Transforming Growth Factor-beta and Lung Tumorigenesis

Sonia B. Jakowlew, PhD
Cancer Training Branch
Center for Cancer Training
National Cancer Institute
Bethesda, MD
jakowles@mail.nih.gov

TRACO September 27, 2018

Lung Cancer in 2018, USA

- Most common cause of cancer deaths in both men and women
- 234,030 diagnosed new cases
  121,680 men; 112,350 women
- 154,050 deaths due to lung cancer
  83,550 men; 70,500 women
- Most cases now occur in ex-smokers
  - Average age 70
- < 15% five year survival rate

Transforming Growth Factor-ß (TGF-ß)

Multifunctional regulator of cellular growth
- Potent inhibitor of normal epithelial cell proliferation
- Widespread tissue expression
- Pivotal role in epithelial homeostasis
- Association with various types of cancers
- Context-dependent inhibition or stimulation of cell proliferation and neoplastic transformation

TGF-ß is an attractive candidate for new therapeutic intervention approaches

Transforming Growth Factors: The Beginning

Transforming Growth Factors: The Beginning

Sarcoma Growth Factor – Polypeptide secreted by Moloney murine sarcoma virus-transformed mouse fibroblasts that stimulated normal rat fibroblasts to form colonies in soft agar (transformation assay).
De Larco & Todaro: PNAS 75:4001, 1978

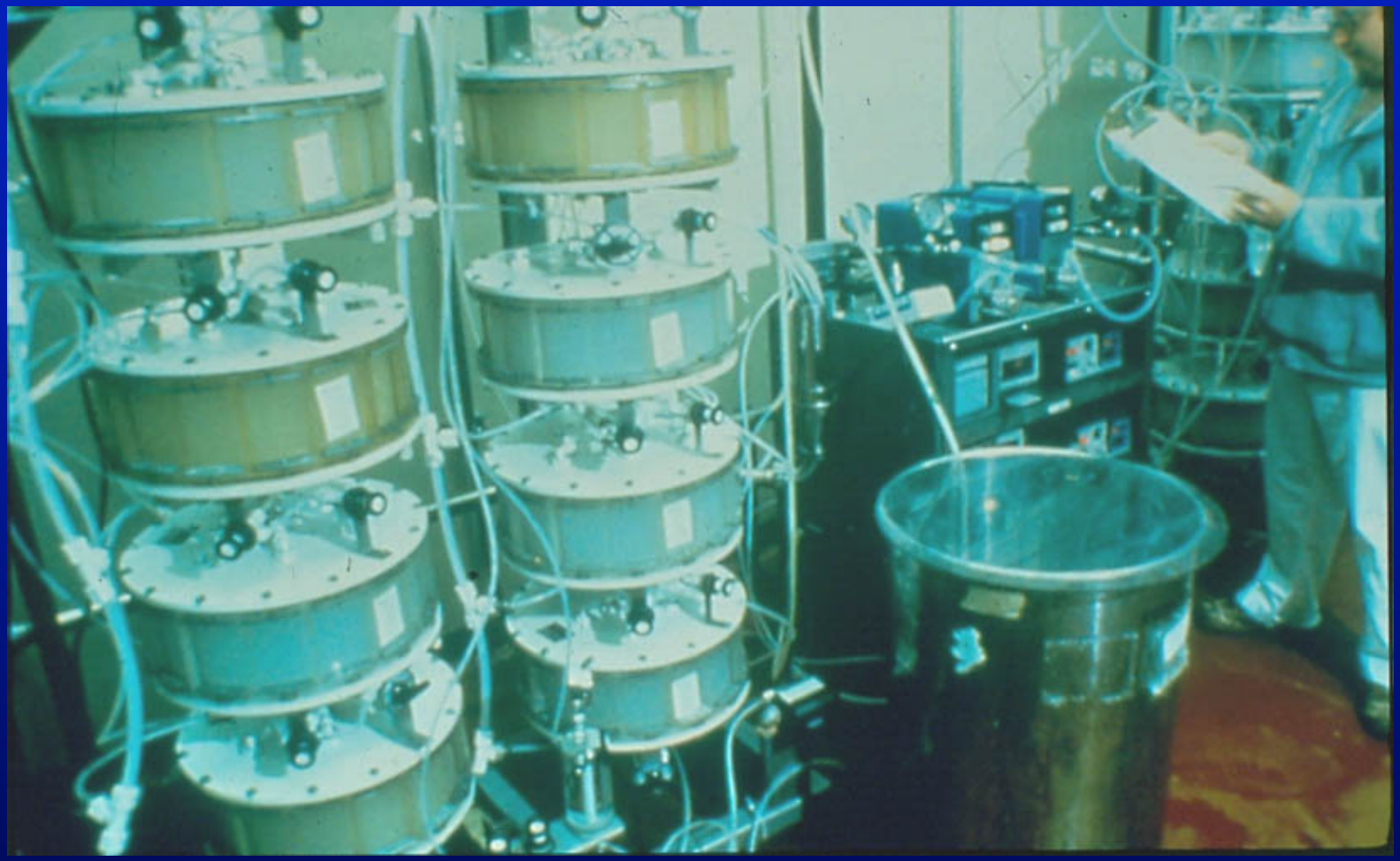
Two classes of TGFs isolated from MSV-transformed cells:
1. Competes with EGF for receptor binding (TGF-α)
2. Does not compete for EGF binding, but colony forming activity is enhanced by EGF (TGF-β)
   Sarcoma growth factor = TGF-α + TGF-β
   Roberts, Anzano,…Sporn: Nature 295:417, 1982

