

Molecular and Cellular mechanisms/biomarkers in PDAC

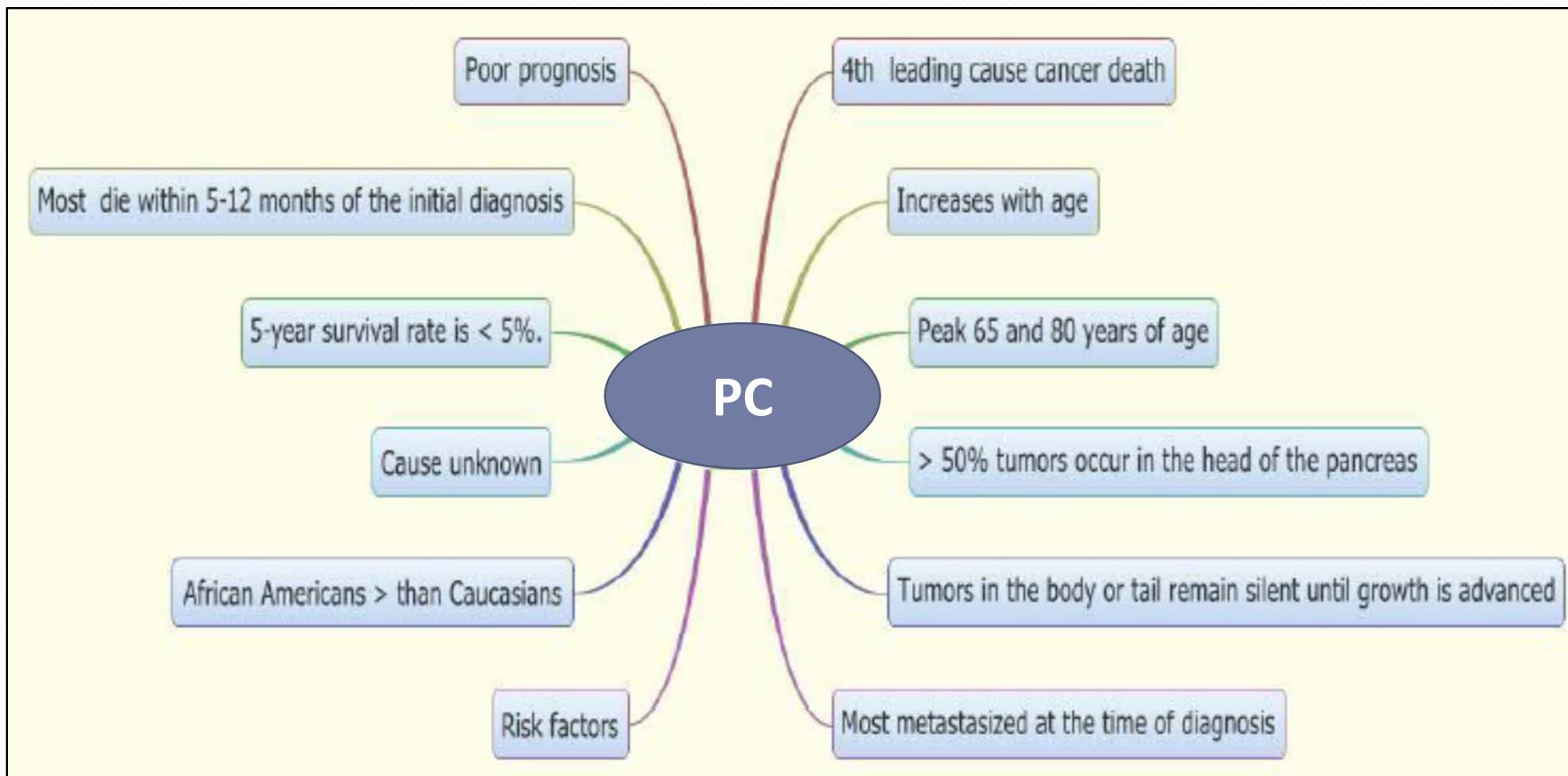
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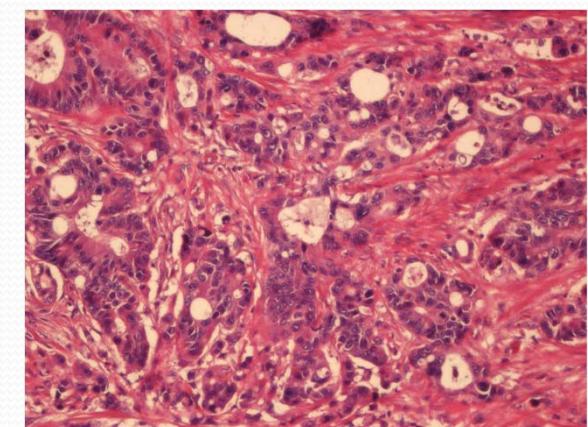
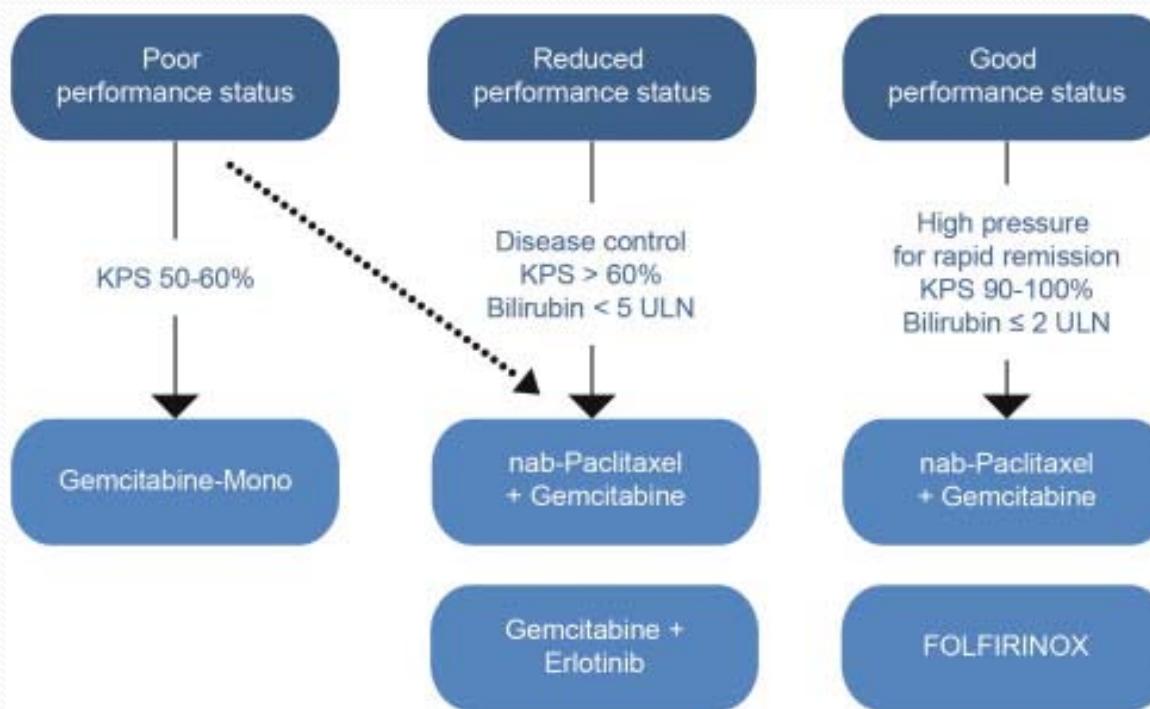
PDAC-AECC: <http://pdacaecc.com>



Pancreatic cancer as unmet clinical need



Pancreatic cancer treatment: urgent need for improvement

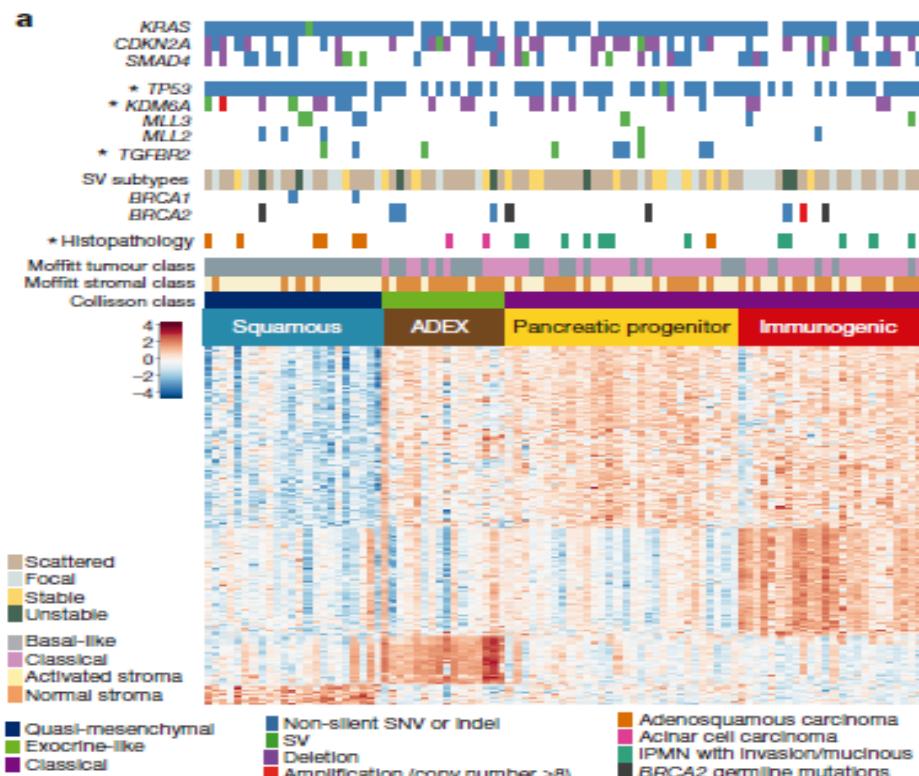


Experimental treatments:

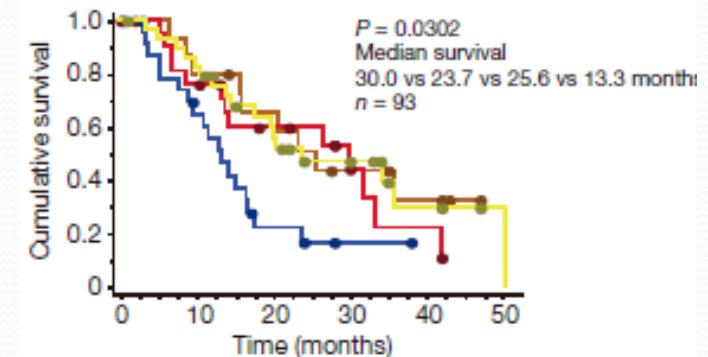
- Hyaluronidase (PEGPH20)
- Hedgehog pathway modulators (Vismodegib)

PC patients stratification: molecular subtypes

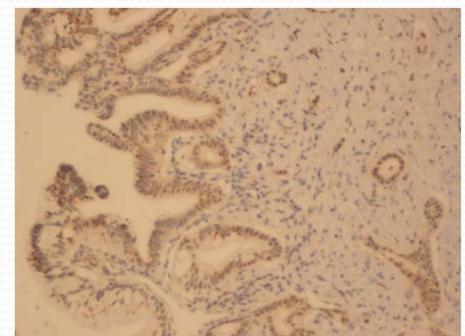
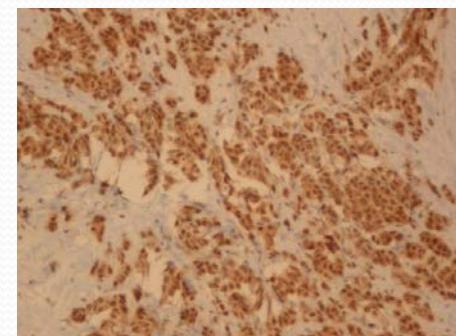
456 human biopsies



