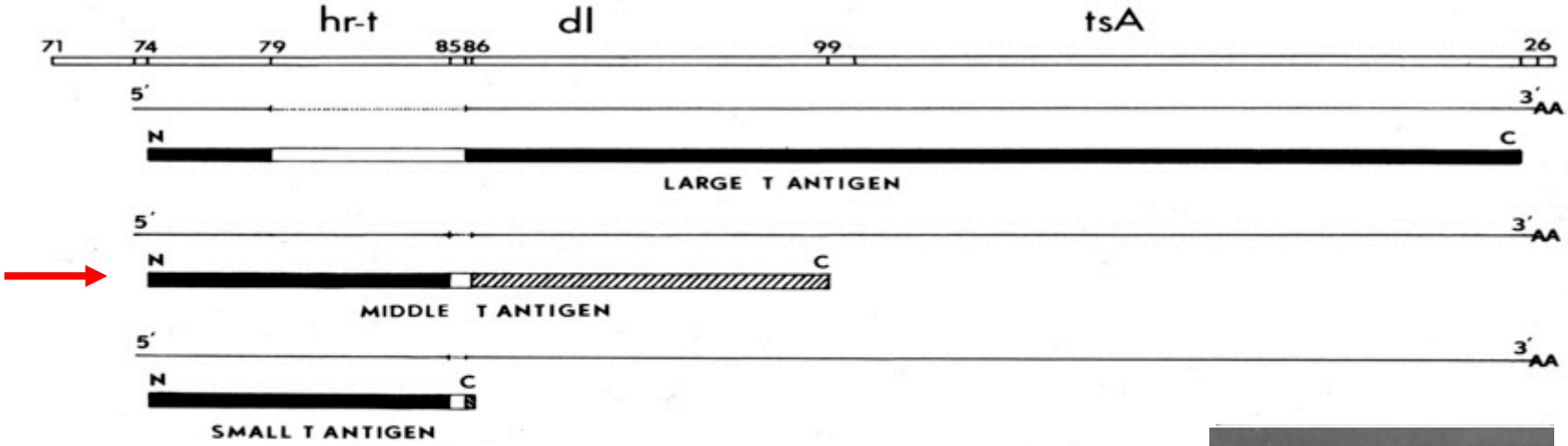
2018 Tang Prize Laureate Lecture

Tyrosine Phosphorylation - From Discovery to Drug Development and Beyond

September 22, 2018

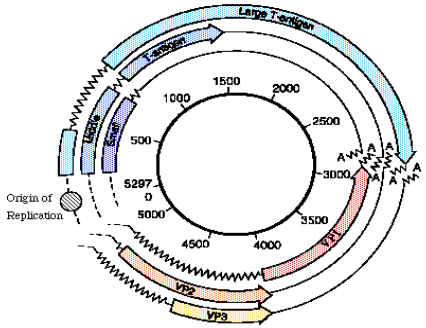
Tony Hunter
Salk Institute

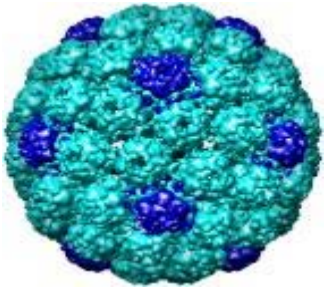
Polyomavirus early region (and Tony) circa 1979



Early

Late

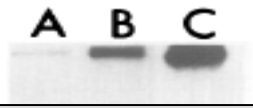


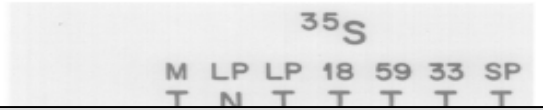


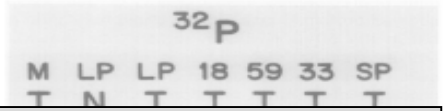
40 nm

~5 kbp circular genome - 6 genes

Polyoma middle T has associated kinase activity







Following up Marc Collett and Ray Erikson's seminal observation that the v-Src RSV transforming protein has an associated protein kinase activity, three groups – Alan Smith and Mike Fried (ICRF), Brian Schaffhausen and Tom Benjamin (Harvard), and my colleagues, Walter Eckhart and Mary Anne Hutchinson, and I – had all found that Py mT has an associated kinase activity and presented our findings at the 1979 CSH Symposium on Tumor Viruses held at the end of May 1979. We agreed to submit our papers to *Cell* when we got back.

Our paper was submitted on June 11, 1979

C ^{32}P kinase assay

at
ng
that kinase activity is important for transformation

It was just as hard to get papers published in 1979!

Paper submitted to Cell - June 11, 1979. Reviews received July 9, 1979

Paper: Eckhart G0623

Reviewer 1

Paper: Eckhart G0623

Reviewer 2

This manuscript reports a protein kinase-like activity is associated with T-sera immunoprecipitates of polyoma T-antigens from virus infected or transformed cells. TsA mutants have little effect upon the detection

Paper: Eckhart G0623

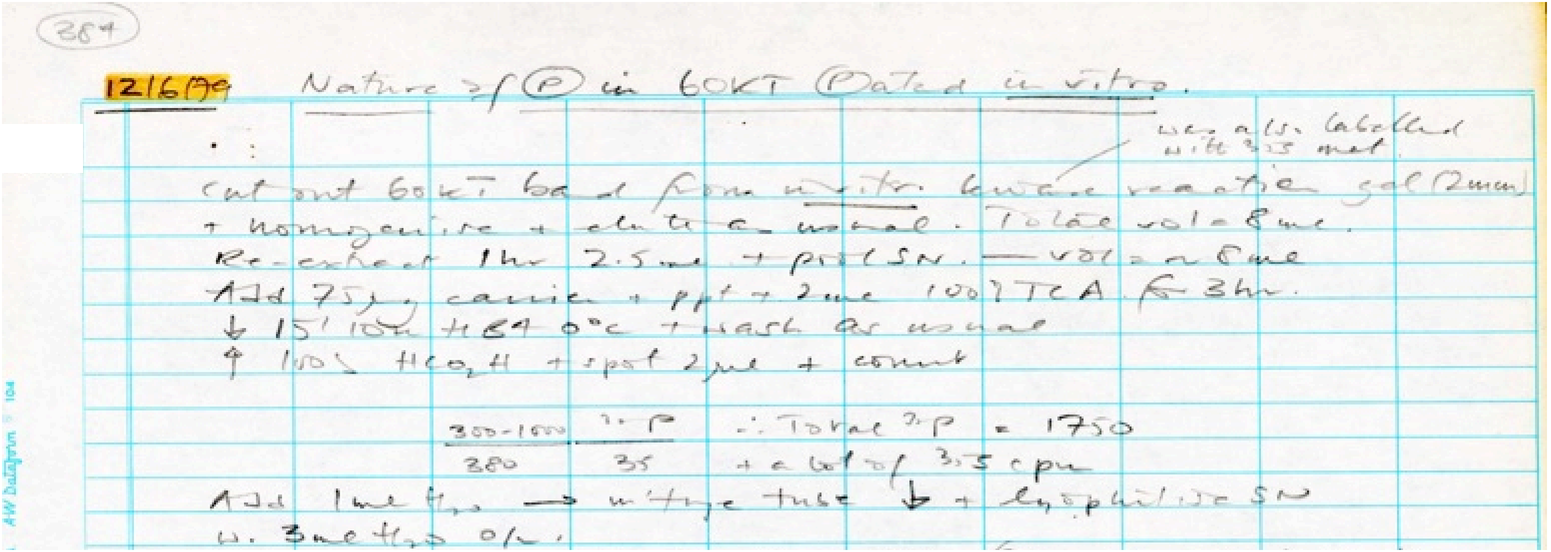
Reviewer 3

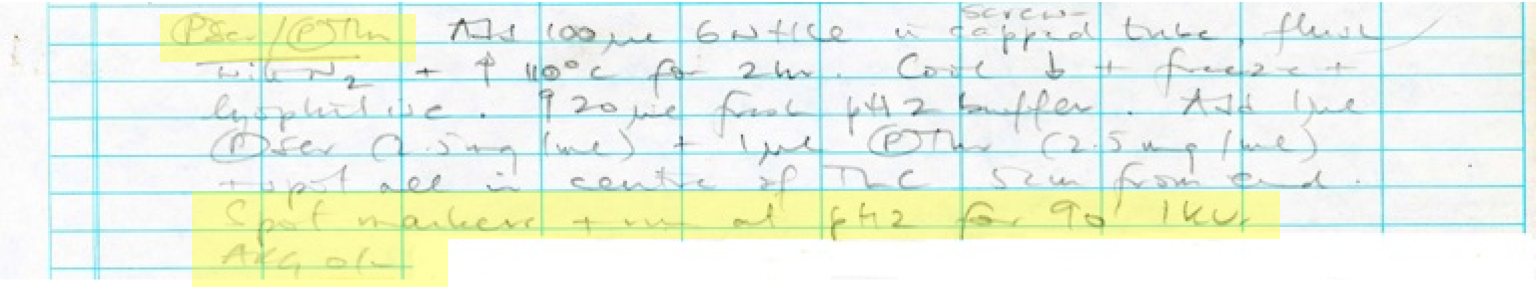
This manuscript deals with the intriguing and somewhat fashionable idea that viral coded proteins involved in transformation may have an associated protein kinase activity. In this particular case the authors present preliminary evidence which is interpreted to indicate that (a) the polyoma medium T from infected and transformed cells is at least the target for phosphorylation and that functional medium T may, in addition, be required for an observed protein kinase activity found in immunoprecipitates using rat anti-tumor sera; (b) large T is also phosphorylated but to a lesser extent, and does not appear to be required for activity; (c) the kinase activity evidently does not use IgG as an efficient phospho acceptor. Unfortunately, very few of these conclusions drawn by the authors are actually clearly substantiated by the data.

Major Comments:

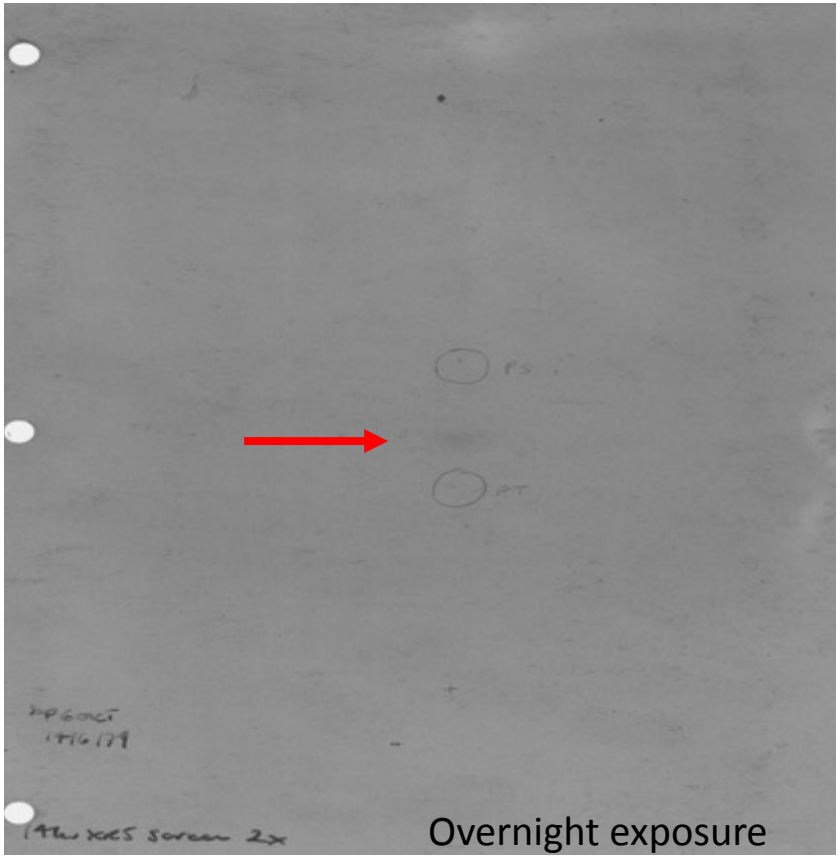
1) It is possible and consistent with some of the data that medium T is the target of a kinase in rat IgG immunoprecipitates. However, without additional biochemical evidence such as fingerprinting of the phospho protein etc., such a conclusion is not wholly warranted. This is especially true because in vivo medium T is apparently not phosphorylated.

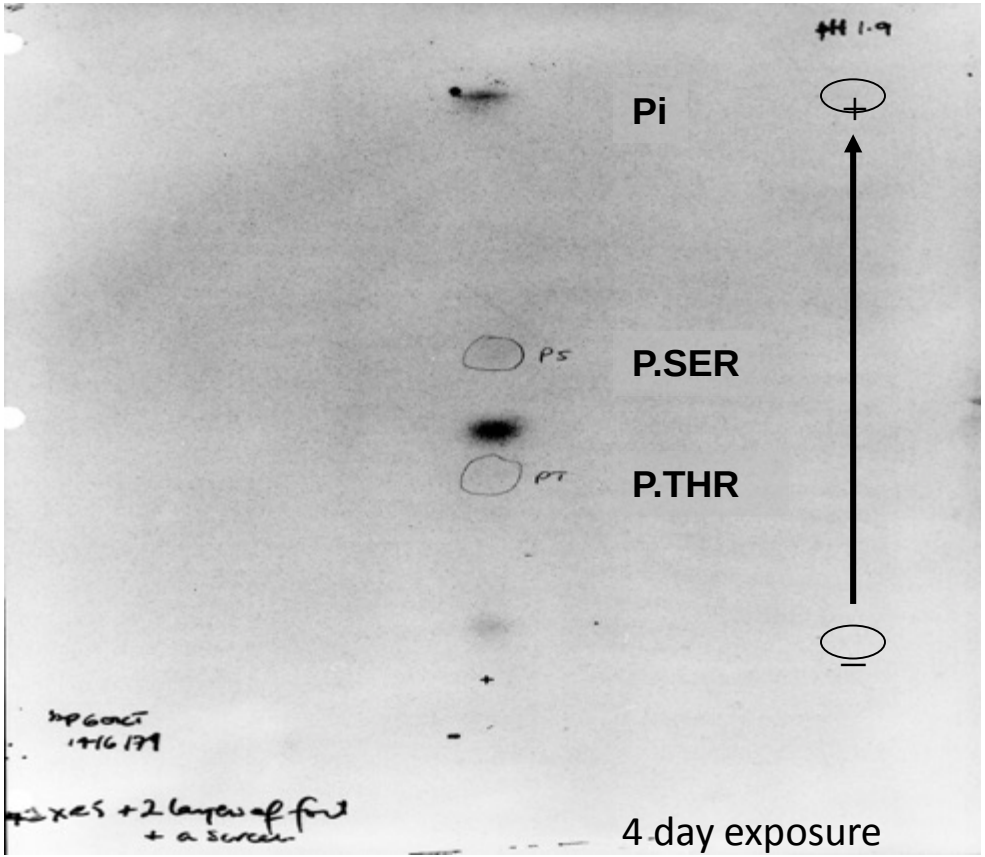
The first sighting of phosphotyrosine - June 1979





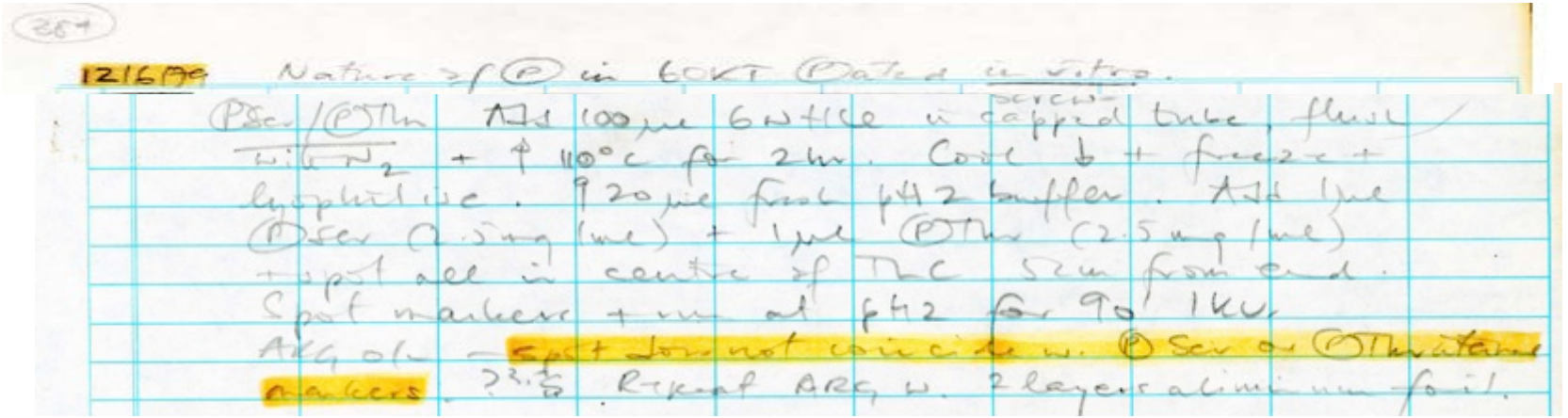
First phosphoamino acid analysis of ^{32}P-labeled polyoma middle T - luckily I used “old” electrophoresis buffer





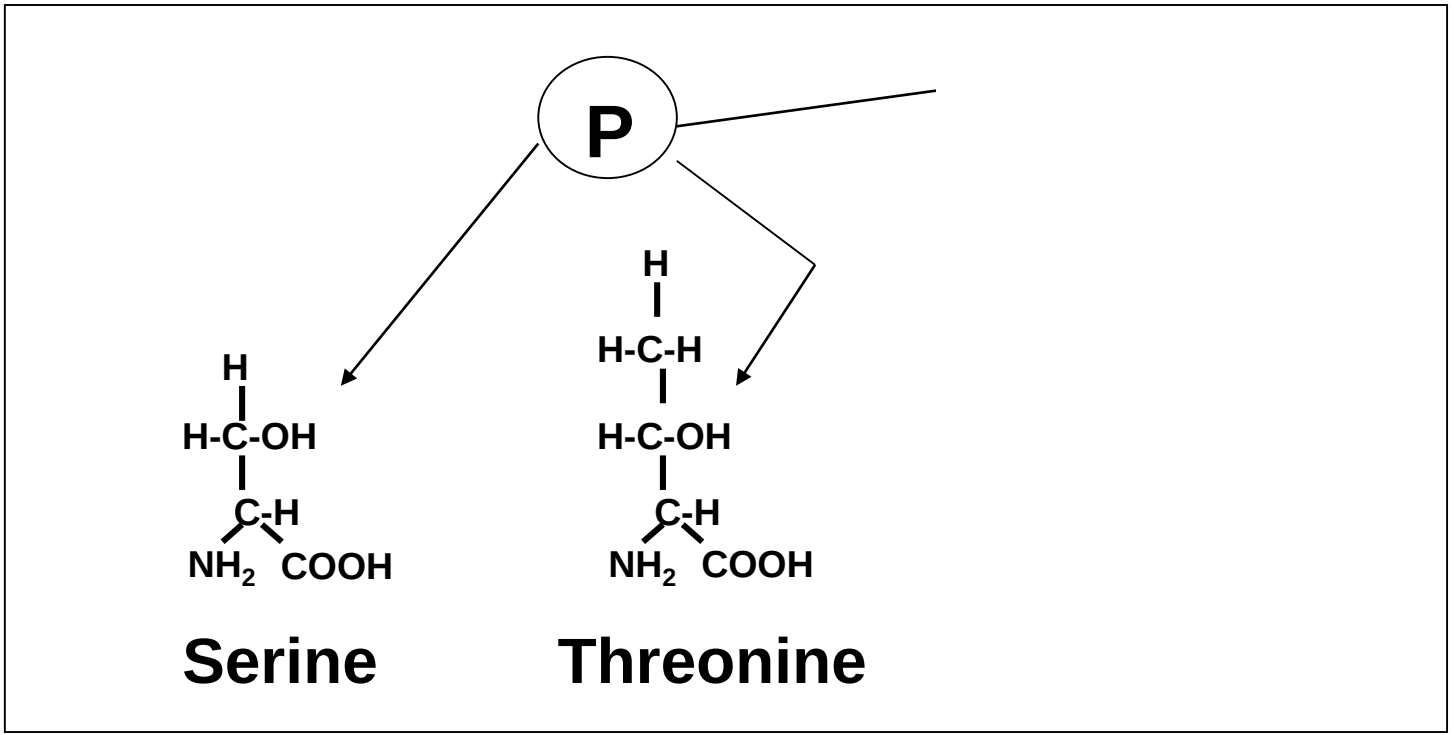
Thin layer electrophoresis at pH “1.9” on June 14, 1979

The first sighting of phosphotyrosine - June 1979

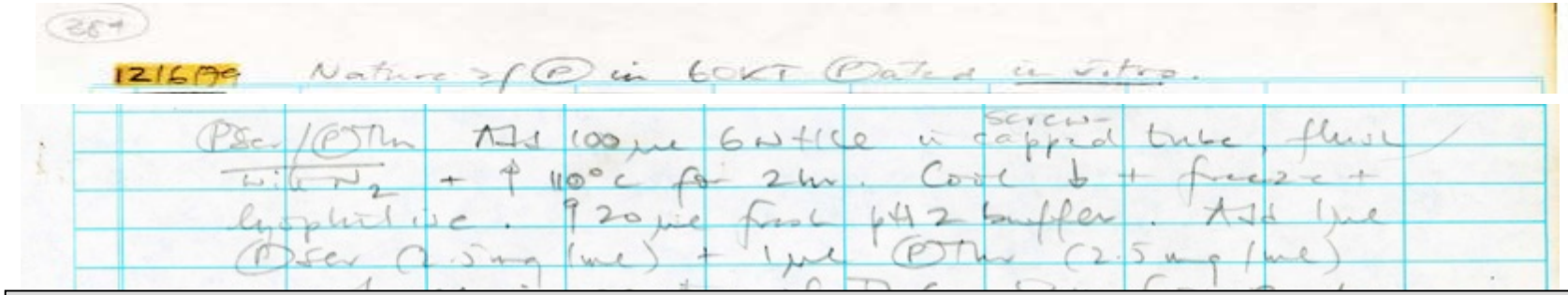


- I repeated the whole experiment on June 24, 1979 with the same result. Since the unknown ^{32}P-labeled compound was stable to acid hydrolysis, it seemed likely to be a phosphate ester, and, because there was only one hydroxyamino acid in addition to serine and threonine, i.e. tyrosine, the most logical explanation was that this was *phosphotyrosine*
- To test this idea, I naively tried to make some P.Tyr by mixing $POCl_3$ and tyrosine in water creating a black tar! However, I extracted a little soluble material, and, on July 2, I ran this at pH “1.9”, finding a faint ninhydrin-staining spot that migrated between P.Ser and P.Thr