1983- Publication of the purification of TGF-ß from:
Human platelets *(Rick Assoian)*
Human placenta *(Chuck Frolik)*
Bovine kidney *(Anita Roberts)*

Scale of TGF-ß1 Purification from Bovine Kidney

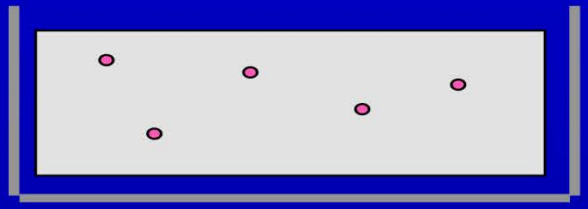
Extract with 8 liters of acid/ethanol
Centrifuge
Precipitate with 32 liters ether +
16 liters ethanol
Redissolve in 2 liters 1M acetic acid
Apply to 80 liter BioGel P-60 column
Collect 1 liter fractions
Lophilize and redissolve for further chromatography
Final Yield = 6 µg TGF-ß1
purification fold = 230,000; recovery = 10%

The Columns for TGF-beta1 Purification



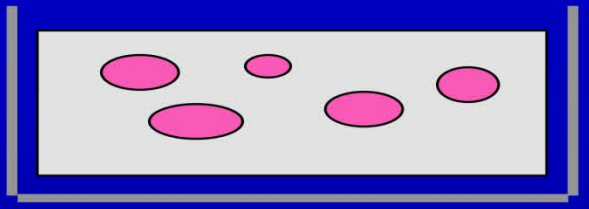
Clonogenic assay

The Assay: Growth of NRK Cells in Soft Agar



If no TGF-ß is present

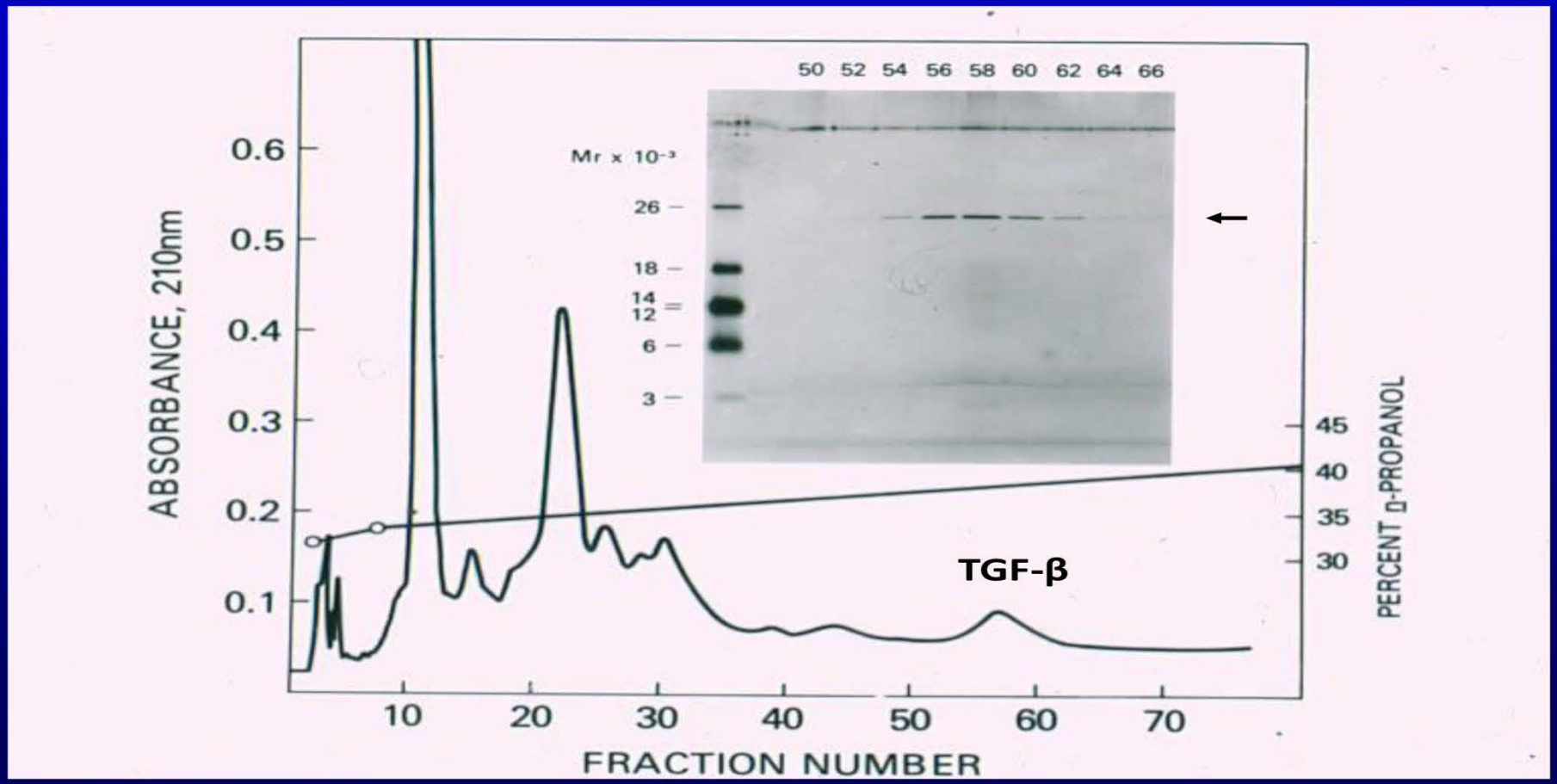
- Plate agar base
- Add mix of media, serum, NRK cells, EGF, sample
- 1 wk/37°/5%CO_2
- Stain
- Count colonies >3100 µm^2 with Omnicon Image Analysis System



