Pancreatic cancer

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Connecting the Cancer Community

TRACO, 2016

Pancreatic Cancer: Current Understanding and Future Challenges

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Laboratory of Human Carcinogenesis

Cancer incidence and mortality

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Pancreatic Cancer Incidence and Mortality

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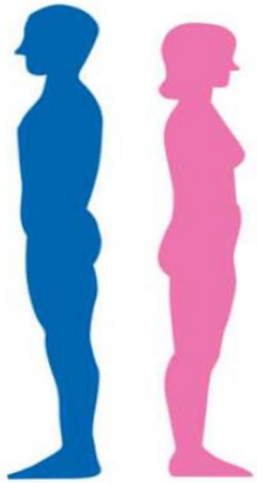
Estimated Deaths

Siegel R et. al., CA Cancer J Clin, 65, 2015

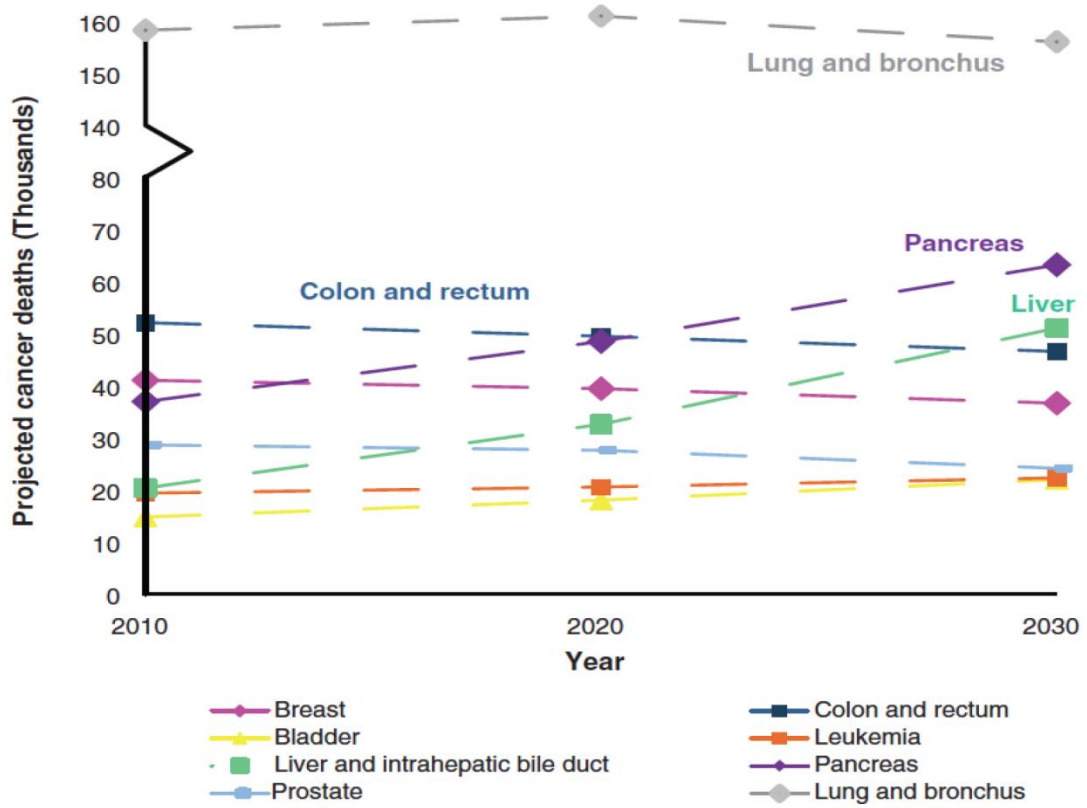
Males				Females		
Lung & bronchus	86,380	28%		Lung & bronchus	71,660	26%
Prostate	27,540	9%		Breast	40,290	15%
Colon & rectum	26,100	8%		Colon & rectum	23,600	9%
Pancreas	20,710	7%		Pancreas	19,850	7%
Liver & intrahepatic bile duct	17,030	5%		Ovary	14,180	5%
Leukemia	14,210	5%		Leukemia	10,240	4%
Esophagus	12,600	4%		Uterine corpus	10,170	4%
Urinary bladder	11,510	4%		Non-Hodgkin lymphoma	8,310	3%
Non-Hodgkin lymphoma	11,480	4%		Liver & intrahepatic bile duct	7,520	3%
Kidney & renal pelvis	9,070	3%		Brain & other nervous system	6,380	2%
All Sites	**312,150**	**100%**		**All Sites**	**277,280**	**100%**



- 4th Leading Cause of Cancer Deaths in the United States.
- Median Survival < 6 Months.
- Estimated 48,960 New Cases and 40,560 Deaths in 2015.
- No Effective Treatment.

Pancreatic cancer deaths are increasing

Pancreatic Cancer: Second Leading Cause of Cancer-related Death by 2030



Rahib, L., et. al., Cancer Res., 74, 2913-21, 2014

Risk factors

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Risk Factors and Inherited Syndromes

Table 1. Risk Factors and Inherited Syndromes Associated with Pancreatic Cancer.*

Variable	Approximate Risk
Risk factor	
Smoking[3]	2–3
Long-standing diabetes mellitus[4]	2
Nonhereditary and chronic pancreatitis[5]	2–6
Obesity, inactivity, or both[6]	2
Non–O blood group[7]	1–2
Genetic syndrome and associated gene or genes — %	
Hereditary pancreatitis (*PRSS1, SPINK1*)[8]	50
Familial atypical multiple mole and melanoma syndrome (*p16*)[9]	10–20
Hereditary breast and ovarian cancer syndromes (*BRCA1, BRCA2, PALB2*)[10,11]	1–2
Peutz–Jeghers syndrome (*STK11* [*LKB1*])[12]	30–40
Hereditary nonpolyposis colon cancer (Lynch syndrome) (*MLH1, MSH2, MSH6*)[13]	4
Ataxia–telangiectasia (*ATM*)[14]	Unknown
Li–Fraumeni syndrome (P53)[15]	Unknown

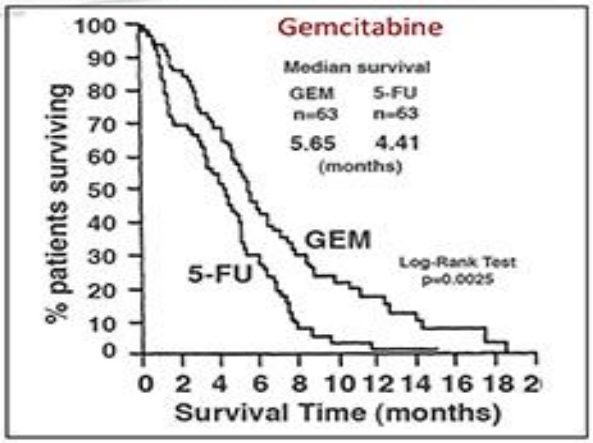
* Values associated with risk factors are expressed as relative risks, and values associated with genetic syndromes are expressed as lifetime risks, as compared with the risk in the general population.

Ryan, Hong and Bardeesy, NEJM, 371, 2014

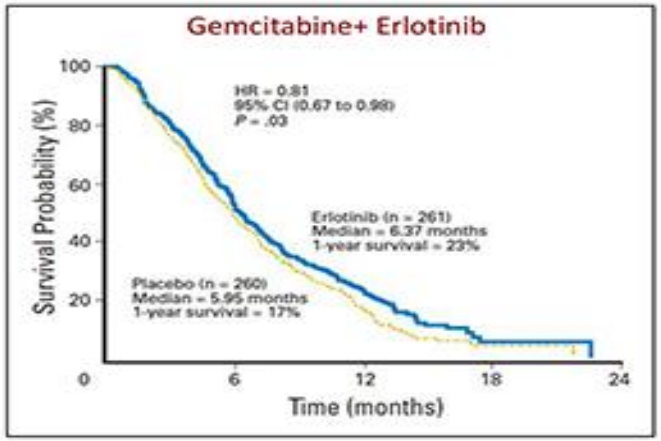
Pancreatic cancer patient treatment

Disappointing Progress in the Treatment of Pancreatic Cancer

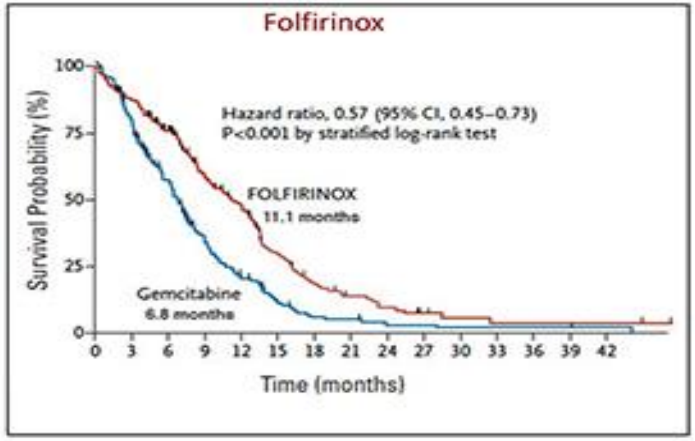
Burris et. al., J. Clin. Oncol., 15, 1997



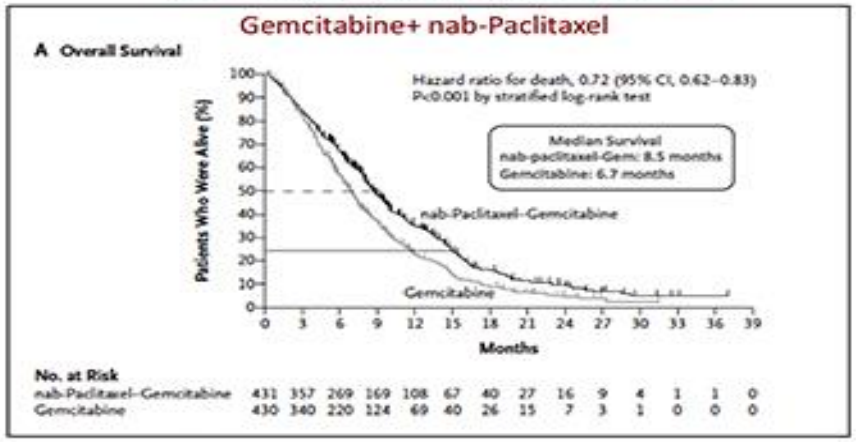
Moore et. al., J. Clin. Oncol. 25, 2007



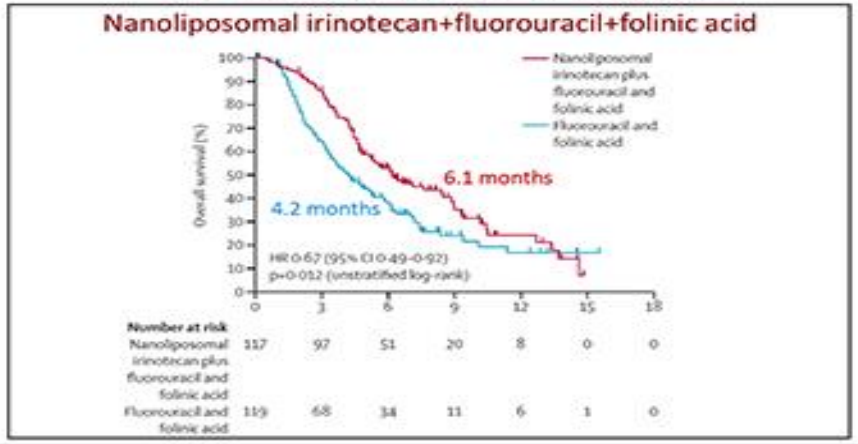
Conroy et. al., NEJM, 36, 2011



Von Hoff, D.D. et. al, NEJM, 369, Oct, 2013



Wang-Gillam A., et. al., Lancet, Nov 20, 2015



Resected pancreatic cancer

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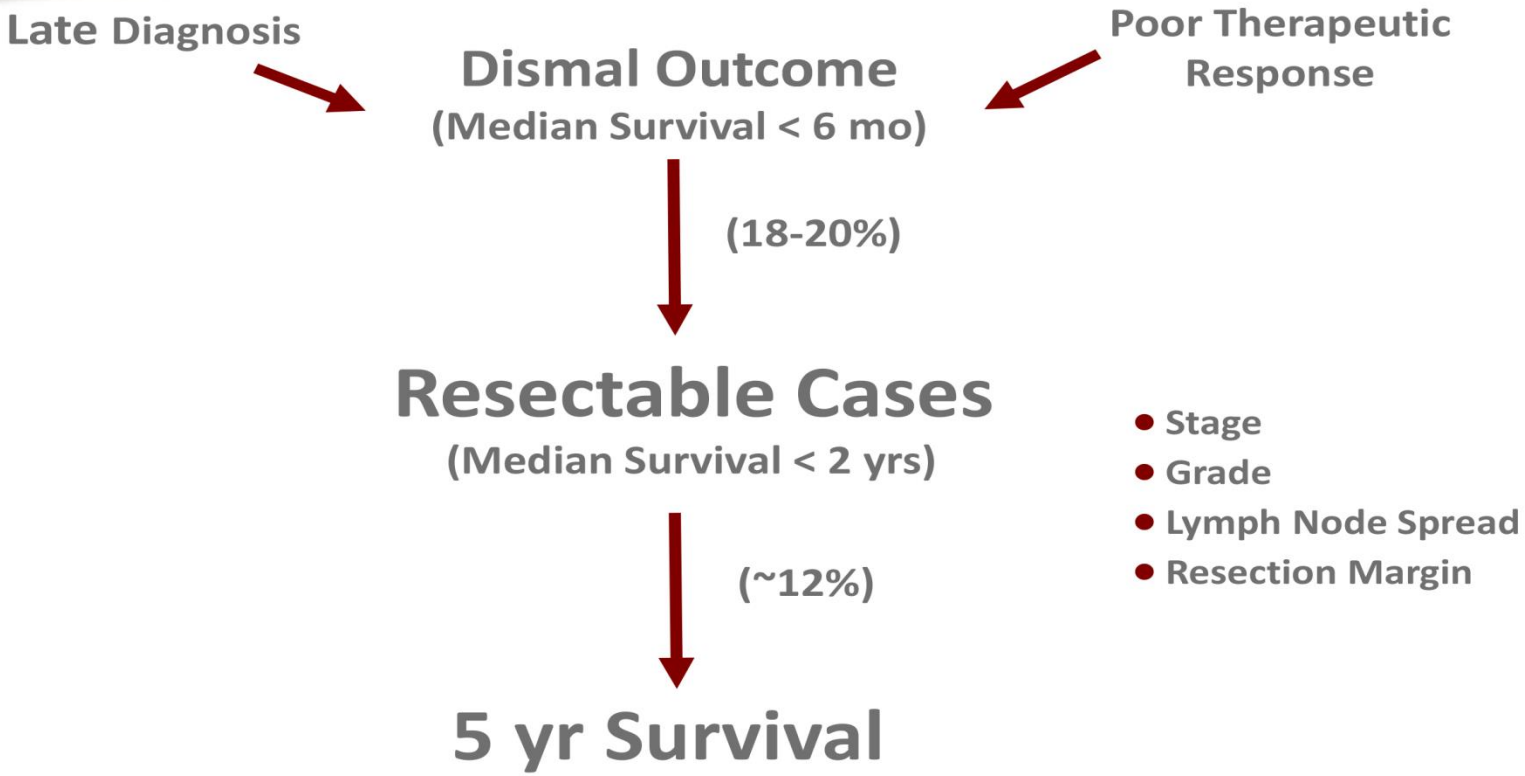
Improved Survival in Resected Pancreatic Cancer Cases



Molecular Differences in Tumors Determine Patient Outcome?

Tumor biology

Understanding Pancreatic Tumor Biology is Key to Improving Disease Outcome

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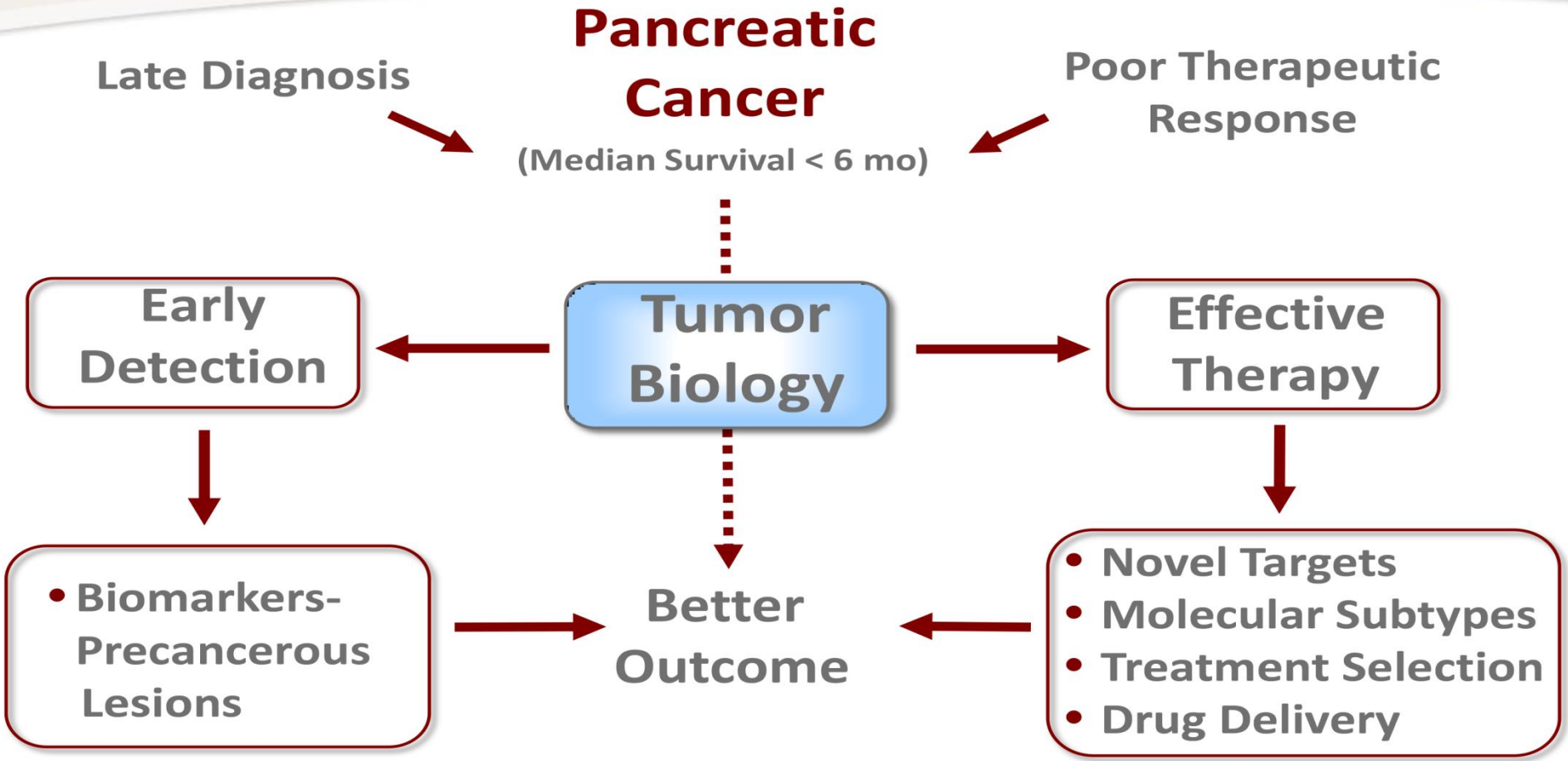