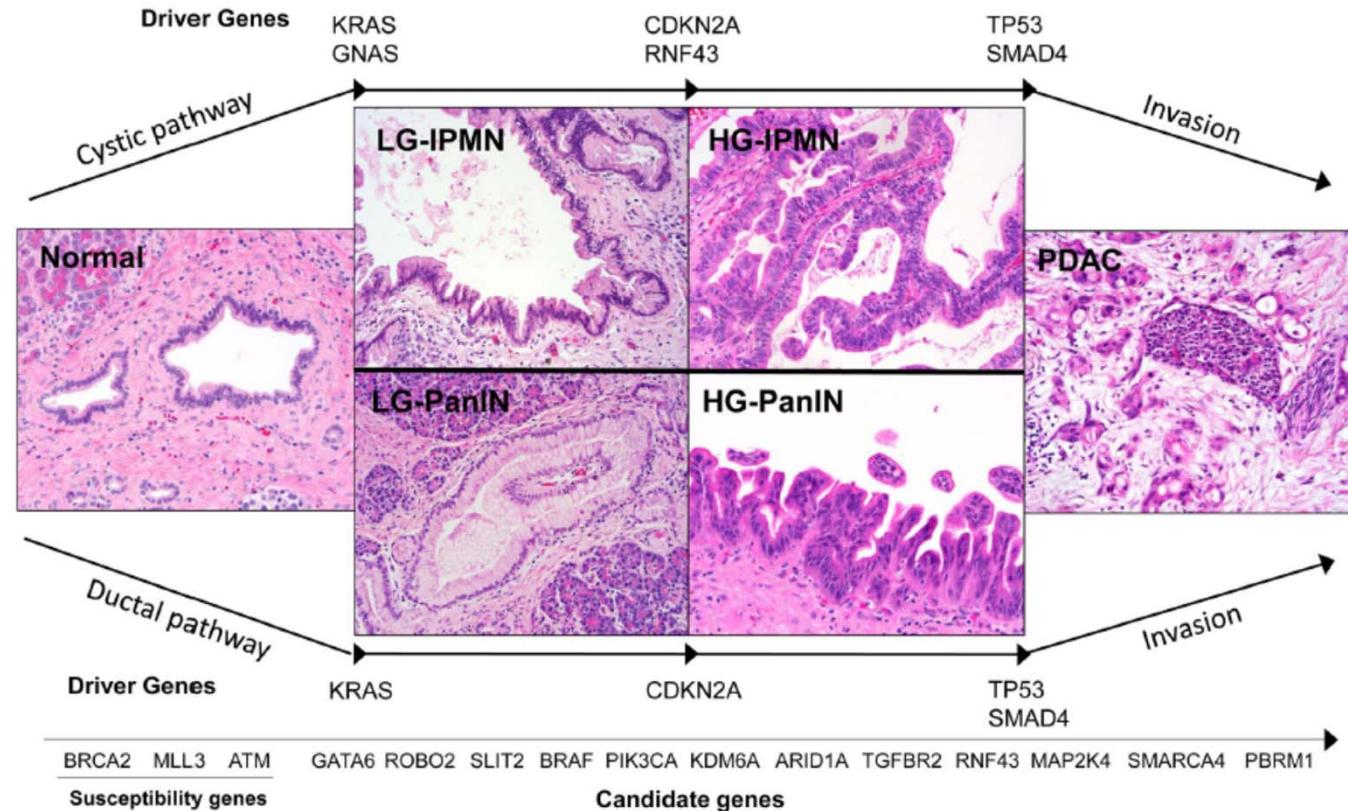
Bailey et al.; 2016, *Nature* 531; 47-65



GATA-6



Biology of pancreas cancer: gene drivers and susceptibility in PC development

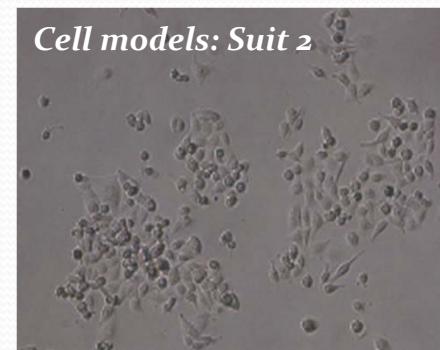


Biology of pancreas cancer: gene drivers and druggable genes as therapeutic targets

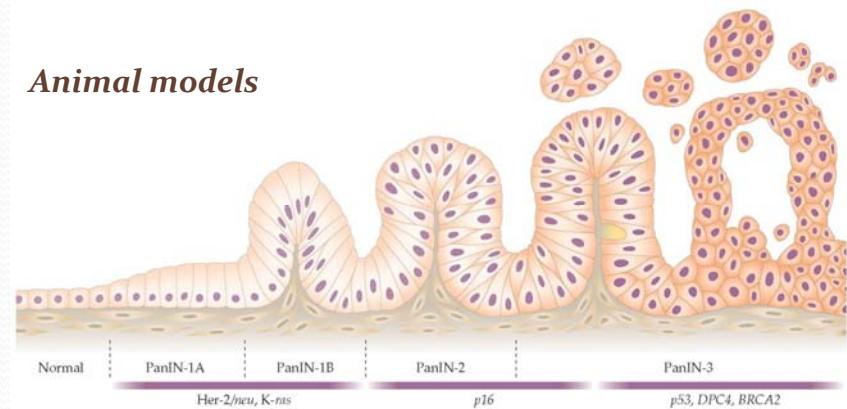
Mutated genes in recent comprehensive sequencing analyses of PDACs

Driver gene	Signaling pathway	Mutation prevalence (%) *	Targeted therapy
<i>KRAS</i>	KRAS signaling	91	
<i>TP53</i>	Cell cycle	61	
<i>CDKN2A</i>	Cell cycle	44 **	CDK4/6 inhibitor
<i>SMAD4</i>	TGF β signaling	40 **	
Candidate driver gene			
<i>GATA6</i>	Wnt/Notch signaling	17 **	Gamma secretase inhibitor
<i>ARID1A</i>	Chromatin remodeling	8	
<i>RNF43</i>	Wnt/Notch signaling	8 **	Porcupine Inhibitor
<i>TGFBR2</i>	TGF β signaling	6	
<i>MAP2K4</i>	KRAS signaling	5	
<i>MLL3</i>	Chromatin remodeling	5	
<i>PIK3CA</i>	KRAS signaling	5 **	mTOR Inhibitor
<i>RBM10</i>	RNA processing	4 **	
<i>ATM</i>	DNA Repair	4	
<i>ROBO2</i>	Axon Guidance	3	
<i>SMARCA4</i>	Chromatin remodeling	3	
<i>PBRM1</i>	Chromatin remodeling	3	
<i>SLC2</i>	Axon Guidance	3	
<i>KDM6A</i>	Chromatin remodeling	3	
<i>BRAF</i>	KRAS signaling	2	BRAF inhibitor
<i>BRCA2</i>	DNA Repair	2	PARP inhibitor

Cell models: Suit 2



Animal models



**Key role of stroma in pancreas cancer:
microenvironment regulation mechanisms**

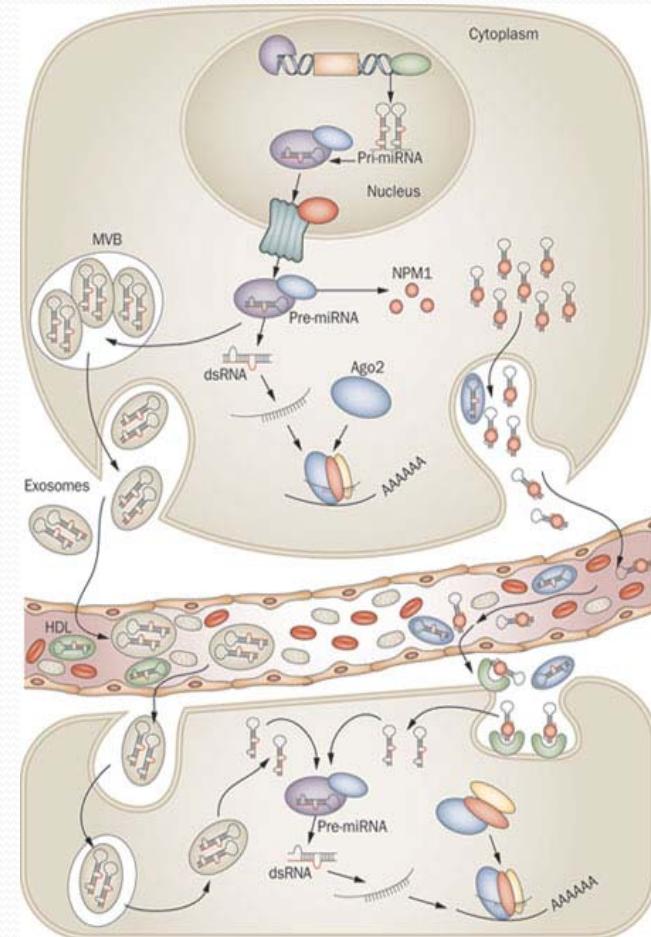
Biology of pancreas cancer: miRNAs as novel mechanisms/microenvironment regulation

- Small endogenous RNA molecules (20-25 nt).
- Post-transcriptional negative regulation of gene expression (90% of genes).
- Target mRNA → recognition of small complementary sequences
- Translational Repression
- mRNA deadenylation and degradation

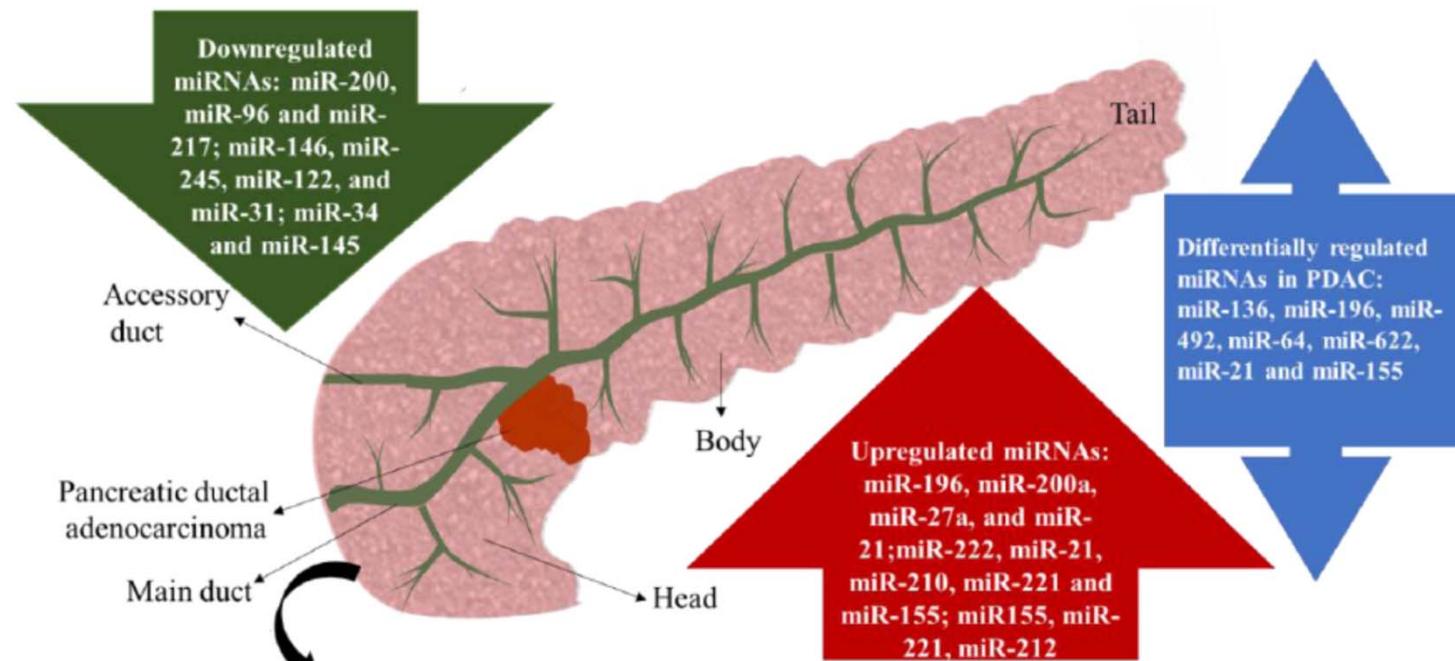
Pathophysiological mechanisms of diseases