If TGF-ß is present

HPLC Purification

The Final HPLC Purification



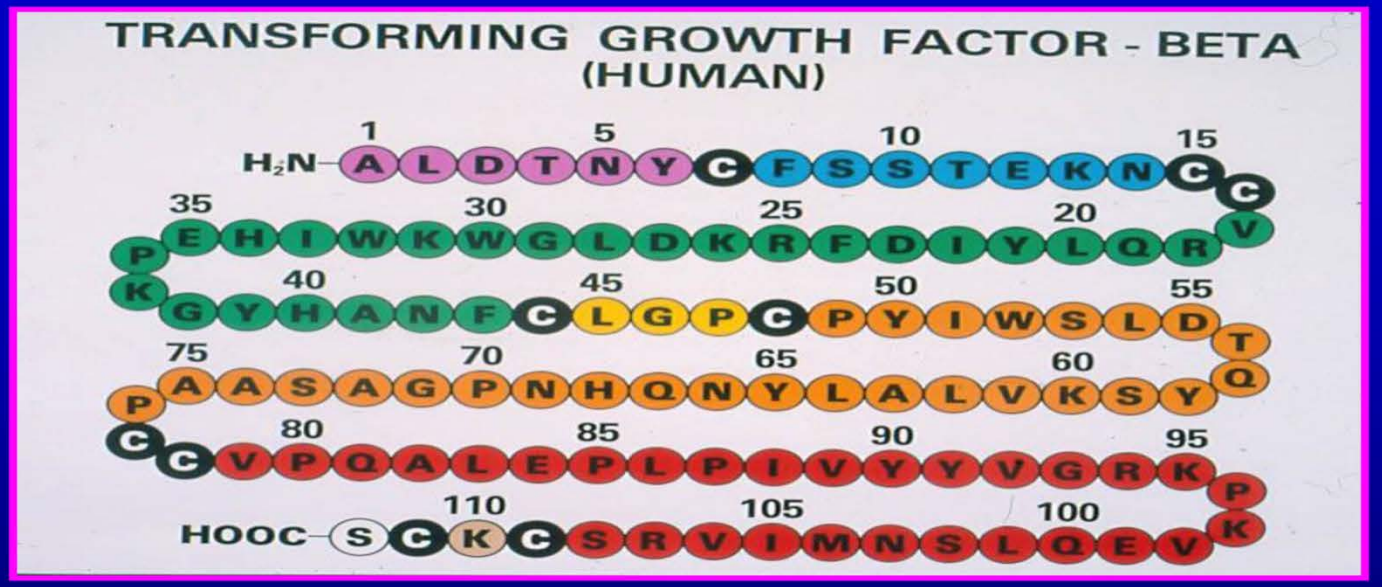
TGF beta

EUREKA!! TGF-ß: Born at NCI

Michael Sporn & Anita Roberts

TGF beta structure

Sequence of mature TGF-ß1 monomer



Pre-pro TGF-ß 391 amino acids

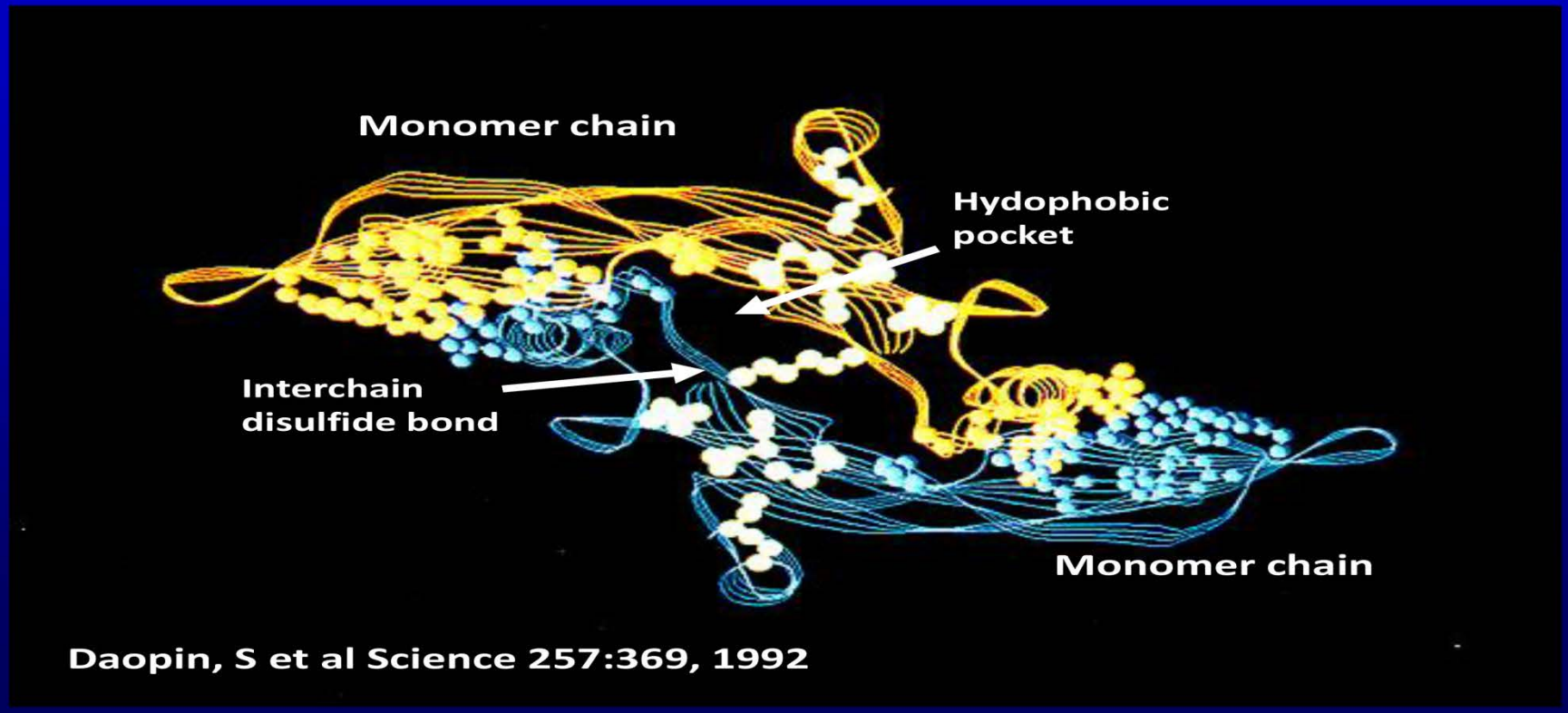
Signal peptide

(latency associated peptide, LAP)