Pancreatic carcinogenesis

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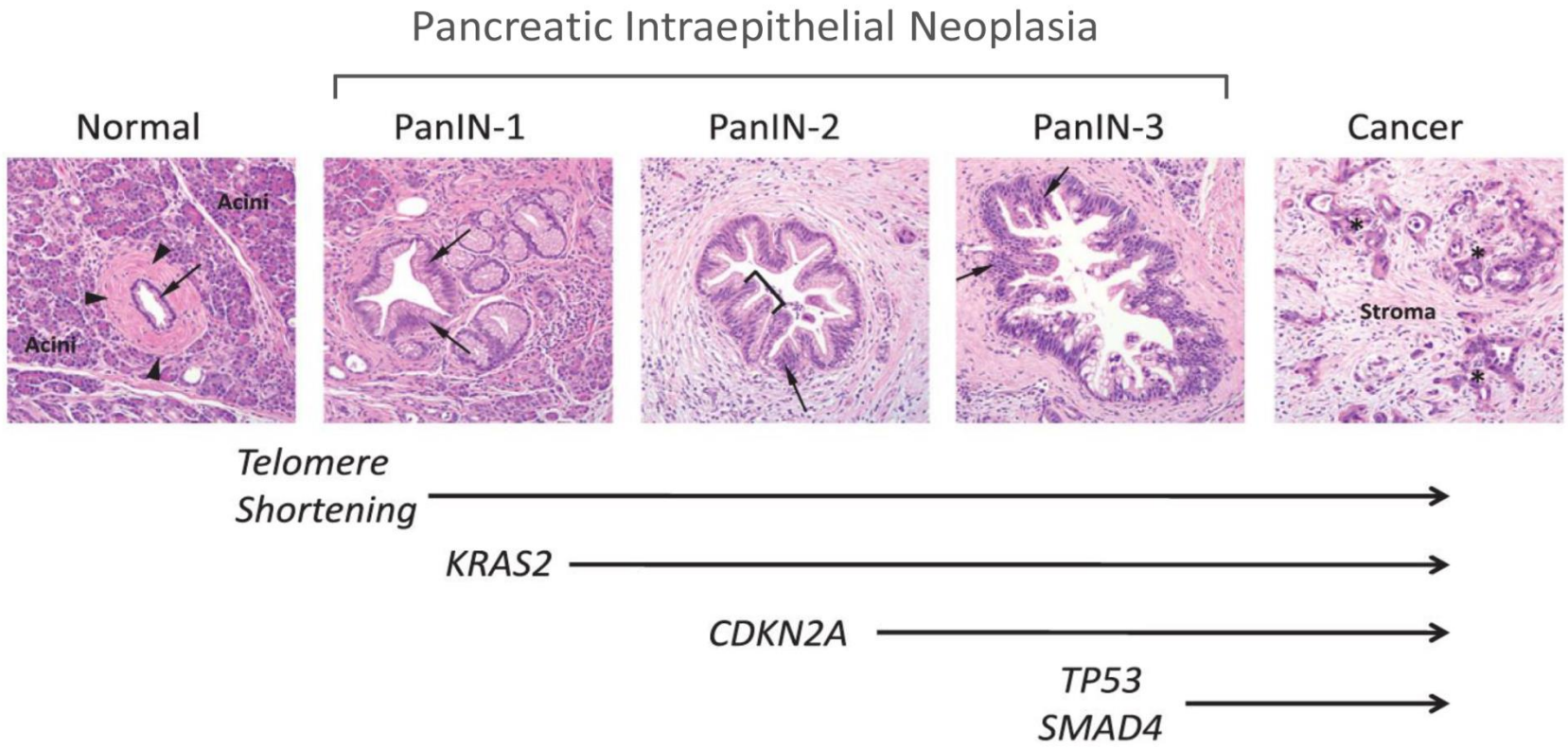
Progression Model of Pancreatic Carcinogenesis



Desmoplastic stroma

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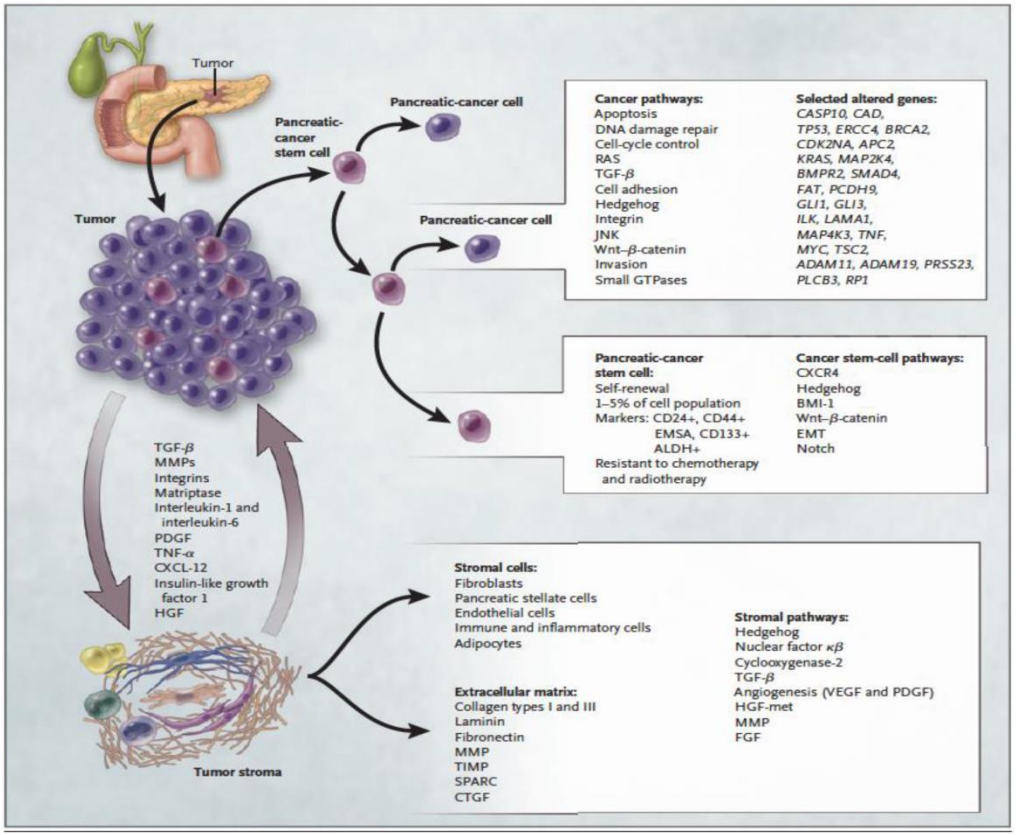
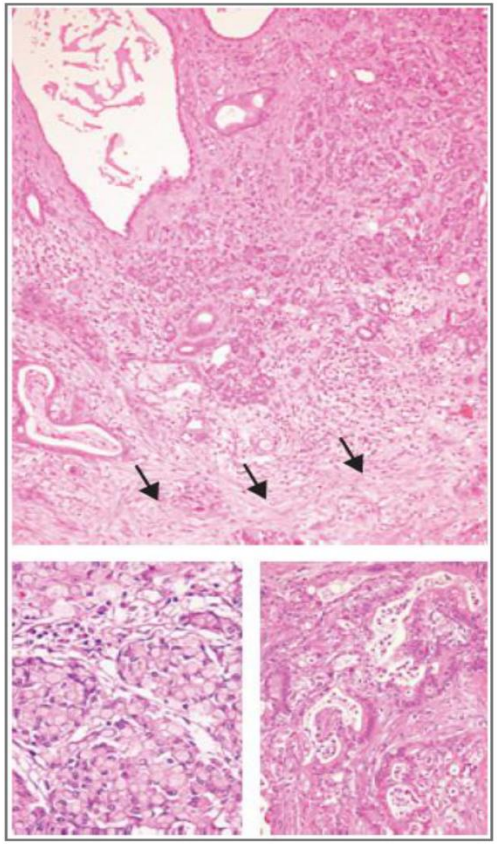
Prominent, Desmoplastic Stroma in Pancreatic Cancer





H/E

From: Hidalgo, M., N Engl J Med, 2010

Biomarkers

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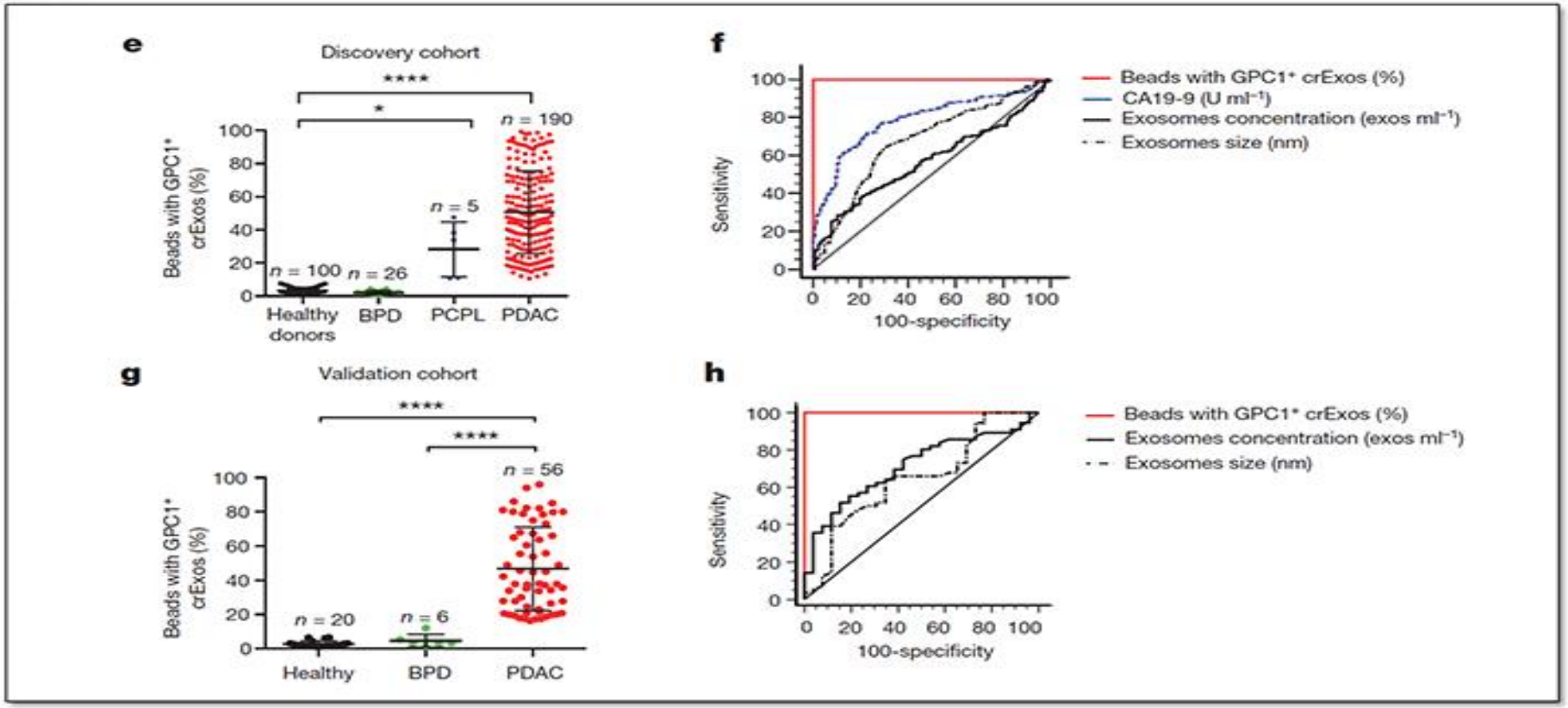
Glypican-1 Positive Circulating Exosomes as a Biomarker for PDAC



Melo, S.A., et al., Nature, 523, 2015

Glycan-1 positive exosomes

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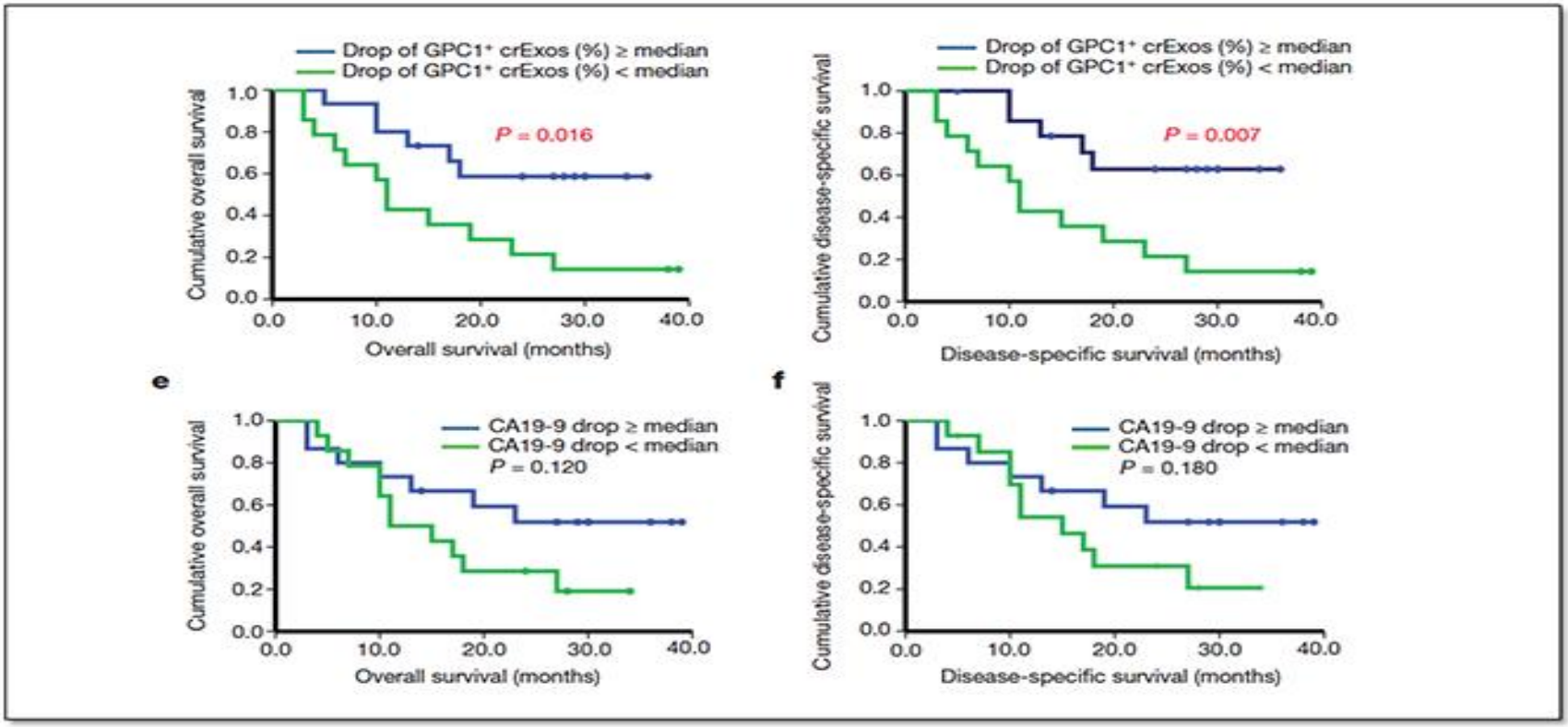
Glypican-1 Positive Circulating Exosomes Predicts Prognosis in Resected PDAC Patients



Melo, S.A., et al., Nature, 523, 2015

Pancreatic cancer and tumor heterogeneity

Tumor heterogeneity and molecular subtyps.

Heterogeneity

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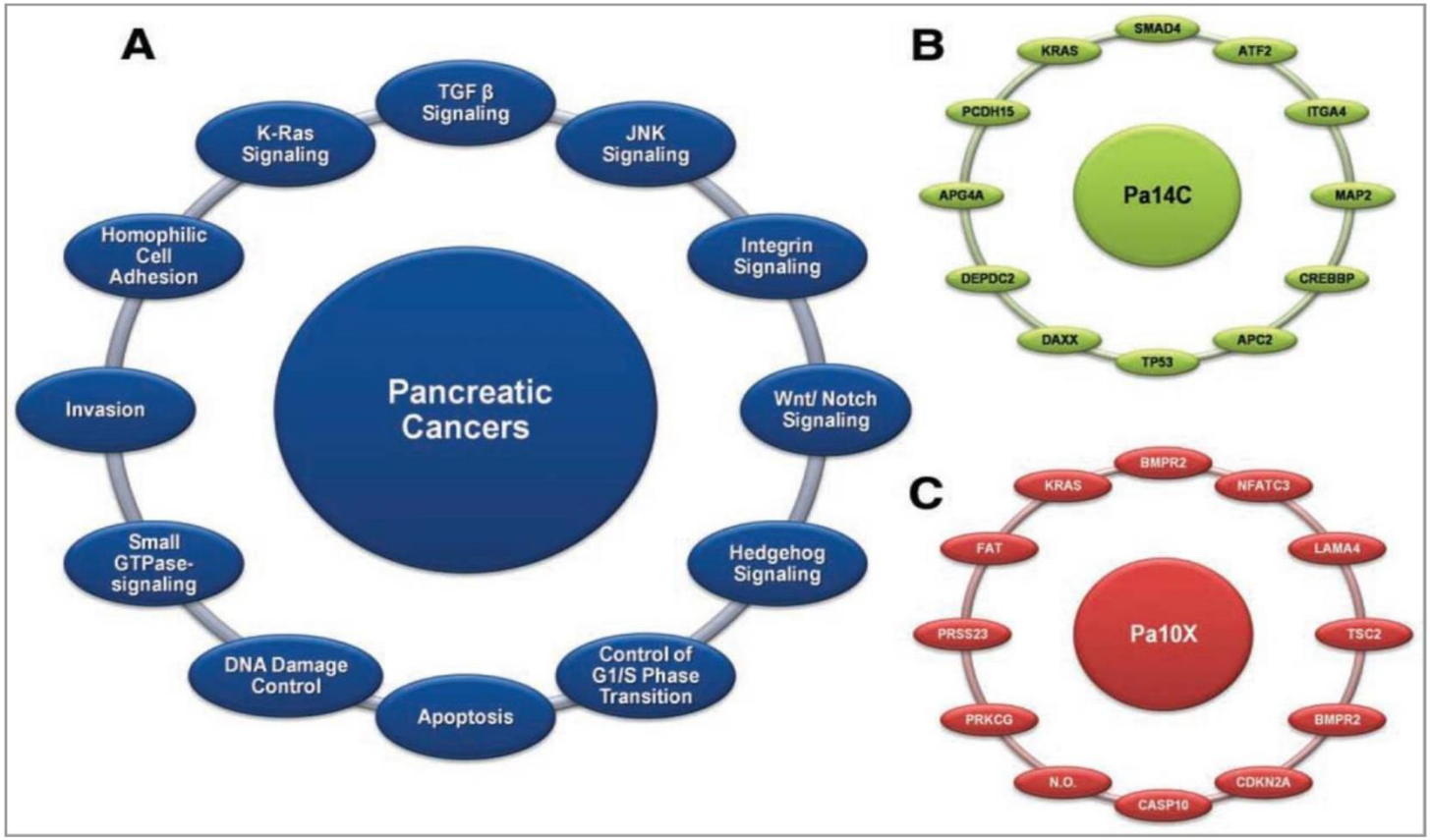
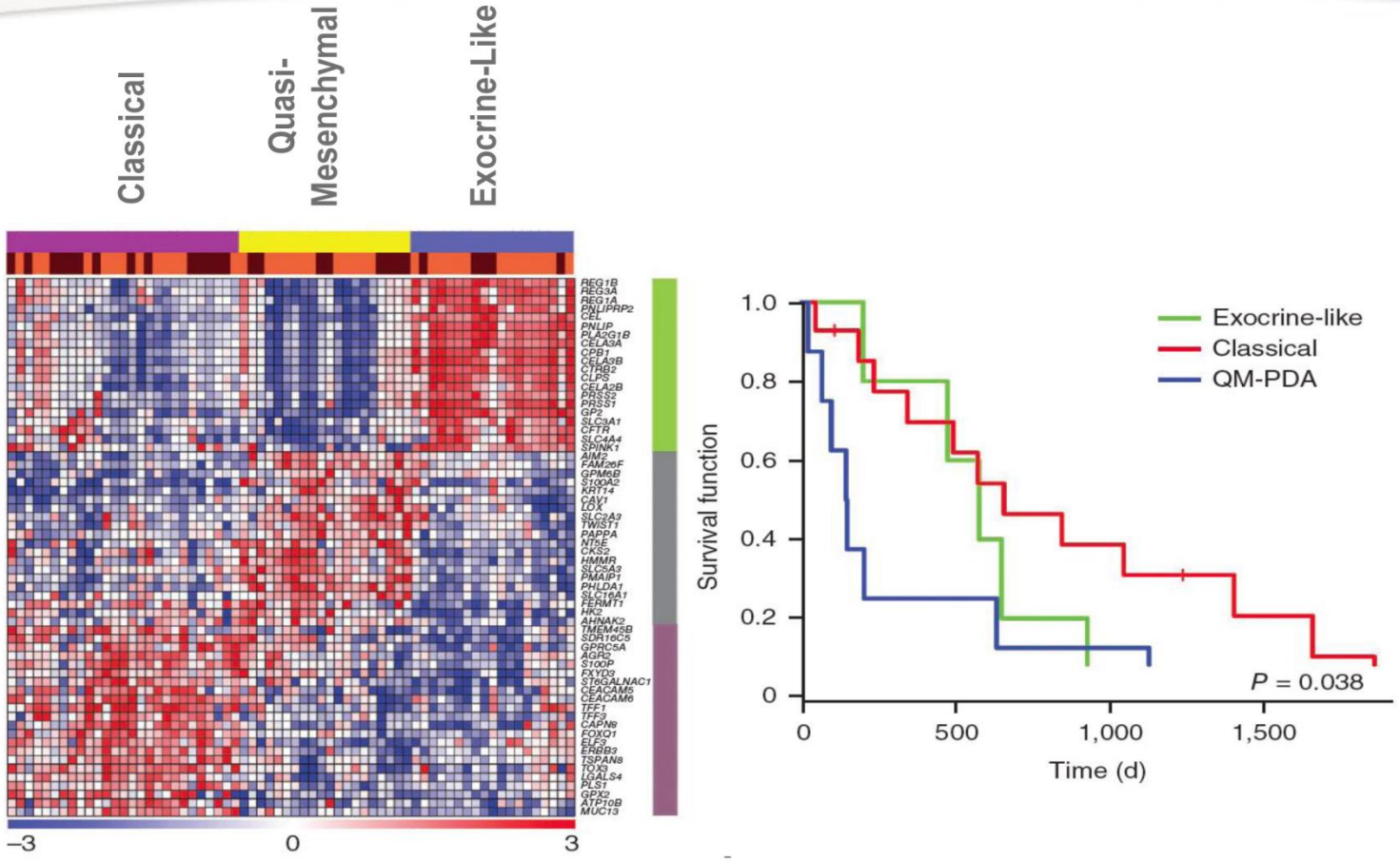
Pancreatic Cancer is Highly Heterogenous