The three hydroxyamino acids in proteins



The first sighting of phosphotyrosine - June 1979

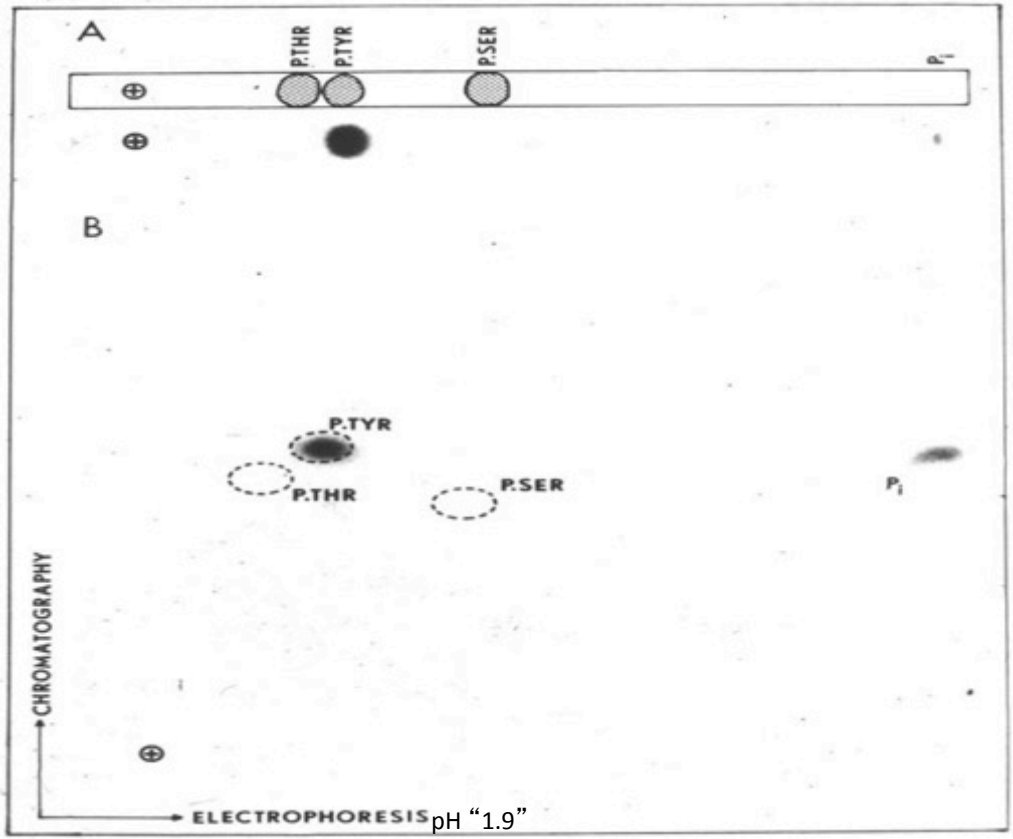


Despite the possibility that I might be on the verge of an important discovery, I left on July 3 to drive up to Idaho to raft the Salmon River, and from there on to Cambridge in England to attend the DNA Tumor Virus Meeting, not getting back to La Jolla until August 6!

- To test this idea, I naively tried to make some P.Tyr by mixing $POCl_3$ and tyrosine in water creating a black tar! However, I extracted a little soluble material, and, on July 2, I ran this at pH “1.9”, finding a faint ninhydrin-staining spot that migrated between P.Ser and P.Thr

Salmon River, Idaho - July 1979

Phosphoamino acid analysis of phosphorylated polyoma middle T



Eckhart, Hutchinson and Hunter, *Cell* 18:925 (1979)

Cell, Vol. 18, 925–933, December 1979, Copyright © 1979 by MIT

An Activity Phosphorylating Tyrosine in Polyoma T Antigen Immunoprecipitates

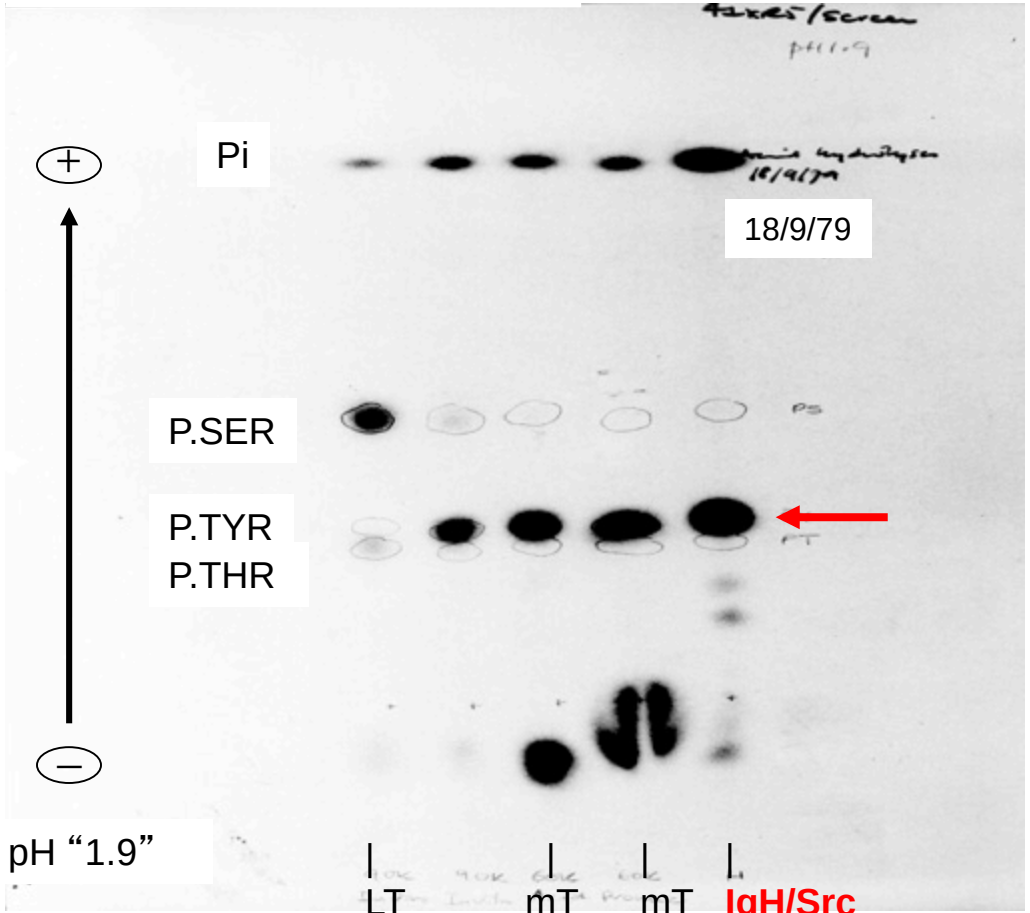
In retrospect, the discovery of P.Tyr depended on the fact that I had been too lazy to make up fresh pH 1.9 electrophoresis buffer, and the pH of the buffer I used had dropped to 1.7, causing P.Tyr and P.Thr to resolve

In 1983, Sara Courtneidge showed that the polyoma mT-associated tyrosine kinase activity is due to associated c-Src rather than being intrinsic

Paper submitted to Cell June 11, revised version submitted September 25, accepted September 27, and published December 1979

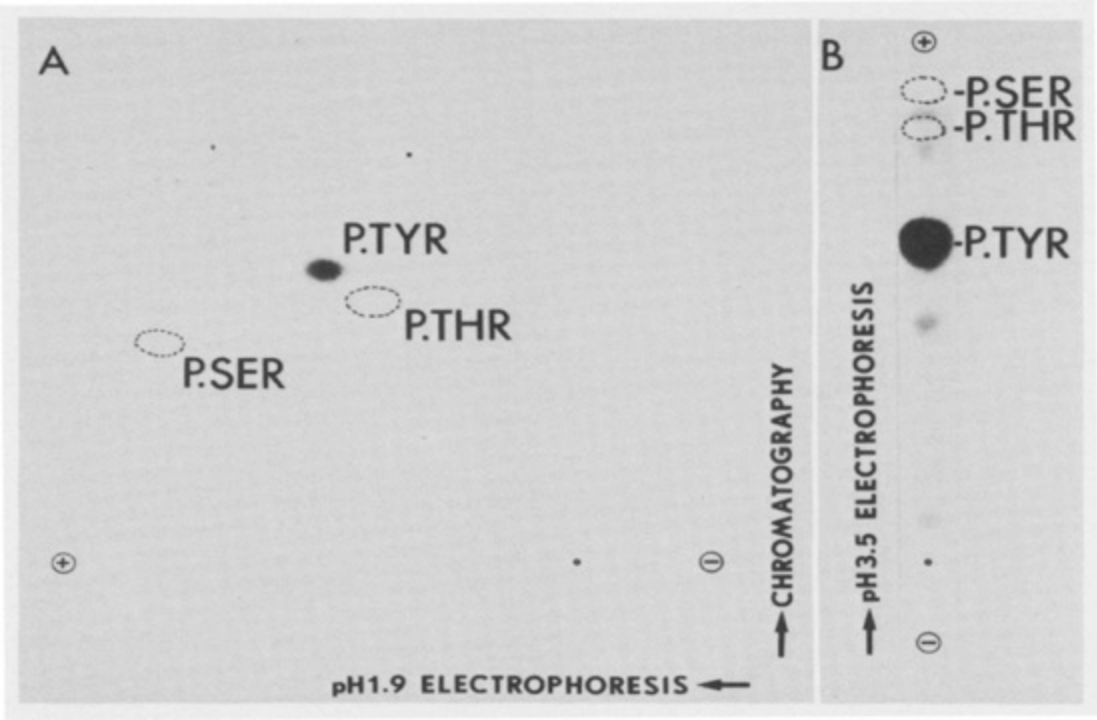
Eckhart, Hutchinson and Hunter, *Cell* 18:925 (1979)
Smith, Smith, Griffin, Fried, *Cell* 18:915 (1979)
Schaffhausen and Benjamin, *Cell* 18:935 (1979)

The second stroke of luck - using v-Src as a control!



Electrophoresis carried out on September 18, 1979

v-Src phosphorylates the Ig H chain on tyrosine

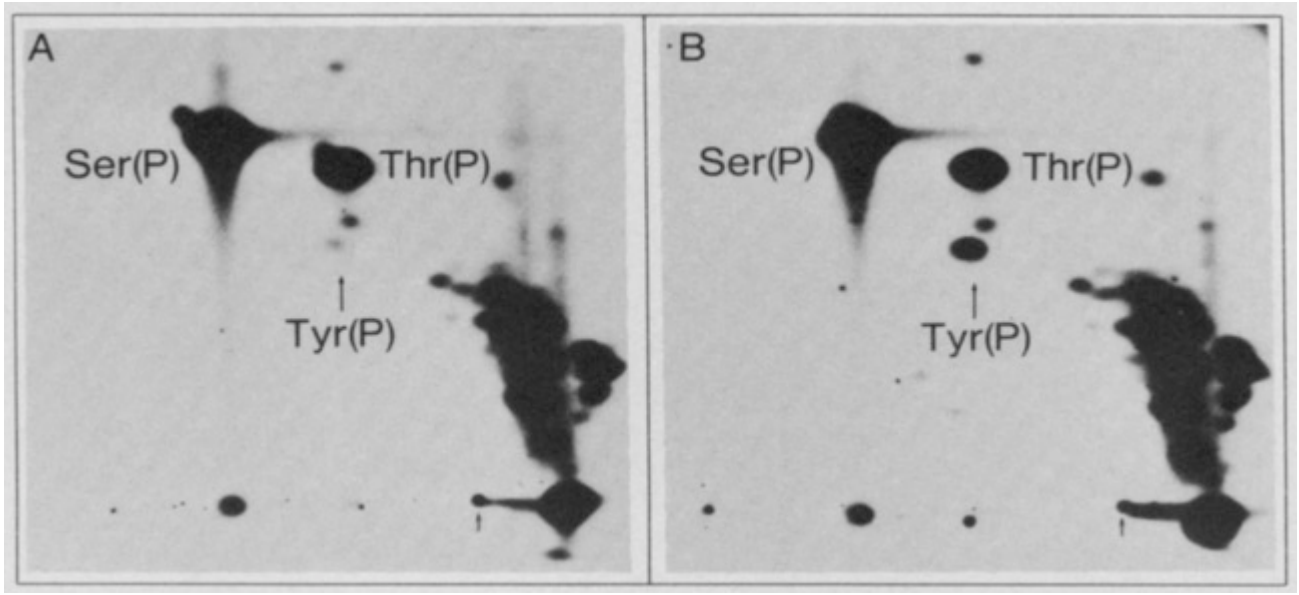


Experiment carried out
September 23, 1979

Hunter and Sefton, *PNAS* 77:1311 (1980)

v-Src increases P.Tyr levels in transformed chick cells

Uninfected v-Src-transformed



Experiment carried out on October 14-17, 1979

^{32}P-labeled control and RSV-transformed chick fibroblasts

Hunter and Sefton, *PNAS* 77:1311 (1980)

The second paper fared a lot better!

#1

#2

#3

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, U.S.A.

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, U.S.A.

Request for opinion on manuscript by **Tony Hunter and Bartholomew M. Sefton**

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, U.S.A.

Request for opinion on manuscript by **Tony Hunter and Bartholomew M. Sefton**

Title **The transforming gene product of rous sarcoma virus phosphorylates tyrosine**

The *Proceedings of the National Academy of Sciences, U.S.A.*, an interdisciplinary journal, intends to publish brief reports of original research of exceptional importance or novelty. I am writing to ask your opinion on the following points, together with any other comments you may offer.

1. Does the evidence justify the conclusions drawn? Yes ☒ No ☐
2. Are the procedures used sufficiently documented so that other competent investigators can repeat the work? Yes ☒ No ☐
3. Is this a paper of particular broad interest to diverse groups of scientists? Yes ☒ No ☐
4. If this is primarily a "method" paper, does the method described markedly increase available sensitivity, specificity, or convenience when compared to existing techniques? Yes ☐ No ☐
5. Is the paper clearly written? Yes ☒ No ☐
6. Is the overall quality of this paper in the top 10th percentile of papers in its field? Yes ☒ No ☐

What is novel or significant about this paper?

Reports a new protein kinase specificity (for tyrosine).

Comments (use additional pages if necessary; send original and two copies).

This is a clear well-written MS of great significance. This unusual specificity for tyrosine will greatly improve chances for identification of the cure kinase targets.

No revision is necessary and immediate publication is strongly recommended. This is very nice work.

Proc. Natl. Acad. Sci. USA
Vol. 77, No. 3, pp. 1311–1315, March 1980
Biochemistry

Transforming gene product of Rous sarcoma virus phosphorylates tyrosine

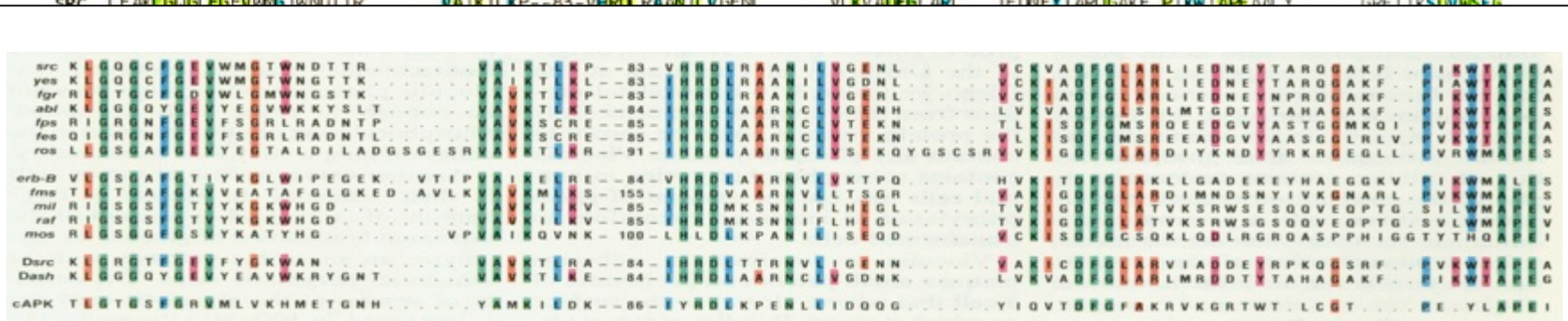
(phosphotyrosine/protein kinase/*src* gene/phosphoproteins)

Amazingly, all the experiments in the *Cell* and *PNAS* papers were done in less than 5 months, and all the v-Src experiments were completed in less than a month

"Discovering the first tyrosine kinase."
PNAS 112:7877 (2015)

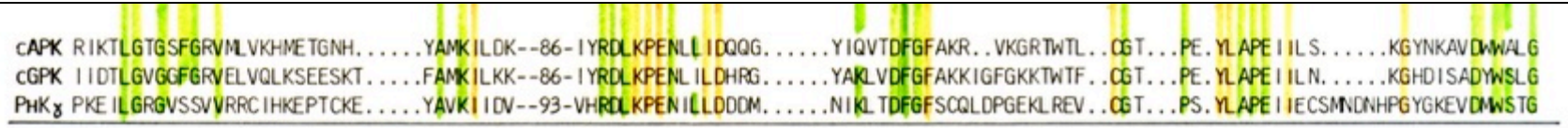
Paper submitted to Bob Holley on November 12, communicated to *PNAS* on December 4, 1979, and published March 1980

The kinome turns 30 - stone age bioinformatics!



Hunter, *Scientific American* 250:70 (1984)

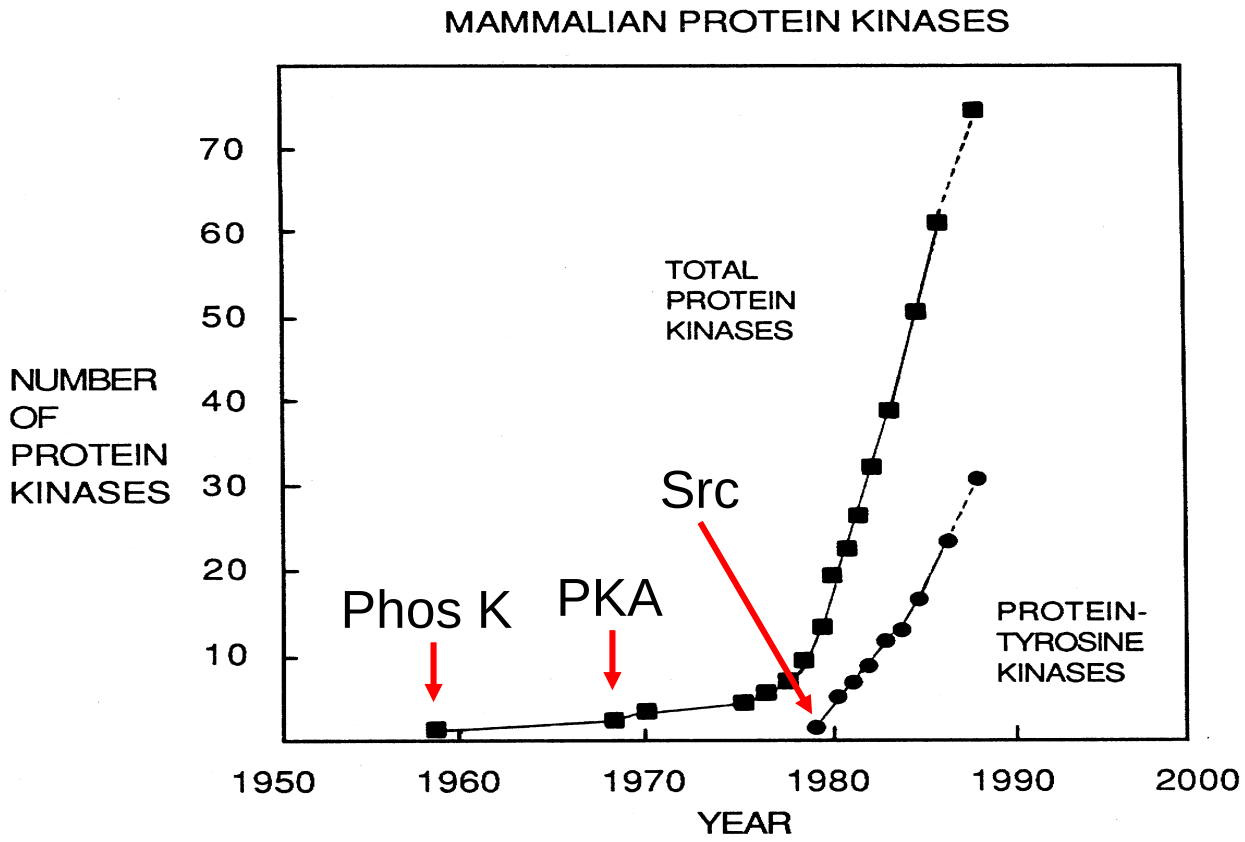
Hunter and Cooper, *Annu Rev Biochem* 54:897 (1985)



Manual alignment - 1984 (BB)