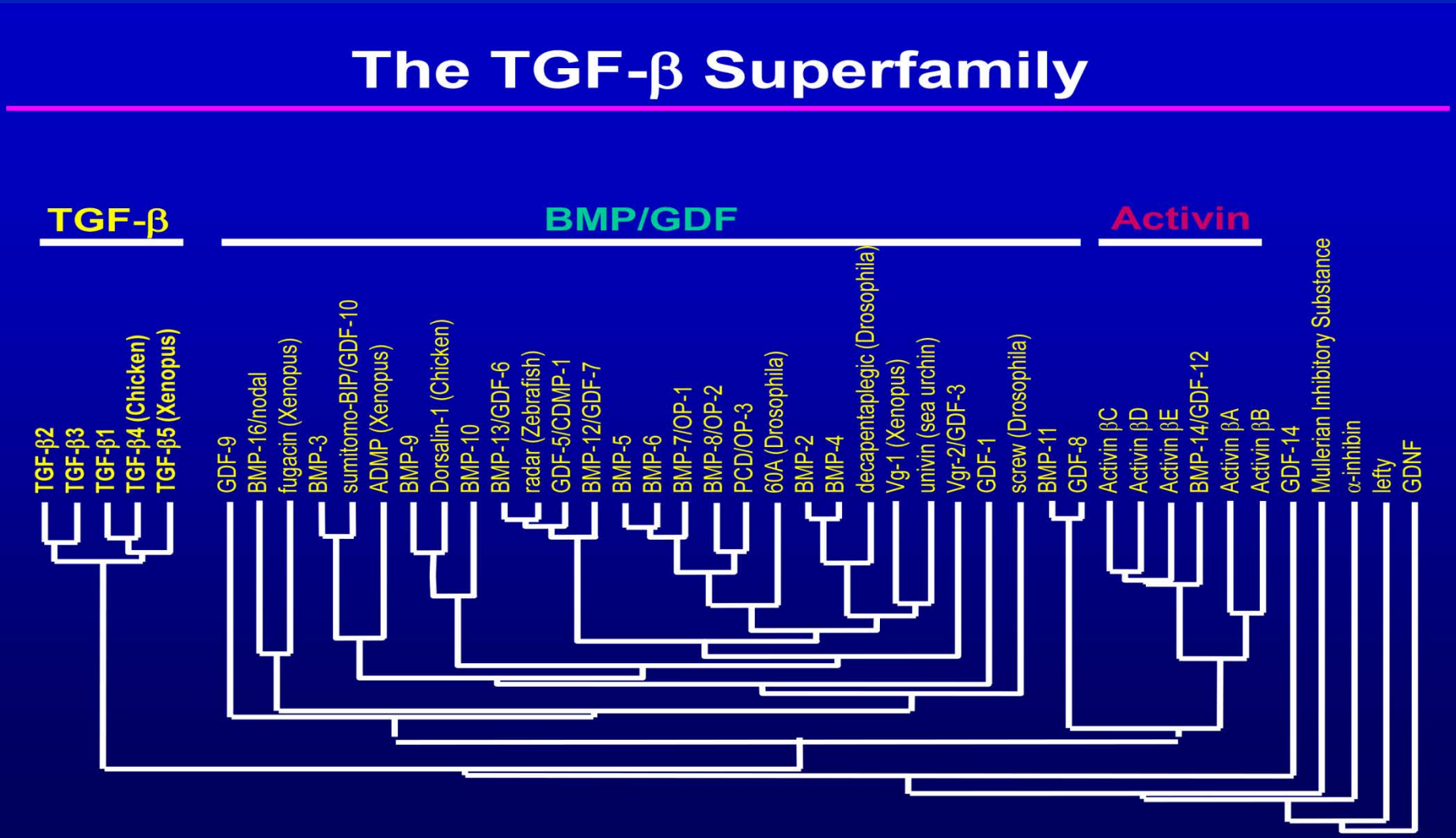
Mature TGF-β
112 amino acids

TGF beta dimer

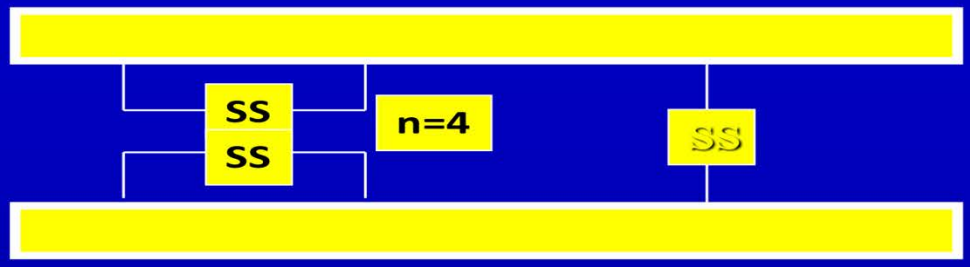
TGF-β: A Homodimer



TGF beta superfamily



Transforming growth factor beta



Transforming Growth Factor-β

- 25,000 MW disulfide-bonded homodimer
- 3 highly homologous isoforms (TGF-β 1, 2 and 3)
- Principal sources - platelets, bone, spleen
- Most cells express TGF-β and its receptors
- Usually secreted in latent, inactive form
- Superfamily of TGF-βs, activins/inhibins, BMPs, GDFs

Major Biological Responses Regulated by TGF-beta

inhibits proliferation

regulates apoptosis

regulates differentiation

regulates immune cell function

stimulates accumulation of extracellular matrix

promotes chemotaxis

The TGF-β Superfamily: Central Control Modules for Many Biological Processes

TGFβ is associated with development, immune system function, reproduction, angiogenesis, agining, response to injury, metabolic regulstion and proliferation.

Model for TGF-β pathway