From: Jone, S. et al., Science, 321, 2008

Molecular subtypes

Are There Different Molecular Subtypes of PDAC?



Collison et. al., Nat. Med., 17, 2011

Chromosomal structure

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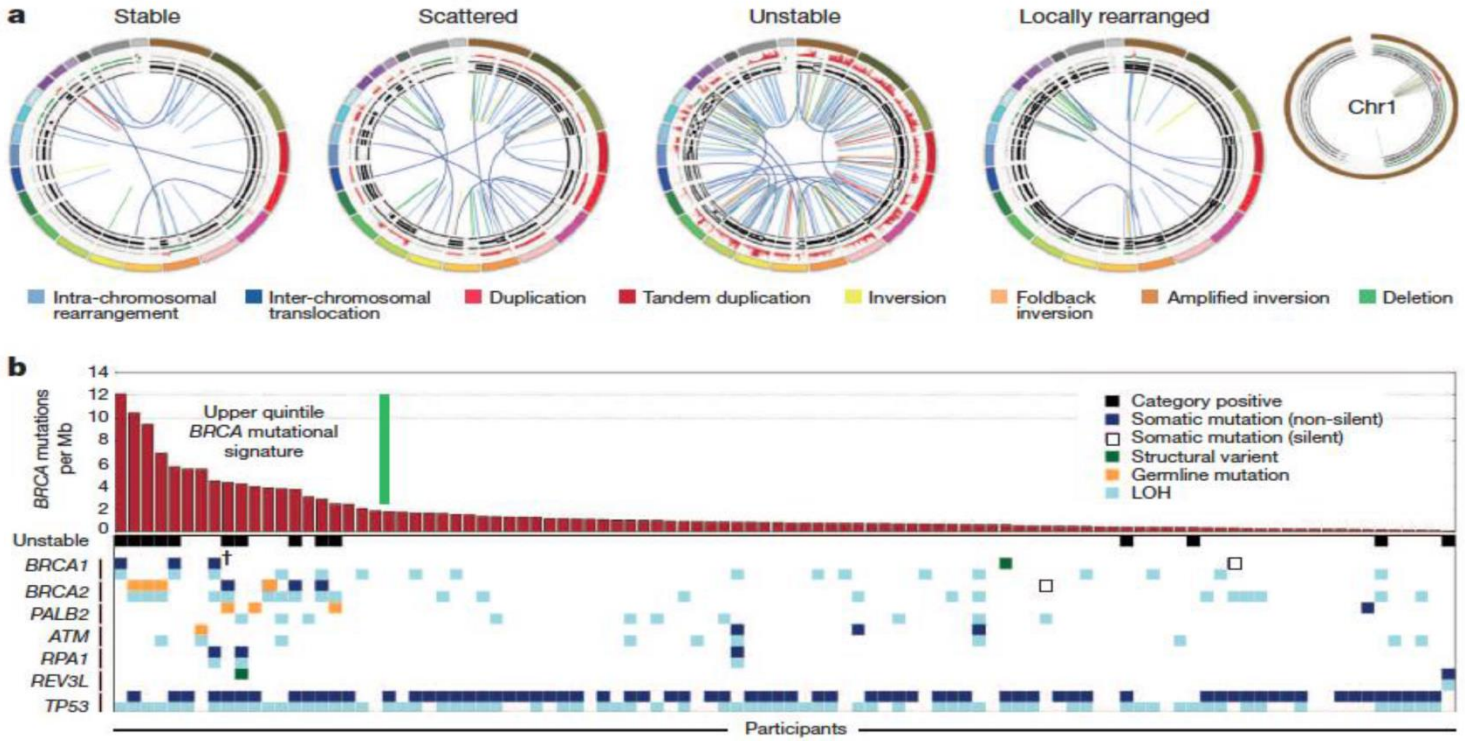
Variations in Chromosomal Structure and PDAC Subtypes



Waddell et. al., Nature, 518, 2015

Stroma specific subtypes

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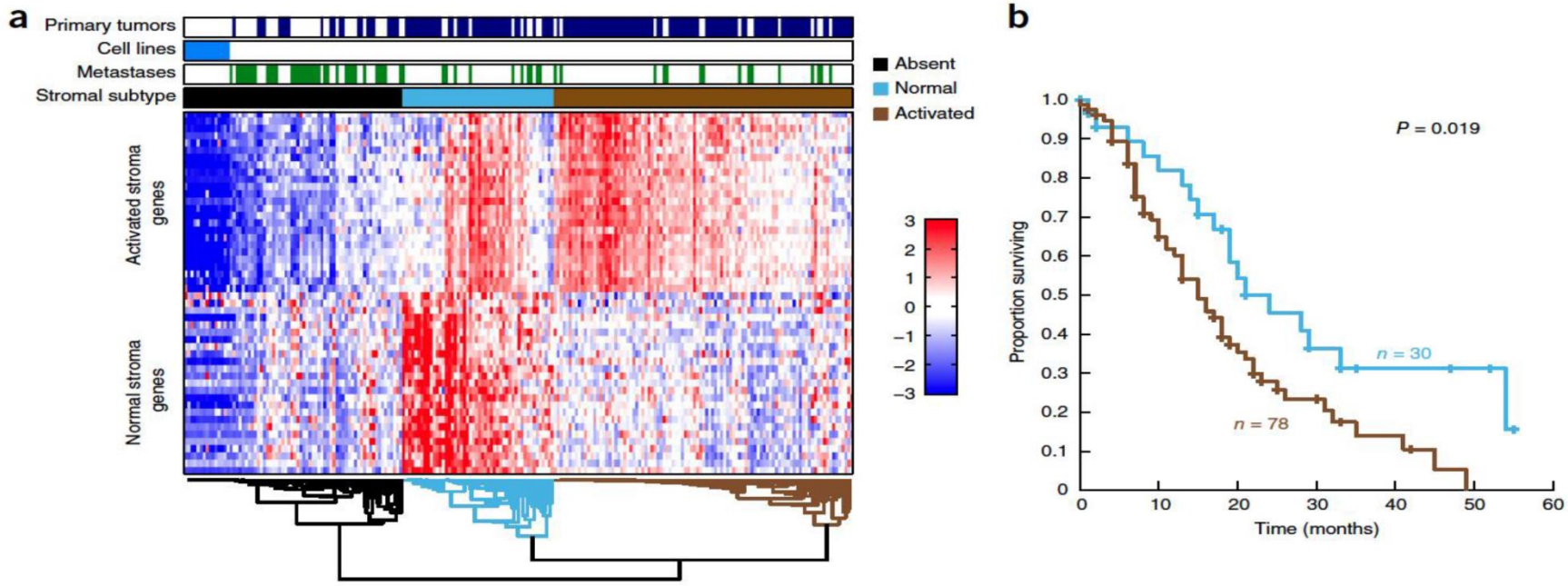
Stroma-Specific Subtypes in Pancreatic Cancer



Moffitt et. al., Nature Genetics, 2015

Four PDAC subtypes

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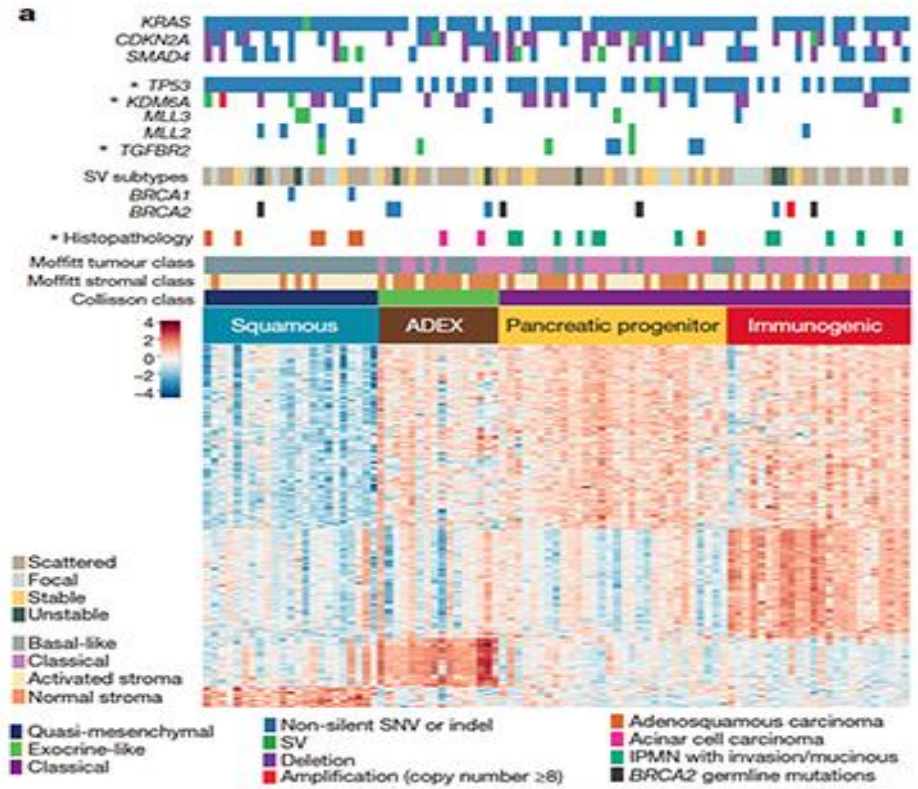
Gene Expression Analysis Identified 4 PDAC Subtypes



(N=456)

Bailey et. al., Nature, 2016

ADEX: Aberrantly differentiated exocrine and endocrine

Metabolic subtypes

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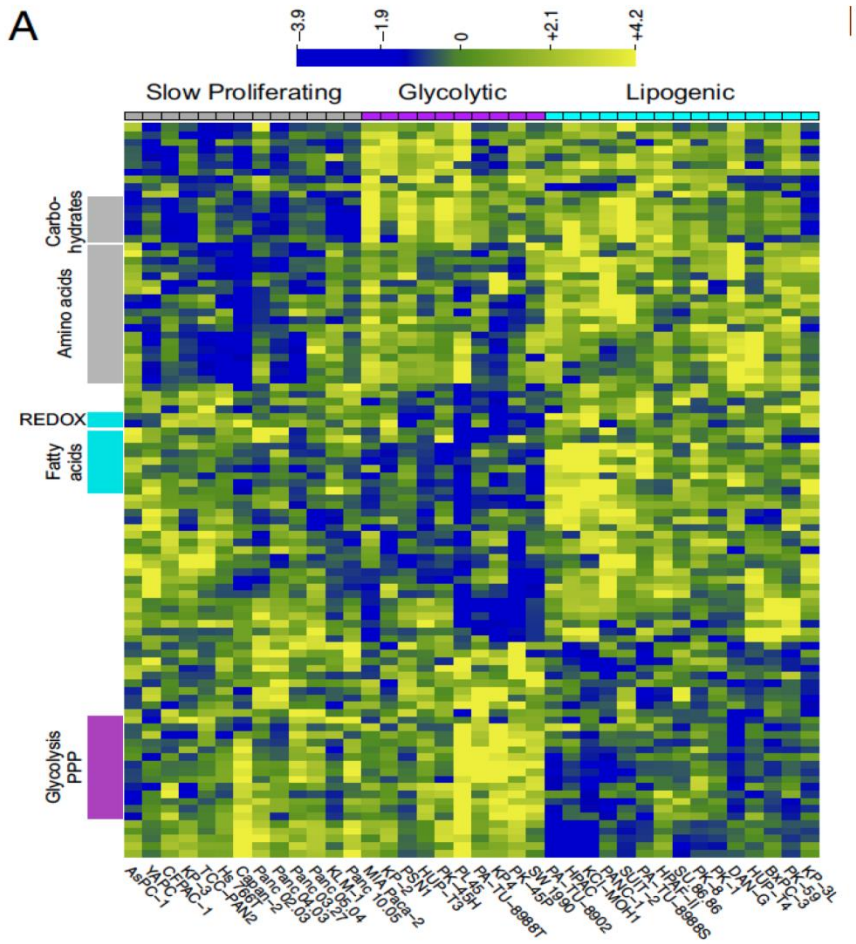
Metabolic Subtypes in Pancreatic Cancer



Daemen et. al., PNAS, 2015

Metabolic programming

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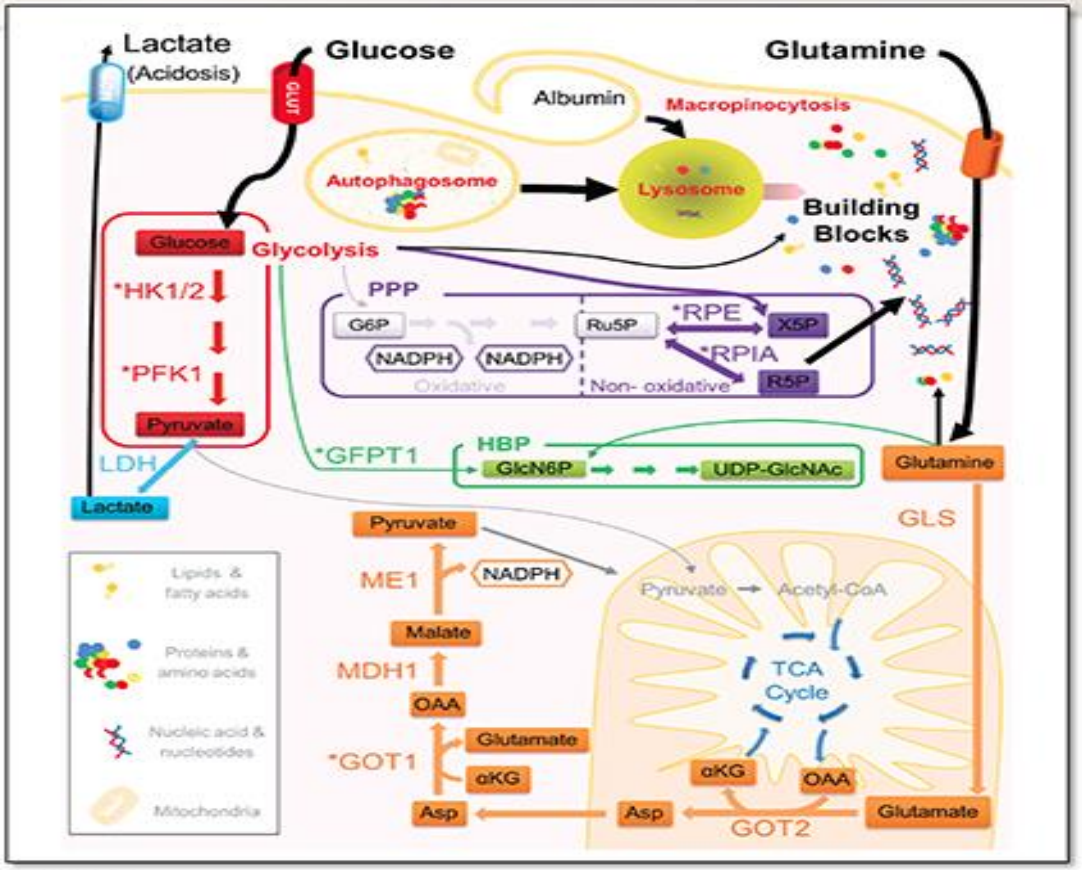
Metabolic Reprogramming in Pancreatic Cancer



Sousa and Kimmelman, Carcinogenesis, 35, 2014

Dessert

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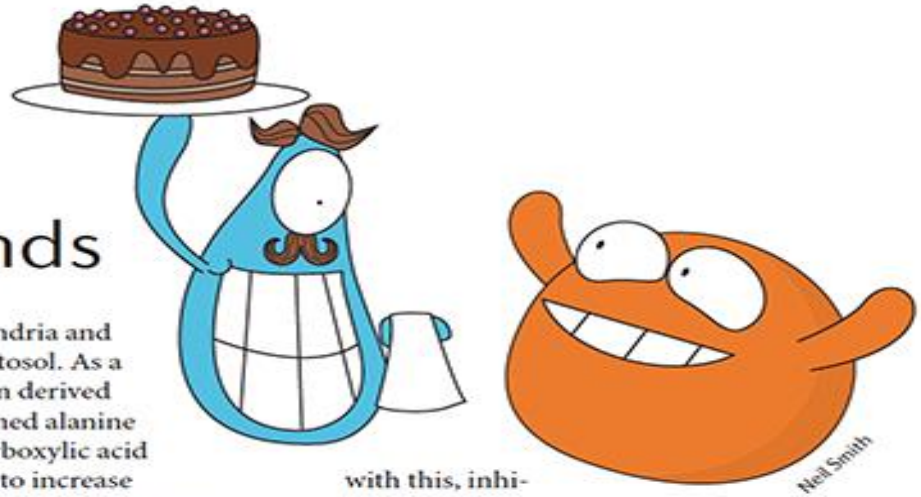
Dessert Anyone?