Barker and Dayhoff. Viral src (Src/Mos) gene products are related to the catalytic chain of mammalian cAMP-dependent protein kinase. *Proc Natl Acad Sci* 79:2836 (1982)

The birth of the kinome: a thousand and one protein kinases



Hunter, *Cell* 50:823 (1987)

Manning, Whyte, Martinez,

Human Kinome 2.0 (2018)

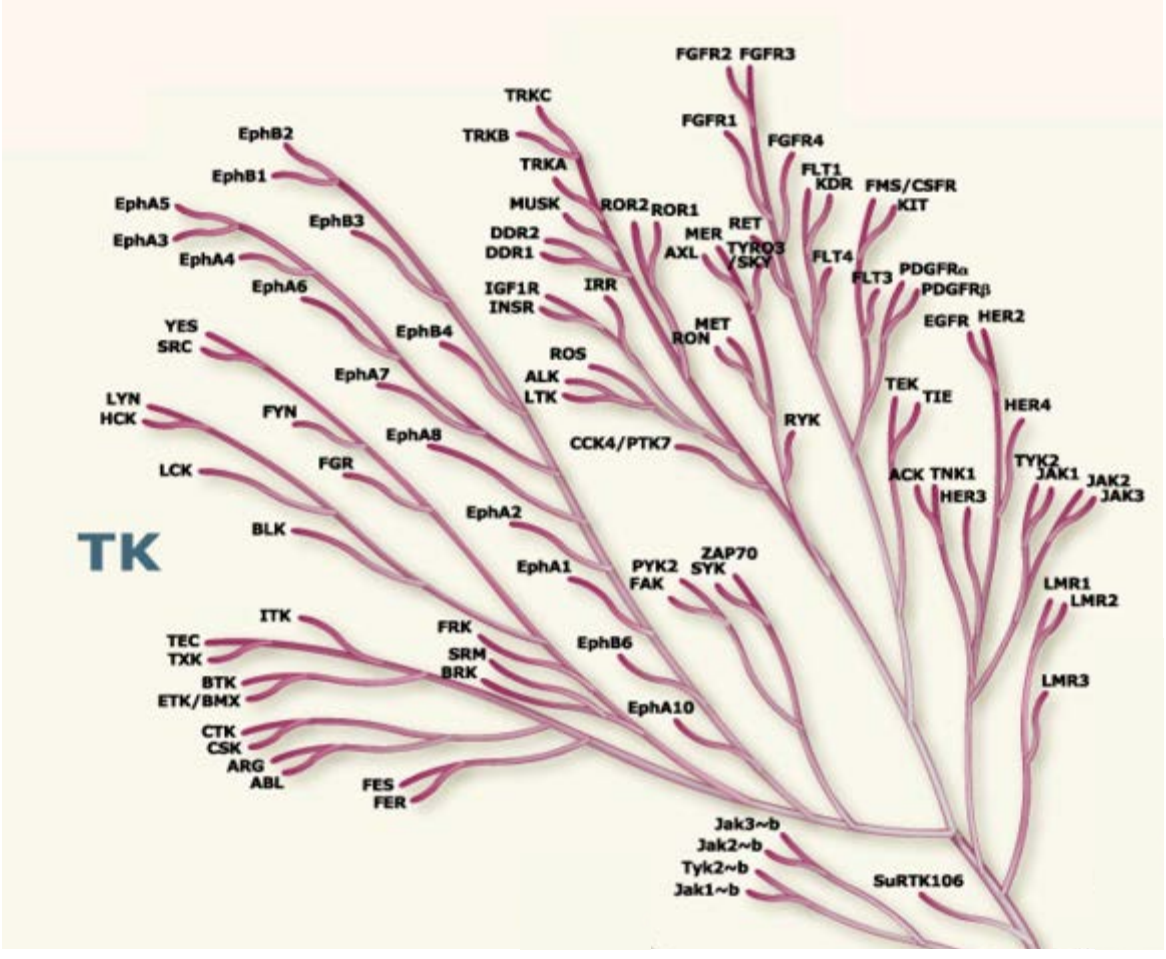
- ~535 protein kinase genes
- 22 new remote/atypical kinases (including: Fam20C, a secreted PK that is the *real* casein kinase, and NME family His kinases)
- No new canonical tyrosine kinases
- A few metabolic kinases that moonlight as protein kinases

Wilson, Prior et al. *Cancer Res* **78:**15-29 (2018) – 535 protein kinases

How many tyrosine kinases are there?

- The finding that v-Src and c-Src phosphorylate tyrosine gave us the first tyrosine kinase in 1979
- By the end of 1980 four tyrosine kinases were known (v-Src, v-Abl, EGF receptor, v-Fps/Fes). In 1984, v-ErbB was shown to be derived from the EGF receptor
- By the end of 1990 over 50 tyrosine kinases had been identified in vertebrates and equal numbers of tyrosine kinases and serine kinases were known, leading to the prediction that there might be several 100 tyrosine kinases in a vertebrate genome and a total of over a 1000 protein kinases
- The complete human genome sequence reported in 2001 reveals that there are **90 tyrosine kinases** out of a total of 518 PKs

Ninety human tyrosine kinases

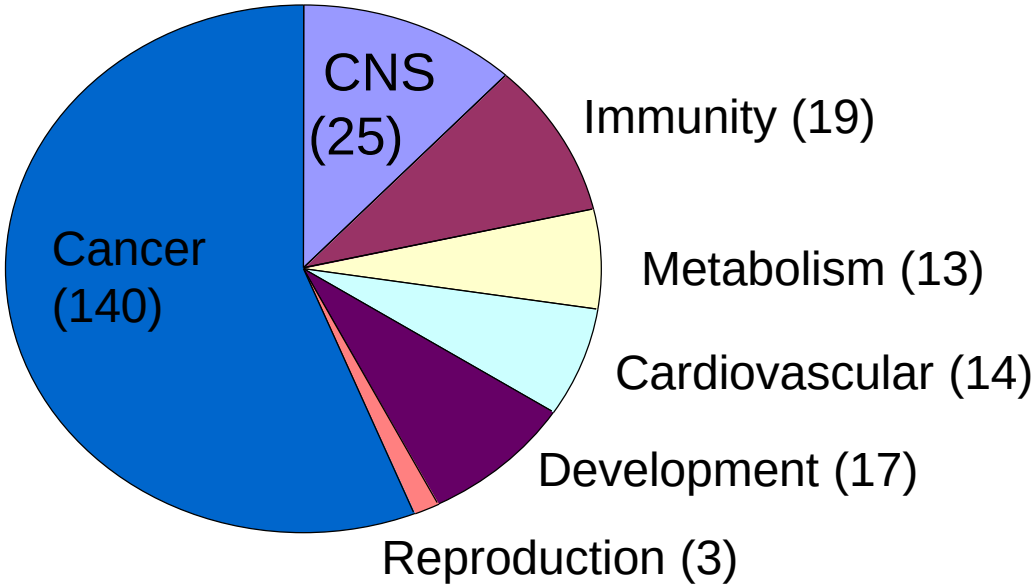


Manning, Whyte, Martinez, Hunter and Sudarsanam *Science* 208:1912 (2002)

Protein kinases and human disease

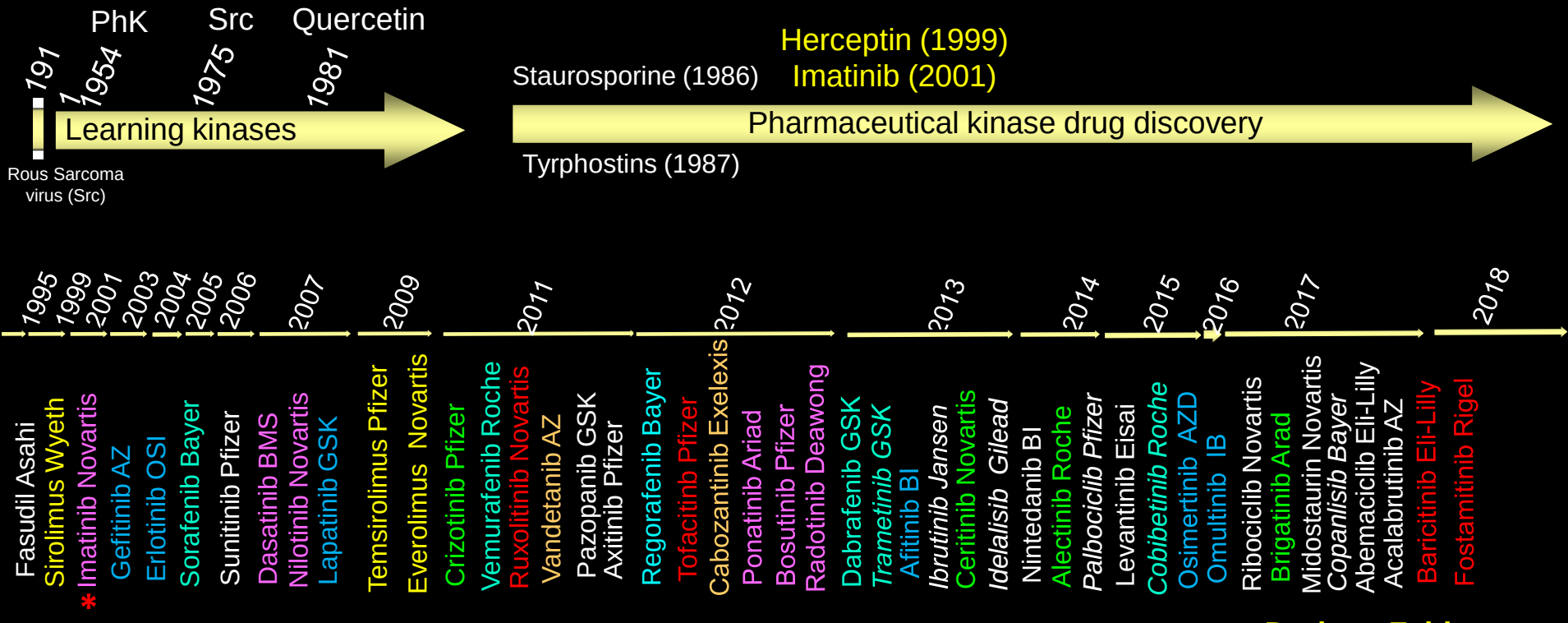
Over 175 protein kinases out of the ~535 human protein kinases have been implicated, either through gain-of-function or loss-of-function mutations, in human disease, especially cancer. The pervasive control functions of protein kinases also make them ideal therapeutic intervention targets, even for diseases where there is no genetic basis

Gerard Manning



Forty five kinase inhibitors (32 TKIs) are approved as cancer drugs

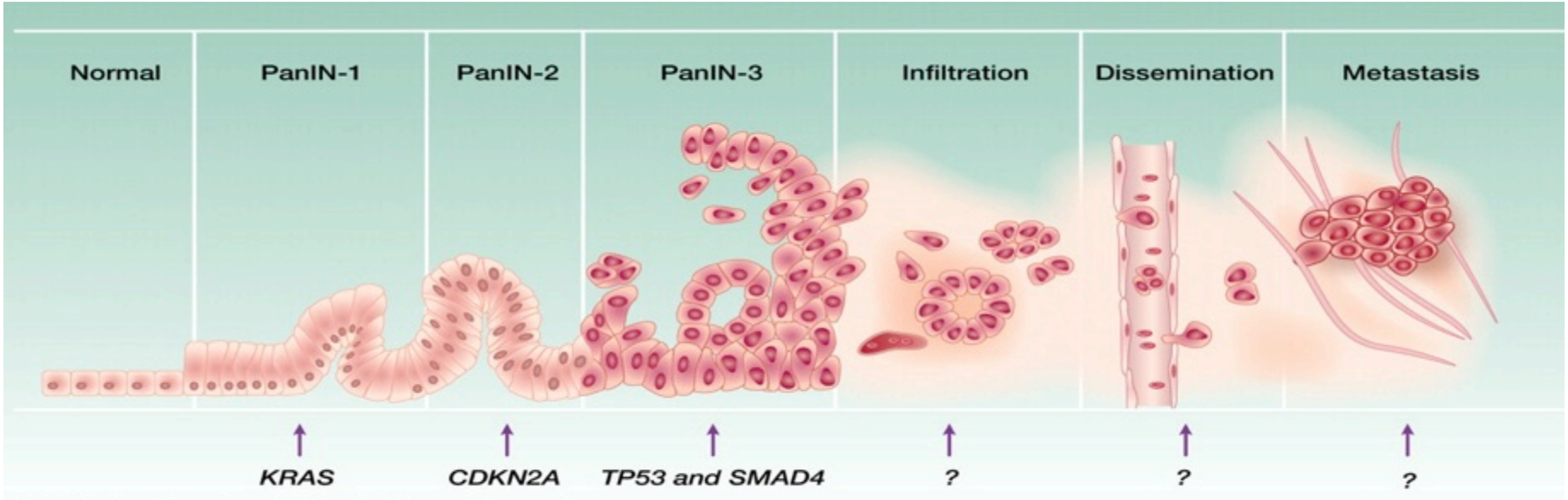
32 years of kinase drug discovery → 45 approved KIs (32 TKIs)



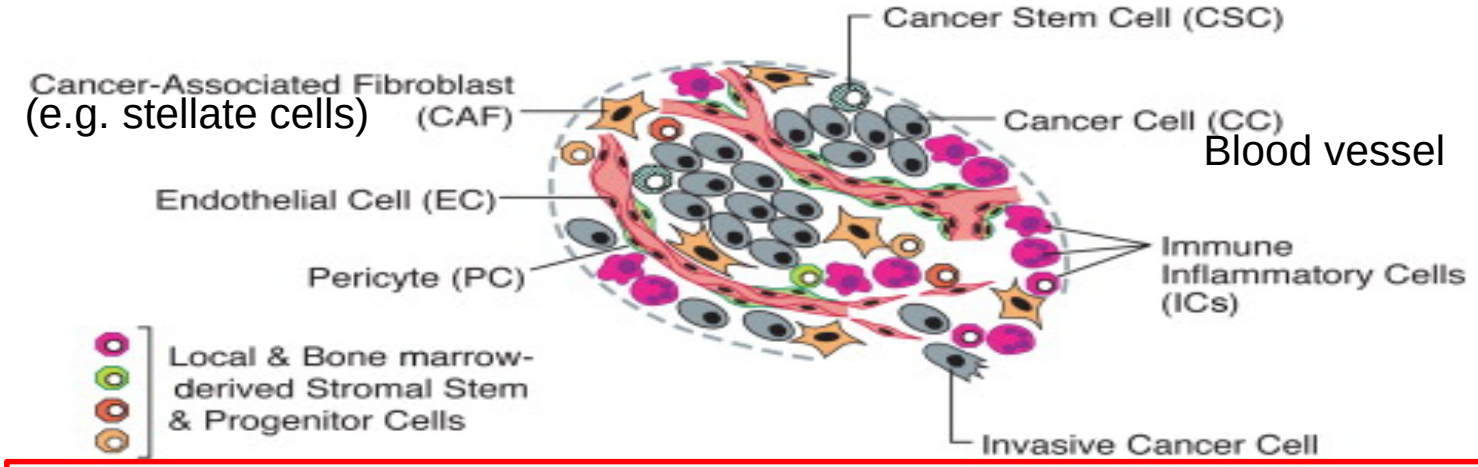
http://www.guidetopharmacology.org/GRAC/LigandListForward?type=Approved&database=all

Doriano Fabbro
(PIQUR Therapeutics)

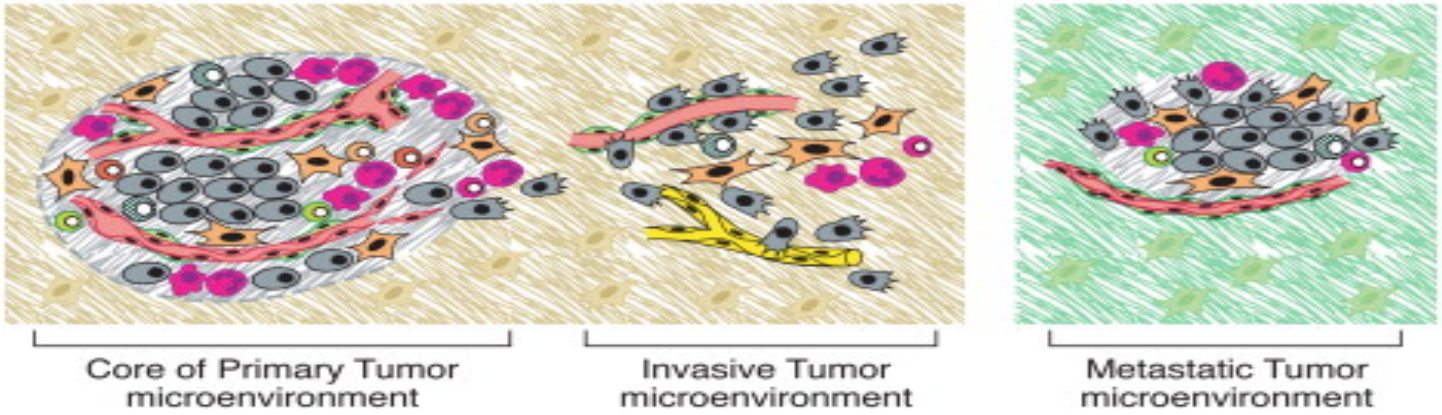
Pancreatic adenocarcinoma progression



The tumor microenvironment

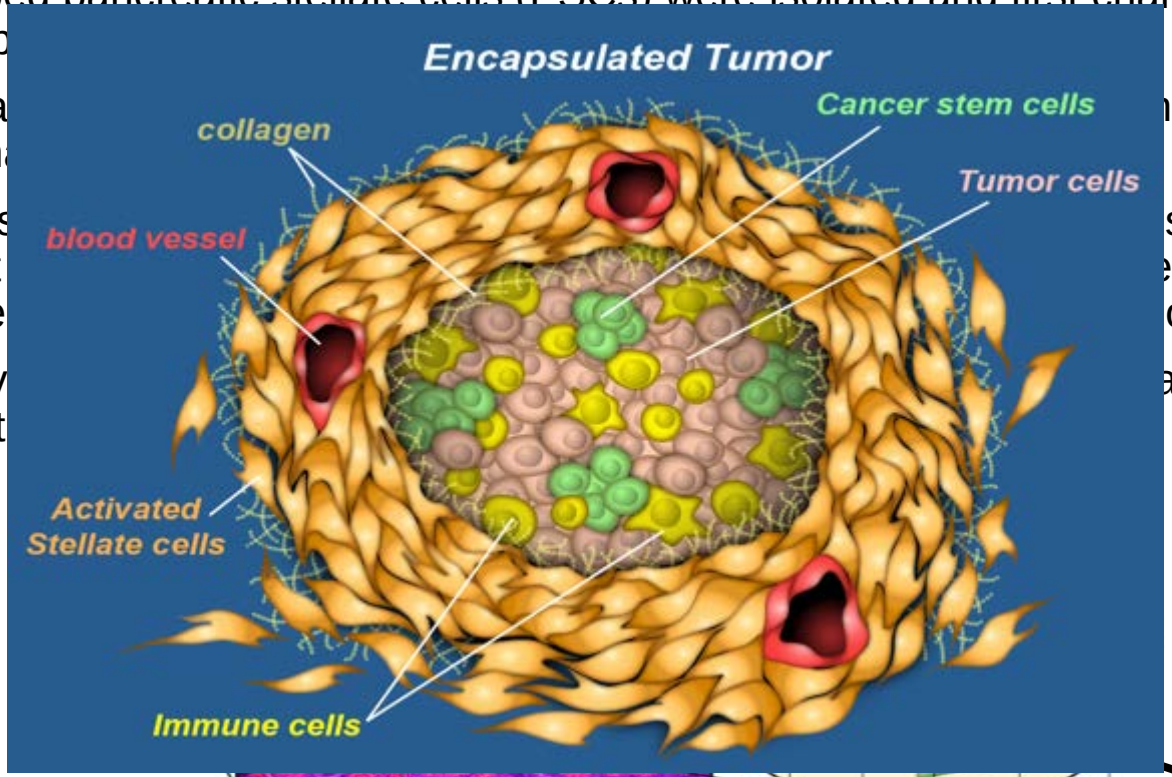


The tumor is a community of cells that talk to and support each other



Pancreatic stellate cells (PSCs)

- Star-shaped pancreatic stellate cells (PSCs) were isolated and first characterized in the 1990s (Ap
- In the hea nd mainly exhibit a periacin
- In respons sform from a quiescent ecretes excessive of cytokines
- PSCs hav athogenesis of pancreatit



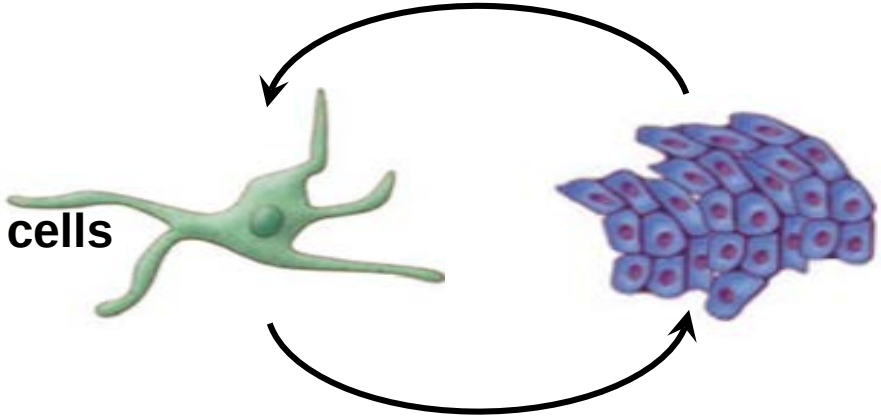
stellate cell

Islet
Acinar cell
Zymogen granule
PaSC
Centroacinar cell
Ductal cell

Omary et al. *JCI* 117:50 (2007)