TGFR I and II form a phosphorylated heterodimer. BMPs cause activation of Smads 1/5/8. Activin TGFβ causes activation of Smads 2/3. A phosphorylated R-S smad 4 complex forms which is biologically active.

Clinical Observations

Clinical Observations

TGF-ß is a tumor suppressor:

- **Germline mutations in TGF-ß pathway components cause familial predisposition to cancer**
  (Smad4 in juvenile polyposis syndrome)
- **TGF-ß pathway components are somatically mutated or deleted in some human cancers**
  (Tß-RII in HNPCC, Smad4 in pancreatic cancer)
- **Reduced expression of TGF-ß1 signaling pathway components or overexpression of endogenous pathway inhibitors are associated with disease progression**
  (Tß-RII, Tß-RI, Smad7, Ski)

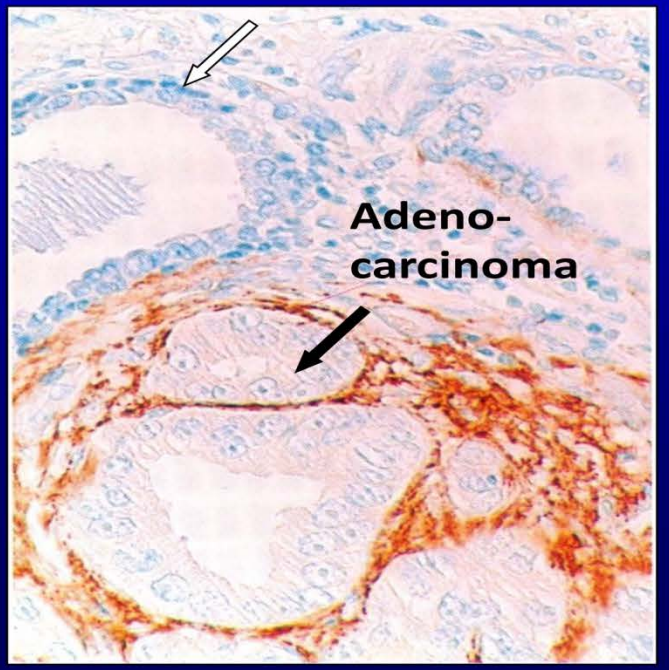
Clinical Observations

Clinical Observations

TGF-ß is a tumor promoter:

- **TGF-ß1 is elevated in many advanced human tumors and correlates with metastasis and/or poor prognosis**

(breast, colon, stomach, liver, pancreas, prostate, lung, kidney, bladder, nasopharynx, melanoma, chondrosarcoma, osteosarcoma)