RESEARCH HIGHLIGHTS

Nature Reviews Cancer | Published online 26 Aug 2016; doi:10.1038/nrc.2016.96



TUMOUR METABOLISM

Feeding your friends

A characteristic feature of pancreatic ductal adenocarcinoma (PDAC) is a strong stromal reaction that is shaped by the activity of pancreatic stellate cells (PSCs). The resultant fibrosis impedes the tumour's access to a blood supply, creating an extremely hypoxic, nutrient-poor environment. the mitochondria and not in the cytosol. As a result, carbon derived from consumed alanine fed the tricarboxylic acid (TCA) cycle to increase oxygen consumption while not affecting glycolysis. Indeed, citrate with this, inhibition of autophagy in PSCs could reduce the growth of

Pancreatic stellate cells

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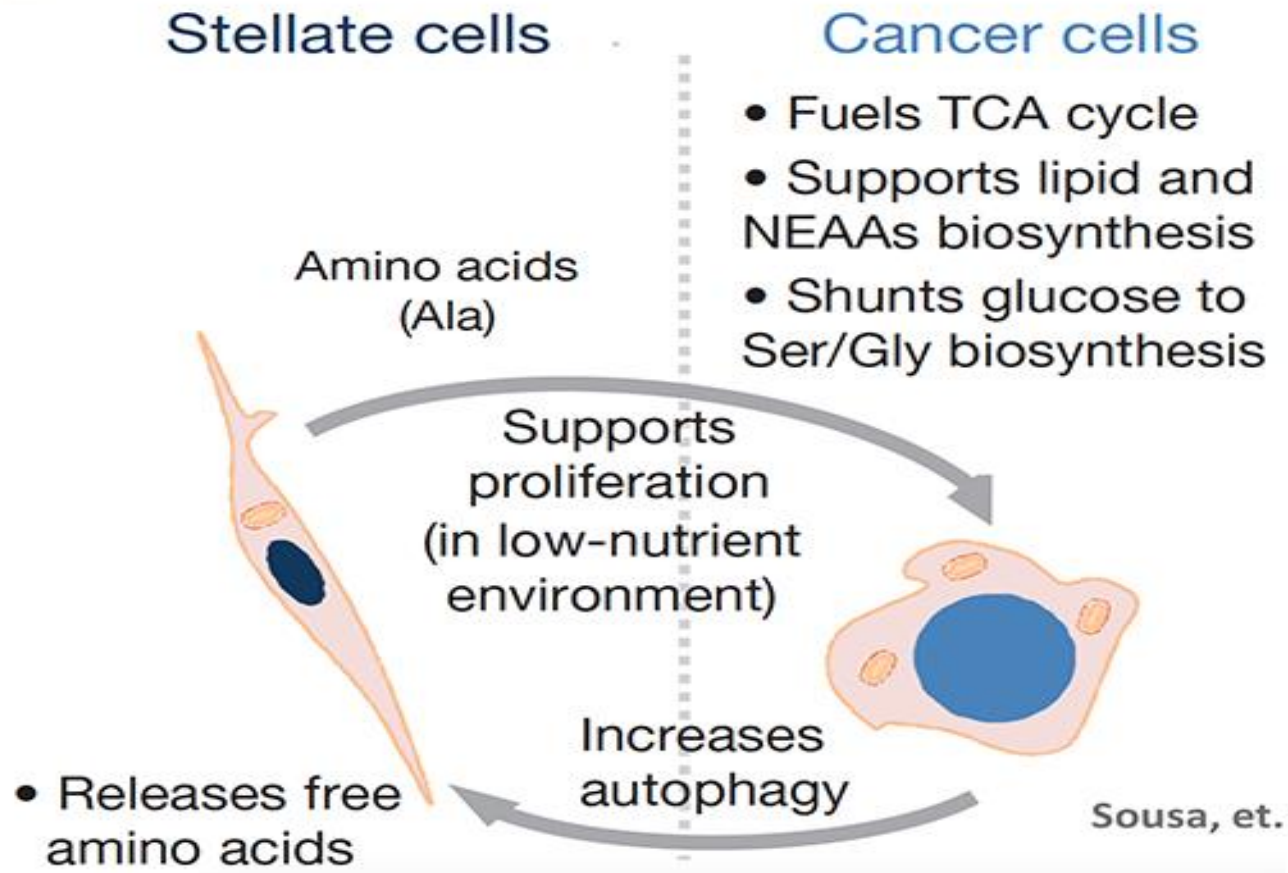
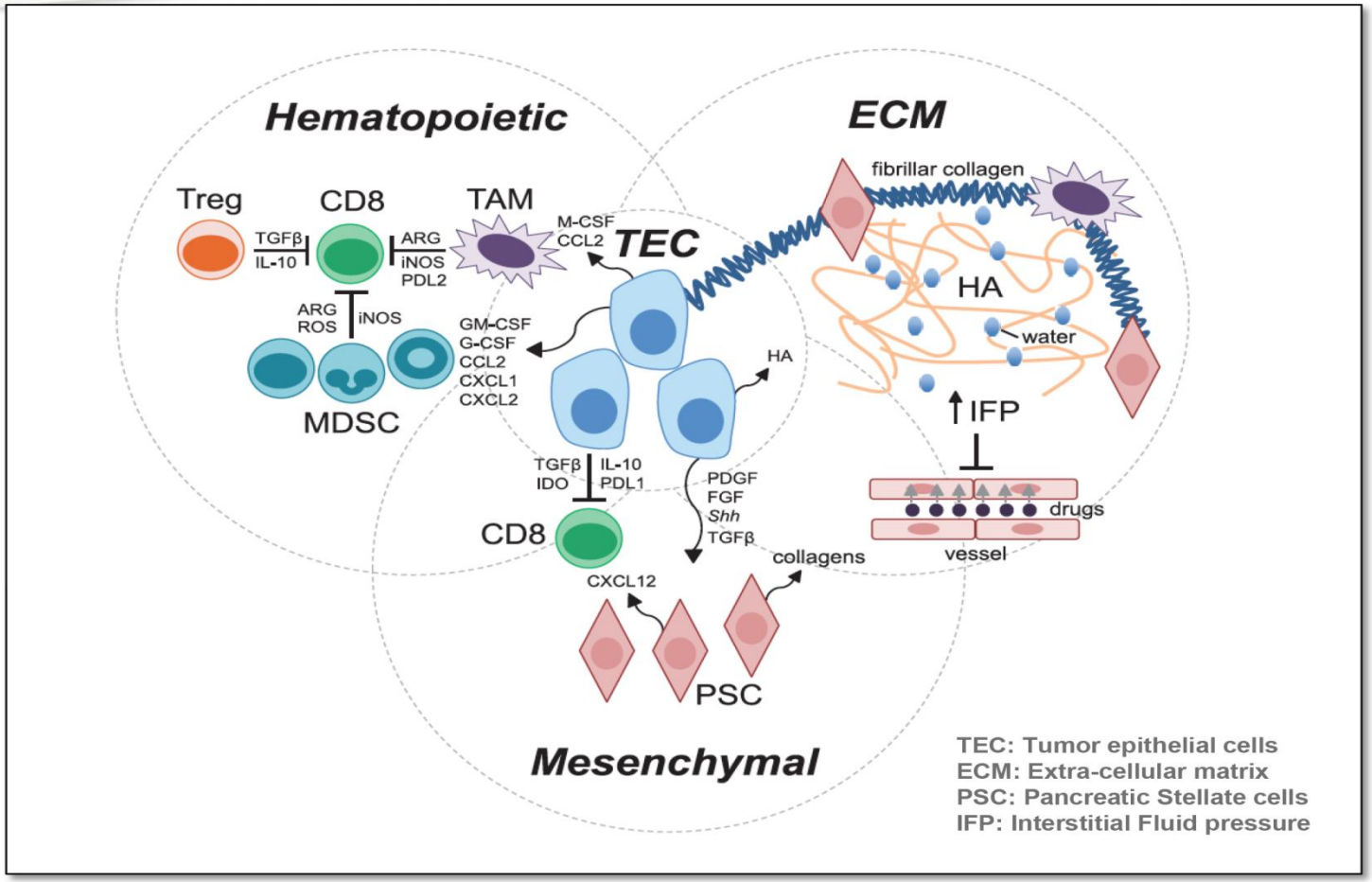
Pancreatic stellate cells support tumor metabolism



Sousa, et. al., Nature, 2016

Therapeutic resistance

Complex Stromal Networks Supporting Pancreatic Cancer Progression and Therapeutic Resistance



Stromnes, I.M., et. al., Carcinogenesis, 35, 2014

Targeting cancer

Treatment Strategies to Improve Disease Outcome

Drug Delivery
and
Effectiveness of Systemic Therapy

↓

Targeting Stroma

Mouse models

Pancreatic Cancer Mouse Model (KPC)

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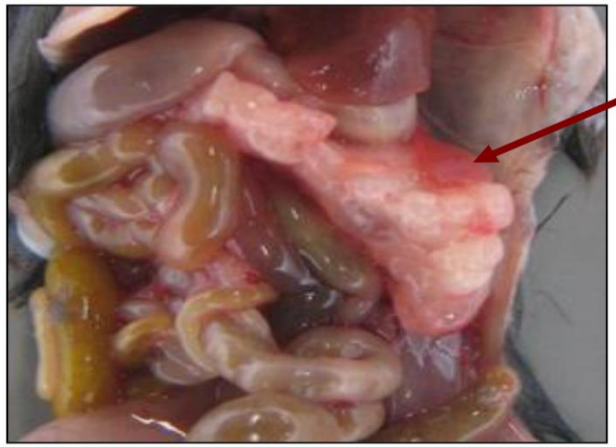
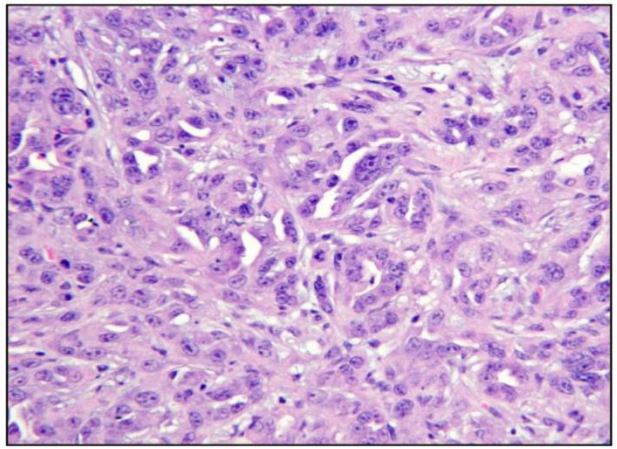
*LSL-Kras-G12D X p53 LSL R172H X Pdx-Cre 1

↓

Pancreatic Ductal Adenocarcinoma (PDAC)

(Median Survival = 4-5 months)





H&E

*Hingorani, S. et. al., Cancer Cell, 2005

Hedgehog signaling

Inhibition of Hedgehog Signaling Depleted Stroma, Enhanced Drug Delivery and Improved Survival in Mice

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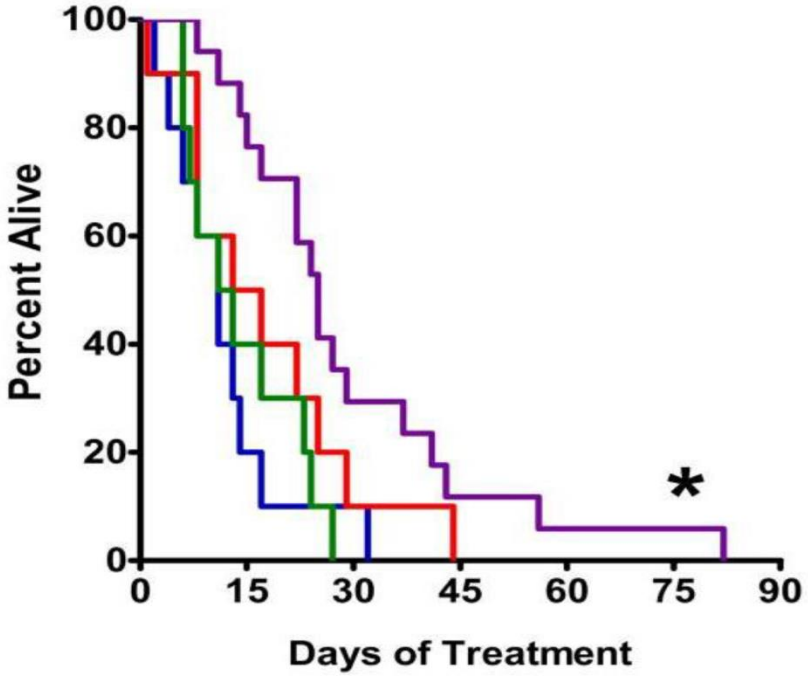

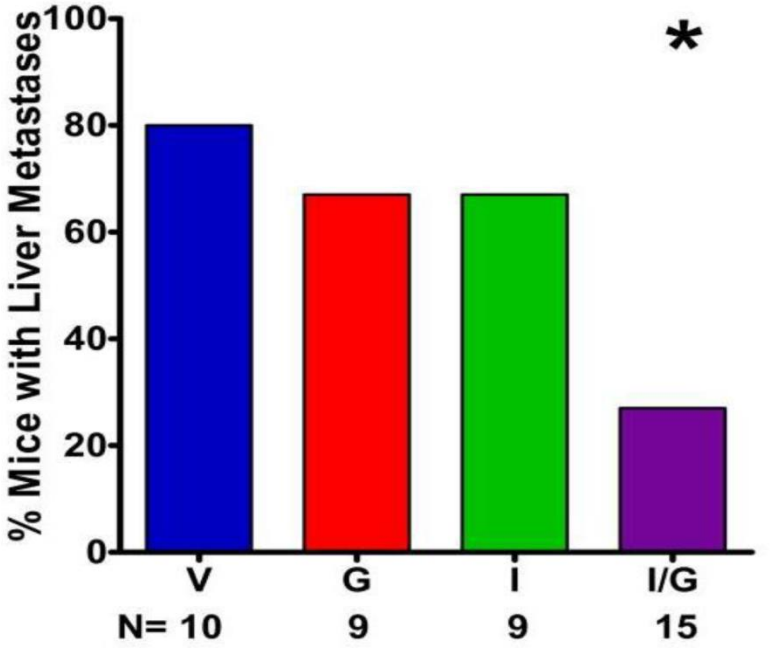

V=Vehicle
G=Gemcitabine
I= IPI-926 (Hedgehog Inhibitor)
I/G= IPI-926/Gem

Olive KP et. al., Science, 324, 2009

Stroma targeting

Enzymatic Targeting of Stroma Enhances Therapeutic Response

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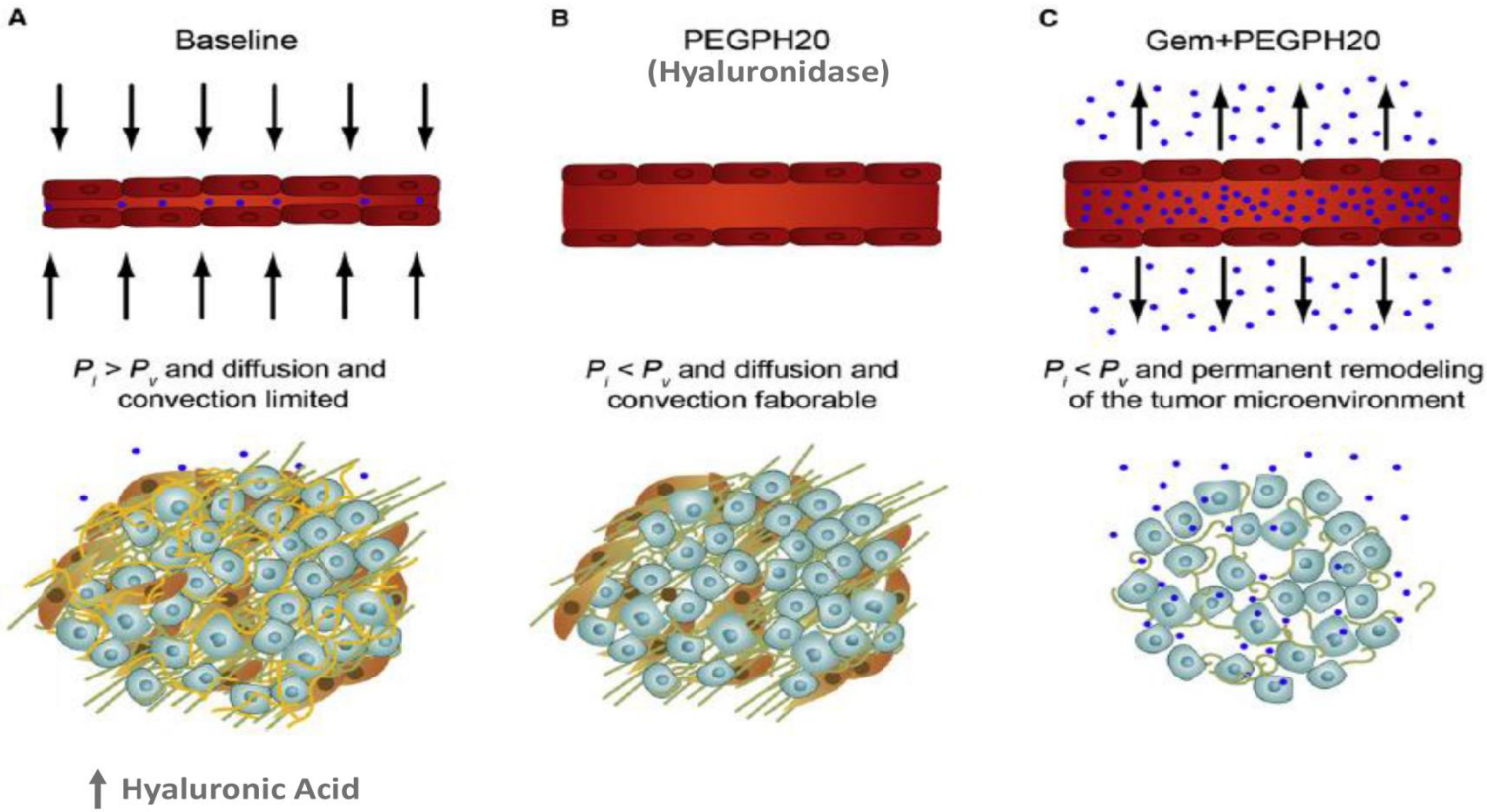