Crosstalk between PSCs and PCCs plays a critical role in PDAC tumor progression

pancreatic stellate cells (PSCs)

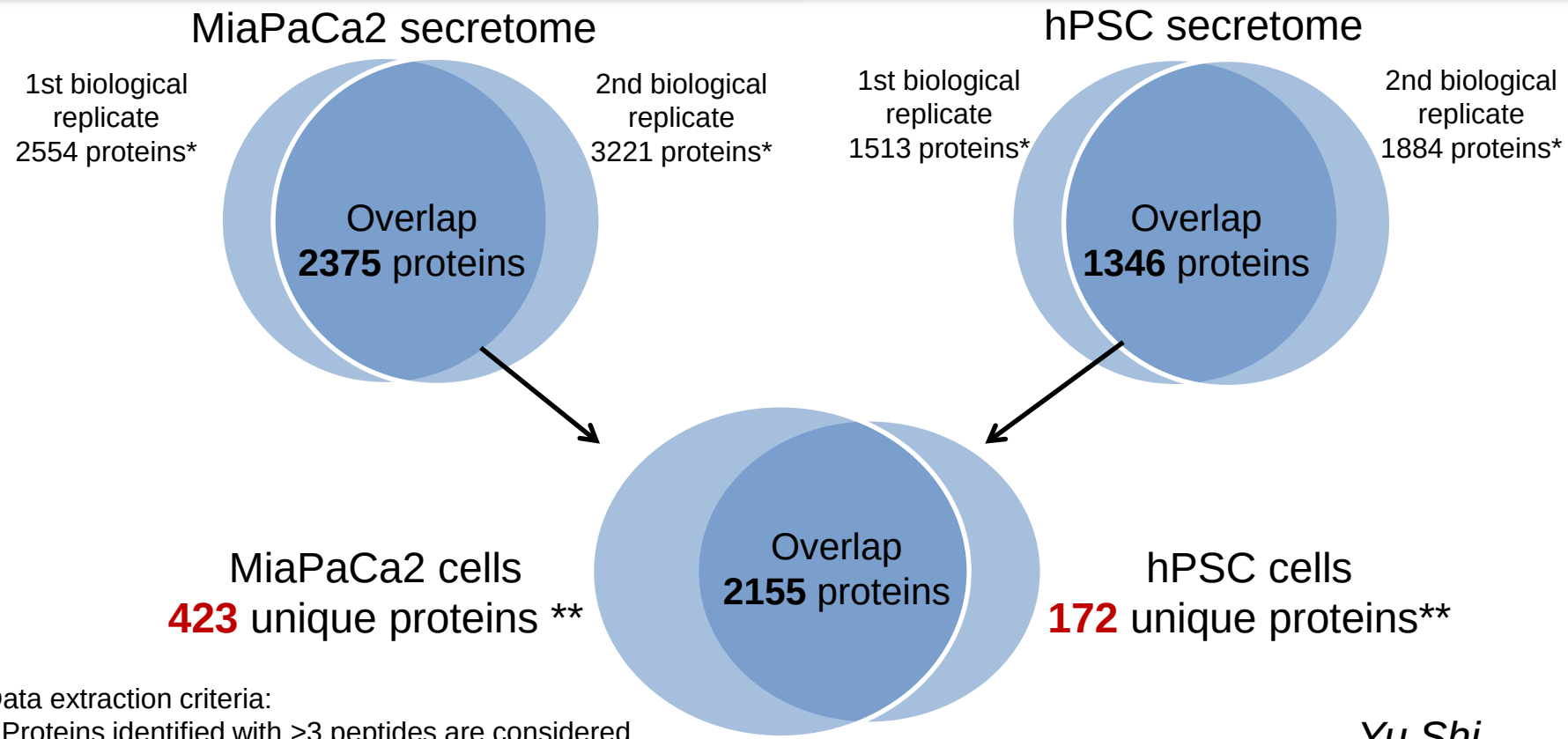


pancreatic cancer cells (PCCs)

Leukemia Inhibitory Factor (LIF)

What paracrine factors do pancreatic stellate cells secrete that can act onpancreatic cancer cells and vice versa?

Profiling the secretome of stellate and cancer cells



Data extraction criteria:
* Proteins identified with >3 peptides are considered
** Only proteins identified with >= 10 peptides and are more than 10 fold are considered as unique proteins

Yu Shi
Ruijun Tian

Proteins secreted uniquely from stellate and tumor cells

	hPSC	MiaPaCa2	Both cells
Growth factors / cytokines / chemokines	CTGF, CCL2, CXCL12, HGF, GDF15, IL6, IL11, LIF, Wnt5a, ANGPTL2	AREG, BMP1, CXCL5, CXCL16, M-CSF, G-CSF, PDGFc, b/a(low), PEDF, VEGFa, VGF	CXCL1, CXCL2/3(low), HDGF, IL8, TGFβ1, VEGFc,
ECM	Collagen Ia1, IV, XII, XV, COMP, EFEMP1/2, FBN1/2, FMOD, FN1, LUM, POSATN, SPARC, SPON2, STC1/2, VCAN	SRRM2	Collagen Ia2, III, IV, V, VI, ECM1, LTBP3
Proteases and inhibitors	MMP1/2/3, ADAMTS1, CST1, MASP1, PAMR1, PLAT, RECK, SERPINs, TFPI2	ADAM15, MBTP1	ADAMTS9, CTSB, CTSD, CSTB, CST3, CPE, PLAU, SERPINE1, TIMP1/2
Receptors / membrance proteins	CDH2, CDH6, CD248/Endosialin, CD90, RARRES1	EGFR, Erb2, EphA2, EphA4, DNER, HGFR, IL27Ra, TGFBR3, TNFR1a	CD44, CD59, NRP1, TNFRSF12A

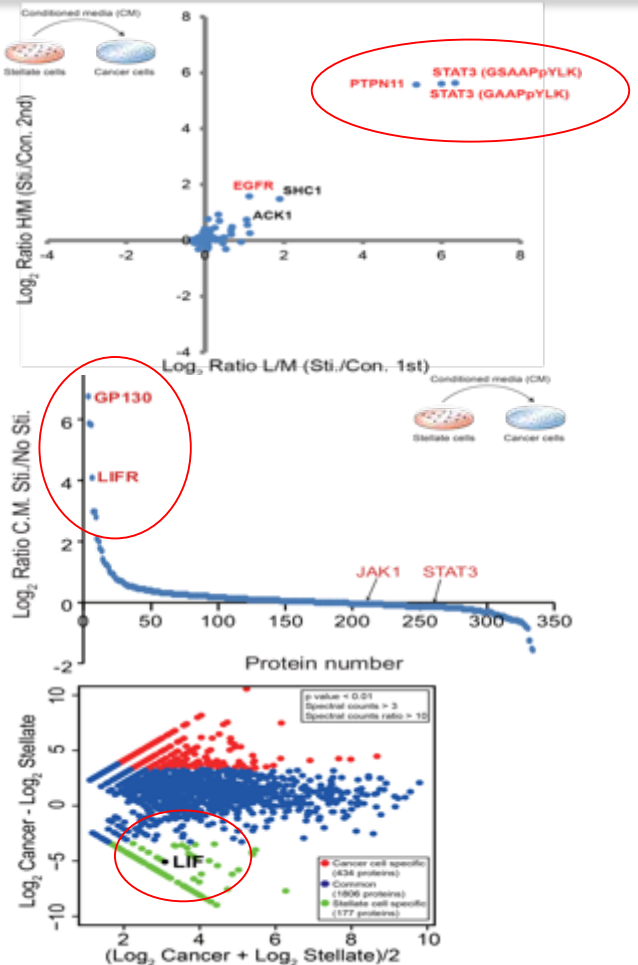
This secretome analysis has now been repeated with 30 PDAC lines and tumor tissue samples

Why we focused on LIF

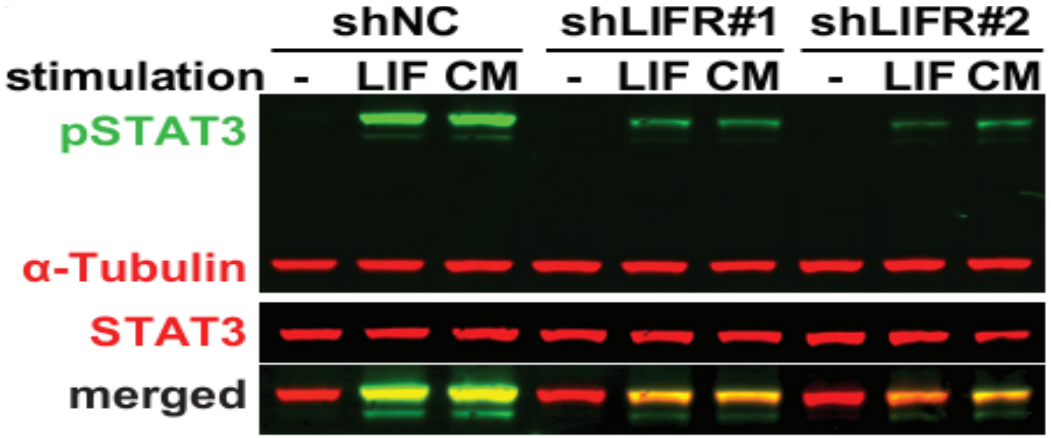
- Pancreatic stellate cell conditioned medium (CM) stimulates pTyr705-STAT3 in PDAC cells

- STAT3 binds to the LIF receptor (LIFR) and its co-receptor GP130 in PDAC cells stimulated with stellate cell CM

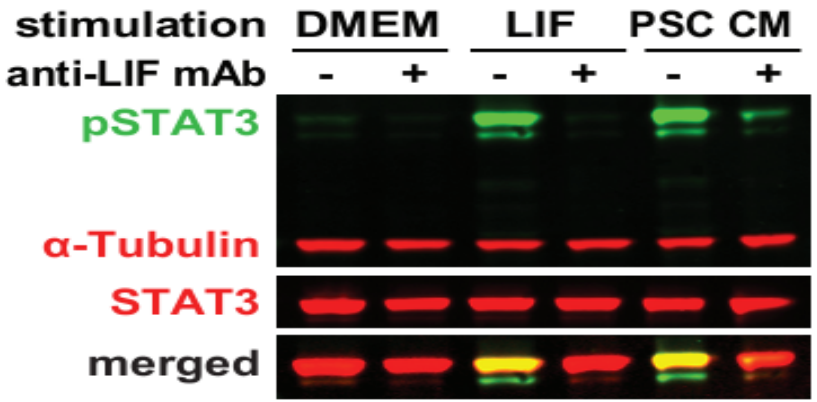
- The stellate cell *<u>secretome</u>* contains high LIF levels, and LIF is a stem cell factor



Why we focused on LIF



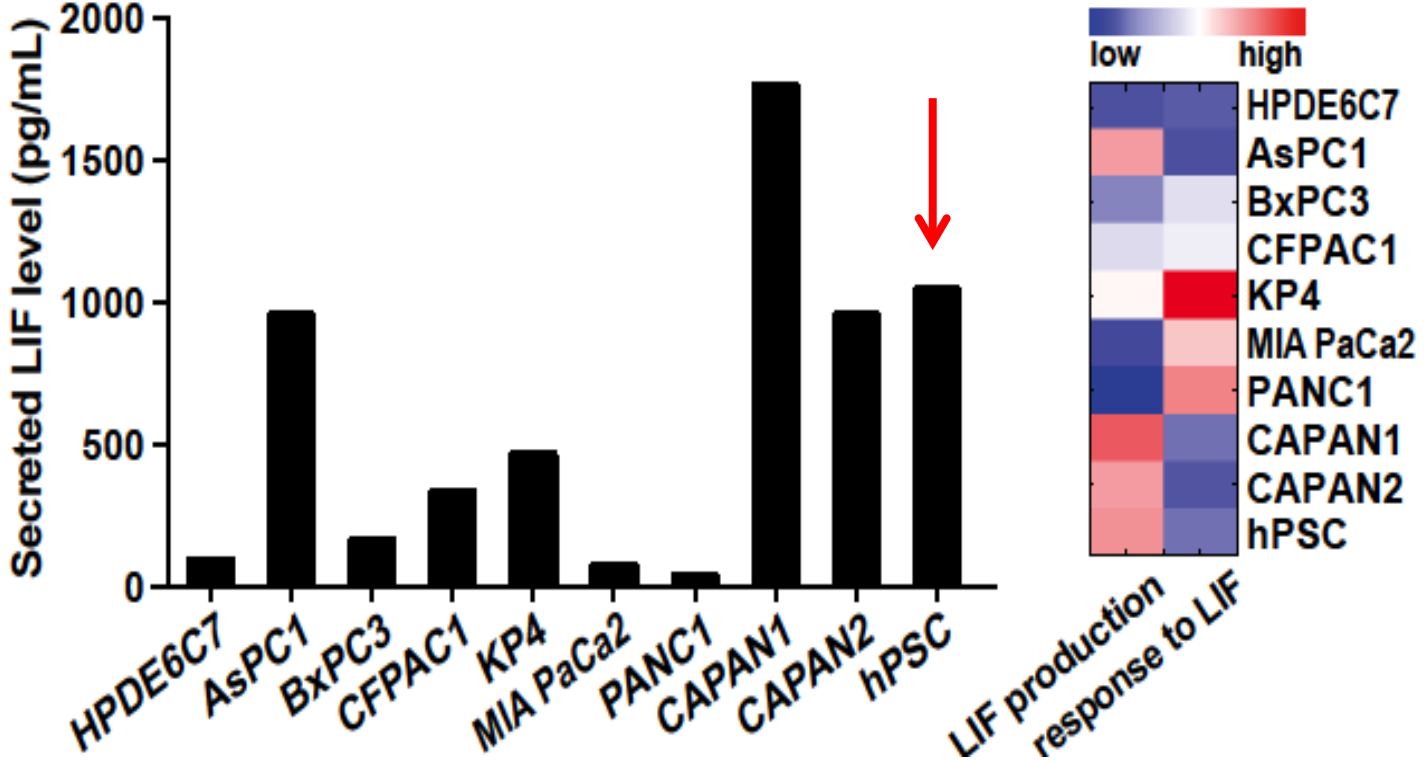
KP4 human PDAC cells
CM = stellate cell conditioned medium



KP4 human PDAC cells
D25 neutralizing anti-LIF mAb

LIF is the major secreted factor activating STAT3 in pancreatic cancer cells

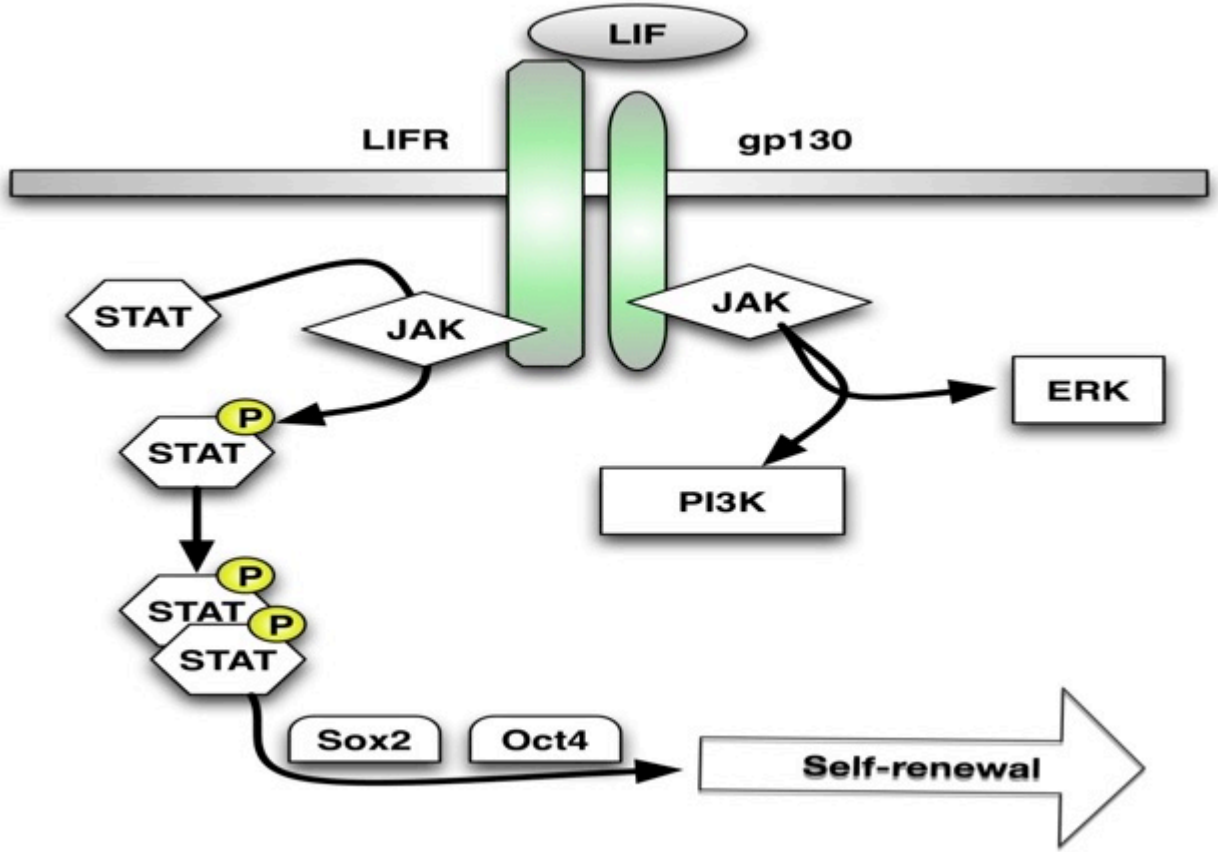
Why we focused on LIF



LIF is highly expressed by pancreatic stellate cells and by some not not all PDAC cells

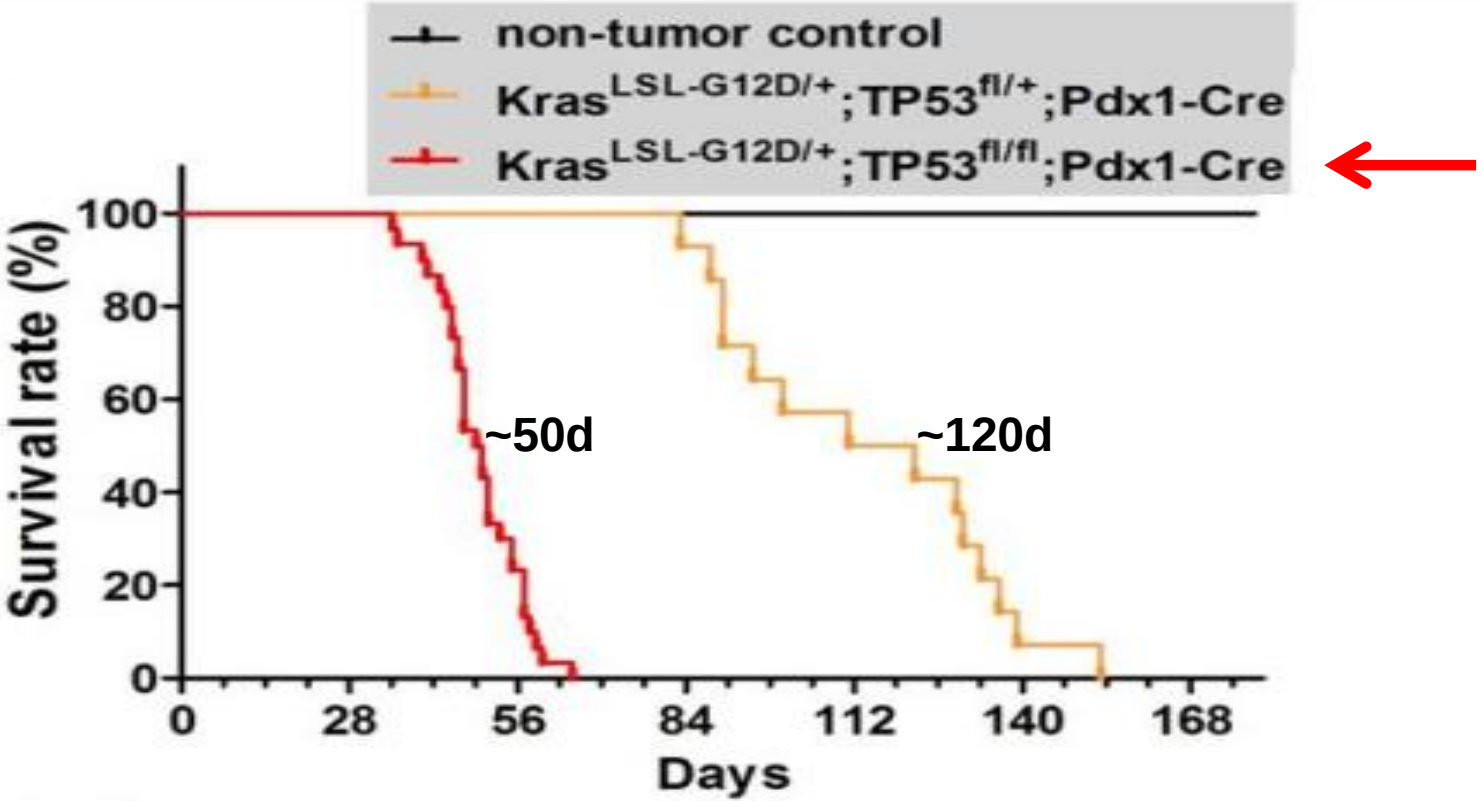
LIF/LIFR signaling

- **Leuk** okines, is a
  pleiot tion and
  also types. *LIF*
  is an
- LIF b dimer with
  the g .-6 family of
  recep cascades
- Effec



Does LIF play an important physiological role in PDAC?