- Carried by:
 - Microvesicles (MVB)
 - Proteins (Ago2)
 - HDL
- miRNA secretion → Highly regulated

Useful Biomarkers of diseases (LB) Microenvironment regulation

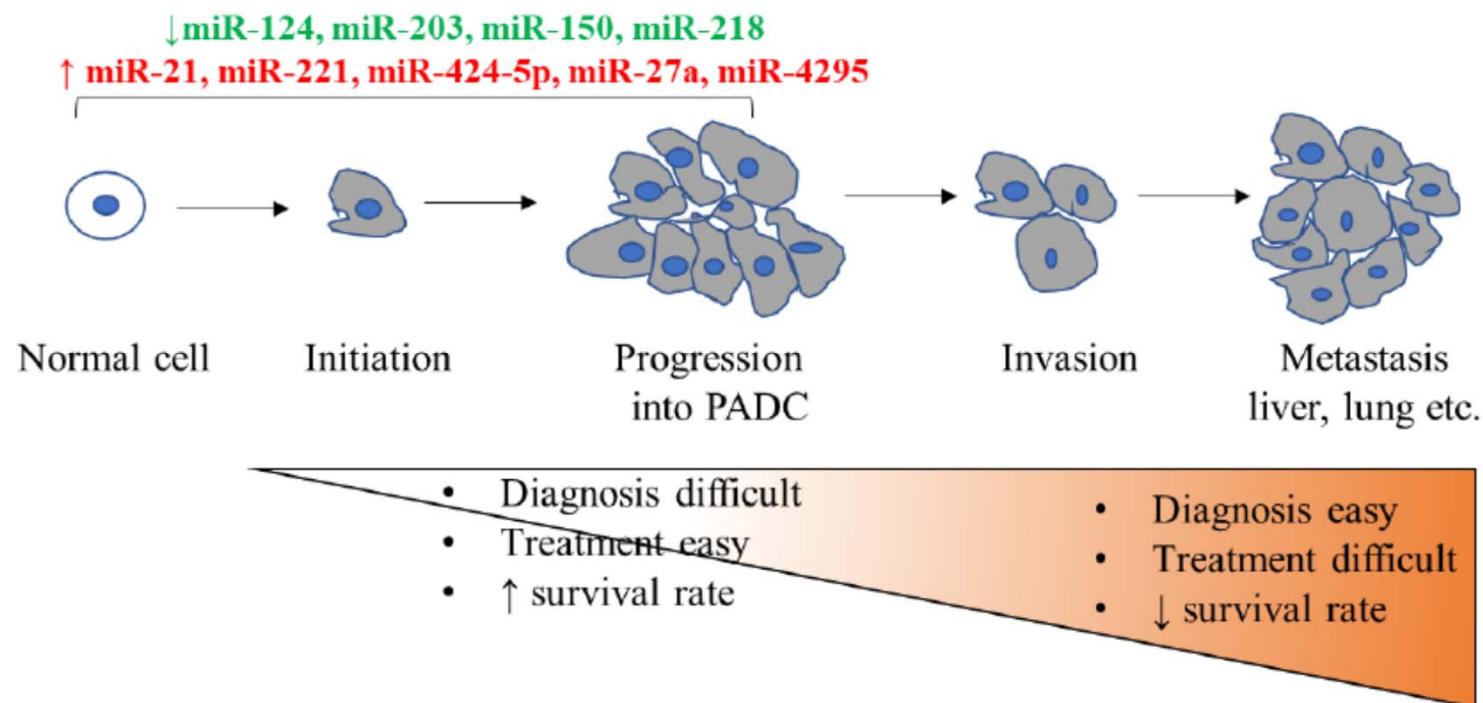


miRNAs link to pancreas cancer

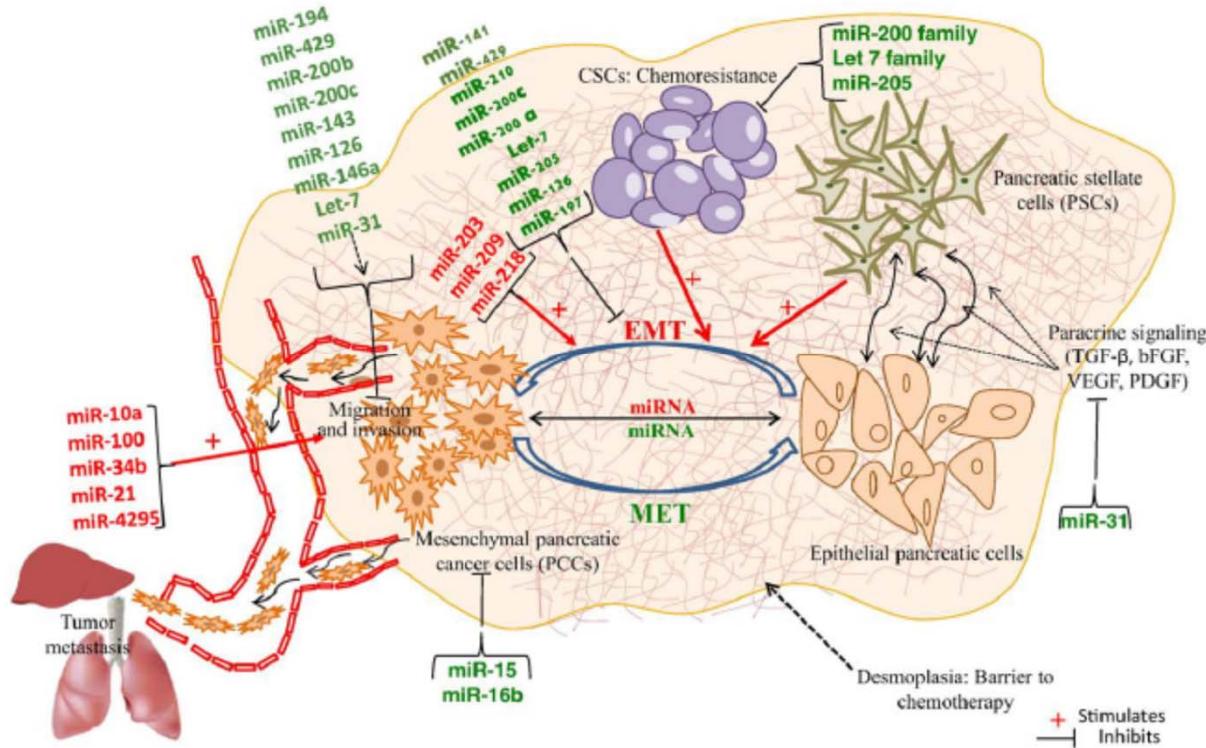


Dysregulated miRNAs in Blood: miR-18a, miR-21, miR-22, miR-24, miR-25, miR-99a, miR-155, miR-185, miR-191, miR-196a, miR-642b, miR-885-5p; in Serum: miR-2

miRNAs in pancreas cancer development: miRNAs in transformation



miRNAs in pancreas cancer metastasis: miRNAs in EMT/MET





Molecular and Cellular mechanisms/biomarkers in PDAC

miRNAs as useful biomarkers in patients management

miRNAs screening in sporadic PC serum samples:



762 miRNAs in 21 patients. Clinical settings

PATIENT CODE	SEX		AGE	TNM	Treatment	Evolution
MI-3	MALE	RES	70	pT3N1Mx	Gemcitabine	uk
MI-4	WOMAN	??	77	pTxNxMx	UK	Exitus
MI-5**	MALE	MET	61	pTxNxMx	Gemcitabine+ AMG479	Exitus
MI-6**	MALE	MET	71	pTxNxMx	Gemcitabine-AMG/Folfox6/Docetaxele	Exitus
MI-7**	WOMAN	MET	-	pTxNxMx	UK	uk
MI-8	WOMAN	LA	64	pTxNxMx	Gemcitabine	uk
MI-9	MALE	MET	64	pTxNxMx	Gemcitabine /FOLFOX 6 /FOLFIRI /5FU	Exitus
MI-10**	MALE	MET	52	pTxNxMx	Folfirinox	alive
MI-11	MALE	RES	64	pT2 N0 M0	UK	alive
MI-12**	MALE	LA	61	PT3 N0 Mx	gemcitabine	alive
MI-13**	WOMAN	MET	83	pTxNxMx	Gemcitabine	uk
MI-14**	MALE	LA	70	pT3 N1 Mx	UK	Exitus
MI-16**	WOMAN	LA	68	pT3 N1 Mx	gemcitabine	Exitus
MI-17	WOMAN	LA	67	pTxNxMx	UK	uk
MI-18	WOMAN	MET	68	pTxNxMx	Gemcitabine/Capecitabine/Erlotinib	alive
MI-19	WOMAN	MET	73	pTxNxMx	Gemcitabine	alive
MI-21	WOMAN	MET	73	pT3 N1 M0	Gemcitabine/Abraxan-Gemcitabine/FOLFOX	alive
MI-23	MALE	LA	60	pTxNxMx	Gemcitabine	alive
MI-24	MALE	MET	58	pTxNxMx	Abraxan-gemcitabine	alive
MI-25	MALE	RES-LA	48	T3N1aMx	Gemcitabine /Gemcitabine /FOLFOX 6/5FU Leucovarin	alive
MI-26	WOMAN	RES	76	pT3 N1 Mx	Gemcitabine	alive

RES: reseable
LA: locally advanced
MET. metastatic

miRNAs selection for validation: 31 miRNAs

31 out of 762 miRNAs significantly modulated and functionally related

miRNA	
hsa/let/7a/5p	hsa/miR/16/5p
hsa/let/7b/5p	hsa/miR/181a/5p
hsa/let/7f/5p	hsa/miR/18a/5p
hsa/miR/101/3p	hsa/miR/18b/5p
hsa/miR/103a/3p	hsa/miR/20a/5p
hsa/miR/106a/5p	hsa/miR/23b/3p
hsa/miR/106b/5p	hsa/miR/24/3p
hsa/miR/125b/5p	hsa/miR/301a/3p
hsa/miR/126/3p	hsa/miR/30b/5p
hsa/miR/132/3p	hsa/miR/32/5p
hsa/miR/142/3p	hsa/miR/324/3p
hsa/miR/144/3p	hsa/miR/342/3p
hsa/miR/145/5p	hsa/miR/454/3p
hsa/miR/150/5p	hsa/miR/574/3p
hsa/miR/151a/3p	hsa/miR/93/5p
hsa/miR/15a/5p	