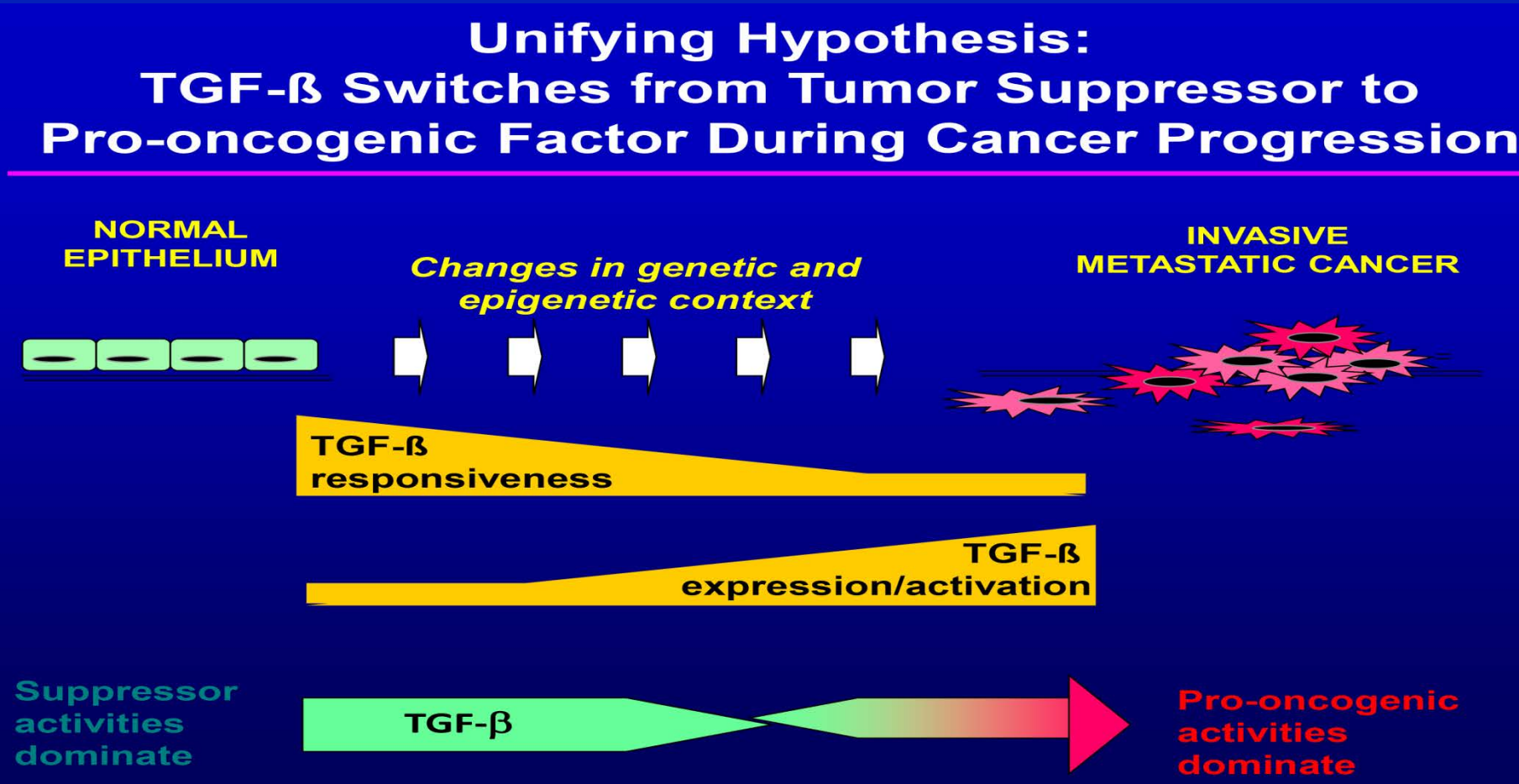
Prostatic adenocarcinoma stained for TGF-ß1:
(Truong et al. Hum Pathol 1993)
TGF-ß sits at the interface between tumor parenchyma and microenvironment

TGF beta in carcinogenesis

TGF-ß in Carcinogenesis - Hero or villain?

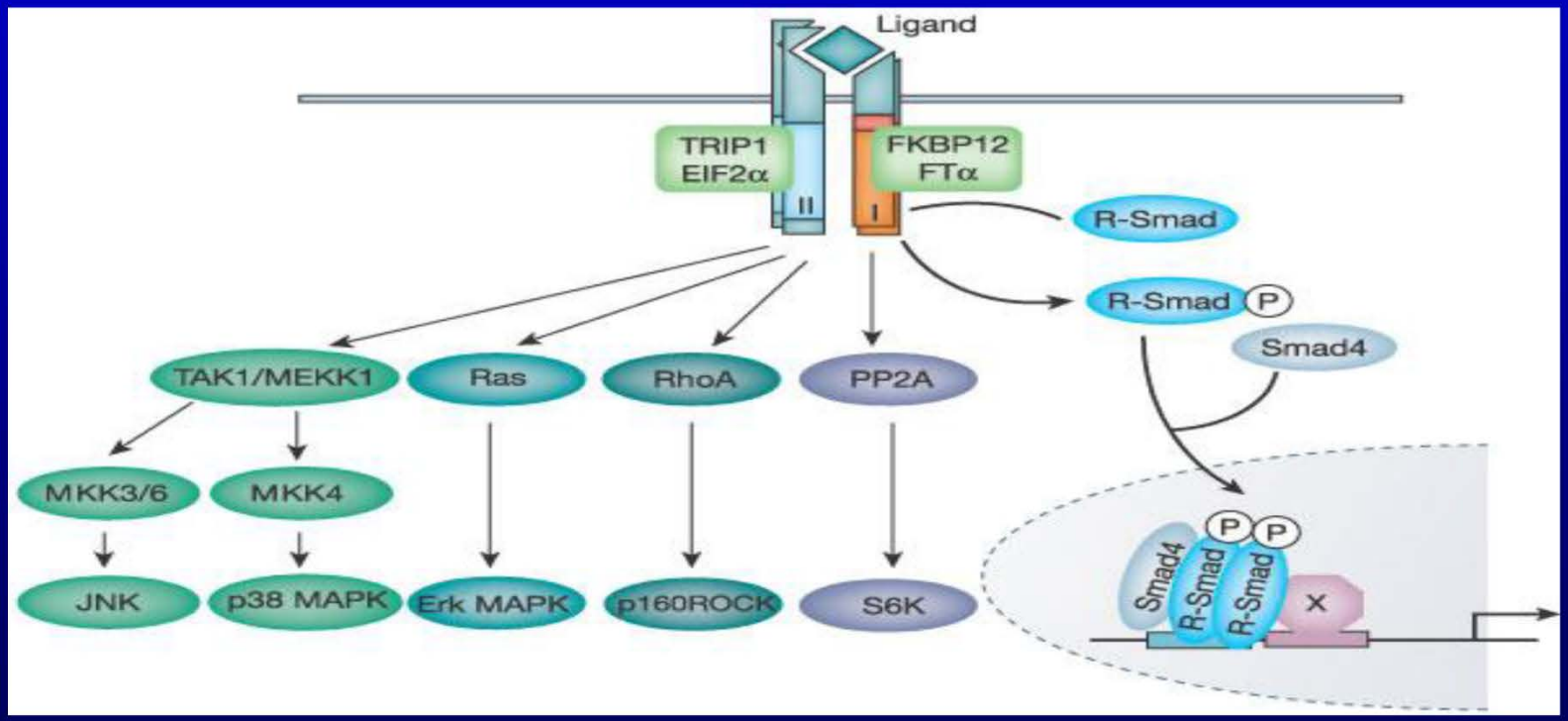
- TGF-ß, a proximal effector of the malignant phenotype
- TGF-ß, potent growth inhibitor and tumor suppressor
- TGF-ß, a pro-metastatic factor

