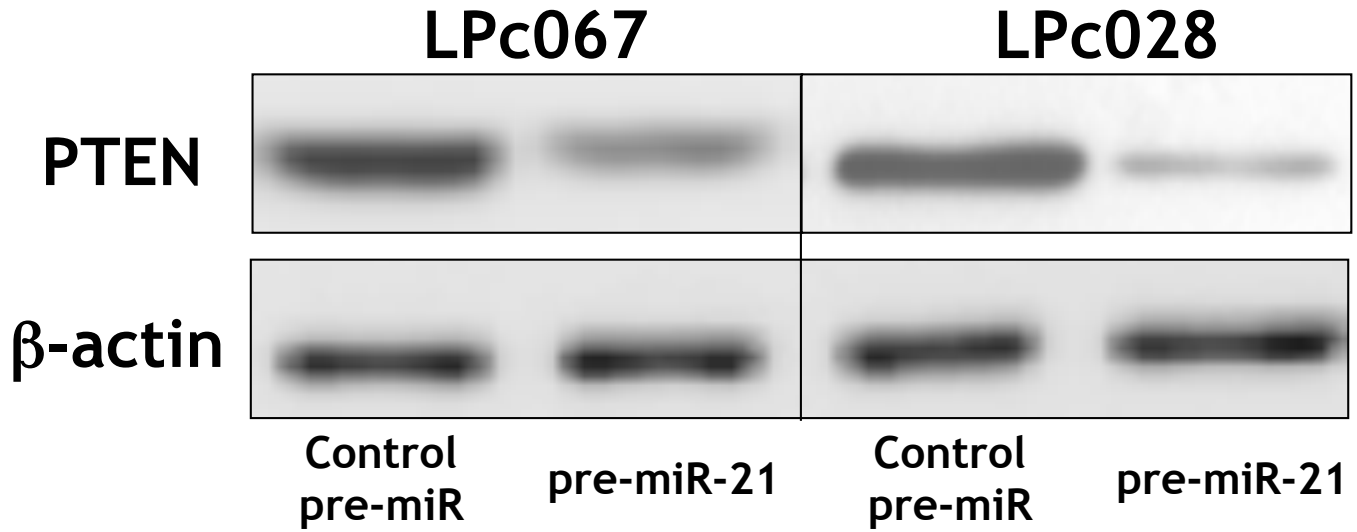
PTEN expression and its modulation by pre-miR-21



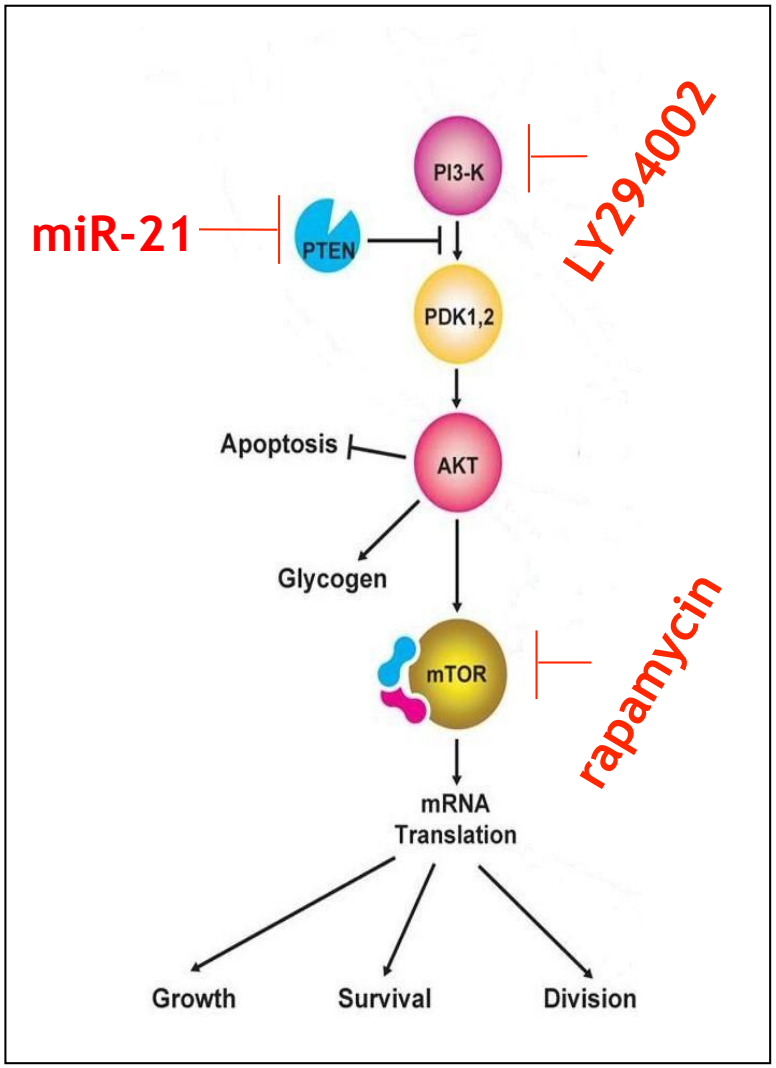
7 PDAC tumors with miR-21 expression above median: PTEN expression -/+