Validation in 131 patients (PANFAM/PANGEN), by individual qRT-PCR

Serum miRNAs correlation with clinical features in sporadic PC: miRNAs as potential useful biomarkers

Location
Head
Body
Tail
Histological Grade
I
II
III
Stage
Localized
Locally advance
Metastatic
pT,pN
Surgery
Relapse
Local Relapse
Distant Relapse
Pancreatitis
Survival (18 Months)

Grade	Hsa-miR-142-3p, hsa-miR-342-3p, hsa-miR-30b-5p, hsa-miR-103a-3p
Stage	Hsa-miR-101-3p, hsa-miR-125-5p, hsa-miR-574-3p, hsa-miR-106a-5p
Surgery	Hsa-miR-101-3p, hsa-miR-574-3p, hsa-miR-106a-5p, hsa-miR-24-3p
Relapse	hsa- miR-106a-5p, hsa-miR-142-3p
Pancreatitis	hsa/miR/20a/5p, hsa/miR/23b/3p
pT,pN	hsa-miR-454-3p, hsa-miR-342-3p, hsa-miR-150-5p
Survival (18 Months)	Hsa-miR 125b-5p, hsa-miR-20a

Useful biomarkers for patient prognosis:**hsa /miR 125b/5p, hsa /miR/106a 5p, hsa-miR-142-3p,**

Valuable information for disease characterization:

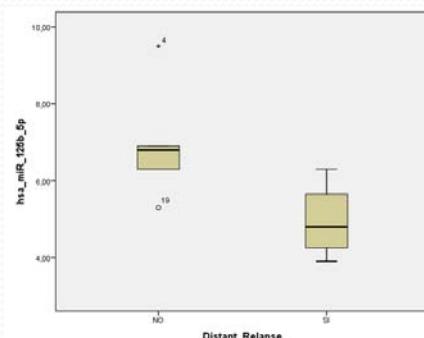
Hsa-miR142-3p, hsa-miR-574-3p, hsa/miR/106a/5p, hsa-miR-150-5p, hsa-miR-101-3p

Serum miRNAs are expressed in PDAC Tissue: miRNAs in PC biopsies

All miRNAs detected in serum are expressed in tissue (Biopsies from patients)

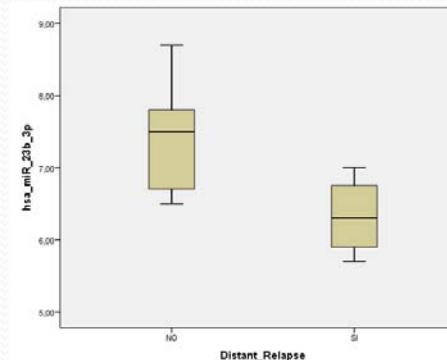
Distance relapse

hsa-miR-125b-5p



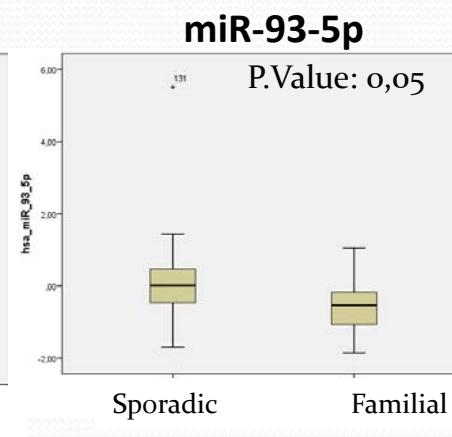
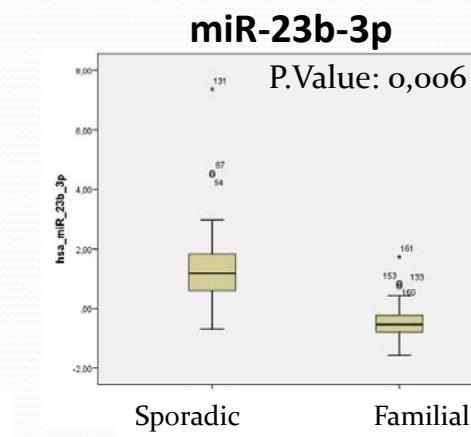
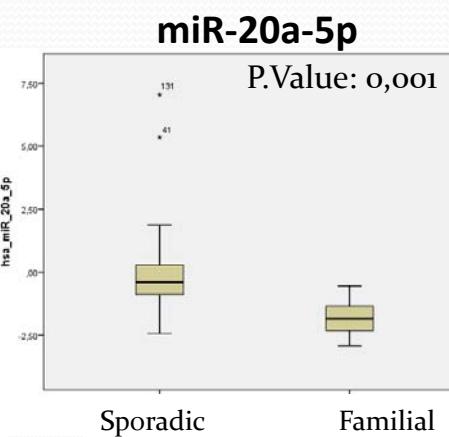
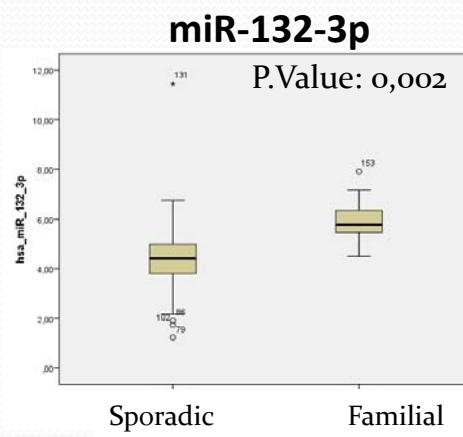
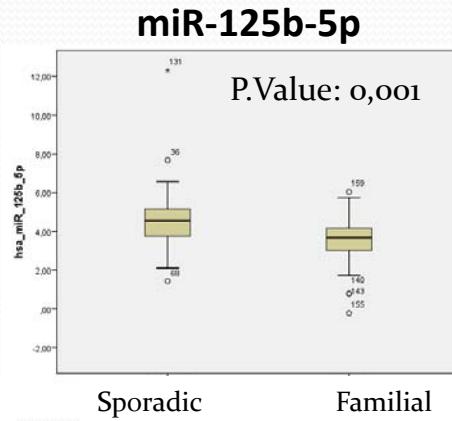
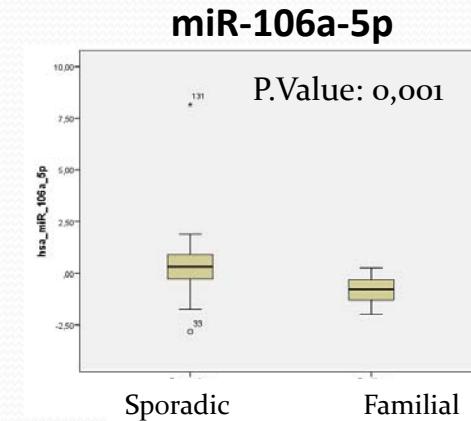
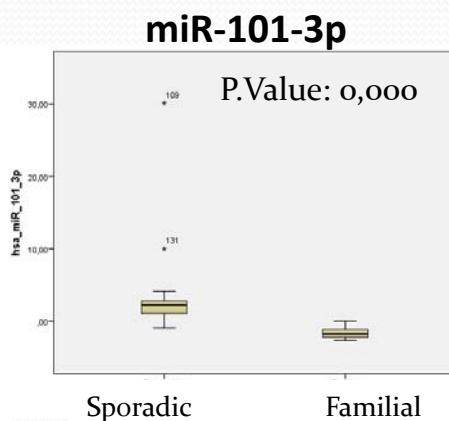
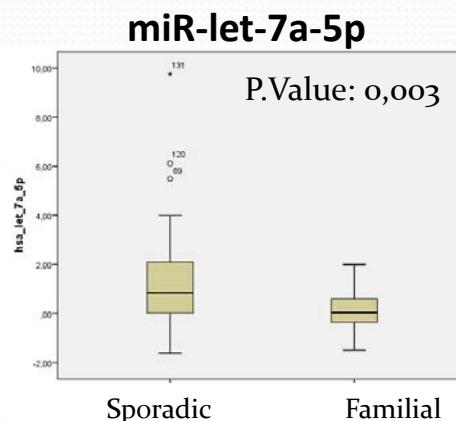
P. value: 0,06

hsa-miR-23



P. value: 0,06

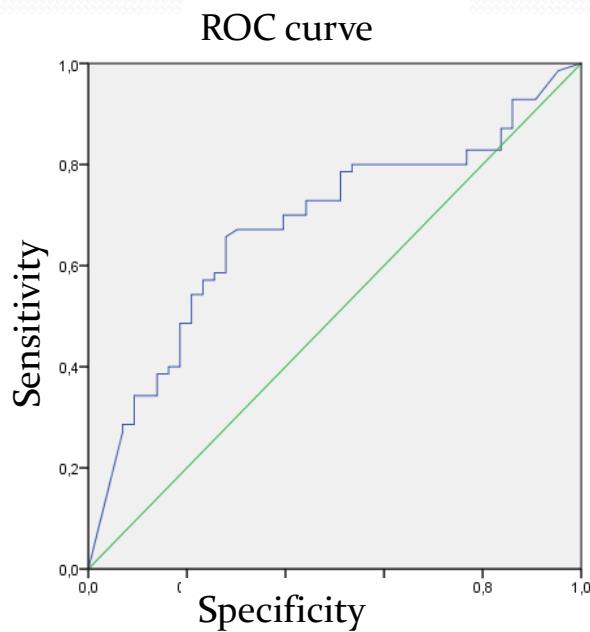
Serum miRNAs discriminating Familial PC vs Sporadic PC





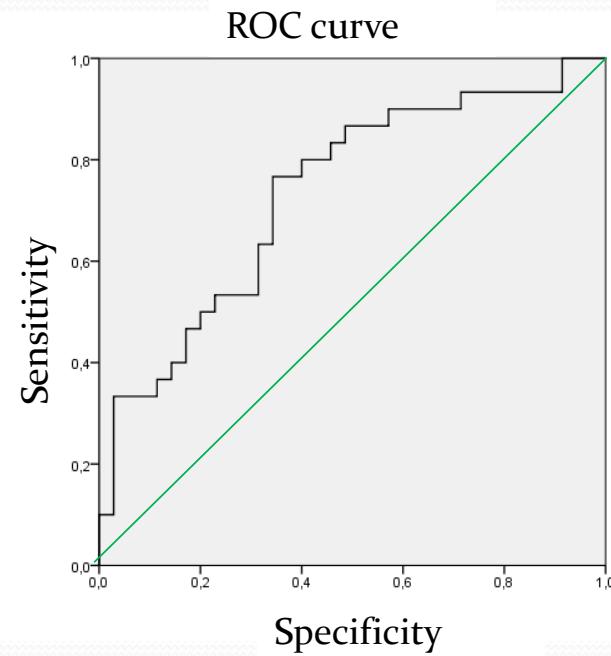
Serum miRNAs as useful biomarkers in PC prognosis: helping CA19.9