Provenzano et. al., Cancer Cell, 21, 2012

Therapeutic response

Enzymatic Targeting of Stroma Enhances Therapeutic Response

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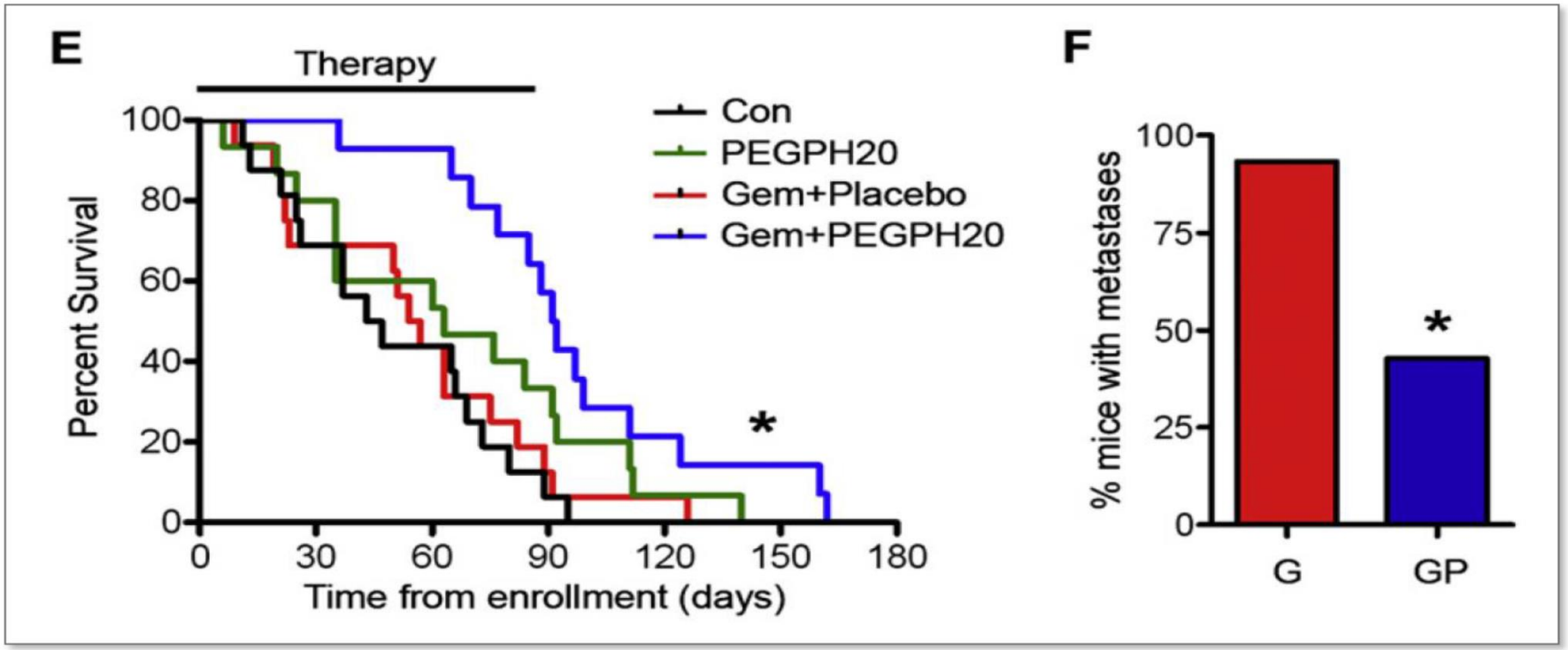

Provenzano et. al., Cancer Cell, 21, 2012

Two Faces of Anti-Stromal Therapy

Stromal-targeting may not (always) have beneficial therapeutic response

Sonic Hedgehog

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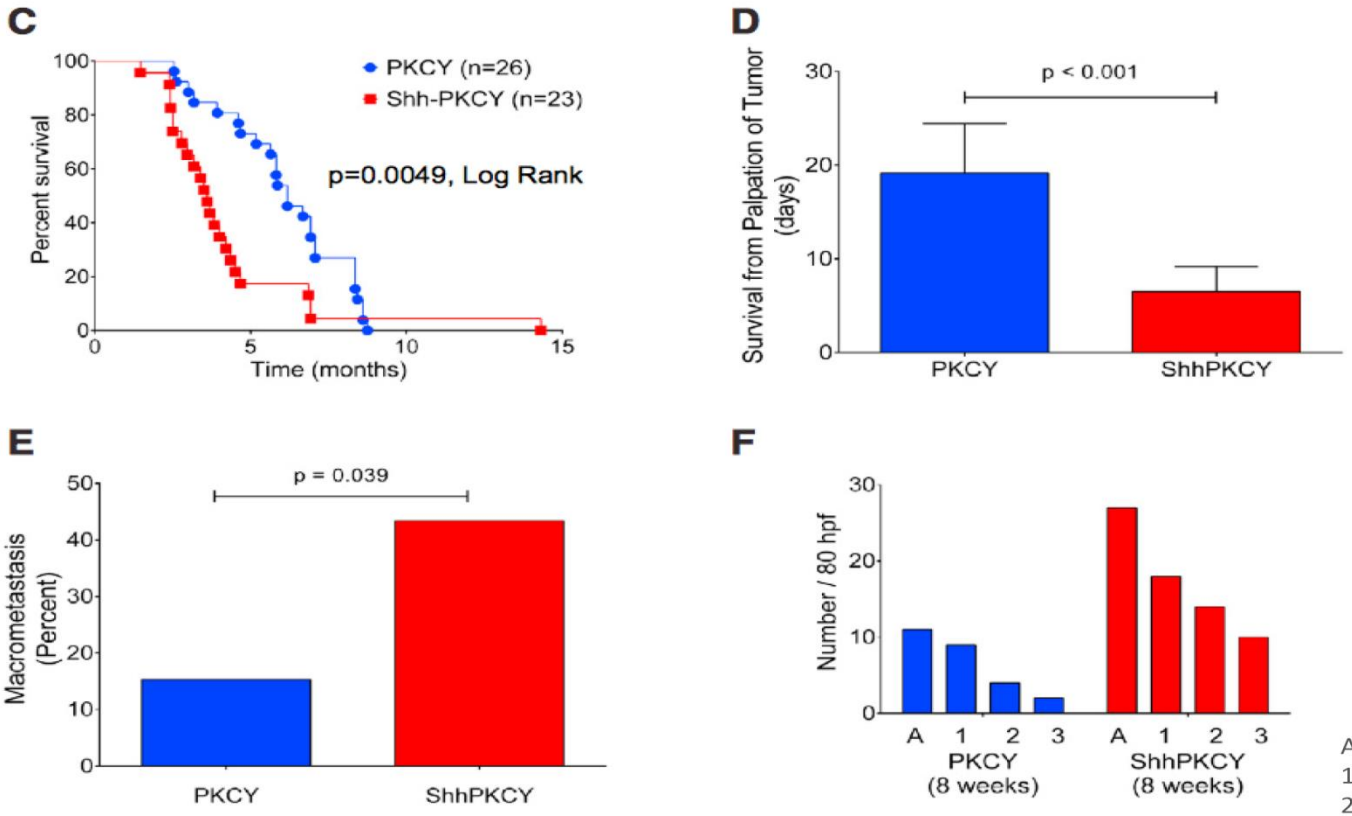
Sonic Hedgehog as a Tumor Suppressor in PDAC

Genetically Engineered Mouse Model



A= Acinar to Ductal Metaplasia
1= PanIN1
2= PanIN2
3= PanIN3

Rhim AD et. al., Cancer Cell, 25, 2014

Myofibroblast Depletion Enhances PDAC

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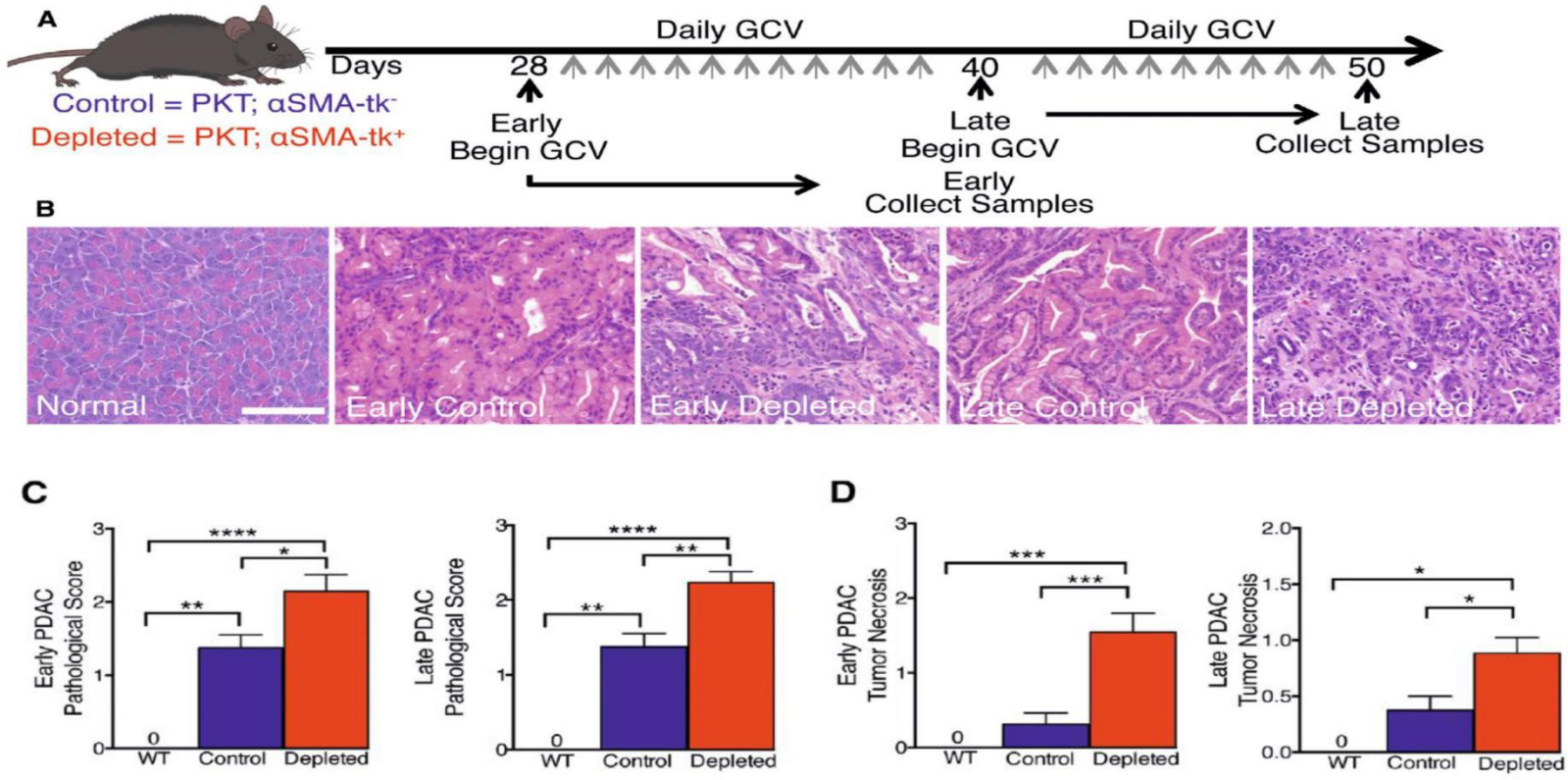
Myofibroblast Depletion Enhances PDAC



Ozdemir, BC et. al., Cancer Cell, 25, 2015

Myofibroblast depletion

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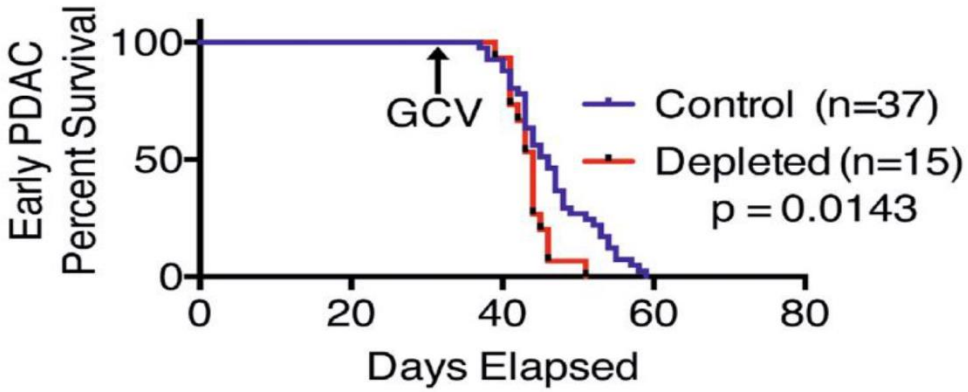
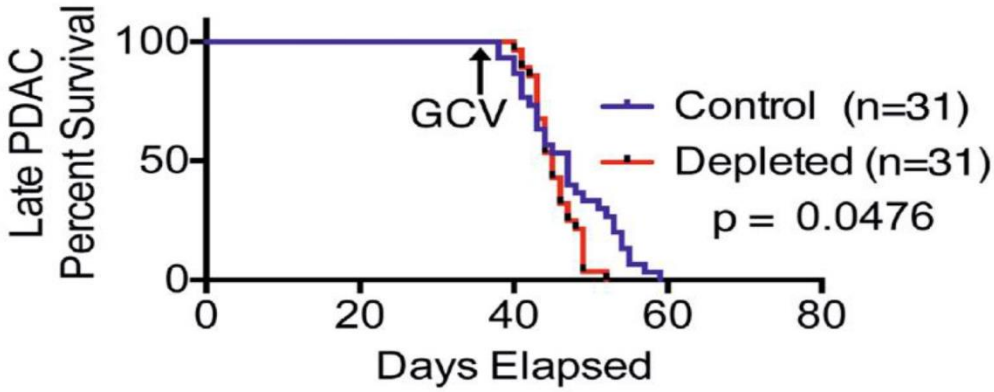
Myofibroblast Depletion Reduces Overall Survival





GCV= genciclovir (Depletes Myofibroblasts in PKT;αSMA-tk+ Mice)

Tumor Stromal Interaction

Complex Tumor-Stromal Interaction in PDAC

Tumor-Stromal interaction is complex and therapeutic approaches targeting stroma needs caution and may require new molecular taxonomy in pancreatic cancer

Mesothelin and Immunotherapy

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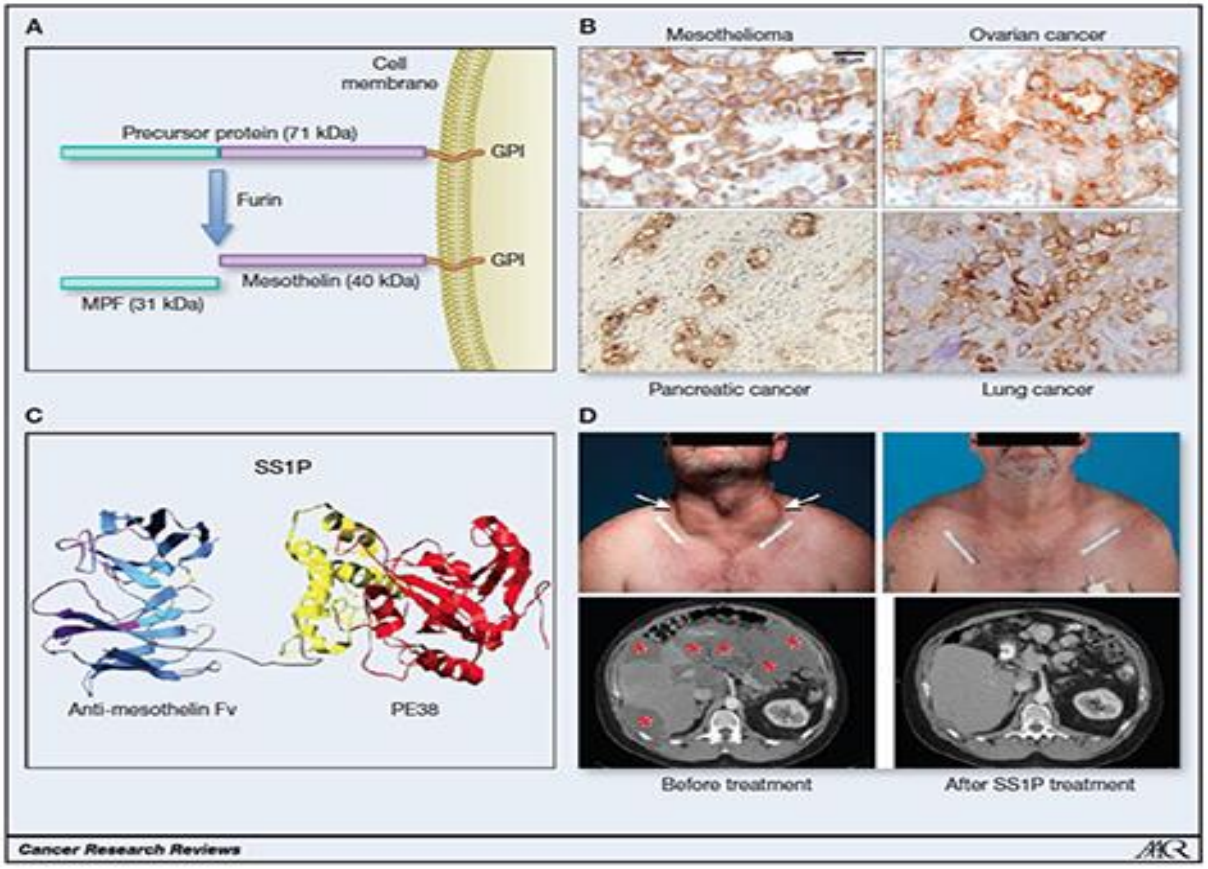
Mesothelin as a Target for Immunotherapy



Pastan and Hassan, Cancer Res., 2014

Mesothelin targeted T cells

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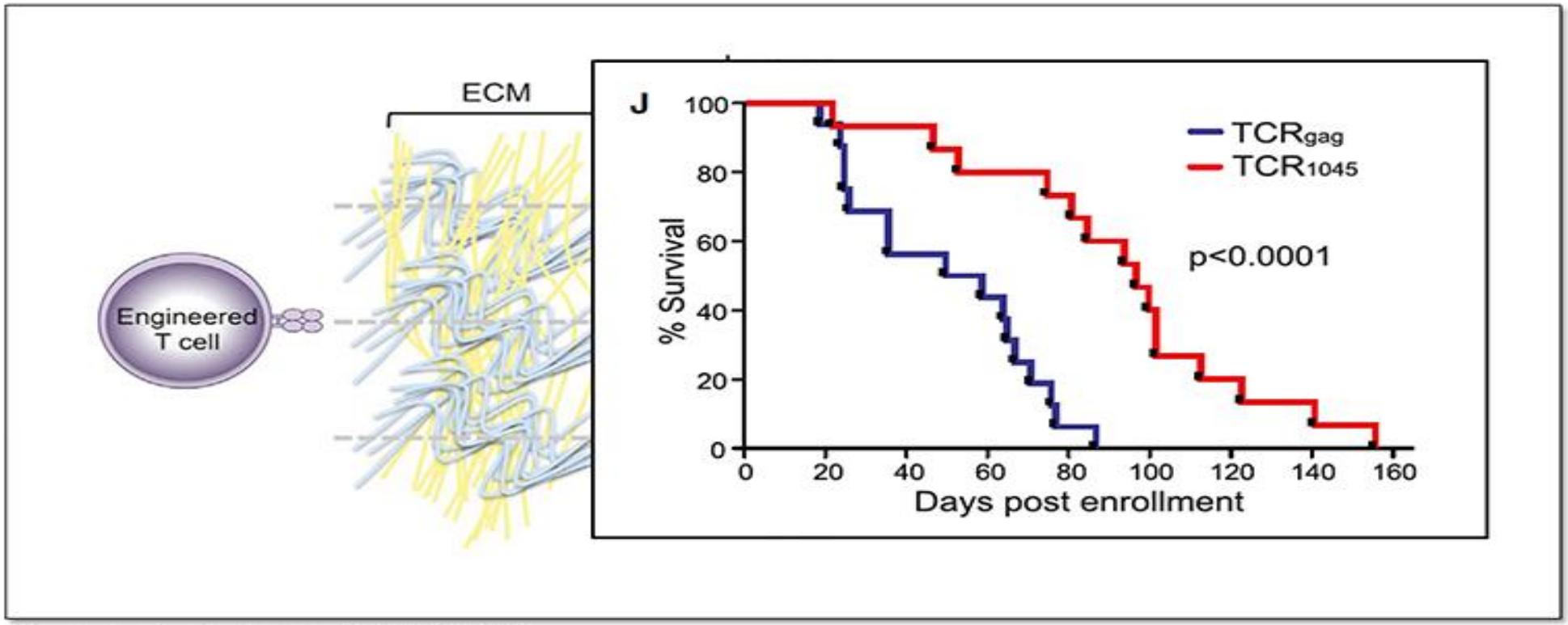
Mesothelin Targeted T Cells Lyse Tumor Cells and Increase Survival in KPC Mouse Model of PDAC