Major Biological Responses Regulated by TGF-beta



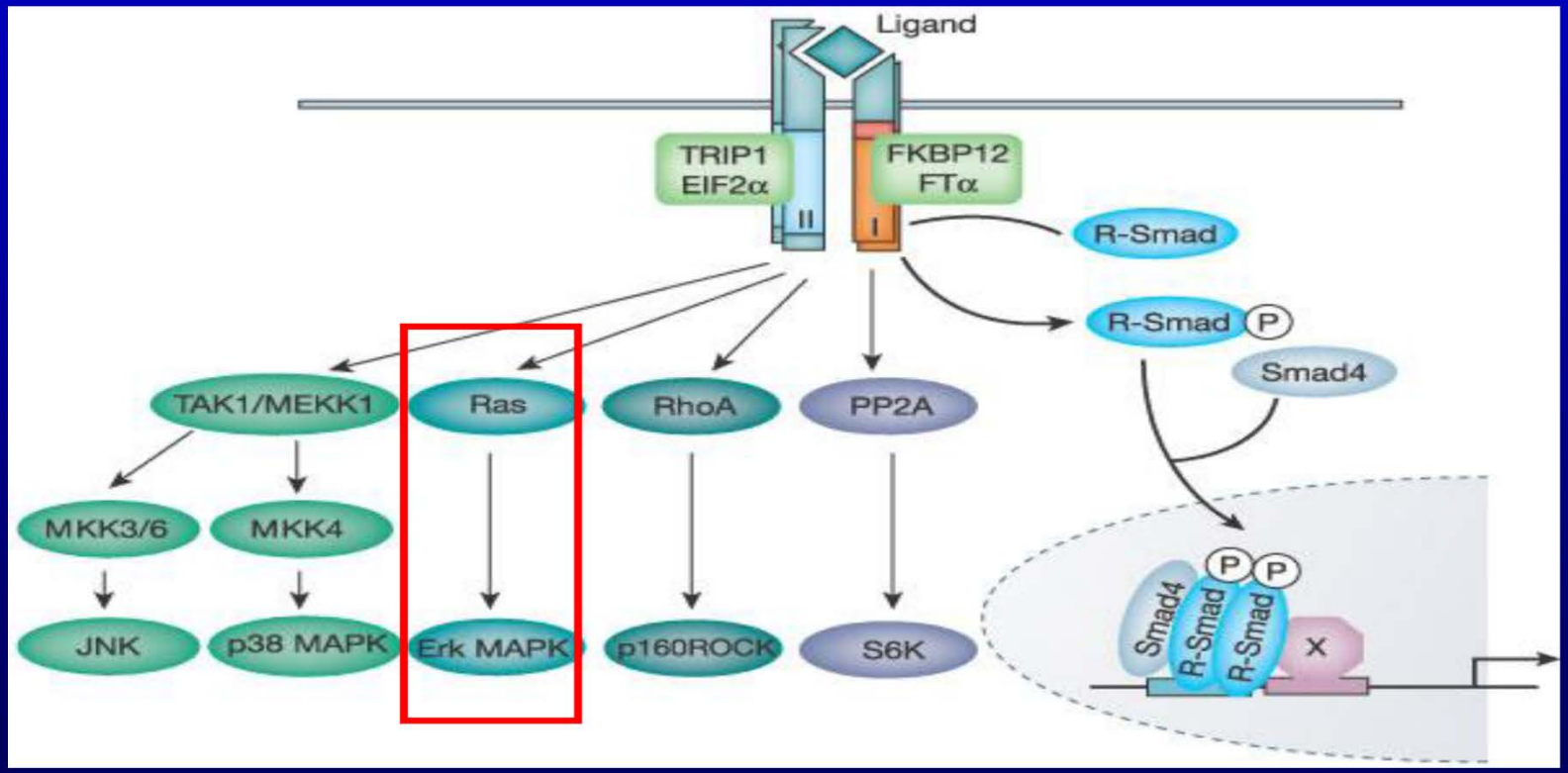
TGF-beta Smad-independent Pathways

TGF-ß Smad-independent Pathways



TGFbeta Smad-independent pathways

TGF-ß Smad-independent Pathways



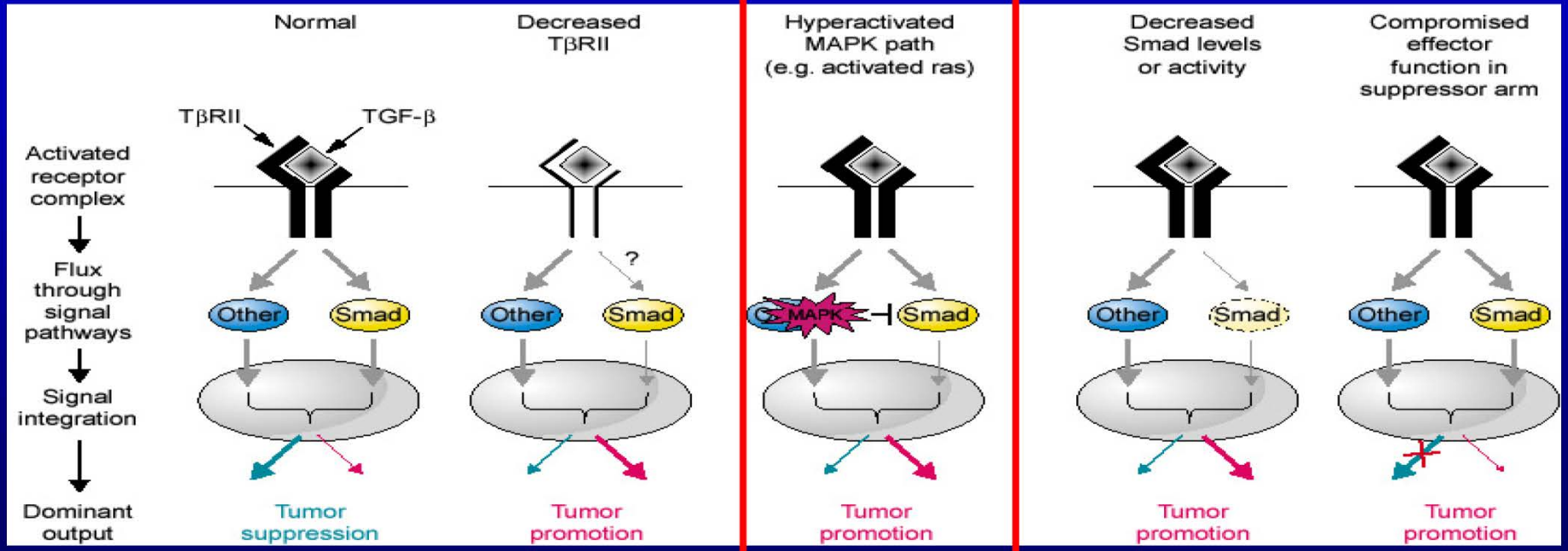
K-ras Protooncogene

K-ras Protooncogene

- K-ras shows an activational mutation in ~25-50% of human lung adenocarcinomas
- Mutation of even one allele of K-ras increases appearance of lung lesions
- There is cross-talk between Smad-dependent pathway and the Ras/MEK signaling
- Activation of the Ras pathway can modulate TGF-β1 signaling through the Smads
- In-vitro studies show that TGF-β1 dominates over mitogenic effects of ras, but activated ras overrides antiproliferative effect of TGF-β1

TGFβ in Tumor Suppression/Promotion

TGF-ß in Tumor Suppression/Promotion



- **Activated Ras/MAPK = Tumor Promotion**

Broad Goal

Broad Goal

- **Determine the role of Transforming Growth Factor-ß in the development and malignant transformation of lung epithelial cells**

Epithelial Carcinogenesis Section