CA 19.9



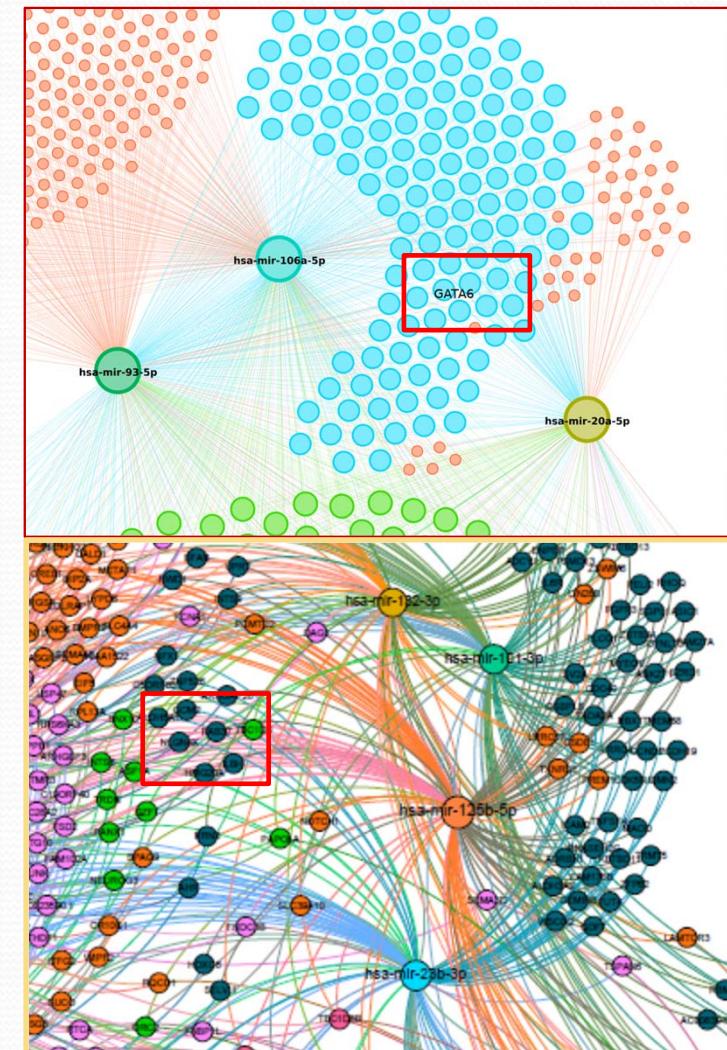
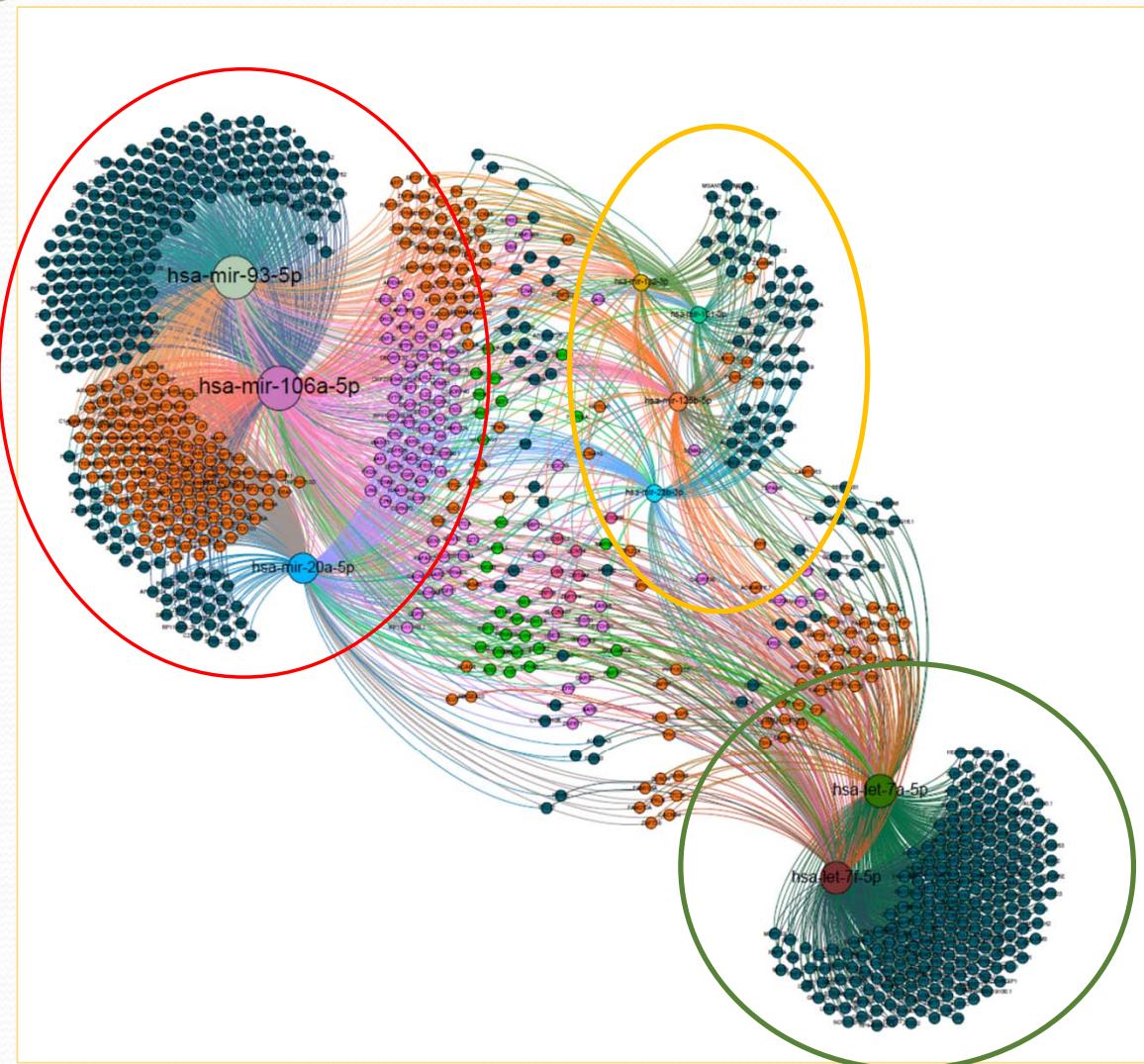
AUC: 0.679

CA 19.9 + miR-125b-5p



AUC : 0.733

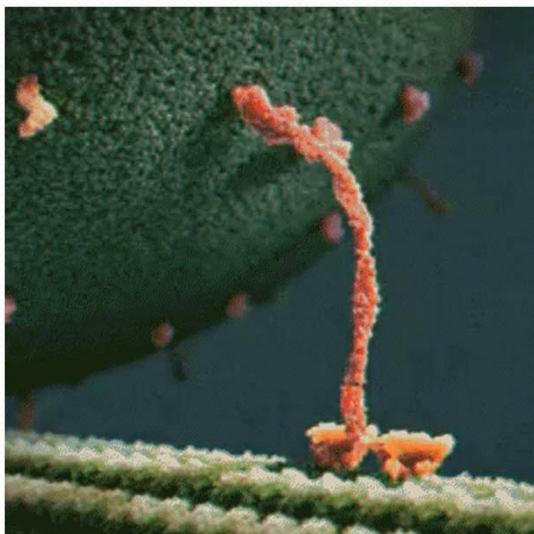
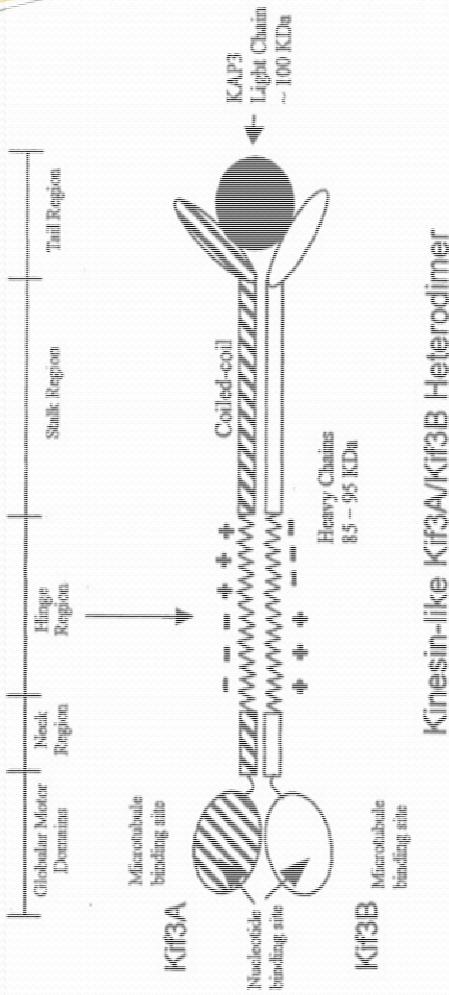
Biological significance: miRNAs act cooperatively in PC: Drivers (Ras), EMT, CSCs



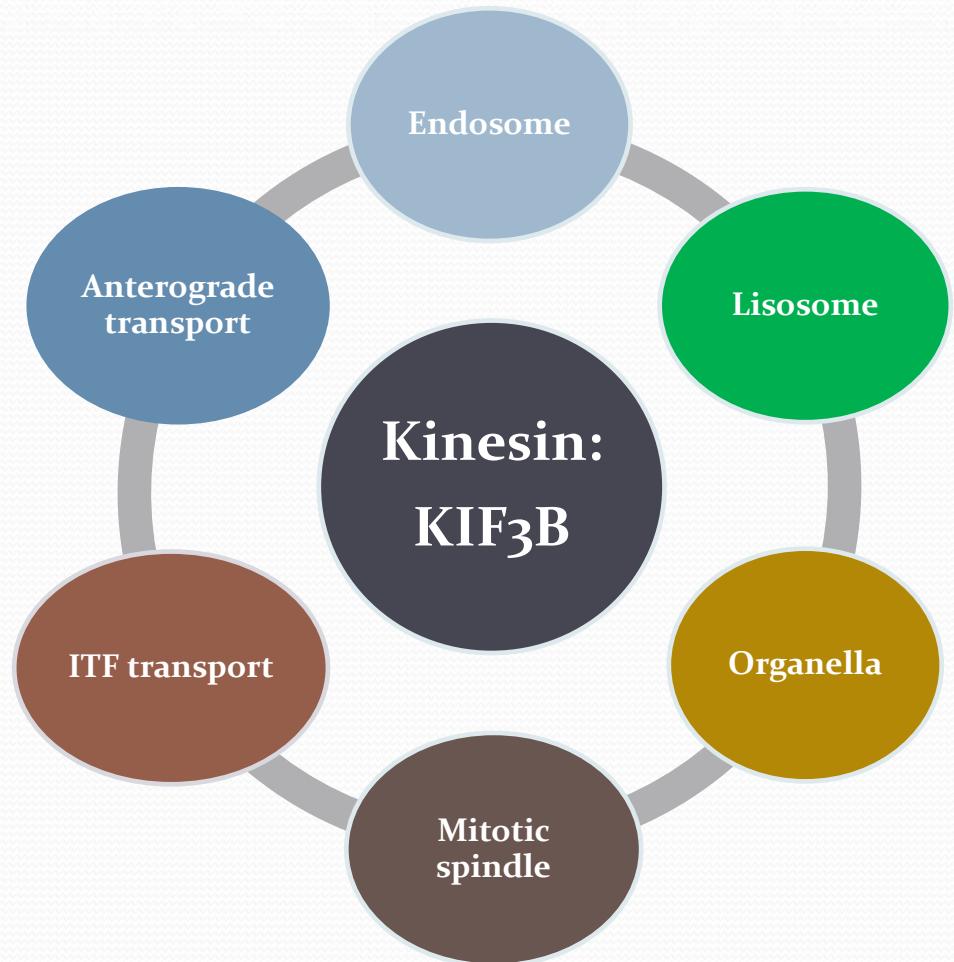


miRNAs target as Novel mediators in PC: novel therapeutic target for treatment development