Stromnes et. al., Cancer Cell, 28, 2015

Organoid

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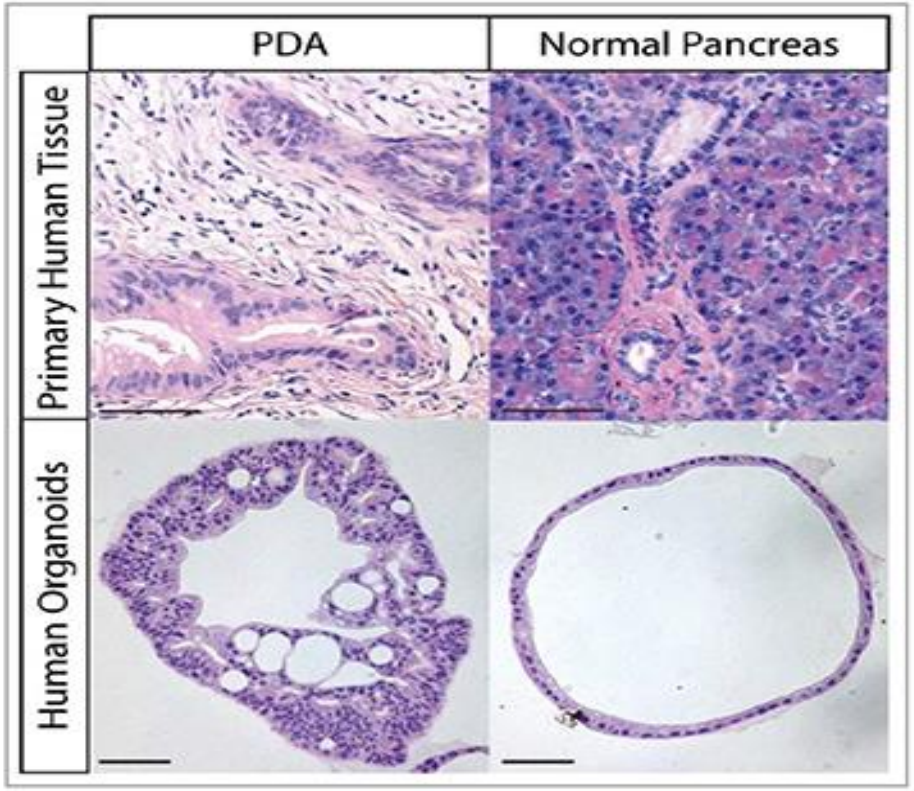
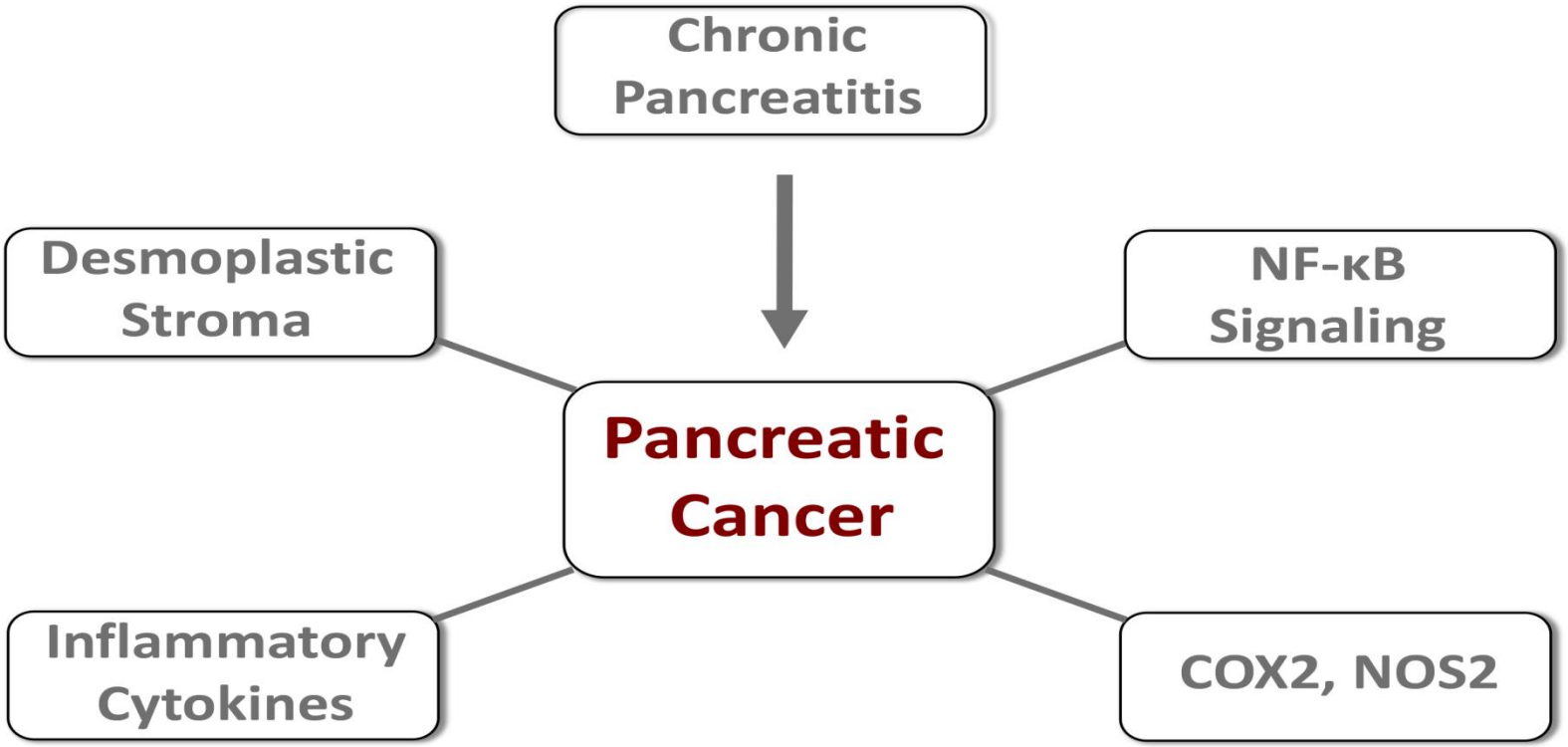
Organoid: A highly promising model for PDAC



Boj et. al., Cell, 160, 2015
Boj et. al., Mol. Cell. Onc., 2016
Hwang et. al., J. Pathology, 238, 2016

Inflammation and Pancreatic Cancer

Inflammation and Pancreatic Cancer



Pancreatic Cancer Development

Inflammatory Changes During Development and Progression of Pancreatic Cancer

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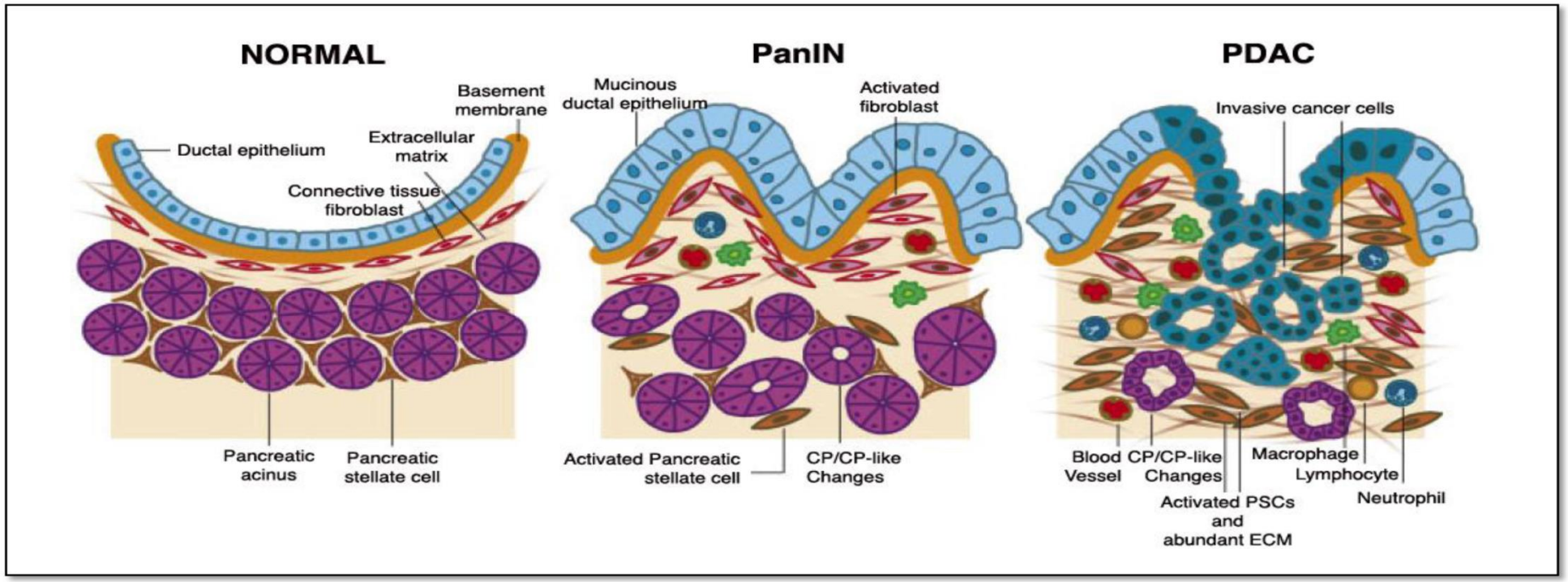

From: Chu GC et al., J. Cell. Biochem, 101, 2007

CP= Chronic Pancreatitis
PSC= Pancreatic Stellate Cells
ECM= Extracellular Matrix

Inflammation and Pancreatic Cancer

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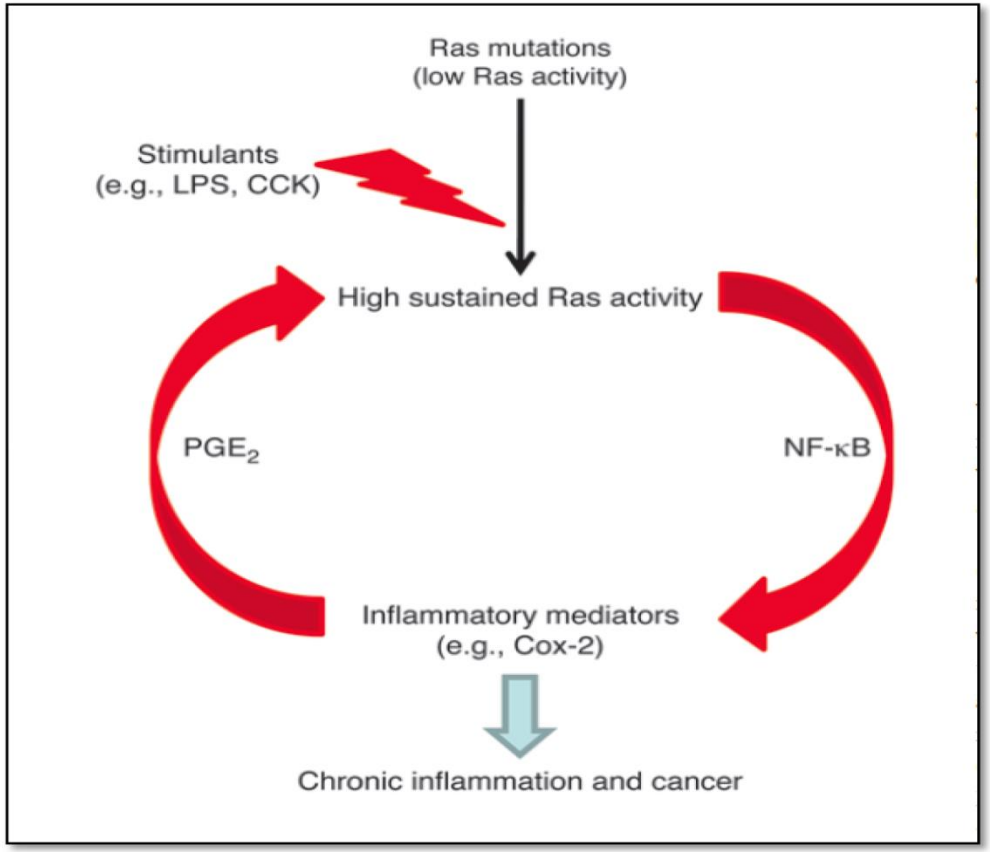
Inflammation Enhances and Maintains a Pathologic Level of Oncogenic KRAS in Pancreatic Cancer



Daniluk et. al., JCI, 2012

MIF and Cancer

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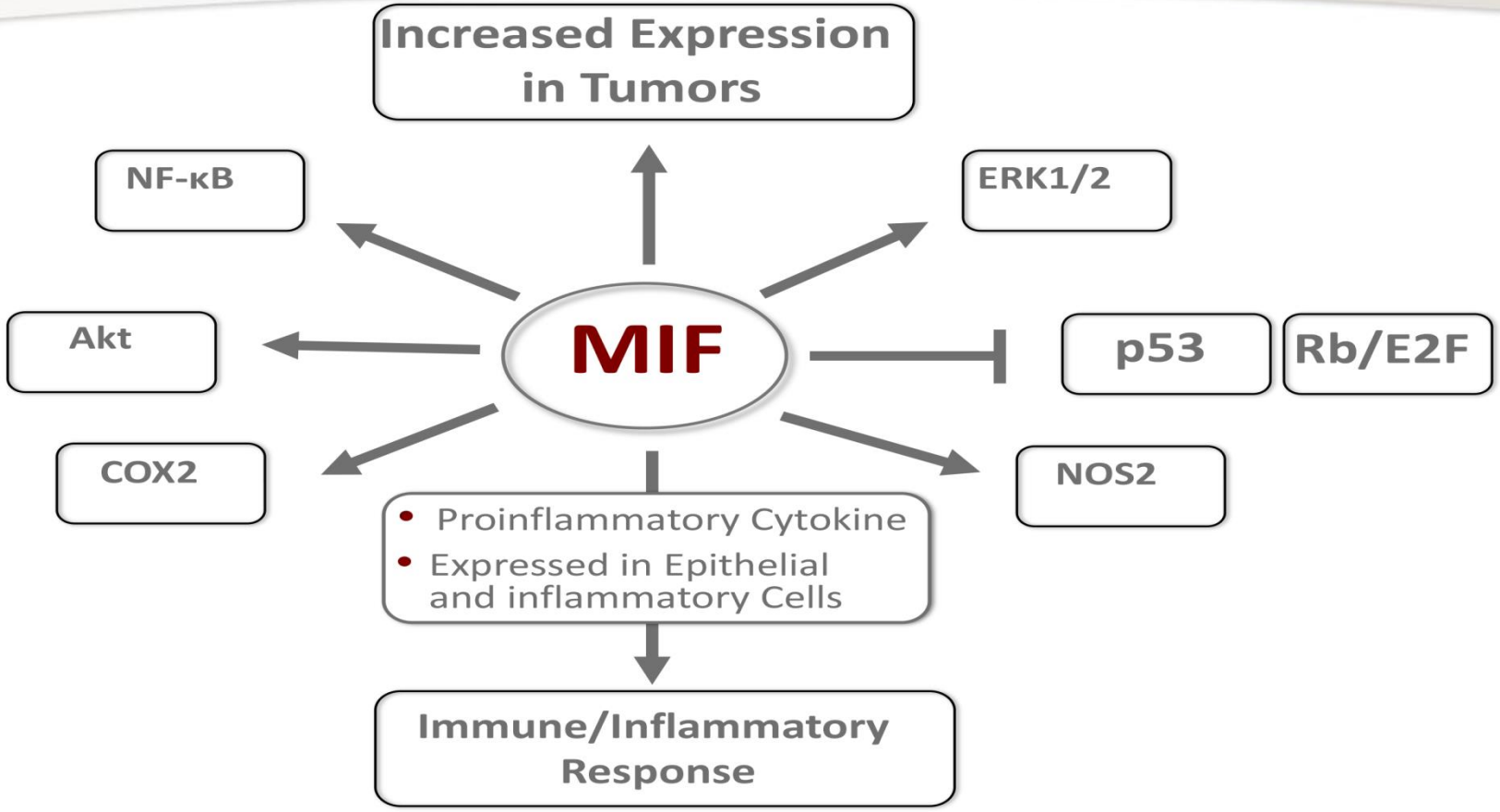
Macrophage Migration Inhibitory Factor (MIF)



MIF, Inflammation and Cancer

MIF, Inflammation and Cancer

J. Exp. Med., 190, 1999

At the Crossroads of Inflammation and Tumorigenesis

By Carlos Cordon-Cardo* and Carol Prives‡

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Macrophage Migration Inhibitory Factor MIF Interferes with the Rb-E2F Pathway

Oleksi Petrenko* and Ute M. Moll*

Immunity, 26, 2007

Perspective

Macrophage Migration Inhibitory Factor: A Probable Link between Inflammation and Cancer

Richard Bucala[1,*] and Seamas C. Donnelly[2,*]

HYPOTHESIS

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MIF Contributes to Pancreatic Cancer Progression and Predicts Disease Outcome.

MIF and PDAC

Increased expression of MIF in tumors from pancreatic ductal adenocarcinoma cases

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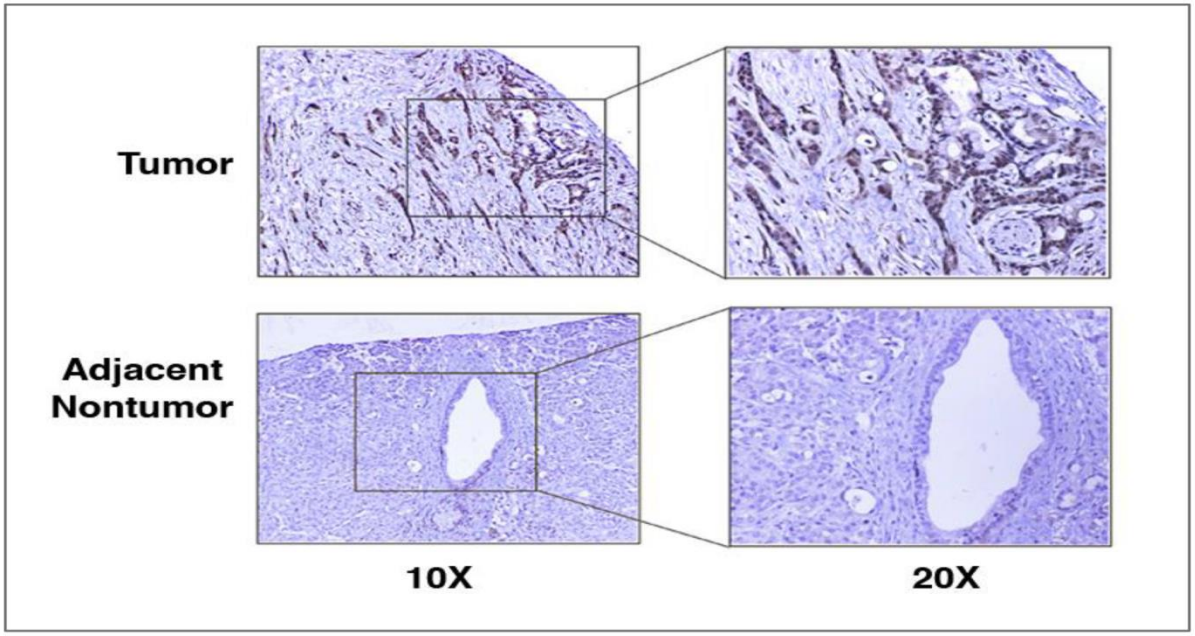