The "*KPC*" Kr*as G12D/p53Δ* mouse pancreatic cancer model



Pdx1-driven Cre expression in pancreatic epithelial cells during development induces expression G12D K-Ras and loss of p53, initiating tumorigenesis

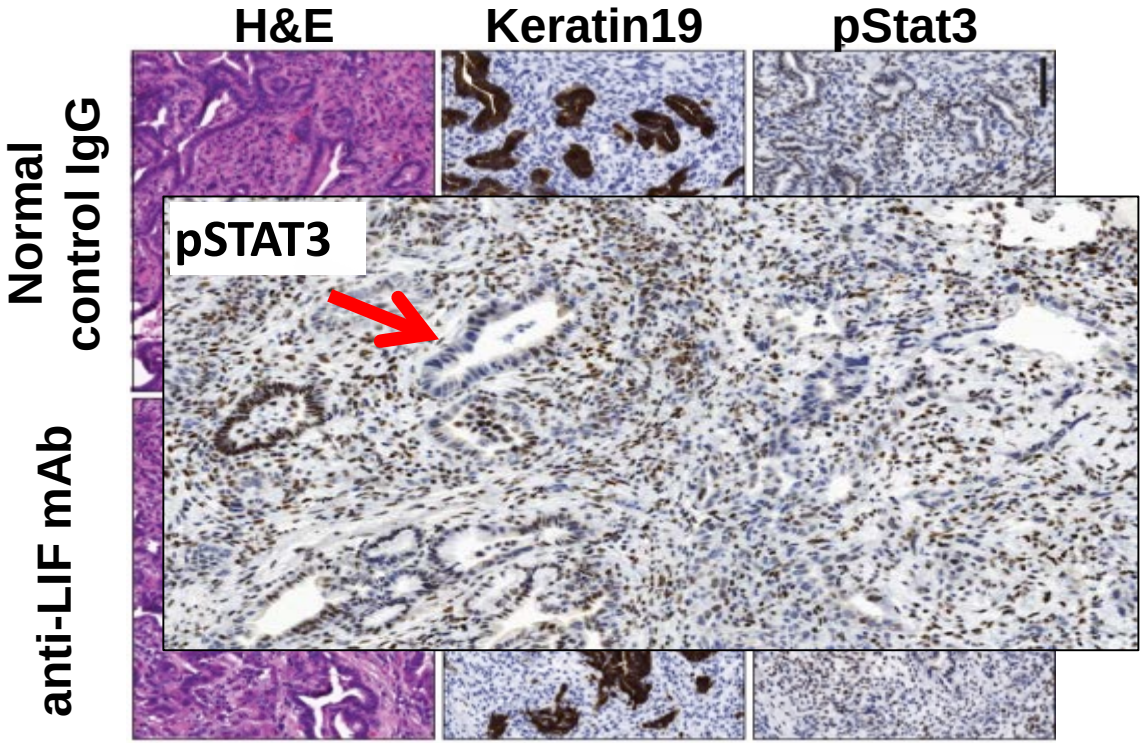
Does LIF play an important physiological role in PDAC?

- Preclinical studies to test the therapeutic effects of LIF signaling blockade with a neutralizing LIF mAb (D25) in $Kras^{LSL\text{-}G12D}$;$TP53^{fl}$;Pdx1-Cre;Rosa26-Luc (KPC-Luc) mice

- Use $Kras^{LSL\text{-}G12D}$;$TP53^{fl}$;Pdx1-Cre;$LIFR^{fl}$ mice to genetically assess the intrinsic role of LIFR signaling in PDAC tumor cells

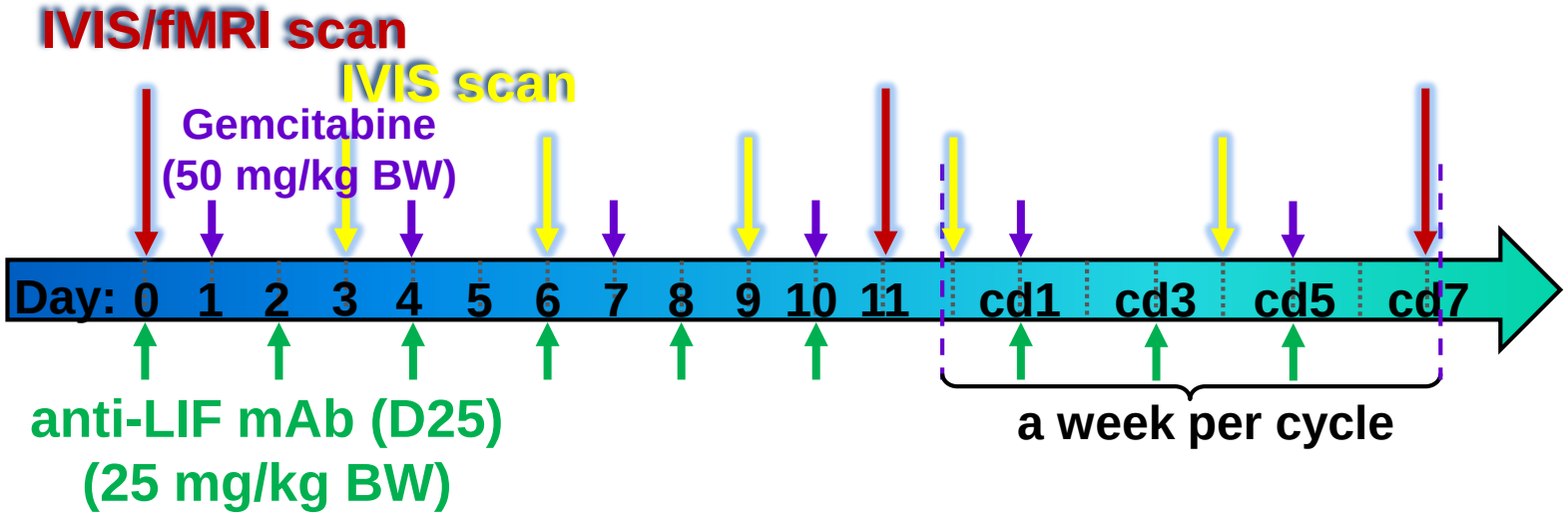
- Evaluate the potential correlation between LIF production and prognosis in PDAC patients

Phospho-STAT3 neutralizing activity of anti-LIF mAb



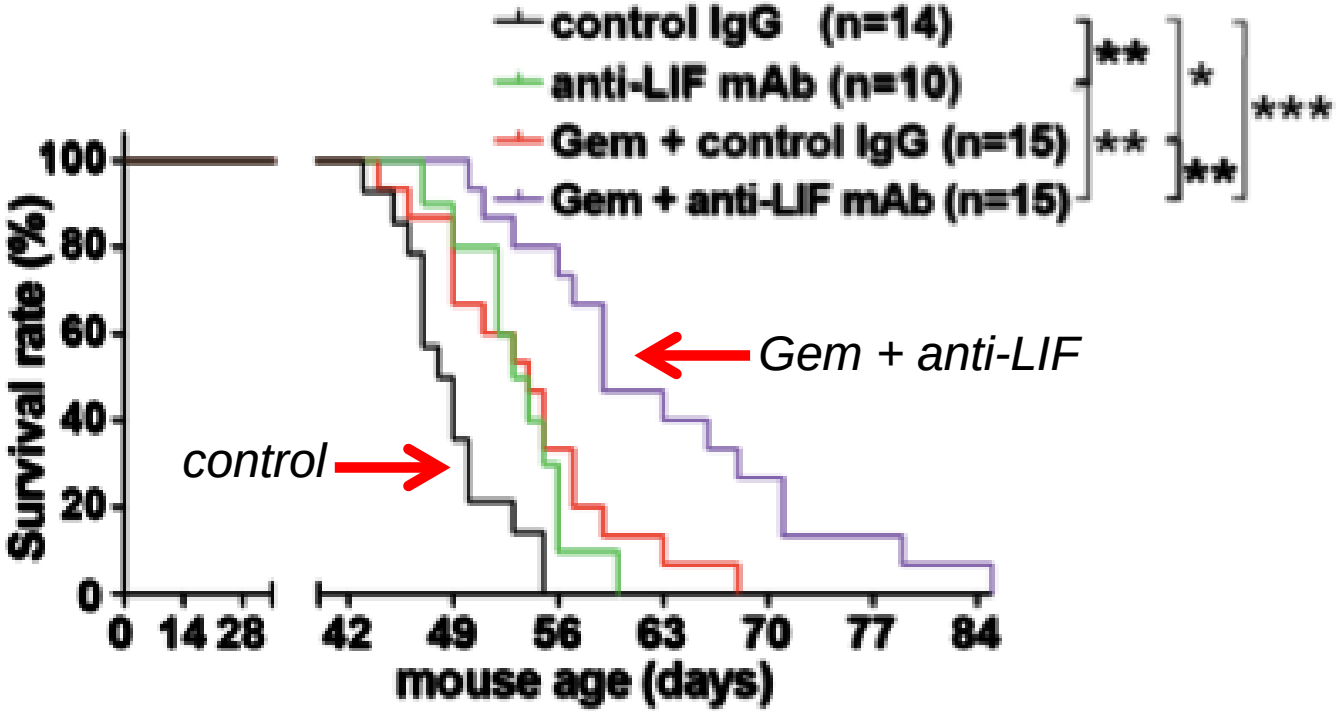
Treatment of KPC PDAC mice with D25 anti-LIF mAb at 25 mg/kg reduces nuclear phospho-STAT3 IHC signal

Preclinical therapeutic treatment protocol



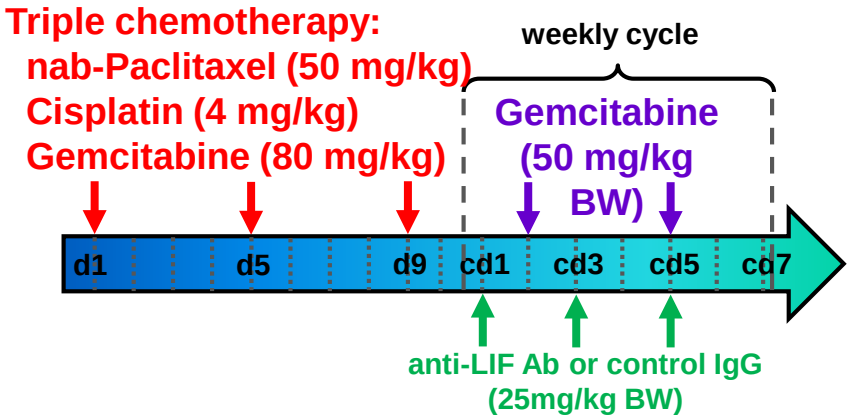
Day 0 = 32 days of age

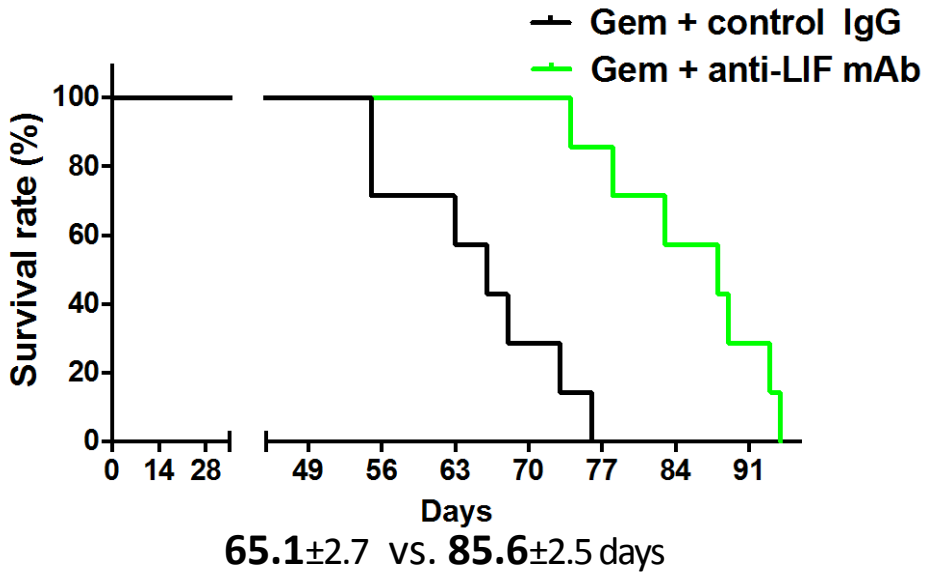
LIF blockade slows down tumor progression and sensitizes chemotherapy to prolong survival



LIF blockade slows tumor progression and enhances the response to gemcitabine in the KPC mouse model of PDAC

LIF blockade has a therapeutic benefit for advanced PDAC in a preclinical study using maintenance KPC mouse model

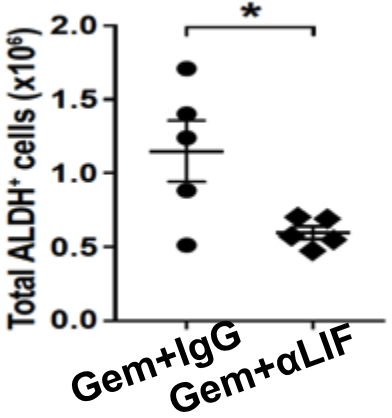


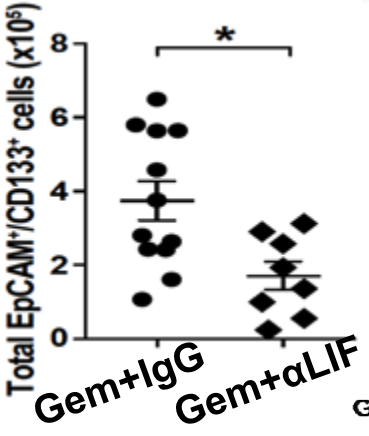


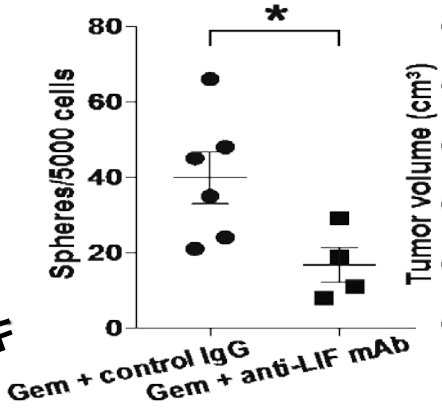
65.1±2.7 vs. **85.6**±2.5 days

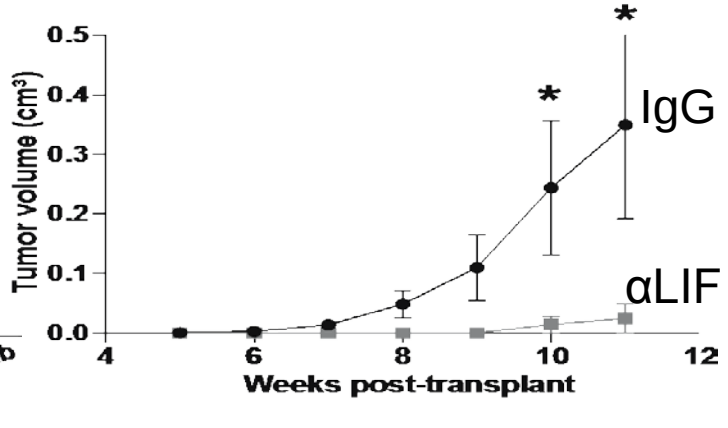
LIF has a role in progression in the KPC mouse model of PDAC

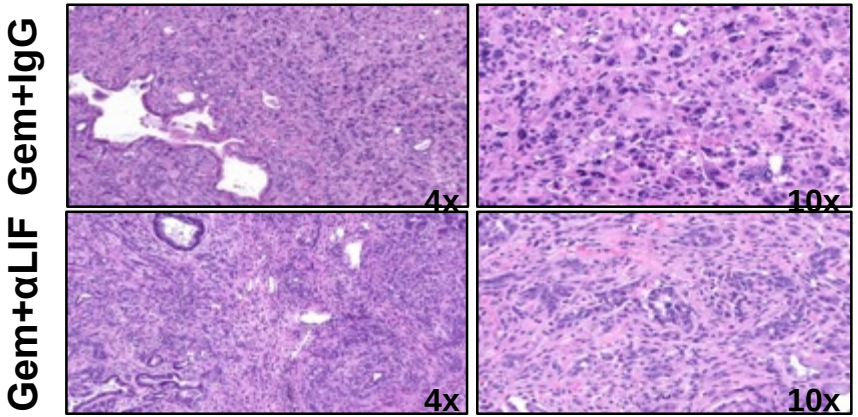
LIF blockade in KPC mice targets the cancer “stem cell” population and promotes tumor differentiation

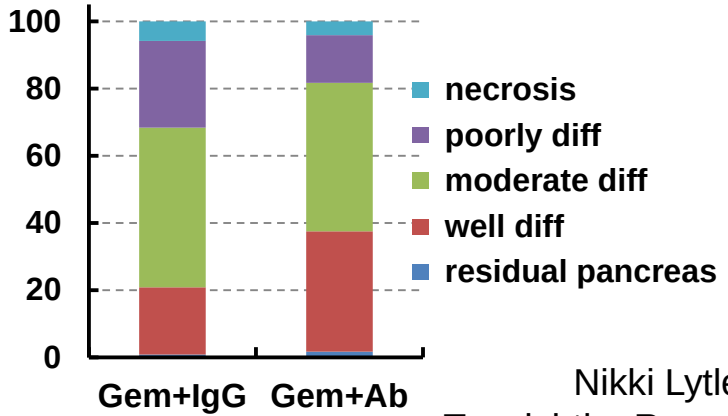












Nikki Lytle
Tannishtha Reya (UCSD)

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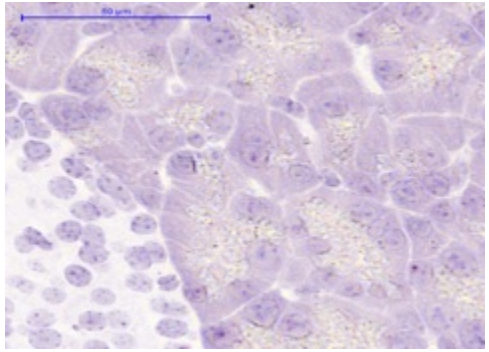
- Evaluate the potential correlation between LIF production and prognosis in PDAC patients

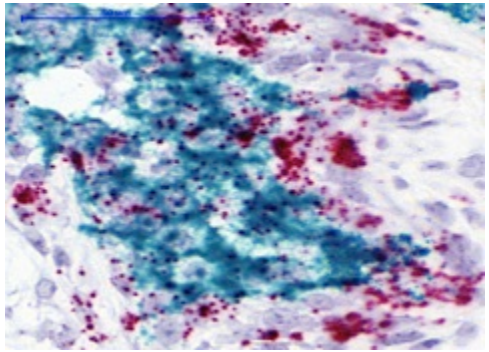
Aberrant *LIF* and *LIFR* expression in PDAC

Lif*/*Keratin19

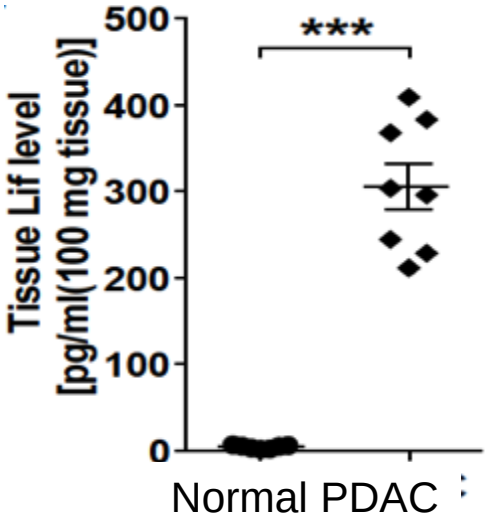
Normal



PDAC

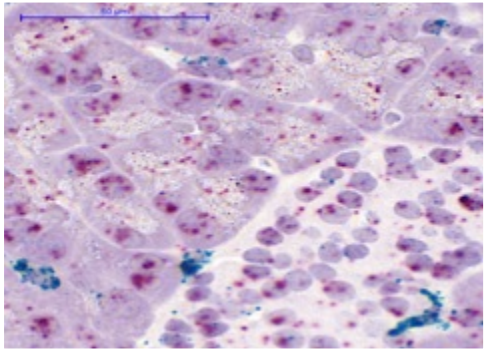


Tumor LIF levels

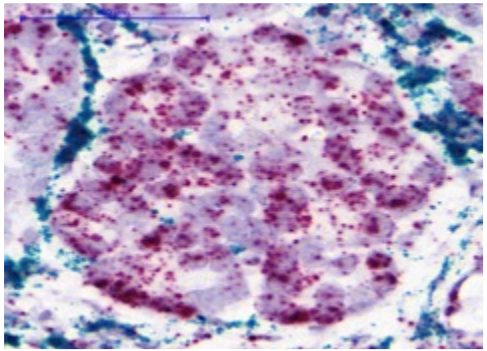


RNAscope two-***color***
RNA in situ hybridization

Lifr*/*Periostin



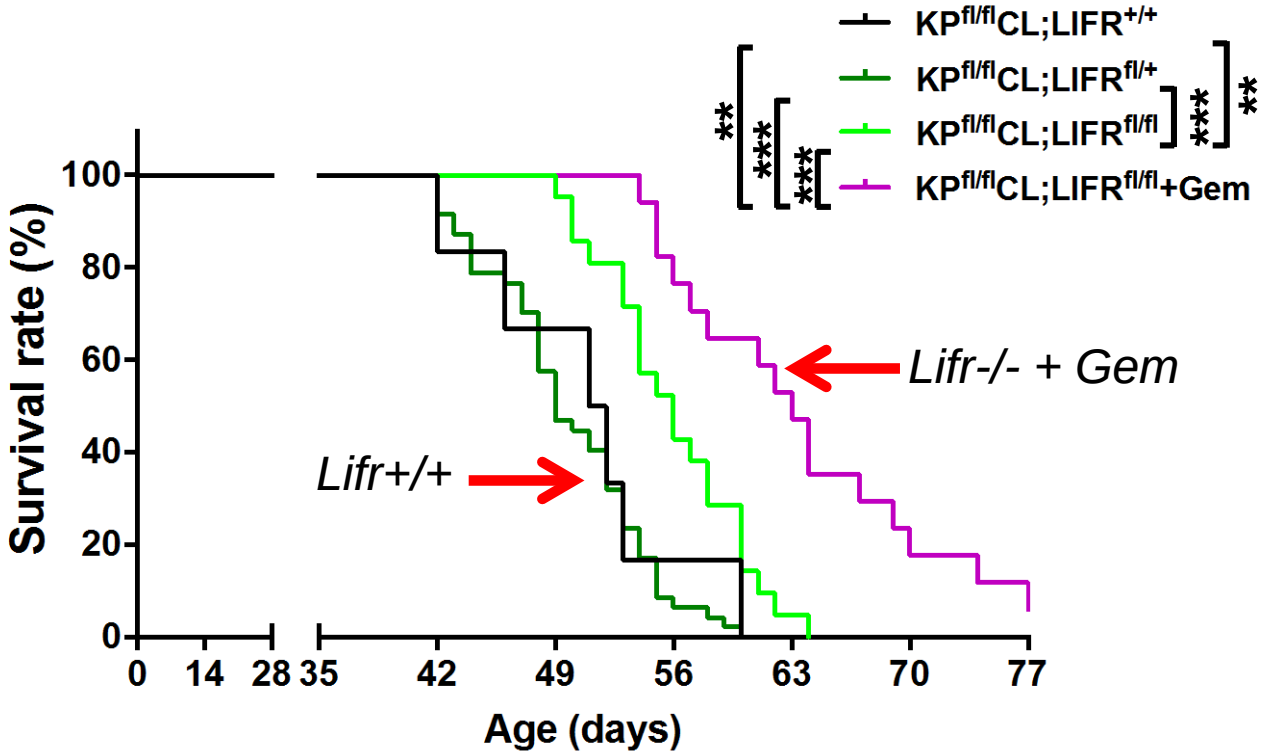
Normal



PDAC

- *LIF RNA is high in stromal cells adjacent to the tumor cells*
- *LIFR RNA is expressed in tumor cells and not stromal cells*