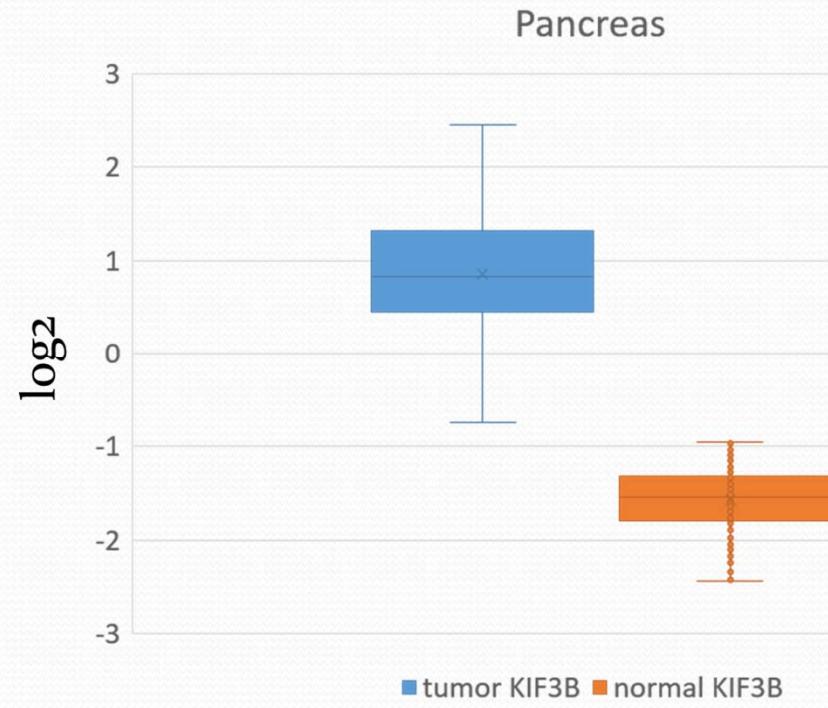
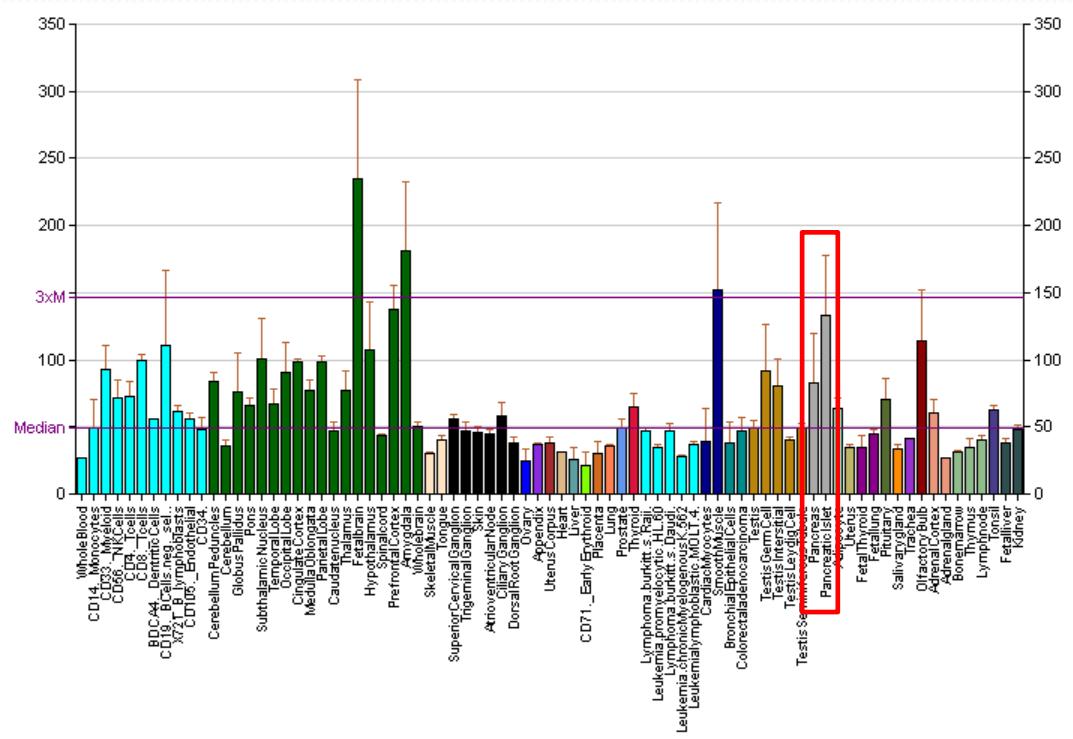
Kinesin Family member 3B: a motor protein in Kinesin complex



Kinesin-like Kif3A/Kif3B Heterodimer



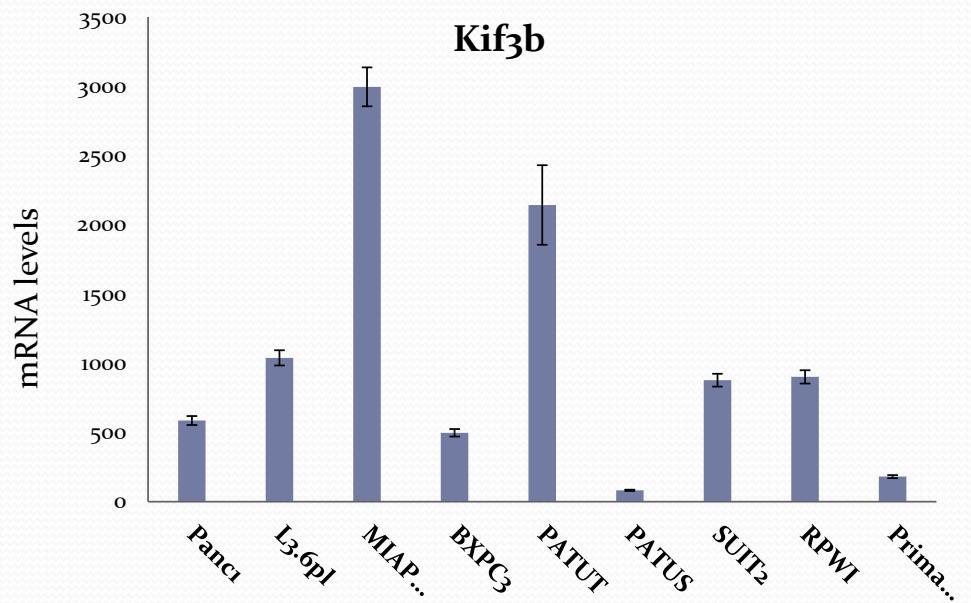
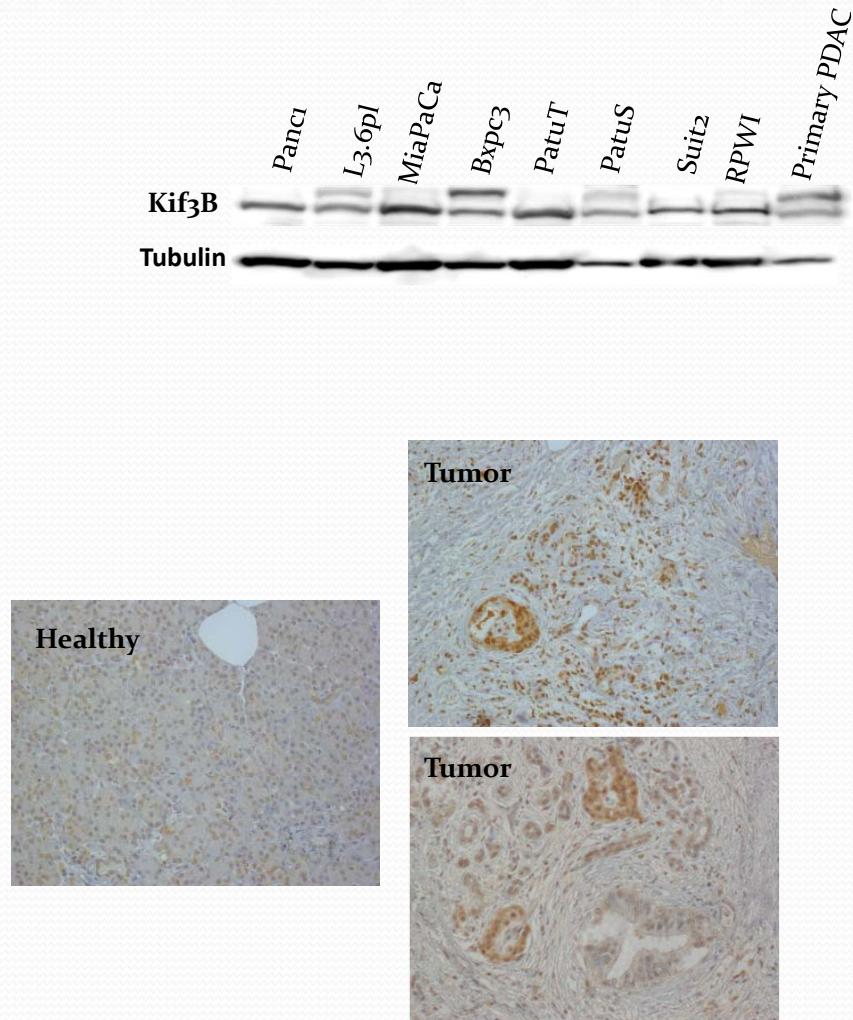
Kinesin Family Member 3B in Pancreas and PDAC



TCGA / TGEX

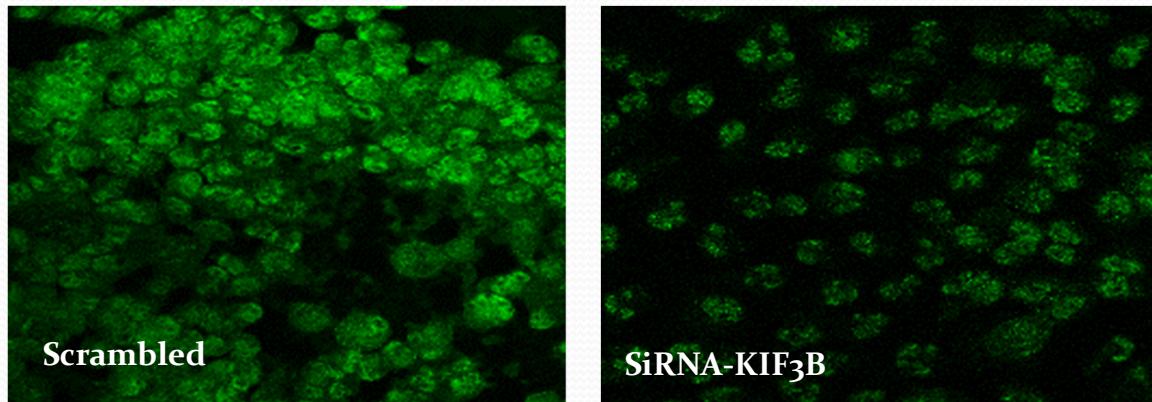
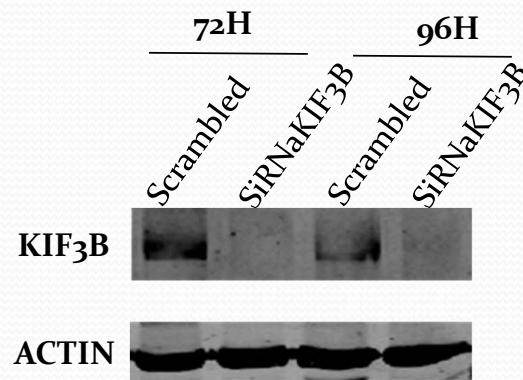
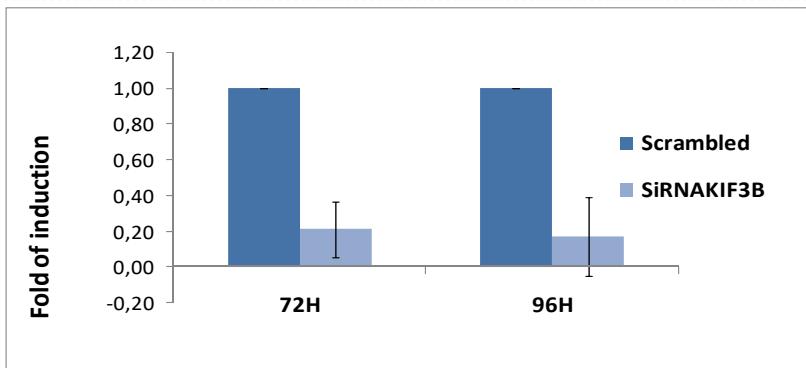
KIF3B expression in PDAC: cells and human tissue