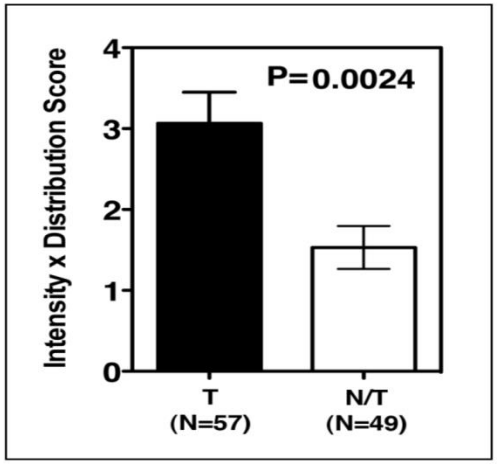

MIF expression and HDAC survival

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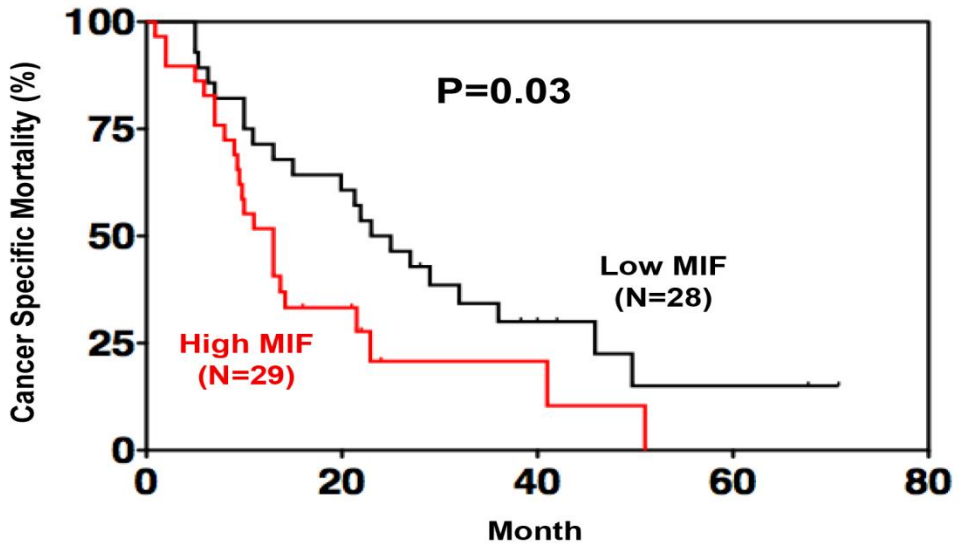
A higher expression of MIF is associated with poor survival in human PDAC

Human Pancreatic Carcinoma Cases



Variables (comparison/referent)	Univariable Analysis		Multivariable Analysis	
	HR (95%CI)	*P*	HR (95%CI)	*P*
MIF (High/Low)	2.21 (1.16-4.22)	0.016	2.26 (1.17-4.37)	0.015
Grading (G3-4/G1-2)	1.86 (1.01-3.45)	0.048	1.90 (1.02-3.54)	0.044
Resection margin (R1/R0)	1.53 (0.82-2.83)	0.178		
Stage (IIB-III/I-IIA)	1.62 (0.79-3.36)	0.191		

Funamizu, N.....Hussain, S.P., Int. J. Cancer, 132, 2012

MIF expression and poor PDAC survival

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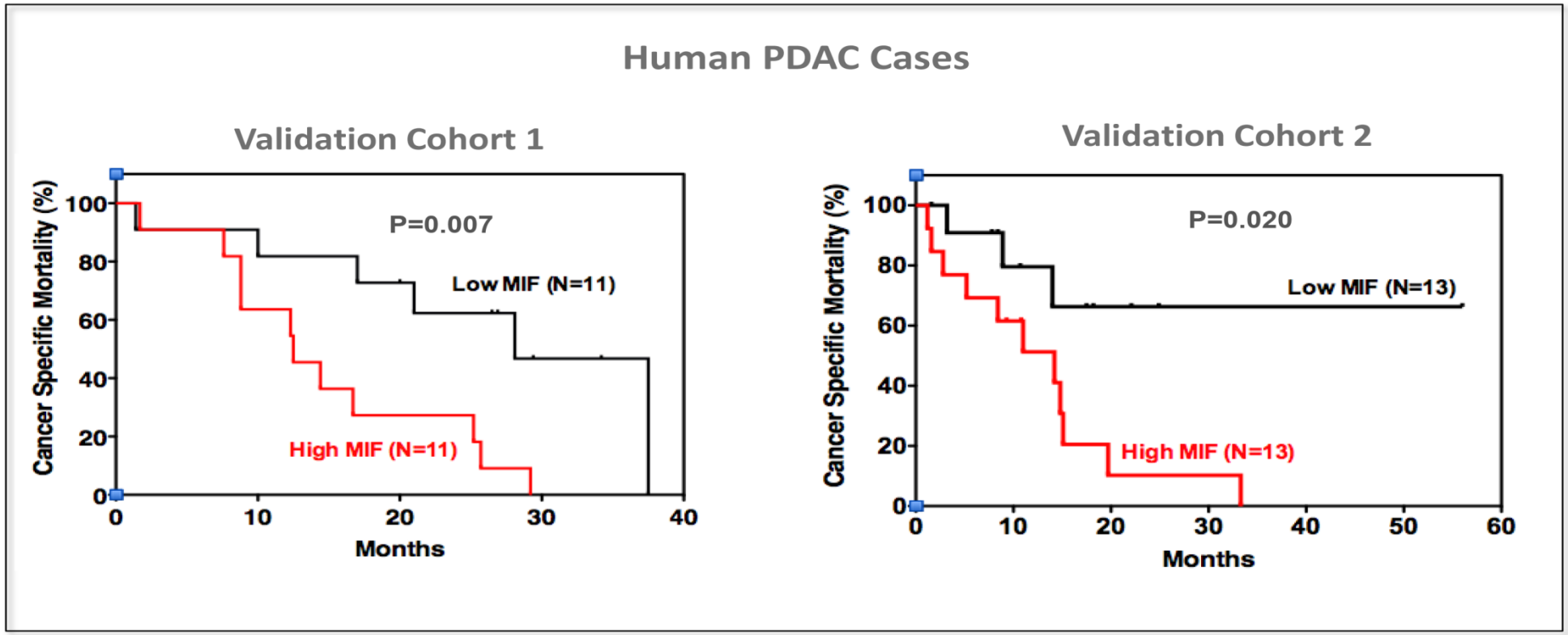
A higher expression of MIF is associated with poor survival in human PDAC

Validation in Independent Cohorts



MIF accelerates tumor growth

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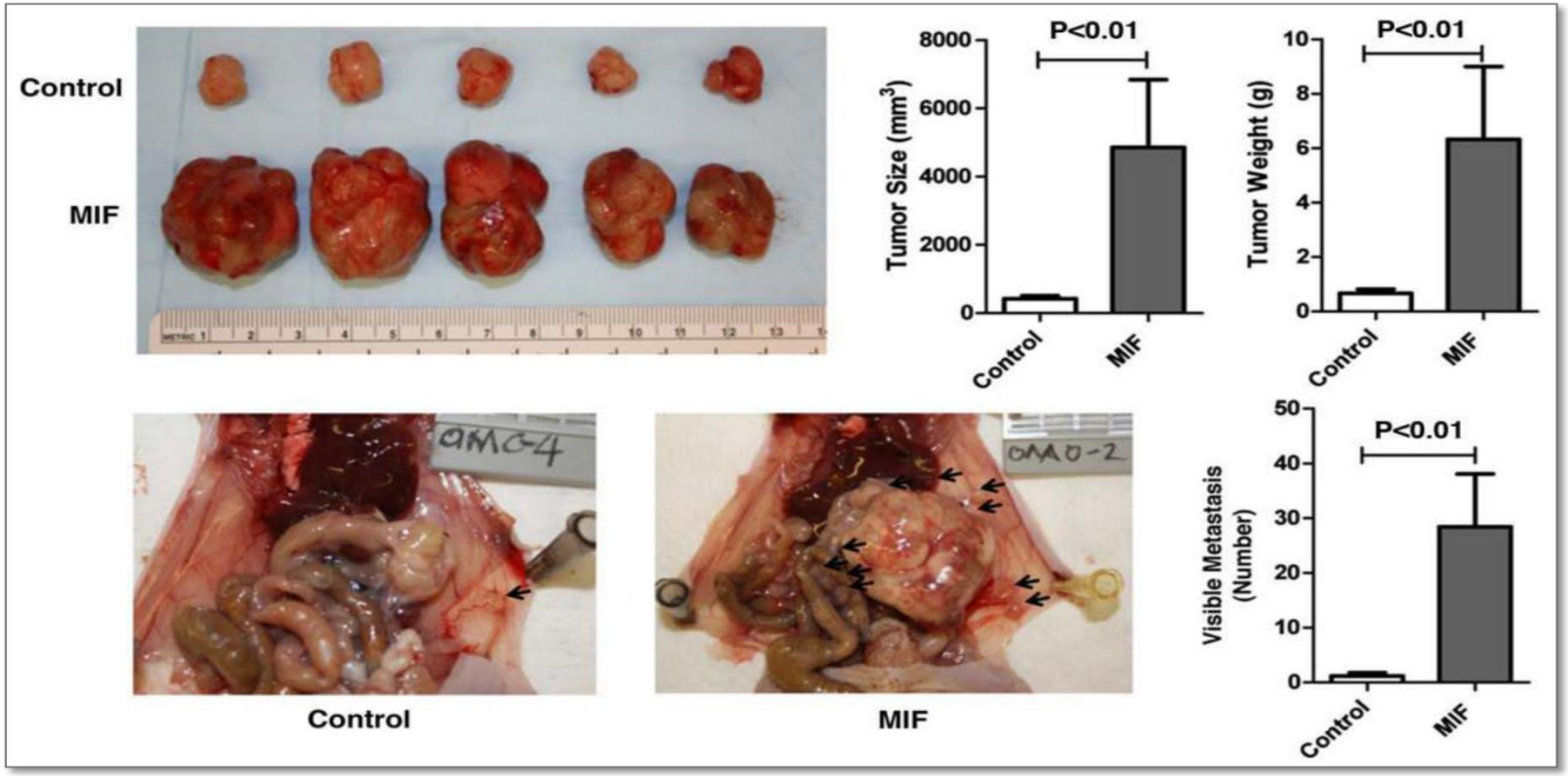
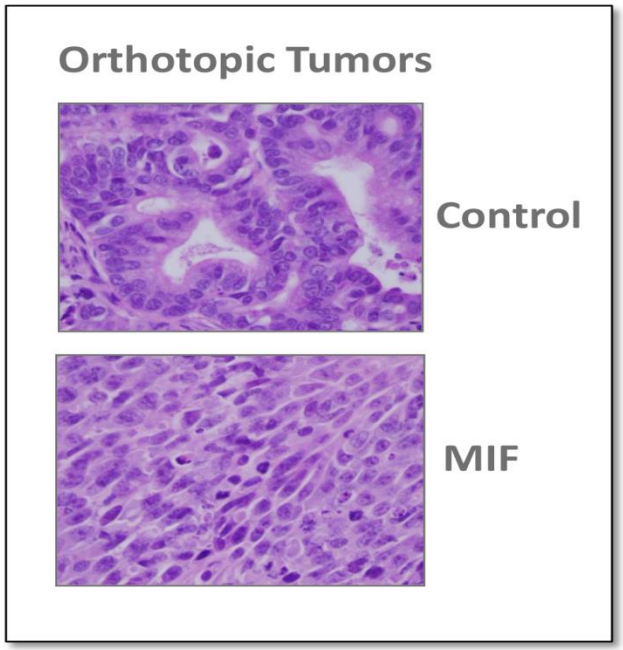
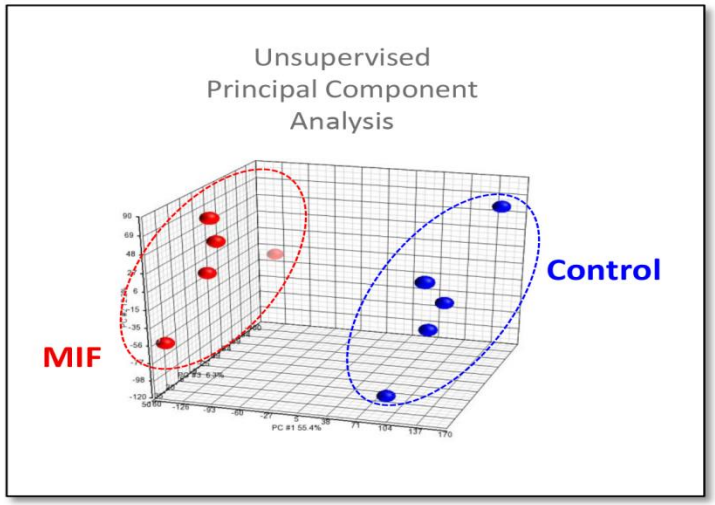
MIF accelerates tumor growth and metastasis In orthotopic xenografts in mice

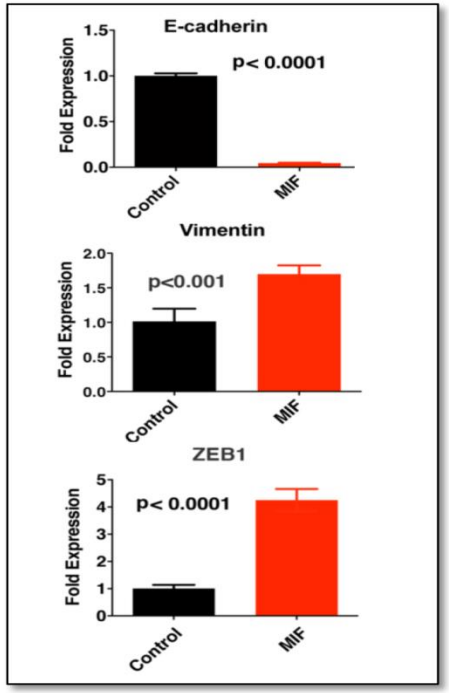


MIF and gene expression

MIF Induces a marked change in global gene expression profile including EMT-related genes in orthotopic tumors







- MIF over-expressing tumors are poorly differentiated.
- MIF induces a change in global gene expression profile.
- MIF over-expressing tumors showed expression of EMT-marker genes.

Exosomal MIF and PDAC

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Exosomal MIF and Liver Metastasis in PDAC

ARTICLES

nature cell biology

Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver

Bruno Costa-Silva[1], Nicole M. Aiello[2], Allyson J. Ocean[3], Swarnima Singh[1], Haiying Zhang[1], Basant Kumar Thakur[1,4], Annette Becker[1], Ayuko Hoshino[1], Milica Tešić Mark[5], Henrik Molina[5], Jenny Xiang[6], Tuo Zhang[6], Till-Martin Theilen[1], Guillermo García-Santos[1], Caitlin Williams[1], Yonathan Ararso[1], Yujie Huang[1], Gonçalo Rodrigues[1,7], Tang-Long Shen[8], Knut Jørgen Labori[9], Inger Marie Bowitz Lothe[10,11], Elin H. Kure[11], Jonathan Hernandez[12], Alexandre Doussot[12], Saya H. Ebbesen[1], Paul M. Grandgenett[13], Michael A. Hollingsworth[13], Maneesh Jain[14], Kavita Mallya[14], Surinder K. Batra[14], William R. Jarnagin[12], Robert E. Schwartz[15], Irina Matei[1], Héctor Peinado[1,16], Ben Z. Stanger[2,19], Jacqueline Bromberg[17,19] and David Lyden[1,18,19]

Pancreatic ductal adenocarcinomas (PDACs) are highly metastatic with poor prognosis, mainly due to delayed detection. We hypothesized that intercellular communication is critical for metastatic progression. Here, we show that PDAC-derived exosomes induce liver pre-metastatic niche formation in naive mice and consequently increase liver metastatic burden. Uptake of PDAC-derived exosomes by Kupffer cells caused transforming growth factor β secretion and upregulation of fibronectin production by hepatic stellate cells. This fibrotic microenvironment enhanced recruitment of bone marrow-derived macrophages. We found that macrophage migration inhibitory factor (MIF) was highly expressed in PDAC-derived exosomes, and its blockade prevented liver pre-metastatic niche formation and metastasis. Compared with patients whose pancreatic tumours did not progress, MIF was markedly higher in exosomes from stage I PDAC patients who later developed liver metastasis. These findings suggest that exosomal MIF primes the liver for metastasis and may be a prognostic marker for the development of PDAC liver metastasis.

MIF-induced disease

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MIF-induced disease aggressiveness in pancreatic cancer

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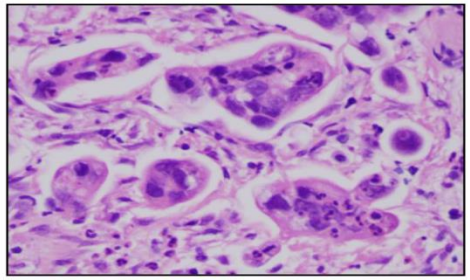
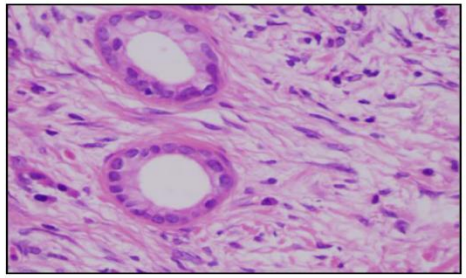
Human PDAC Cases

H/E



High MIF



Low MIF

Shouhui Yang

Questions

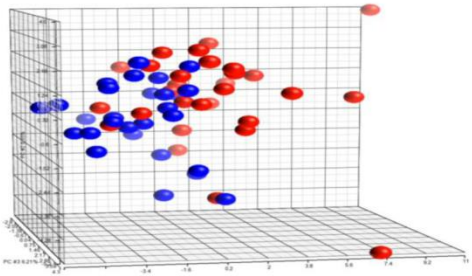
- Molecular Distinctions
- Mechanistic and Functional Role of MIF in Tumor Progression

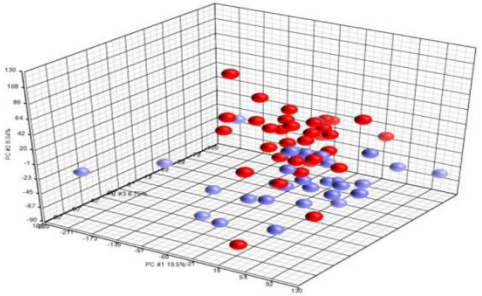
OMICS

miRNA

mRNA

- High MIF
- Low MIF





miRNA profiling

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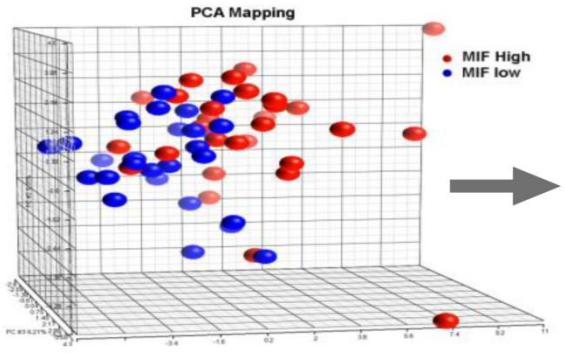
miRNA profiling of MIF-high and MIF-low tumors

- **Hypothesis:** MIF regulates miRNAs associated with tumor progression and disease aggressiveness in patient with pancreatic cancer



Nanostring miRNA Analysis

Differentially Expressed miRNAs
MIF-high vs MIF-low

53 Differentially Expressed miRNAs
MIF-high Vs. MIF-Low
($p<0.05$)

miRNAs Associated with Survival (Kaplan-Meier Analysis)

5 miRNAs Associated with Survival
- miR-301b
- miR-15b
- miR-10b
- miR-93
- miR-590-5p

Validation (Multiple Cohorts of PDAC)