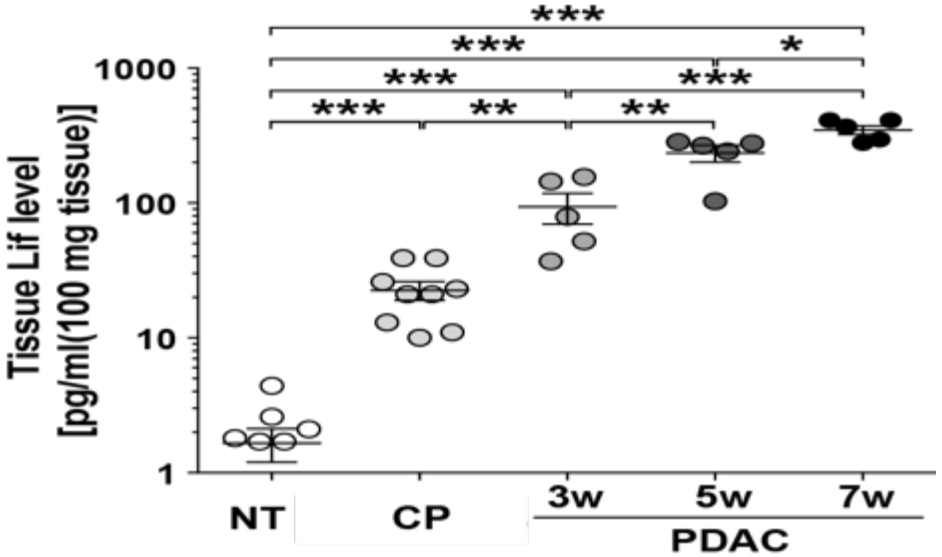
Pancreatic cancer cells are the main target of LIF action



Deletion of both Lifr alleles in pancreatic ductal cells significantly increases survival of KPC-Luc mice without Gem and sensitizes tumors to Gem

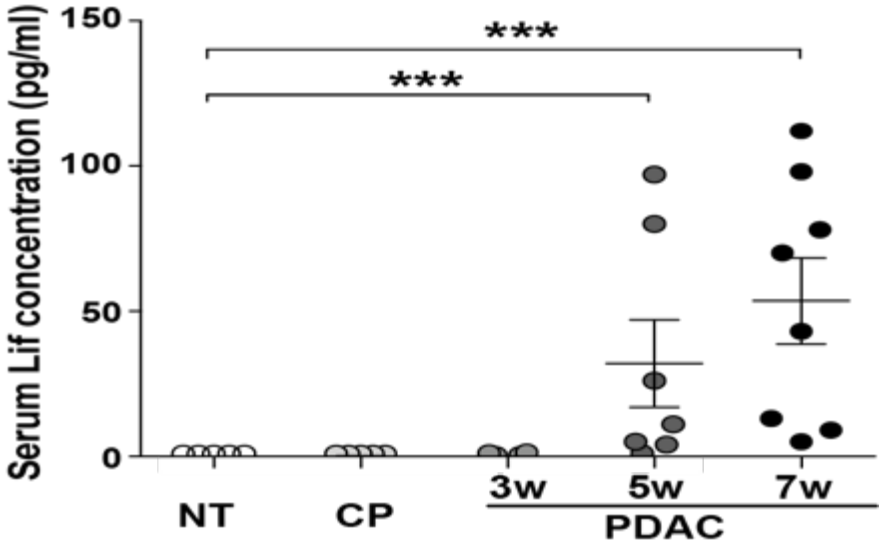
LIF is elevated at early stage disease and correlated with progression in the mouse PDAC model

Tissue



(chronic pancreatitis)

Serum

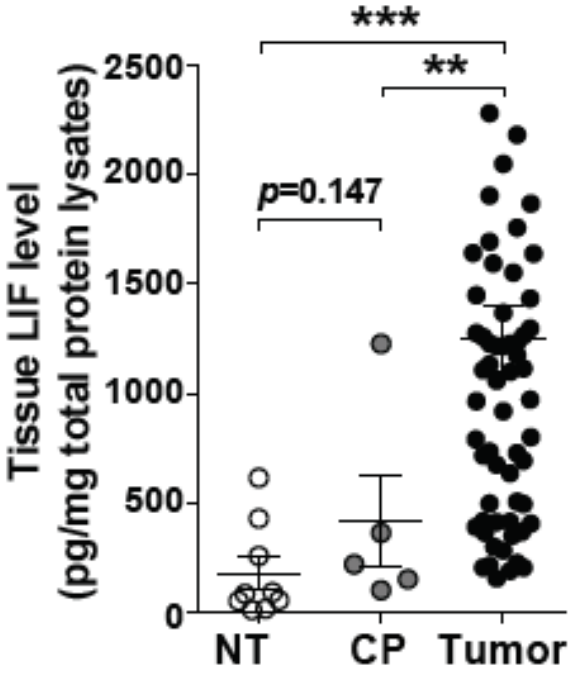


KPC PDAC model mice

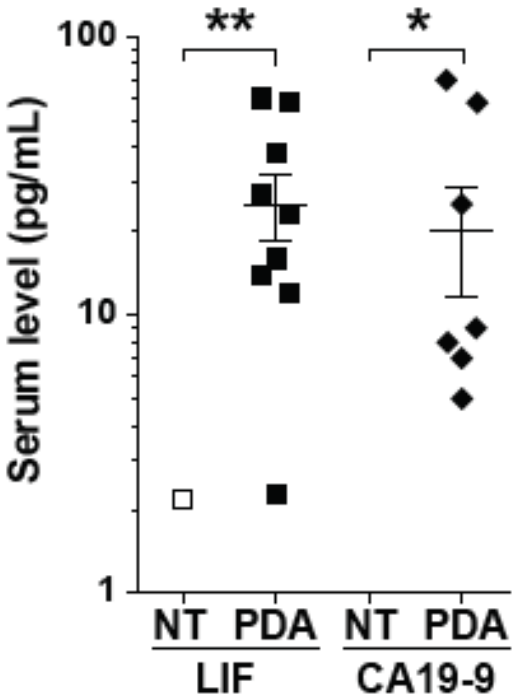
Luminex xMAP® multiplexing ELISA assays

LIF protein levels are elevated in human PDAC tumor tissues

Human normal/tumor tissue

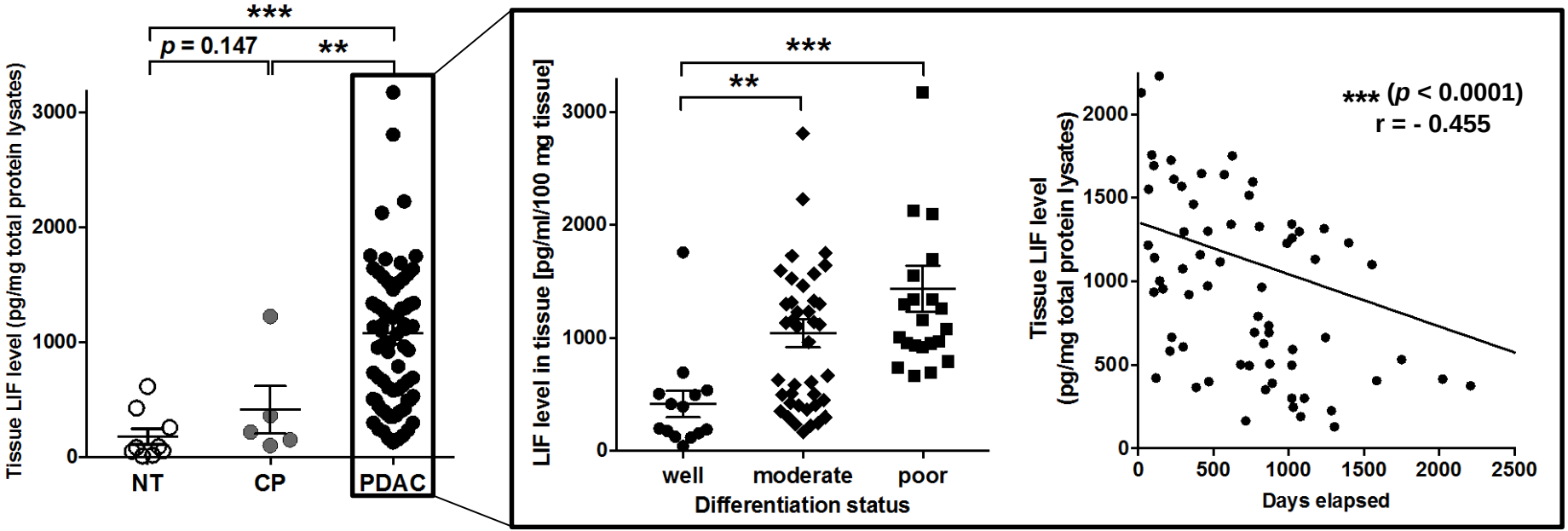


Human patient serum



Luminex xMAP® multiplex ELISA assays

LIF levels correlate with disease state in human PDAC

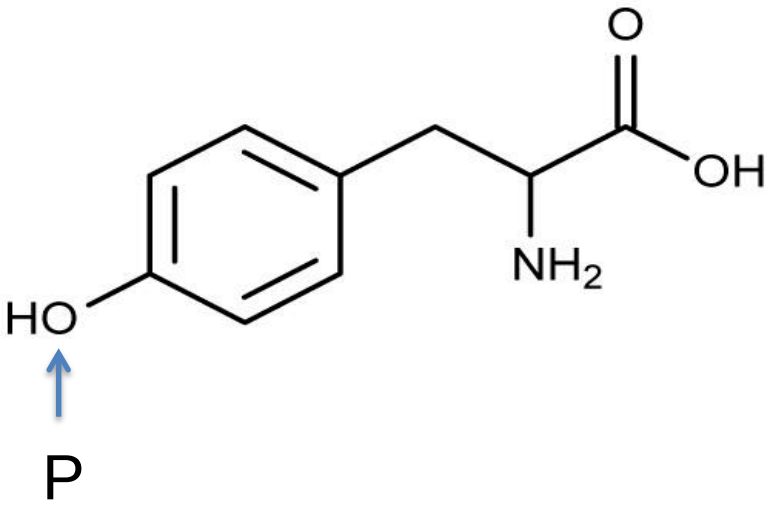


77 human pancreatic tumor samples were analyzed by ELISA

Does LIF play an important physiological role in PDAC?

- Anti-LIF neutralizing mAb can prolong survival of a mouse model of pancreatic cancer
- LIF levels in serum/tumor samples are correlated with stages of PDAC progression in the KPC mouse model
- There is a correlation between LIF levels in serum/tumor samples, and the corresponding prognosis/overall survival in human PDAC patients (LIF as a potential biomarker for early detection?)
- The next step is to develop humanized anti-LIF mAb for human pancreas cancer clinical trials in combination with standard of care
- Northern Biologics (Toronto) has developed a humanized anti-LIF mAb MSC-1, which will shortly begin trials in highly refractory cancer patients (I do not have a financial interest in Northern Biologics)

Phosphotyrosine



Tyrosine

Phosphate is linked to the 4-OH position as a phosphoester (heat stable)

But six other amino acids can be phosphorylated in addition to Ser, Thr and Tyr:

His, Arg, Lys, Cys, Glu, Asp

History of histidine phosphorylation of proteins

- Histidine phosphorylation is well documented in bacterial “two-component” signaling pathways that are used for chemotaxis, osmosensing, etc.
- Stimulus ⟶ pHis in a receptor/sensor protein (P-enzyme) ⟶ pAsp in a response regulator protein ⟶ signal output
- pHis is also found in eukaryotes. Metabolic enzymes such as phosphoglycerate mutase (PGAM), succinyl CoA synthase (SCS), and ATP citrate lyase (ACLY) use a pHis enzyme intermediate. **But pHis is also found in other proteins, e.g. histone H4**
- NME1/2 (NDPK-A/B) are the only reported histidine kinases
- PHPT1, LHPP and PGAM5 are pHis phosphatases
- NME family enzymes (10 members) use a **1-pHis** enzyme intermediate to transfer phosphate from ATP to an NDP (or to a His residue in a protein)

ATP + nucleoside diphosphate (GDP) → ADP + nucleoside triphosphate (GTP)

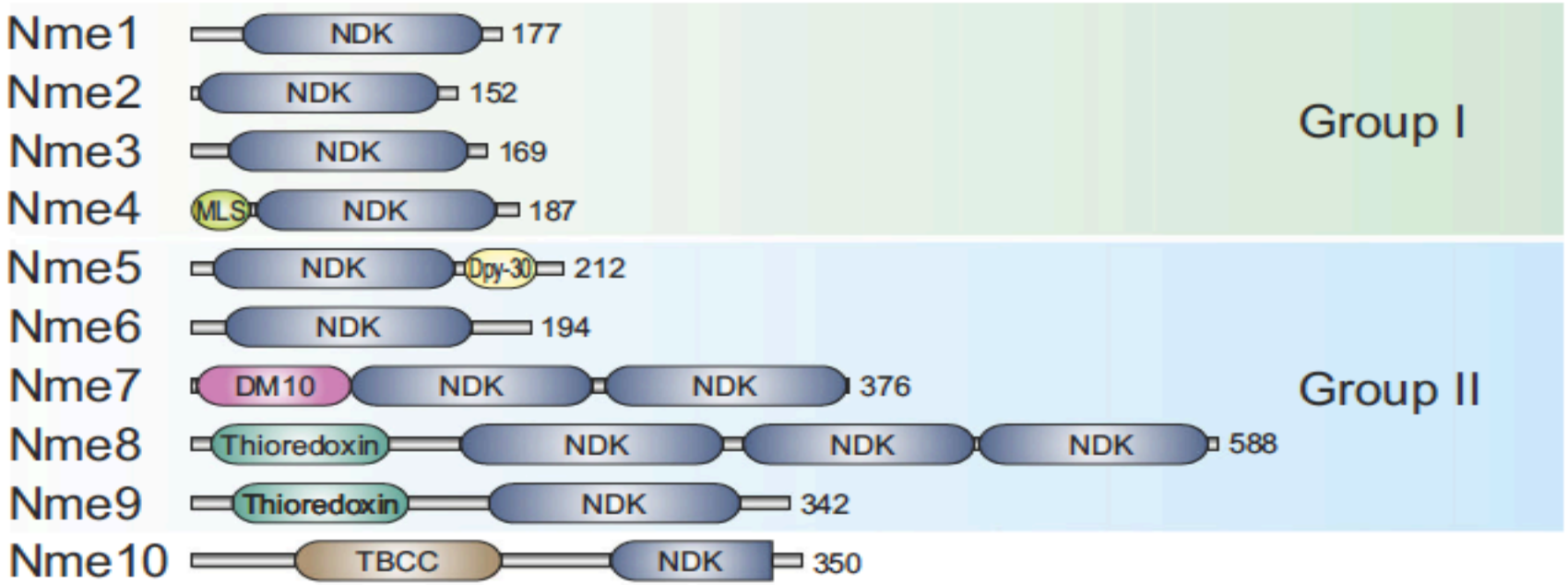
- *Levels of NME1 are reduced in metastatic cells*

History of histidine phosphorylation

- pHis was first d
- Histidine phosp omponent" signaling pathways that a

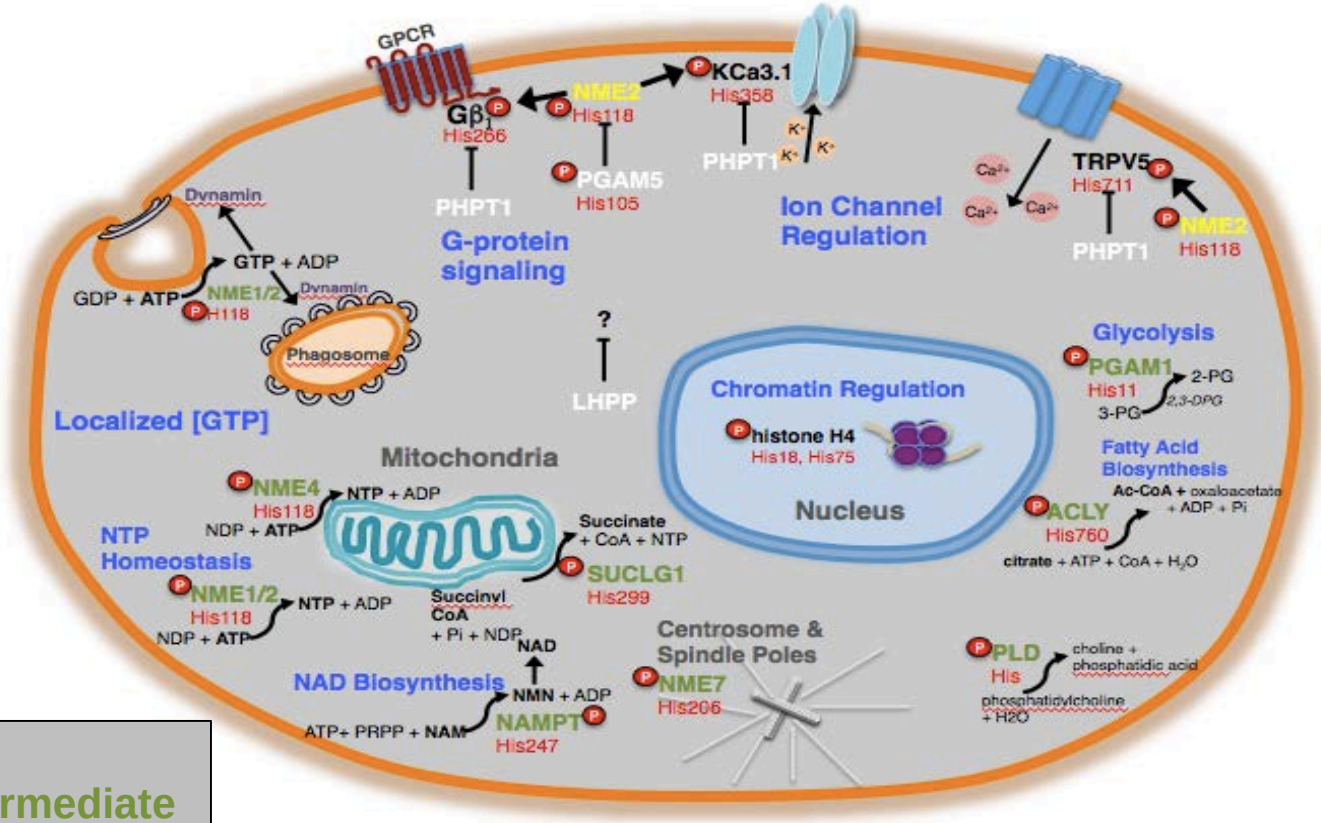
Identification of Phosphohistidine in Digests from a Probable Intermediate of Oxidative Phosphorylation*

P. D. Boyer, M. DeLuca, K. E. Ebner, D. E.



- *Levels of NME1 are reduced in metastatic cells*

Histidine phosphorylation has many functions

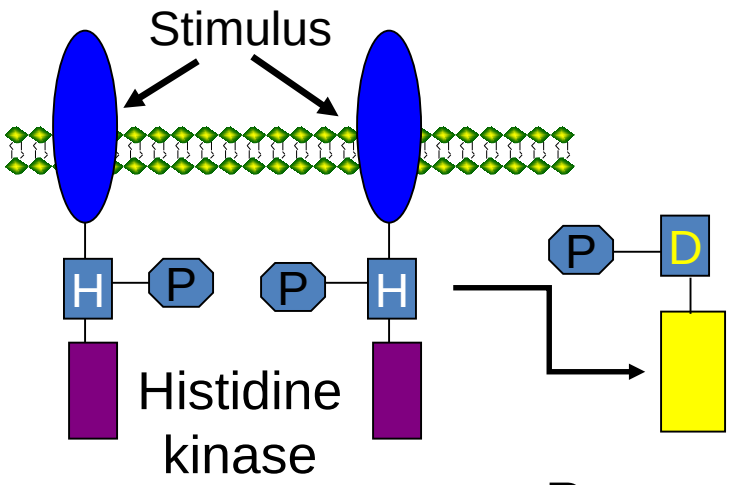


Kinase
Enzyme Intermediate
Phosphatase
Substrate

Fuhs and Hunter, *Curr Opin Cell Biol* 45:8-16 (2017)