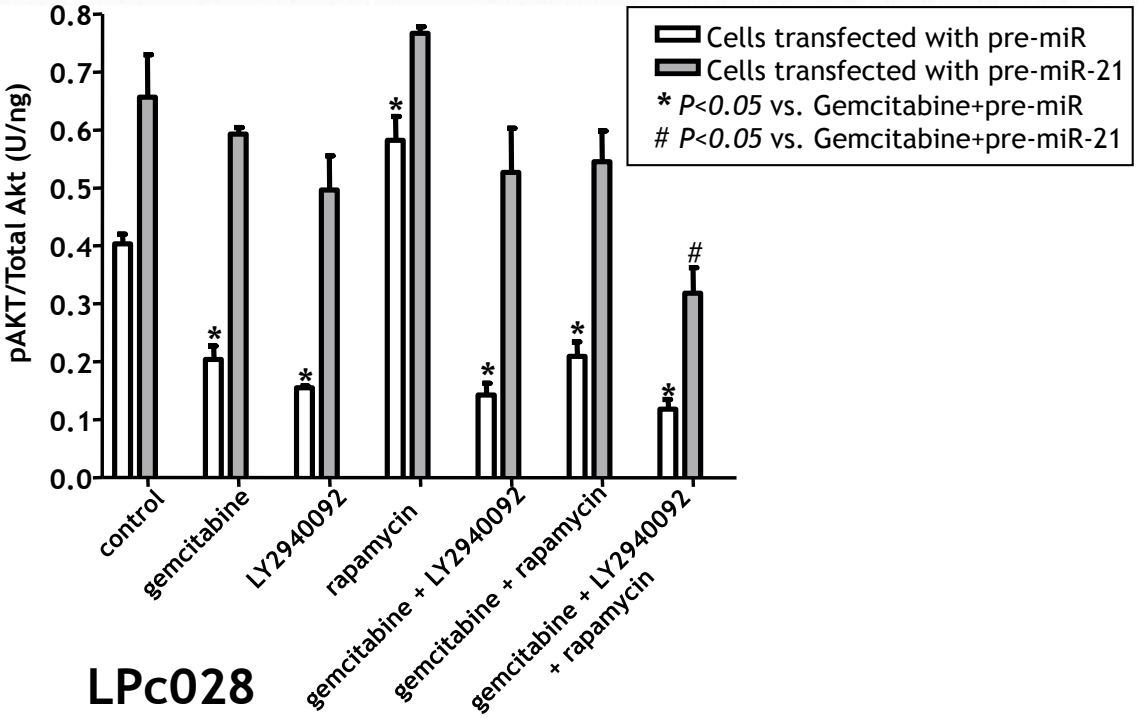


7 PDAC tumors with miR-21 expression below median: PTEN expression ++/+++

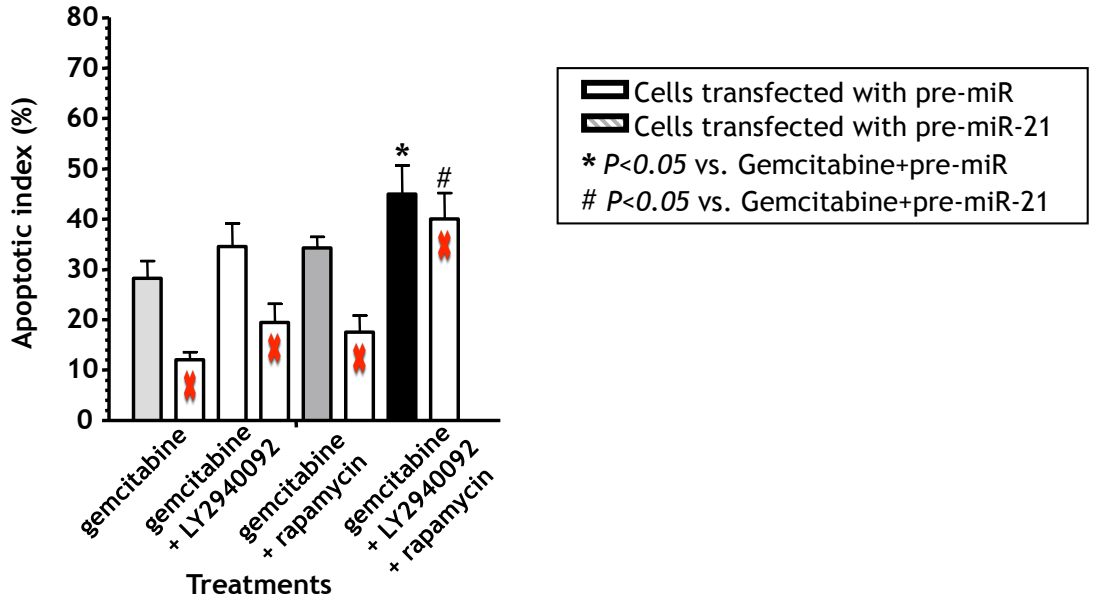


Modulation of Akt phosphorylation

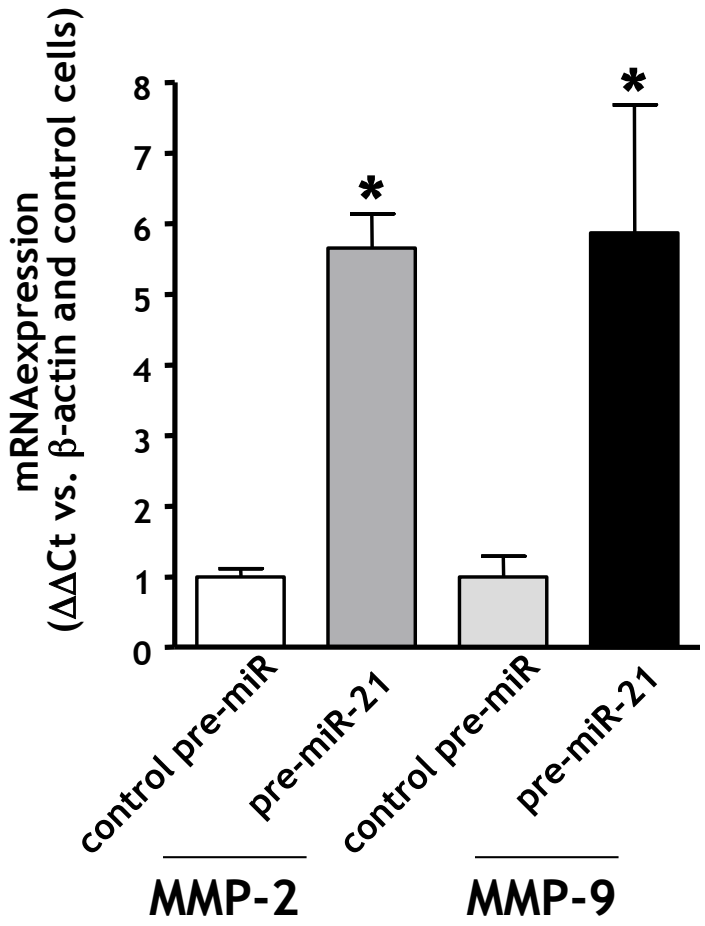


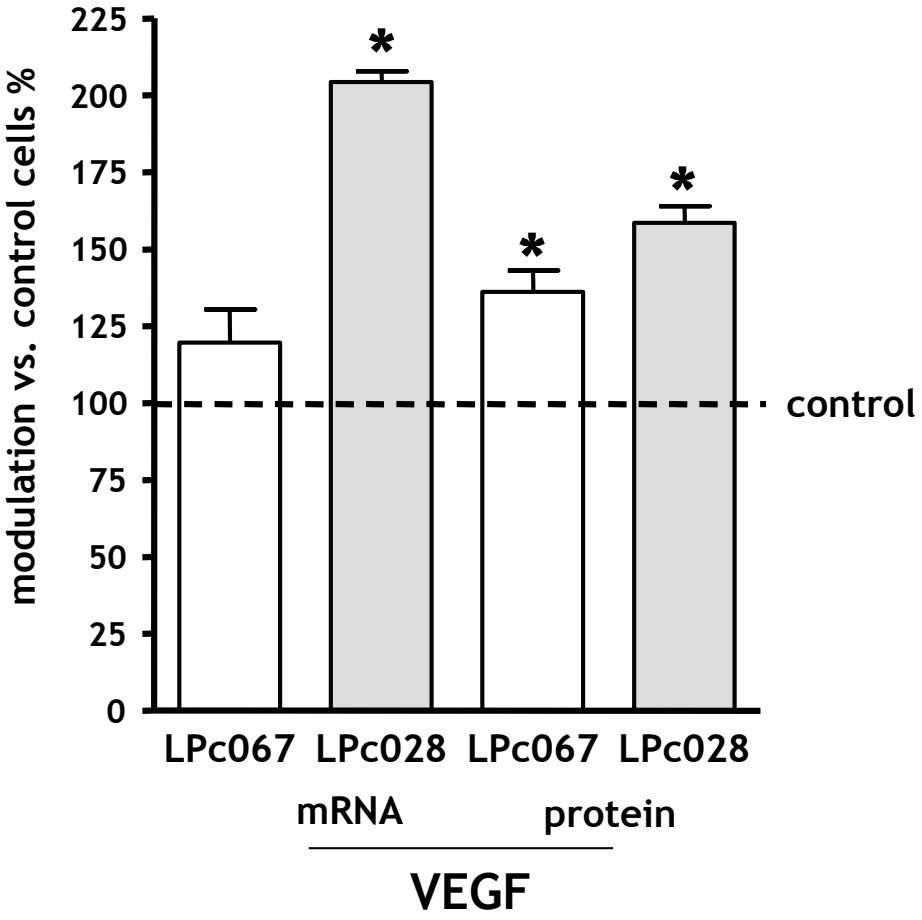


LPc028



Other targets: MPM-2/-9 and VEGF





LPc067

*P<0.05 vs. control

Conclusions

- MiR-21 expression was correlated with clinical outcome in patients with PDAC both in the adjuvant and in the palliative setting

- Preclinical studies showed that miR-21 expression in primary cultures correlated with expression in their respective tissues, and with gemcitabine resistance in all the PDAC cells

- Modulation of apoptosis, PTEN expression, Akt phosphorylation, and expression of genes involved in invasive behaviour, may contribute to miR-21 role in gemcitabine chemoresistance

The consistency of the accumulating preclinical and clinical data suggest that PDAC are more aggressive and resistant to gemcitabine if they have high expression of miR-21, which therefore represents a promising target for prognostic and therapeutic approaches