VISION



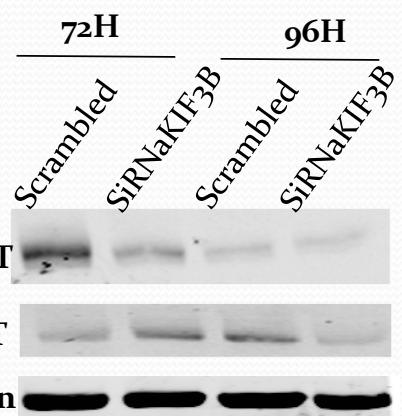
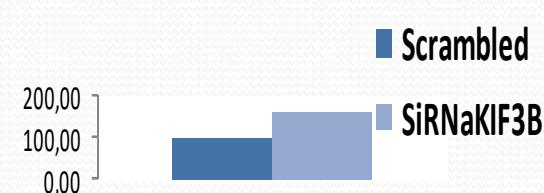
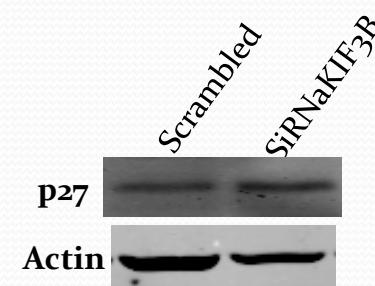
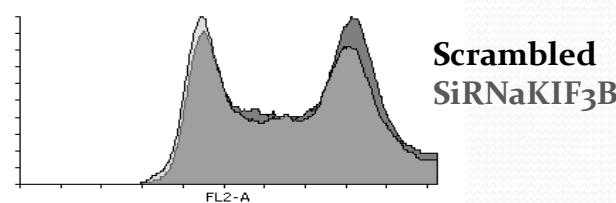
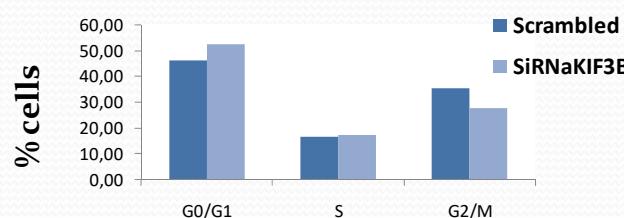
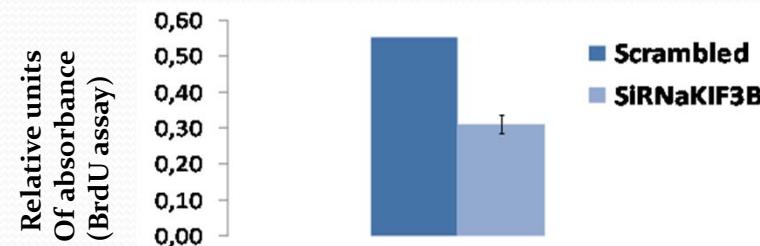
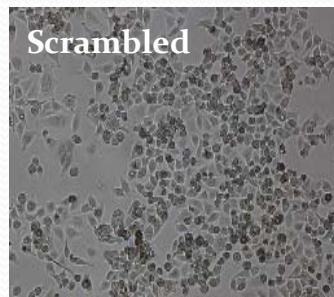
KIF₃B interference in Suit2 cells

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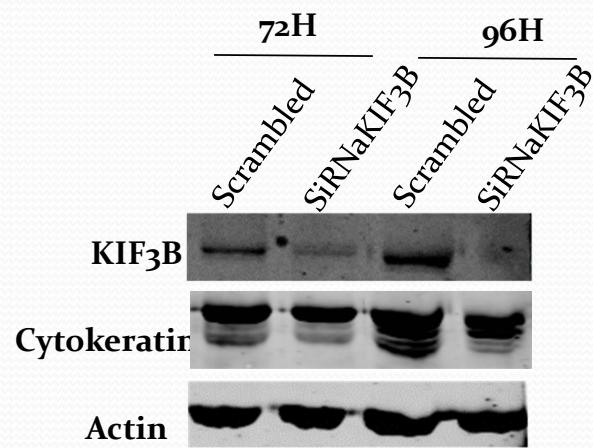
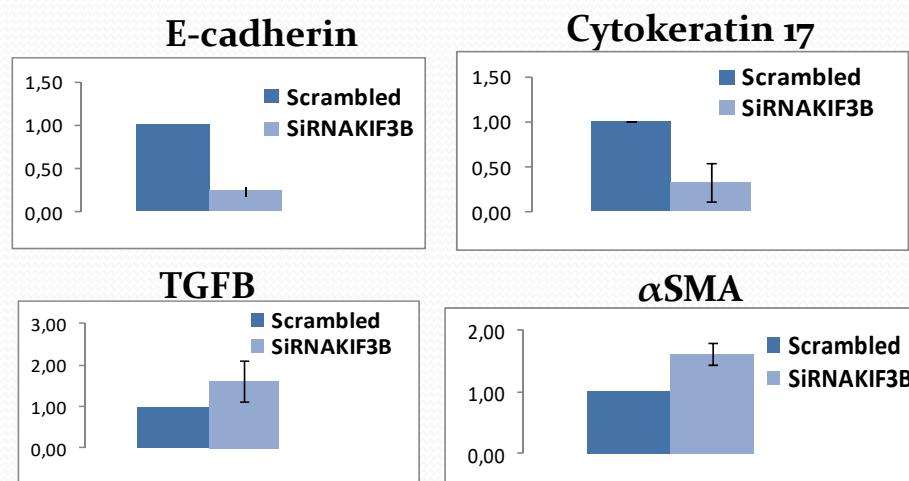
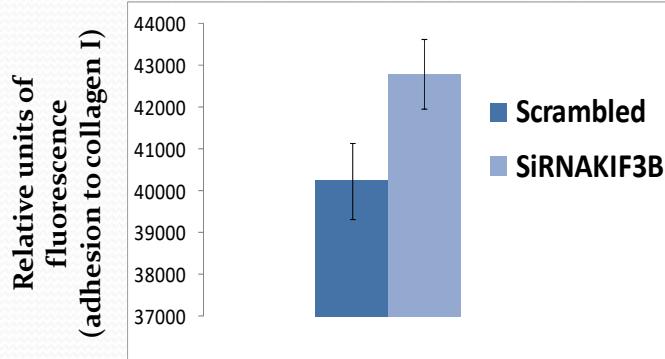
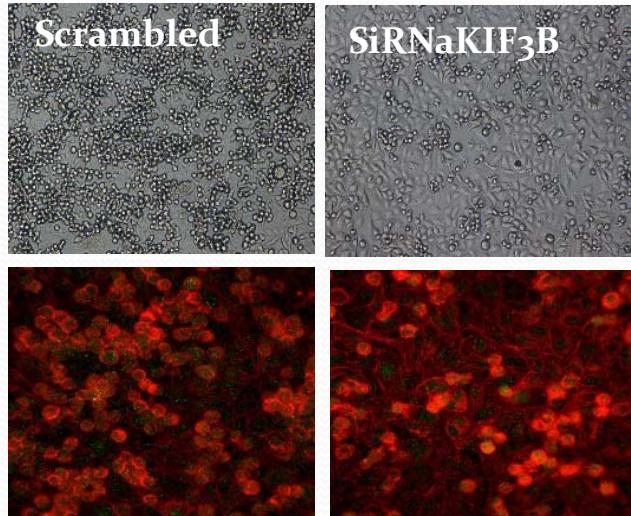


KIF3B knock-down reduces Suit2 proliferation

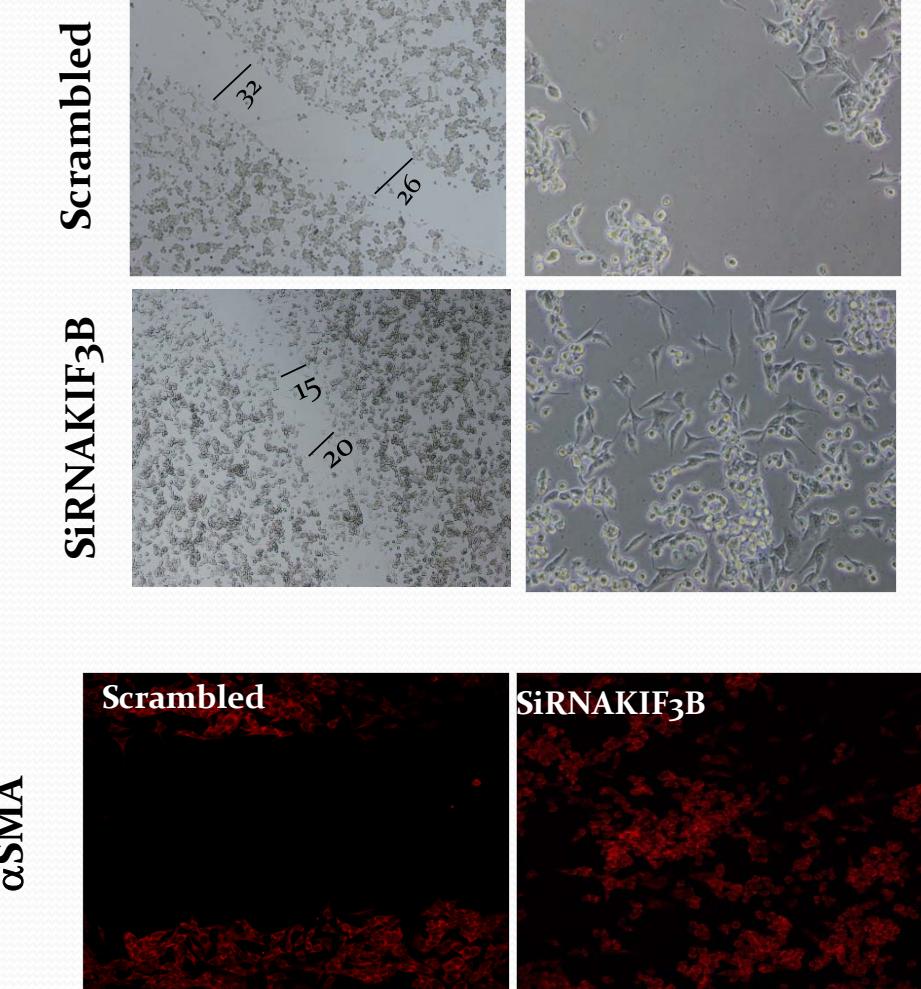
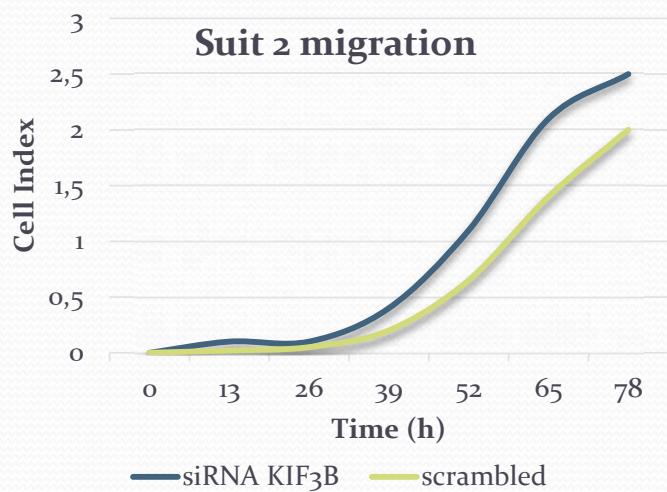
VISION