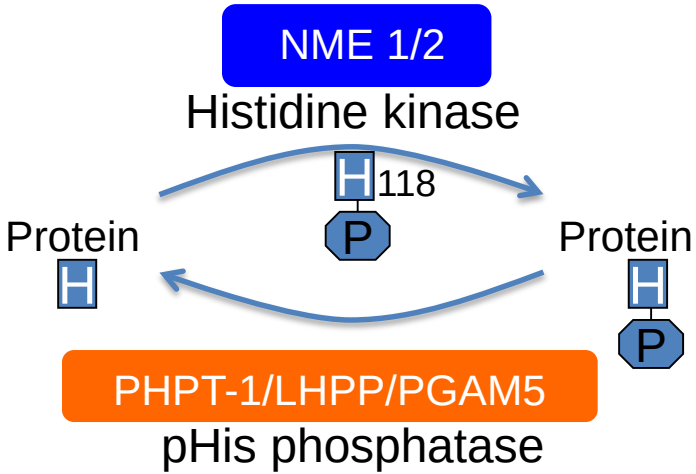
Histidine phosphorylation

Bacteria



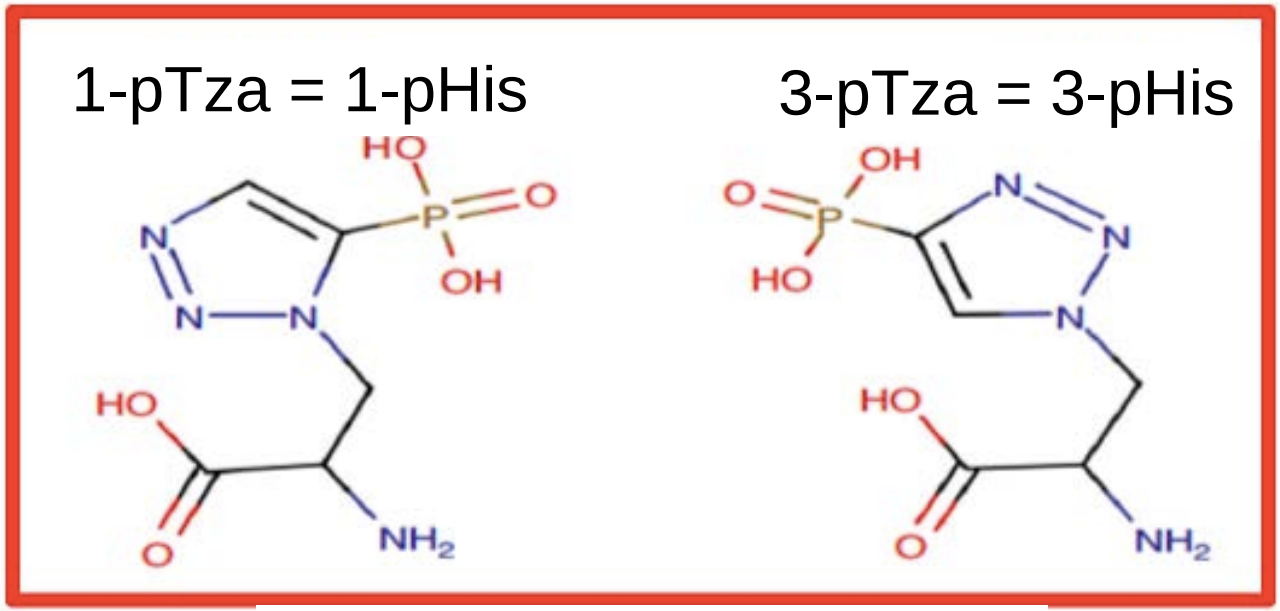
pHis – pAsp phosphorelay

Mammals



- Ca^{2+}-activated K^{+} channel KCa3.1 - pHis358 in C-tail increases opening
- TRPV5 channel activity/Ca^{2+} flux increased by pHis711 in C-tail
- β subunit of G proteins (pHis266 activates)
- Histone H4 (pHis18 unknown function)

Stable phosphohistidine analogues to make anti-pHis antibodies similar to anti-pTyr antibodies

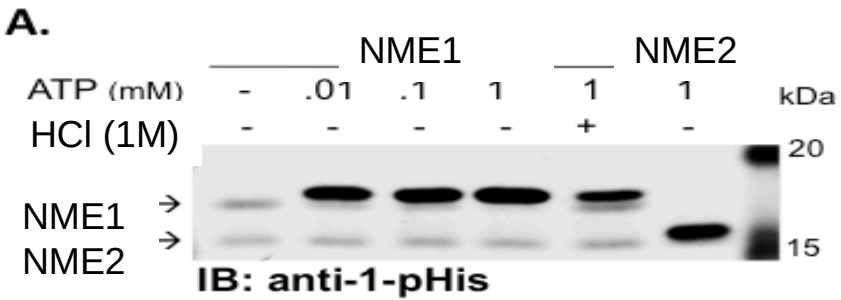


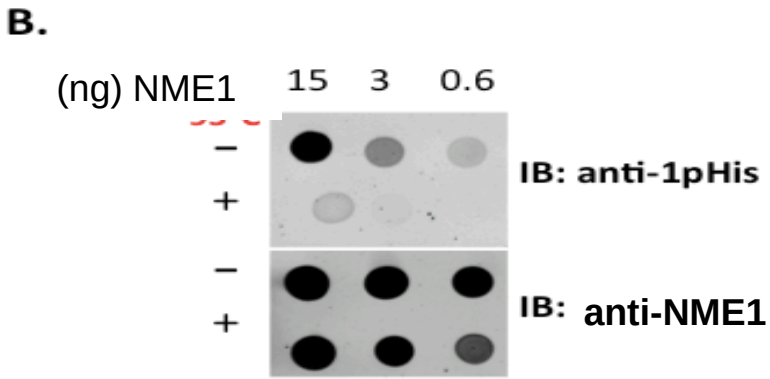
(pTza = phosphoryltriazolylalanine)

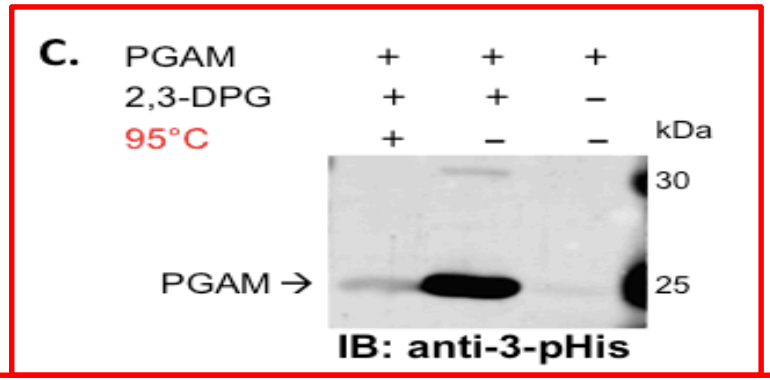
pTza analogues incorporated into degenerate Ala/Gly 11-mer peptides to immunize rabbits and generate **sequence-independent** anti pHis-antibodies

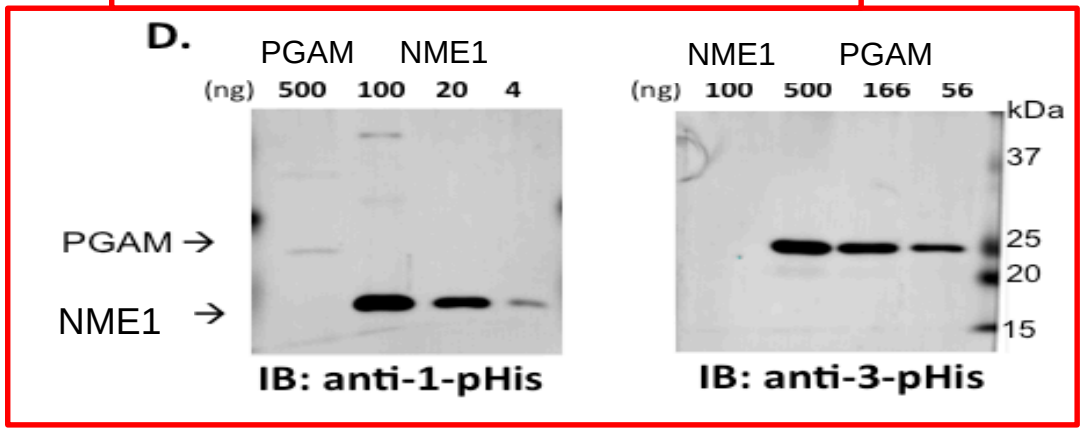
Steve Fuhs

Anti-1-pTza and 3-pTza polyclonal antibodies







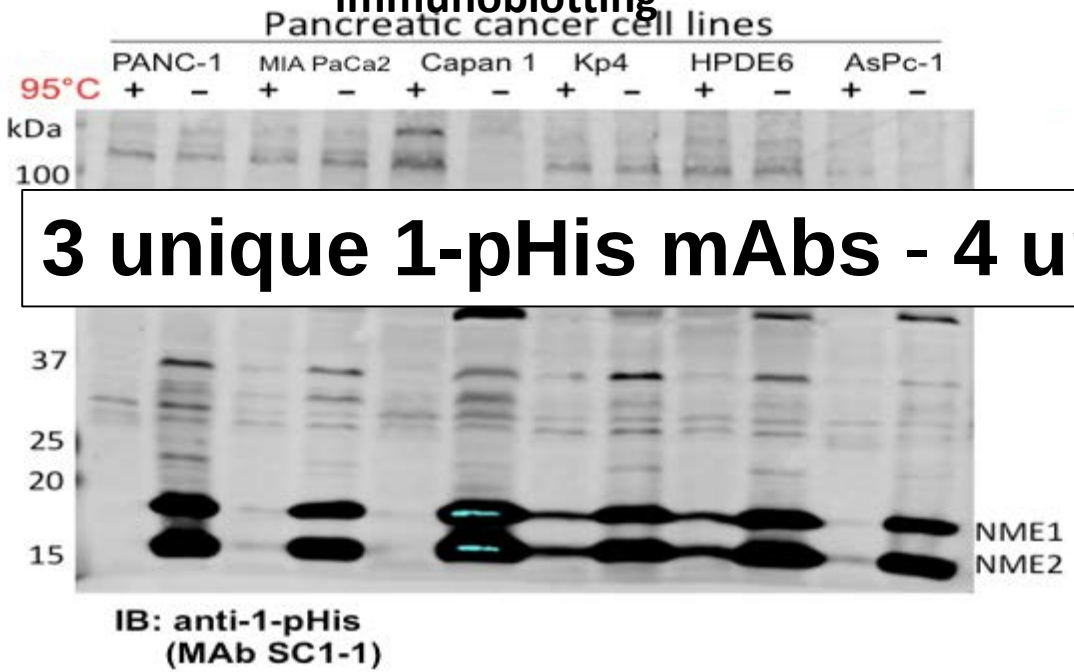


Anti-1-pTza rabbit antibodies detect *only phosphorylated* 1-pHis NME1

Anti-3-pTza rabbit antibodies detect *only phosphorylated* 3-pHis PGAM

Anti-1-pTza and anti-3-pTza monoclonal antibodies

Anti-1-pTza monoclonal antibody immunoblotting



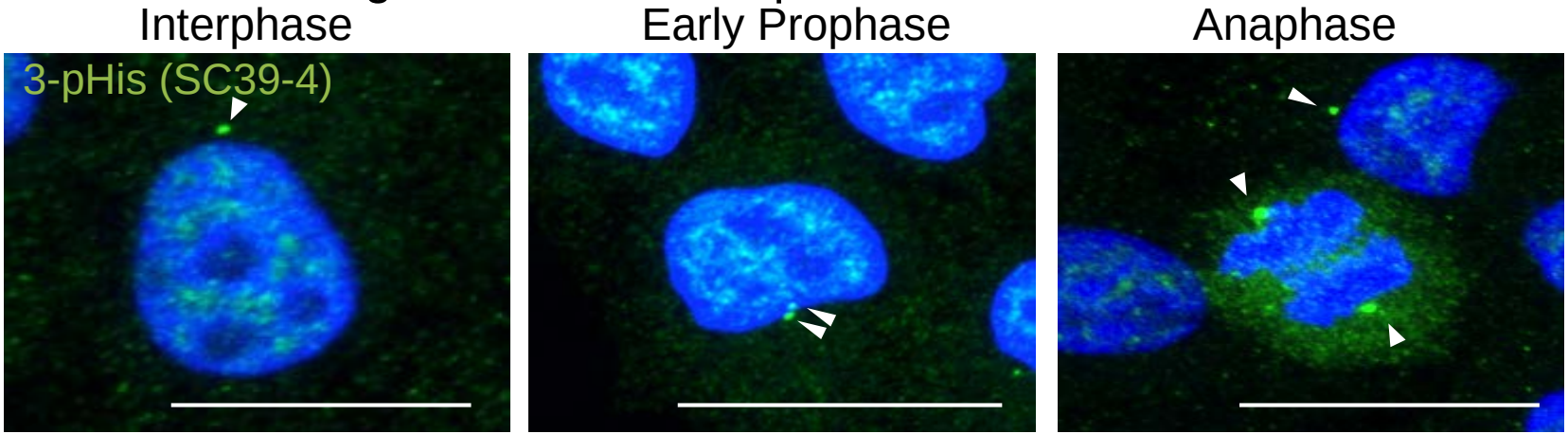
3 unique 1-pHis mAbs - 4 unique 3-pHis mAbs

Fuhs et al. *Cell* 162:198-210 (2015)

HeLa cell anti-3-pHis mAb IF exhibits spindle and centrosome staining

Centrosomes and **spindle poles** stain during interphase and mitosis

Punctate staining of **nuclei** in interphase



3-pHis mAb SC39-4; DAPI

4% PFA, 15 min (pH 7.4); 0.1% Triton (pH 9)

White arrowheds = spindle poles/centrosomes

with Li Ma

Fuhs et al. *Cell* 162:198 (2015)

Proteins enriched by pHis mAbs

In total, **786 proteins** were enriched >2-fold by either 1-pHis (280 unique) or 3-pHis (156 unique) mAb affinity columns in the control versus the pH 6/boiled denatured lysate sample

Top GO Biological Process by p-value

The sites of pHis in these proteins need to be mapped to be certain that they are truly targets for regulation by histidine kinases in the cell

Ribosome biogenesis	**35**
Cell cycle related	**97**

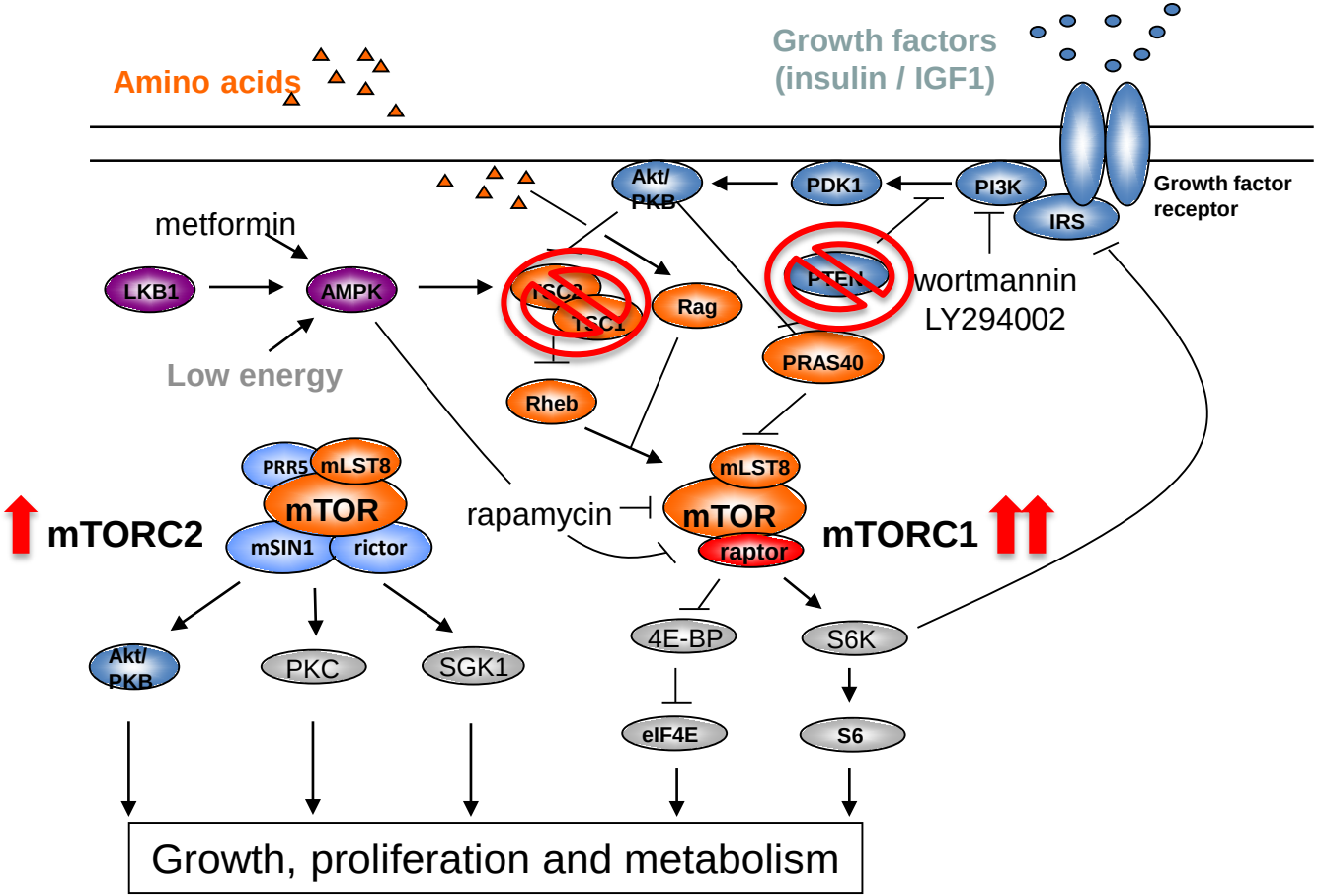
Histone H4, NME1/2, PGAM, ACLY were enriched, as expected

Open questions about histidine phosphorylation

How does His phosphorylation regulate protein activity, and what functions are regulated by His phosphorylation

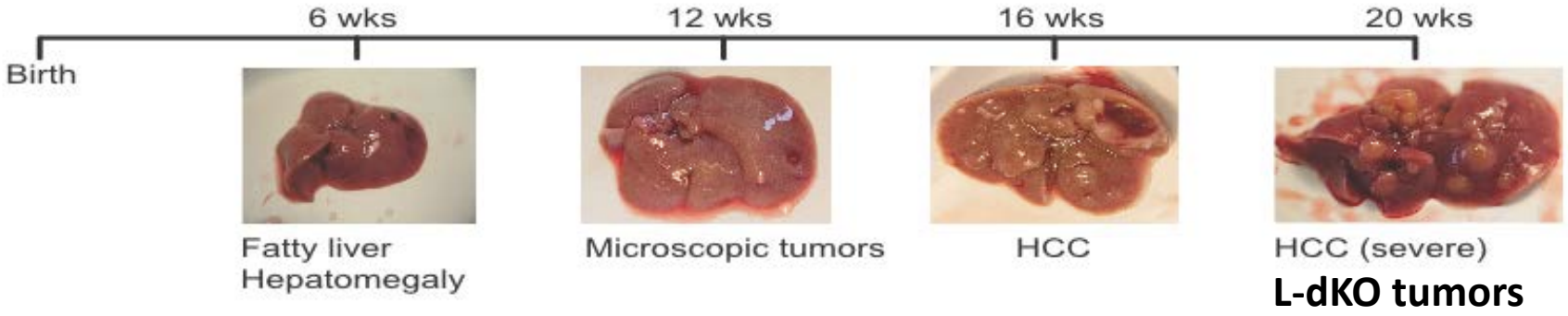
- *Is His phosphorylation used for short term regulatory responses, because of its chemical instability?*
- *Are there pHis-specific binding domains, like SH2 domains, which transmit signals?*
- *Does pHis act through local charge effects on proteins (the change is +1 to -2)?*
- *Is His phosphorylation regulation of divalent metal ion binding a general principle?*

PTEN/TSC1 double knockout activates mTORC1 and mTORC2



Sravanth Hindupur and Mike Hall (Biozentrum, Basel)

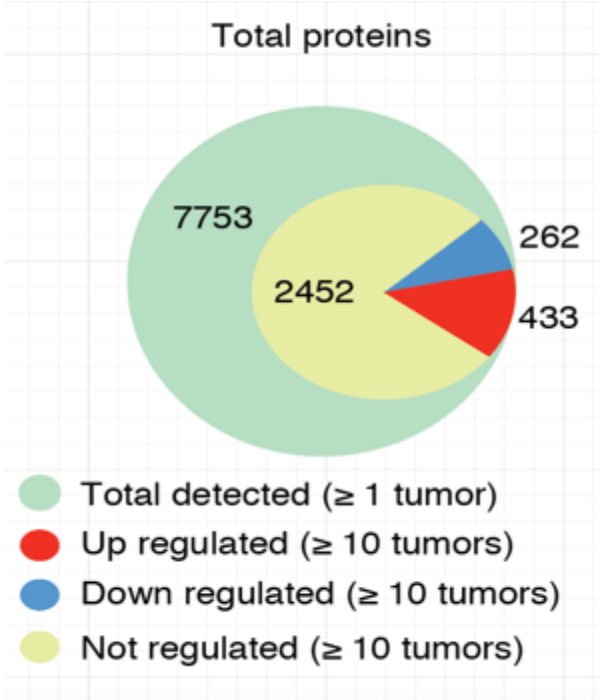
Liver-specific Tsc1/Pten double KO mice develop HCC by 16-20 weeks