MIF axis in Pancreatic Cancer

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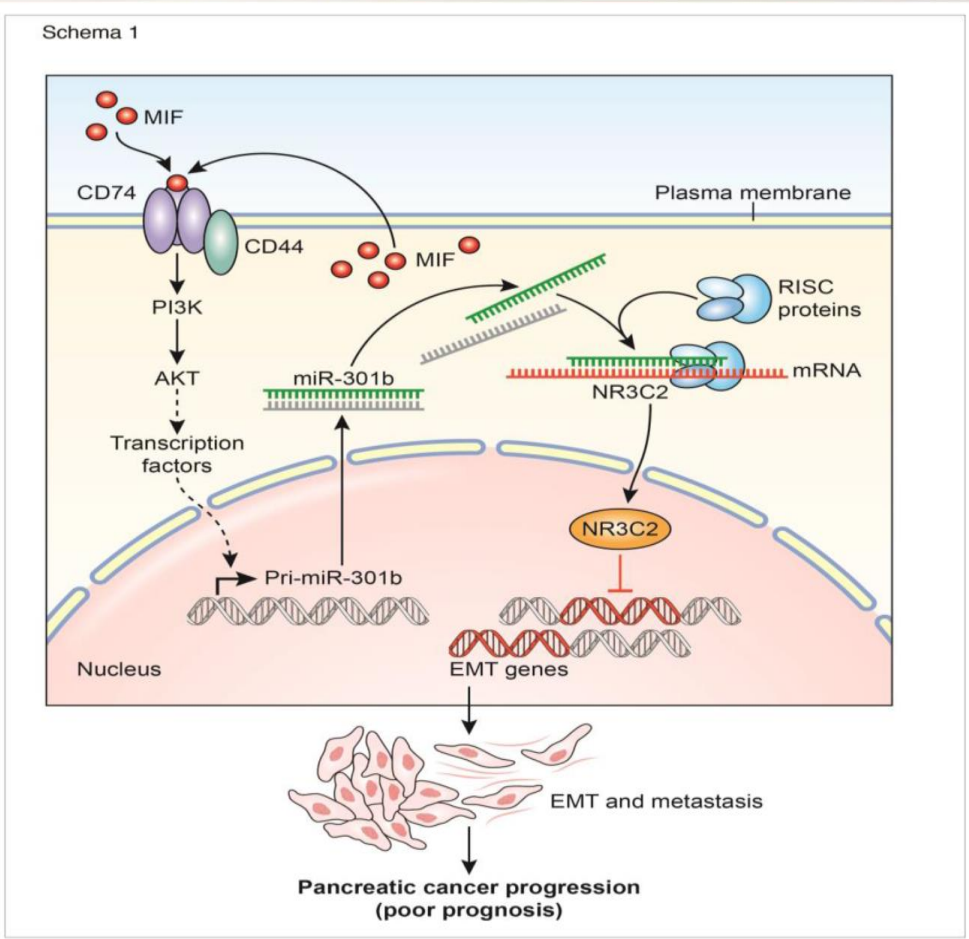
MIF/miR-301b/NR3C2 Axis in Pancreatic Cancer



HYPOTHESIS

MIF/mir-301b/NR3C2 Signaling is a Potential Therapeutic Target in Pancreatic Cancer

Pancreatic Tumors Express MIF

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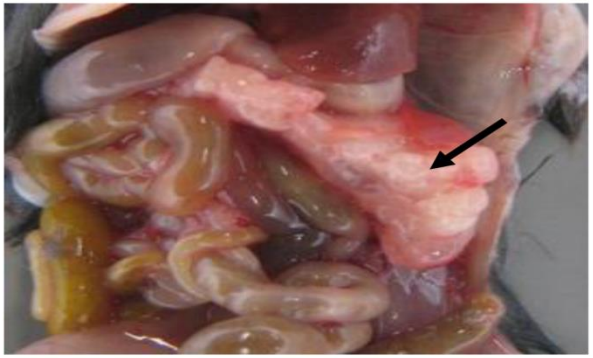
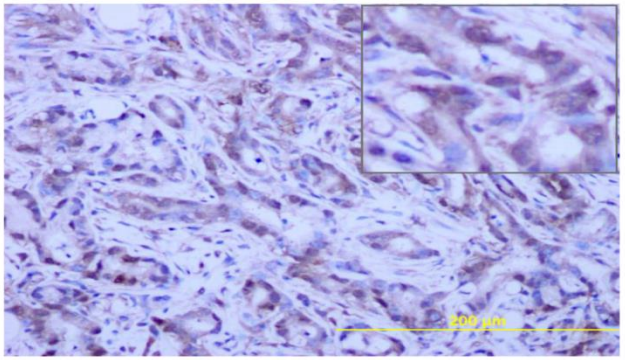
Pancreatic tumors in KPC mice express a high level of MIF

(KPC: $KRAS^{G12D}$; $P53^{R172H}$; Pdx-1-Cre)

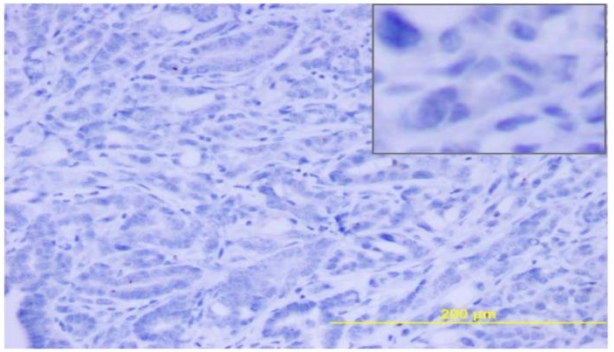


KPC

MIF Immunostaining



KPC



$KPC/MIF^{-/-}$

MIF deletion in genetically engineered mouse model of pancreatic cancer

MIF deficiency enhances survival

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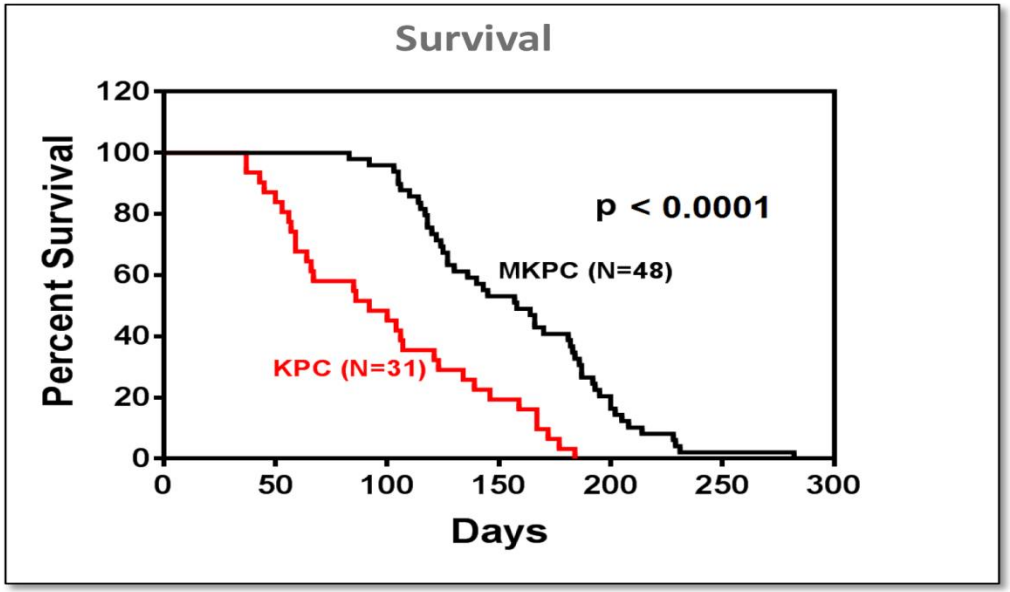
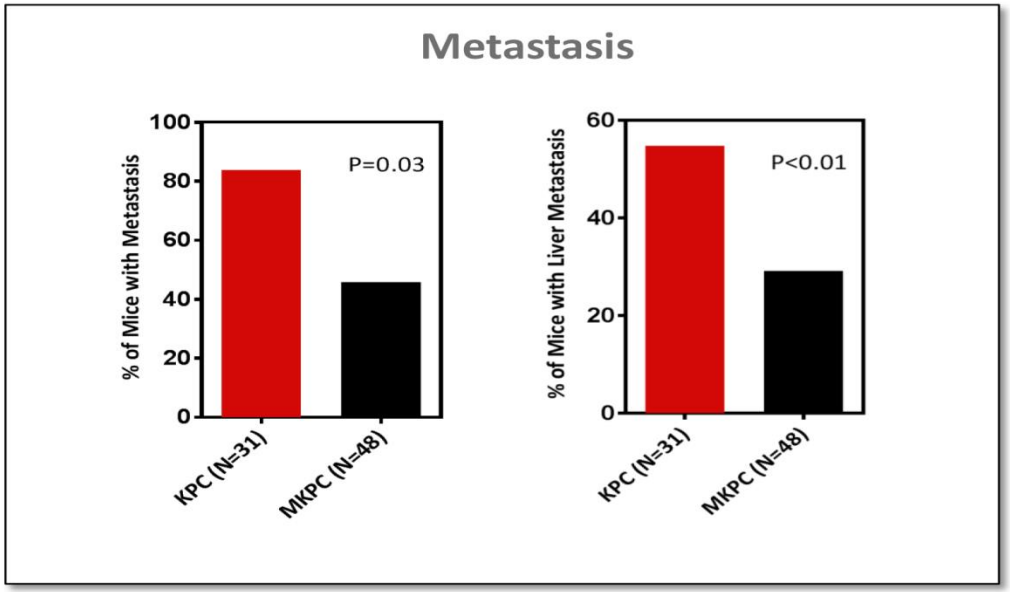
MIF-deficiency enhanced survival and reduced metastasis in KPC mice

KPC Mouse Model





MKPC= MIF-deficient KPC mice

MIF deficiency reduces EMT

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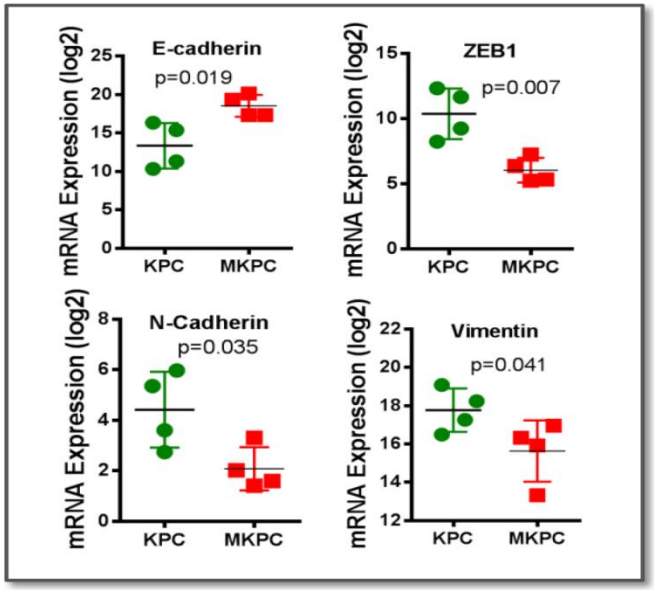
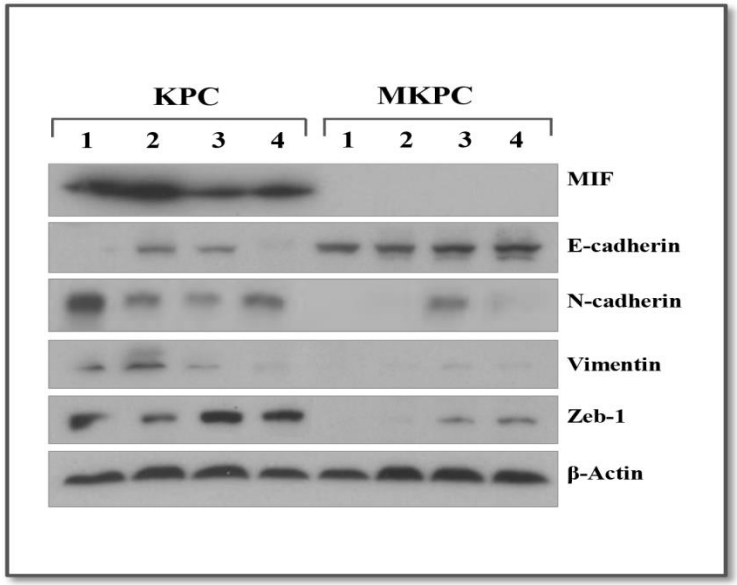
MIF-deficiency reduced EMT in KPC mice

Primary Tumor Cells





MIF deficiency increases NR3C2 expression

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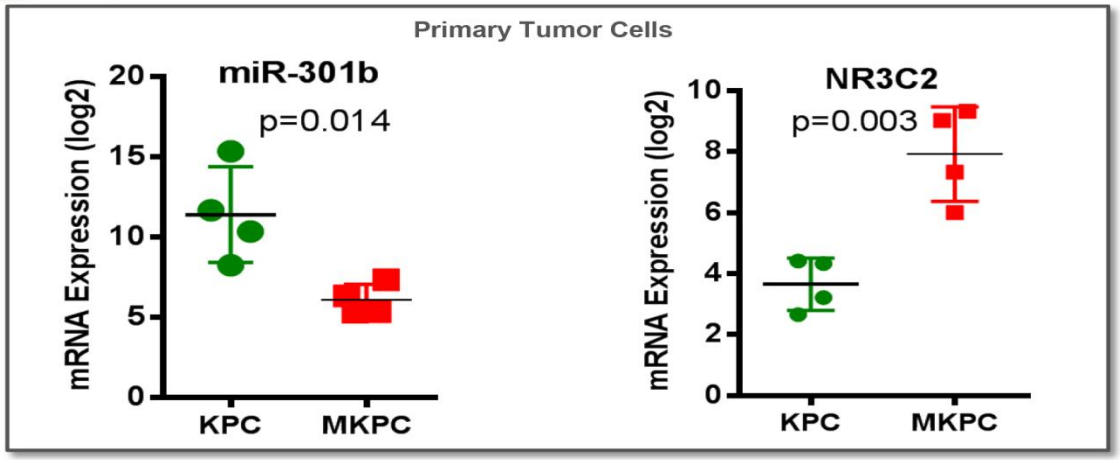
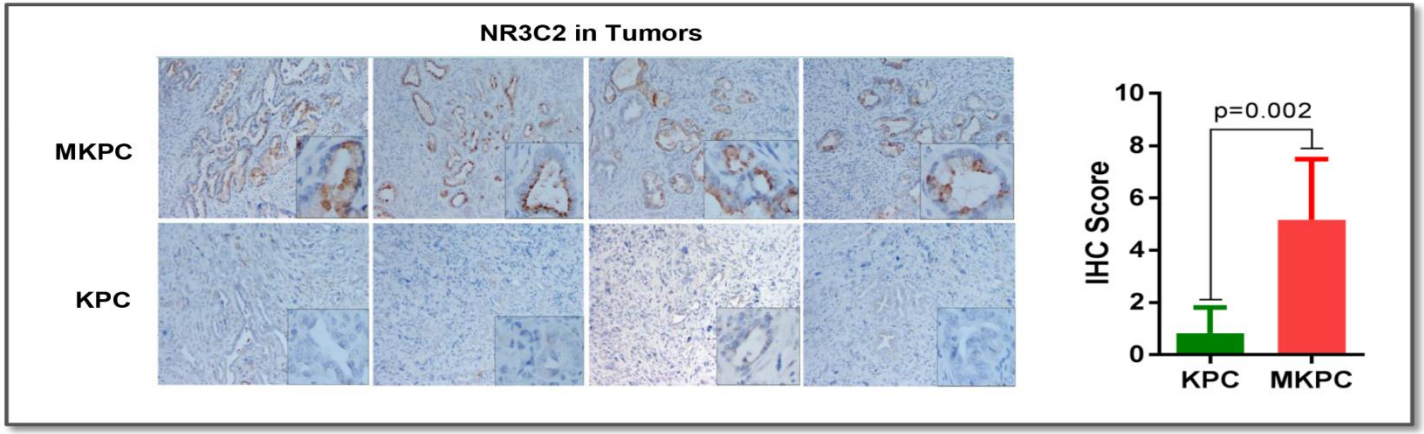
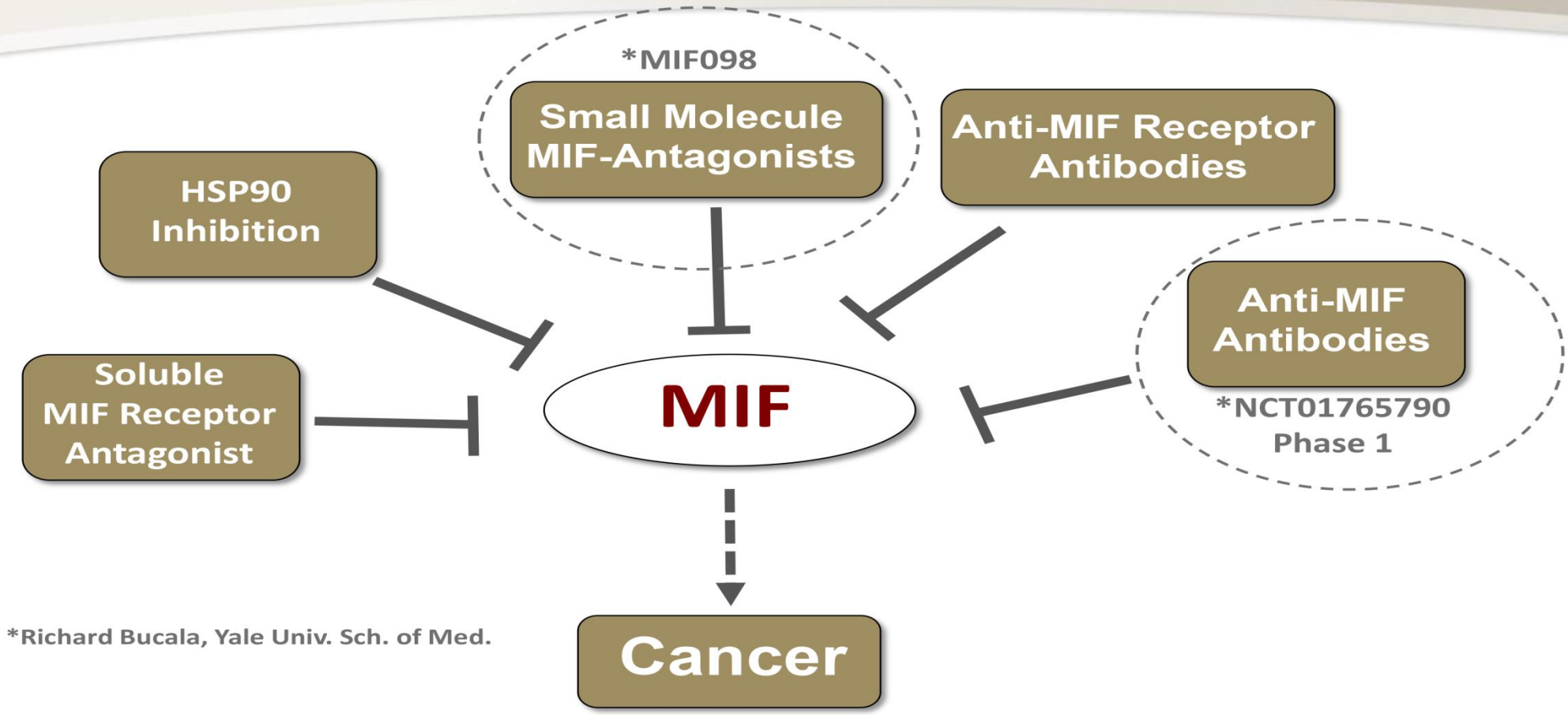
MIF-deficiency decreases miR-301b and increases NR3C2 expression





MIF inhibition strategies

Strategies for MIF inhibition



*Richard Bucala, Yale Univ. Sch. of Med.

SUMMARY

SUMMARY

- A higher MIF expression is associated with poorer outcome in PDAC patients.
- MIF enhances growth and metastasis of tumor xenografts in mice.
- MIF-driven signaling inhibits NR3C2 by upregulating miR-301b.
- NR3C2 is a negative regulator of EMT.
- MIF-deficiency increased survival and reduced metastasis in KPC mice.
- MIF/mir-301b/NR3C2 signaling is a potential therapeutic target.

Understanding pancreatic tumor biology

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Understanding Pancreatic Tumor Biology is Key to Improving Disease Outcome