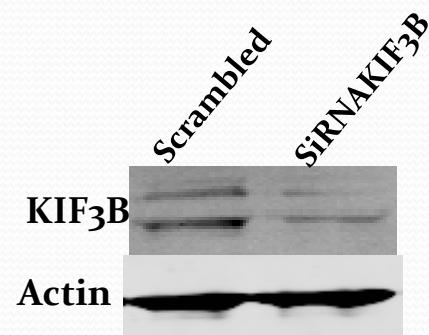
KIF3B knock-down promotes EMT in Suit2



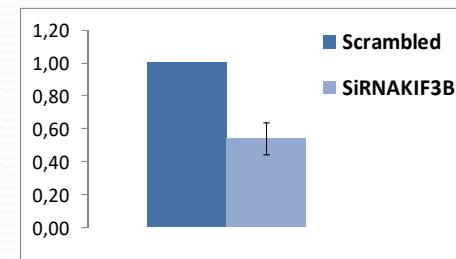
KIF3B knock-down promotes migration in Suit2



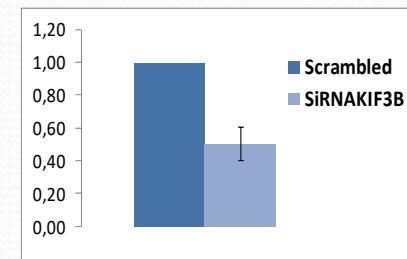
KIF3B knock-down promotes EMT in RPWI cells



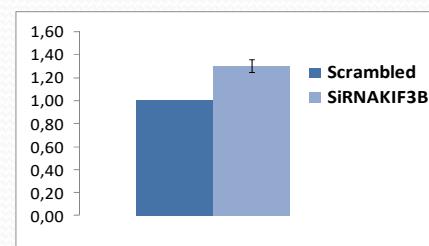
E-cadherin



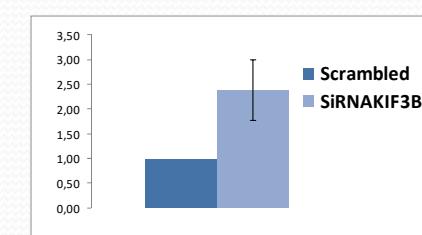
Cytokeratin 17



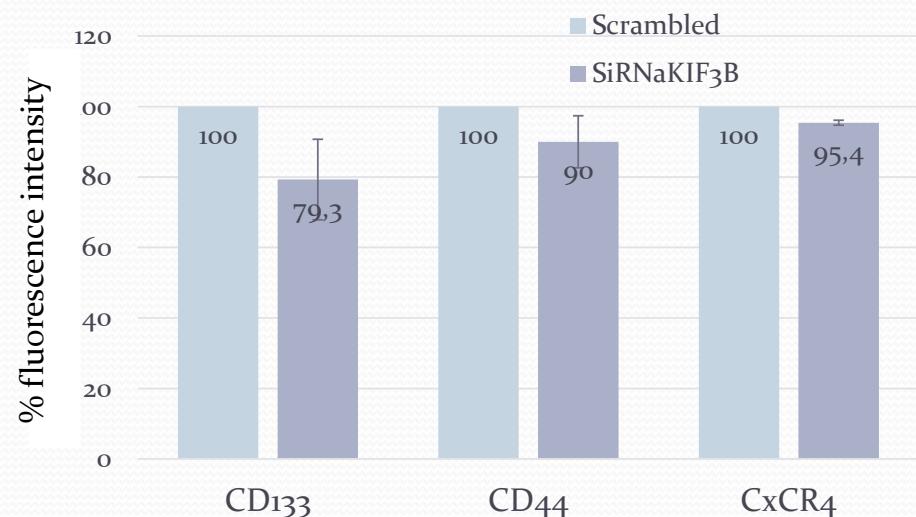
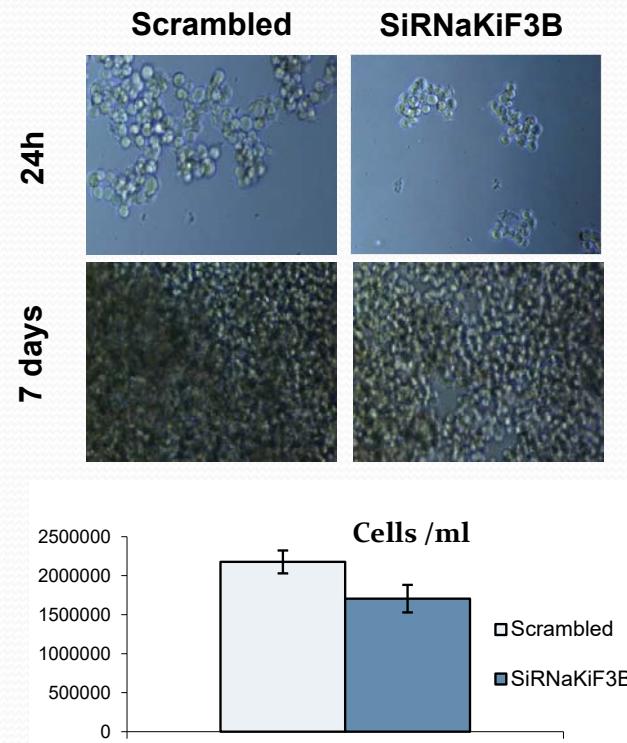
α SMA



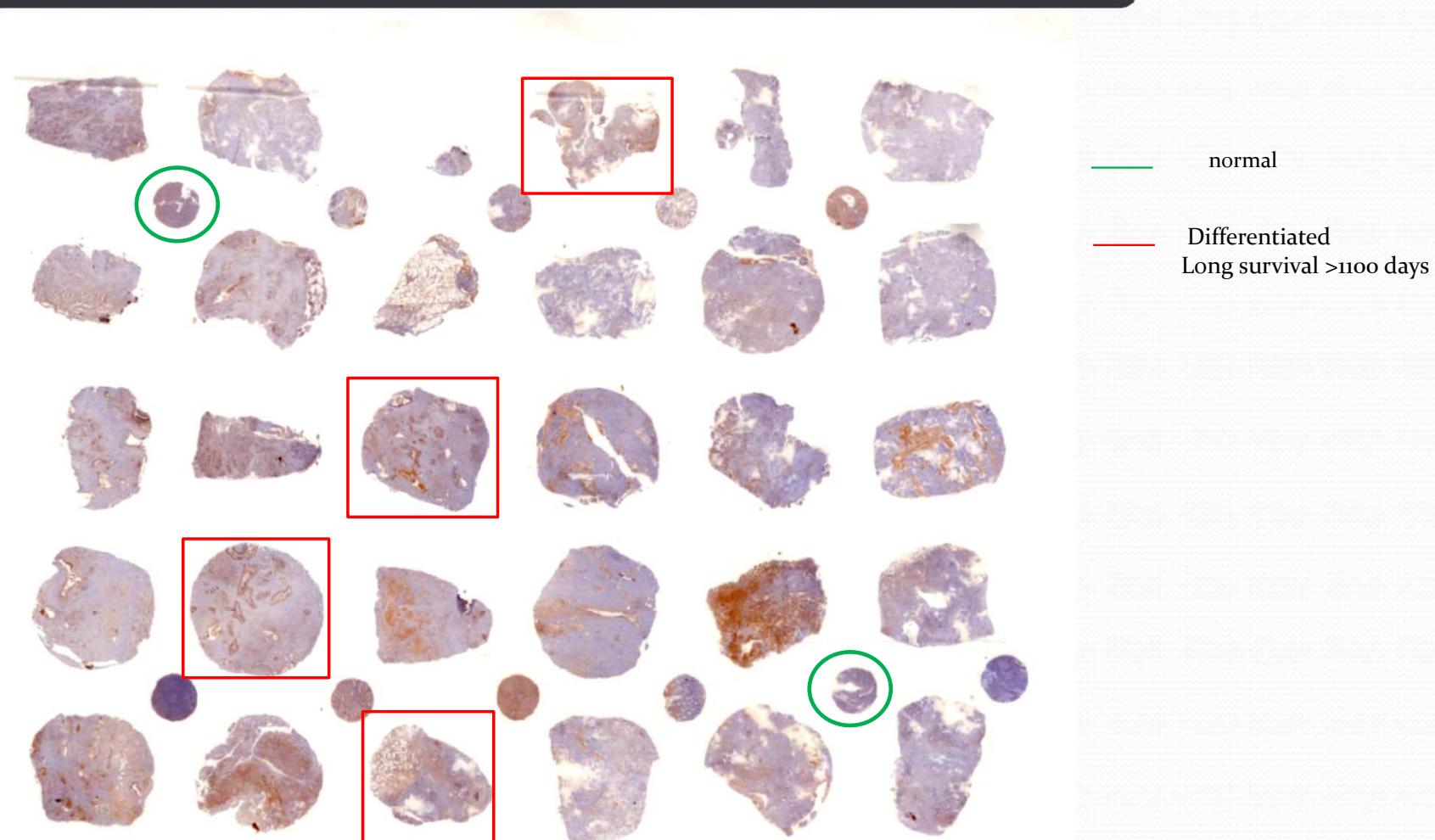
TGFB



KIF3B knock-down reduces tumor spheroids formation Cell plasticity



KIF3B in biopsies could associate to patient survival



Summary

- Molecular and cellular mechanisms studies are critical for PC improvement
 - Depper characterization of drivers and susceptibility genes
 - miRNAs are novel mechanisms in PC
 - miRNAs are useful biomarkers in PC prognosis and management
 - miRNAs could be useful biomarkers for molecular characterization of disease .
 - miRNAs could contribute to better characterized Sporadic and Familial PC
 - miRNAs and targets could shed light into PC molecular mechanisms
 - KIF3B could be critical mediator of EMT and cell plasticity in PC.
- ✓ PC personalized management and treatment election based on better understanding of mechanisms and accurate biomarkers identification and validation

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