Pathology report (20 wks)	Control	L-PTEN KO	L-TSC1 KO	L-dKO
Architecture	Normal	Normal	Normal	Abnormal
Hepatosteatosis	No	30% of liver parenchyma (Micro + Macro steatosis)	No	5-10% of liver parenchyma
Nuclear polymorphism	No	Yes		Yes
Cancer	No	No	No	Severe HCC; Multi nodular; Ductal proliferation

Sravanth Hindupur and Mike Hall (Biozentrum, Basel)

Proteome analysis of L-dKO tumors: combined increase in NME1/2 and decrease in LHPP levels predict altered histidine phosphoryation



His kinases →

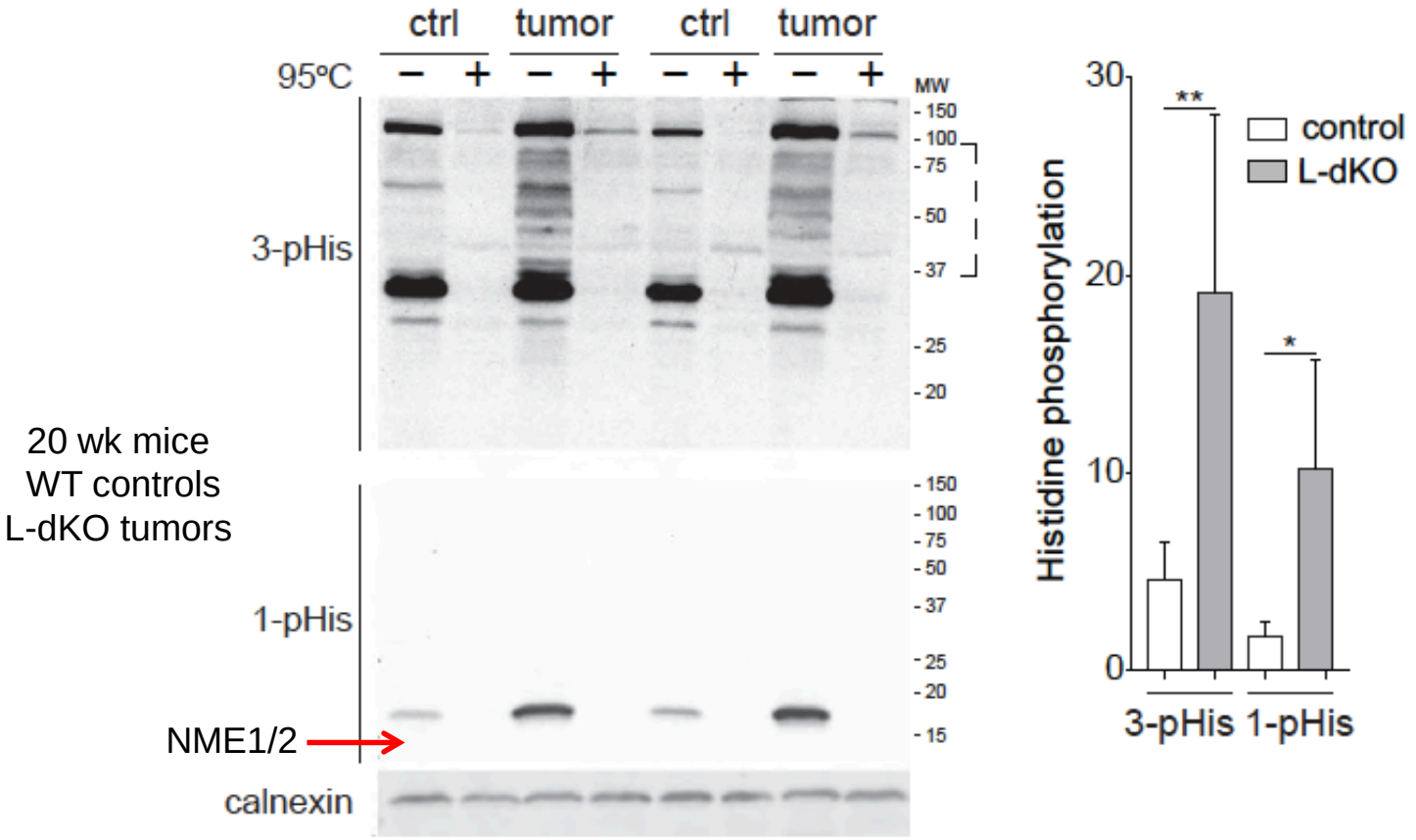
Kinases		Phosphatases	
Up	Down	Up	Down
NME1	AK2	IMPAD1	**LHPP**
NME2	IDNKP	PTPN23	G6PC
EGFR	FKB1	PPP3CA	ACP5
AAK1		PPP1R12A	EPHX2
AMPK		PPP1R14B	
PKM2		PSPH	
MAPK3			
GSK3B			
GSK3A			
PAK2			
CHKA/B			
PFKP			
CKB			
PACSIN2			
GALK2			
PIP4K2C			
PAPSS1			

← pHis phosphatase

3 tumors each from 4 mice (20 wks)

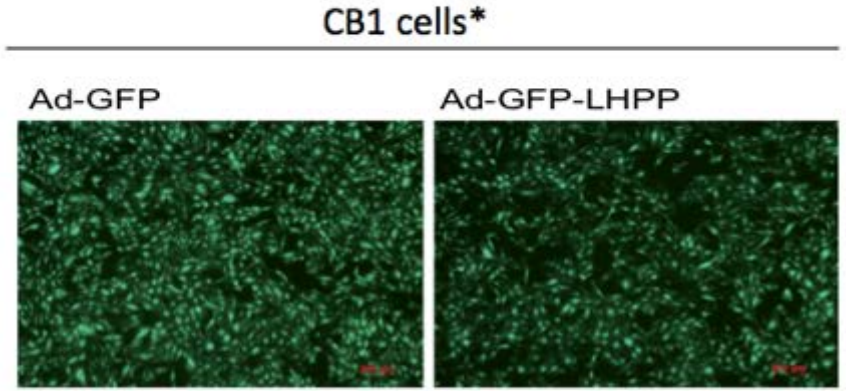
Hindupur et al. *Nature* **555**:678 (2018)

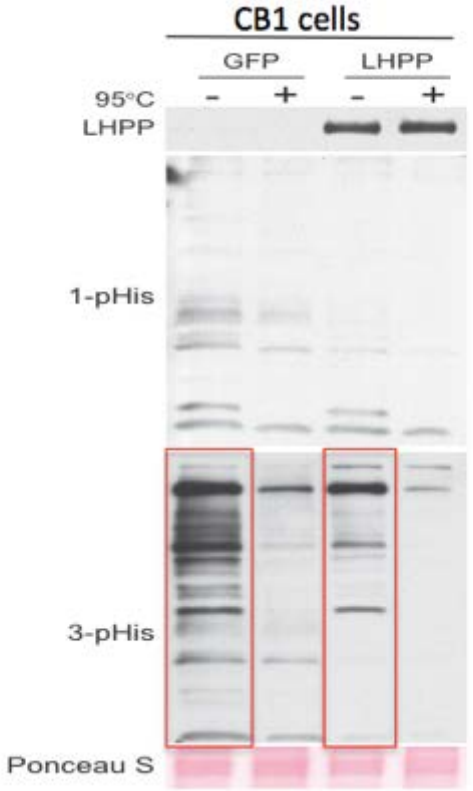
Increased phosphohistidine (3-pHis) in tumors with low LHPP



Hindupur et al. *Nature* **555**:678 (2018)

LHPP overexpression in mouse L-dKO hepatoma cells reduces global phosphohistidine (3-pHis)

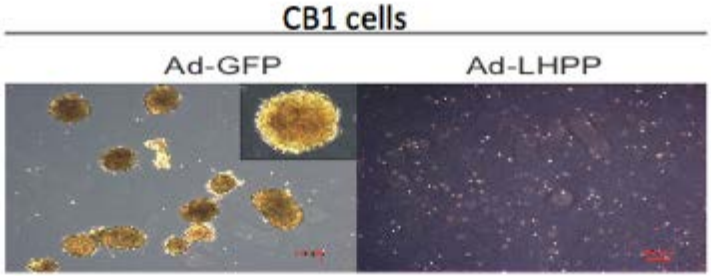




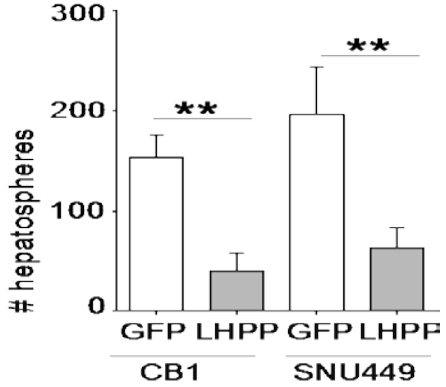
*CB1 cells derived from L-dKO tumor

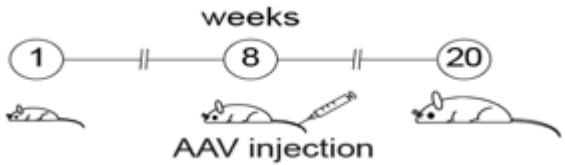
Hindupur et al. *Nature* **555**:678 (2018)

LHPP is a tumor suppressor in vitro and in vivo (mice)

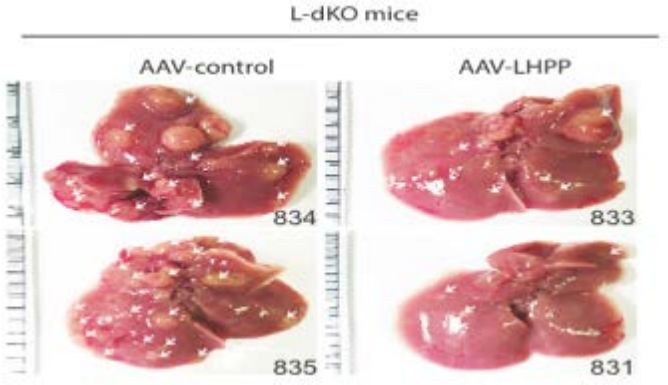


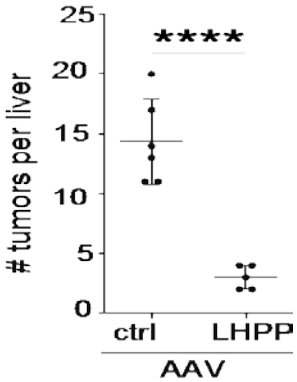
LHPP expression reduces hepatosphere formation





20 week mice injected with AAV at 8 weeks





LHPP expression reduces liver tumor formation

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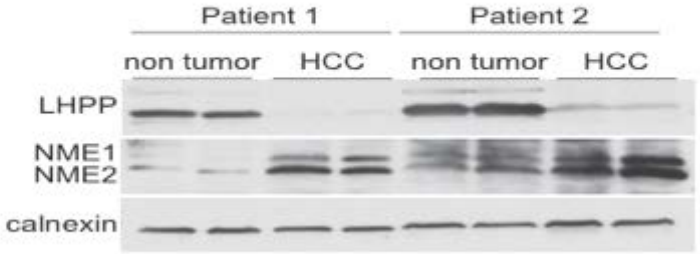
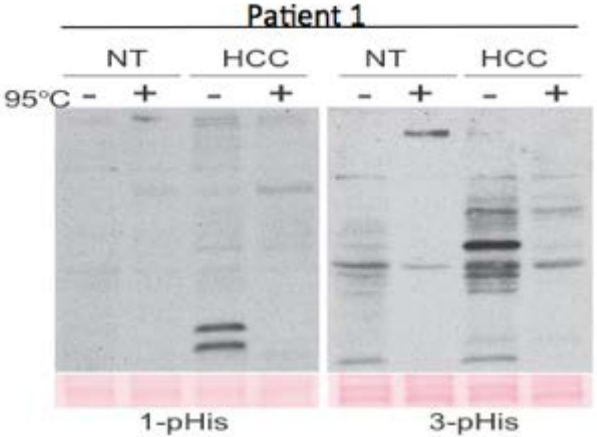
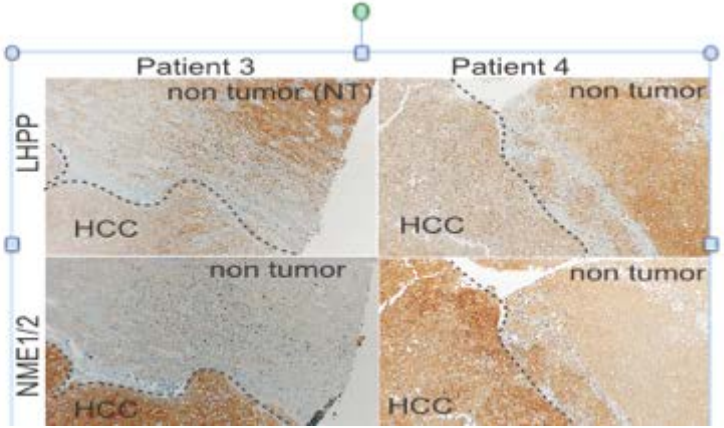
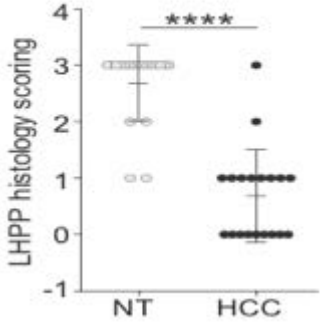
LHPP low (and NME1/2 high) in human tumors



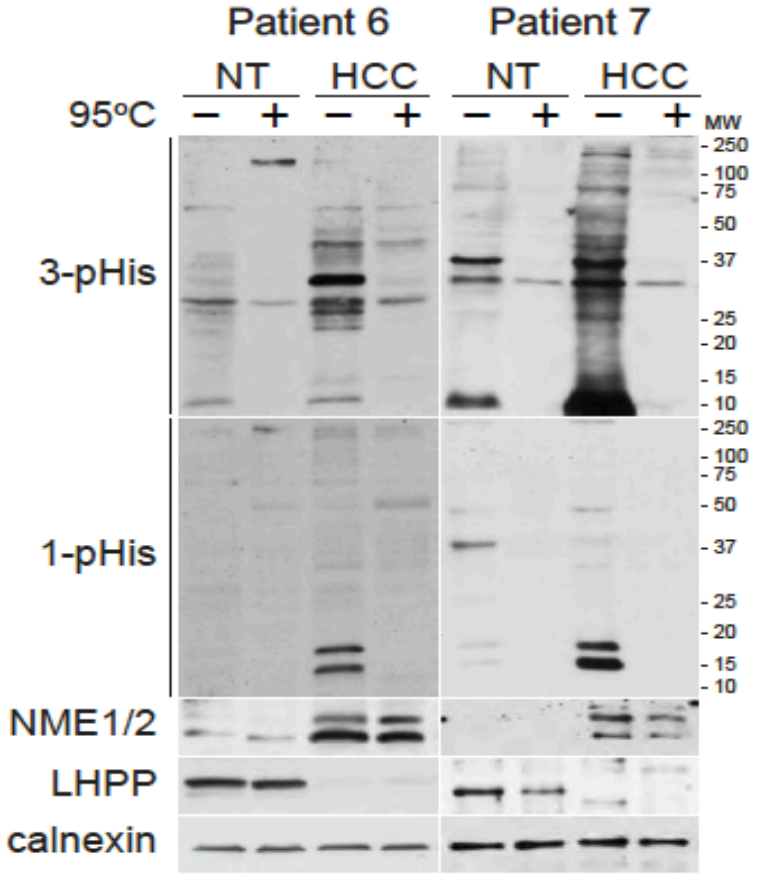


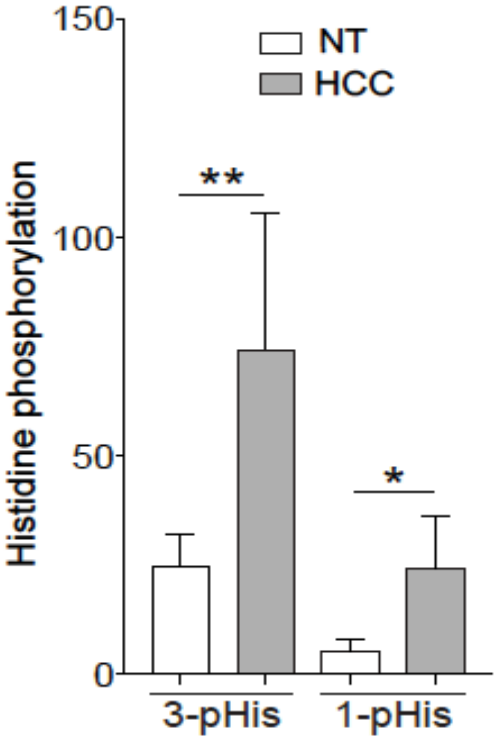




Tissue microarray

Elevated 3-pHis levels in human HCC tumor proteins suggest a role for histidine phosphorylation in liver tumors





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Conclusions

- Our results suggest that the LHPP pHis phosphatase acts as a tumor suppressor in liver cancer
- Consistent with this, HCC patients with low LHPP RNA levels have worse prognosis
- Identification and functional characterization of 3-pHis proteins elevated in HCC is required to establish that LHPP acts as a tumor suppressor by limiting histidine phosphorylation, i.e. which are the key pHis proteins?
- *Can inhibitor drugs be developed to target the key His kinases for treatment of hepatocellular carcinoma? Is His phosphorylation important in other cancer types?*

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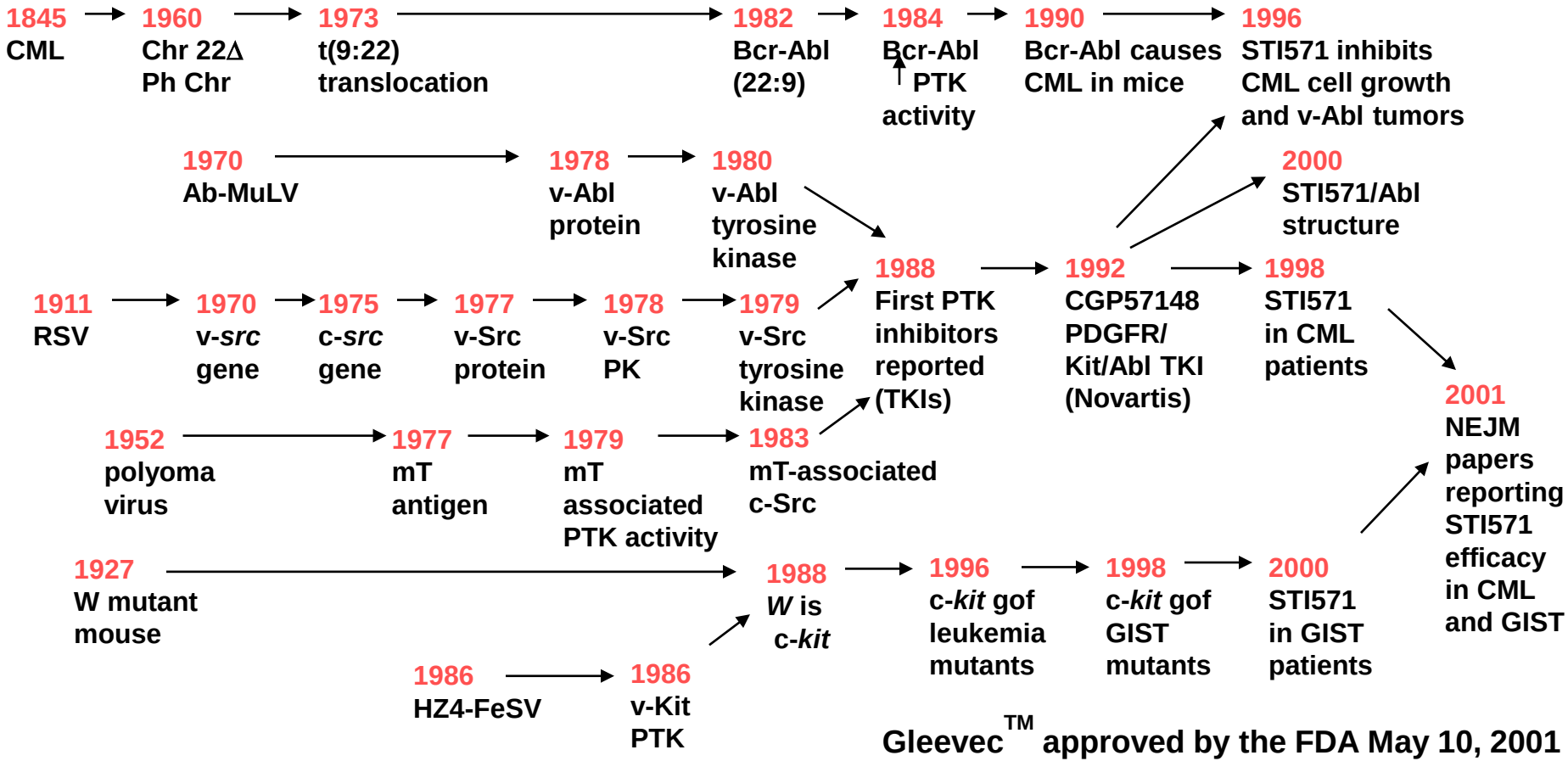

Gleevec™ approved by the FDA May 10, 2001

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and the old buffer!