Cell and Cancer Biology Branch
Center for Cancer Research
NCI

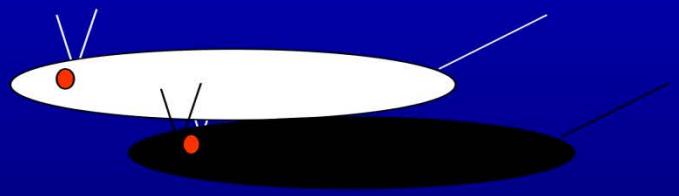
Objectives

Objectives

- Examine the effect of TGF-β1 deletion and K-ras mutation alone and in combination on lung tumor incidence and pathology
- Determine early events in the development of lung lesions and their progression
- Identify potential signal transduction pathway changes with tumorigenesis

Mouse models

Mouse Models



- A/J
- C57BL6 TGF-ß1 HT
- AJBL6 TGF-ß1 HT
- TGF-ß1 HT/K-ras LA

Question

Question

- Does lung tumorigenesis affect the TGF-ß signaling pathway?
- Does the TGF-ß signaling pathway affect lung tumorigenesis?

A/J Mouse Model

A/J Mouse Model

- Susceptible to chemically-induced lung tumors
- Tumors develop in a time-dependent manner
- Hyperplasia, adenoma and carcinoma
- Carcinomas are histologically similar to human lung adenocarcinomas
- Same molecular mutations in both human and mouse lung tumors (ie., over-expression of ras, loss of p53)

Ethyl Carbamate is:

metabolized by CYPE1 to vinyl carbamate and vinyl cabamate epoxide as well as

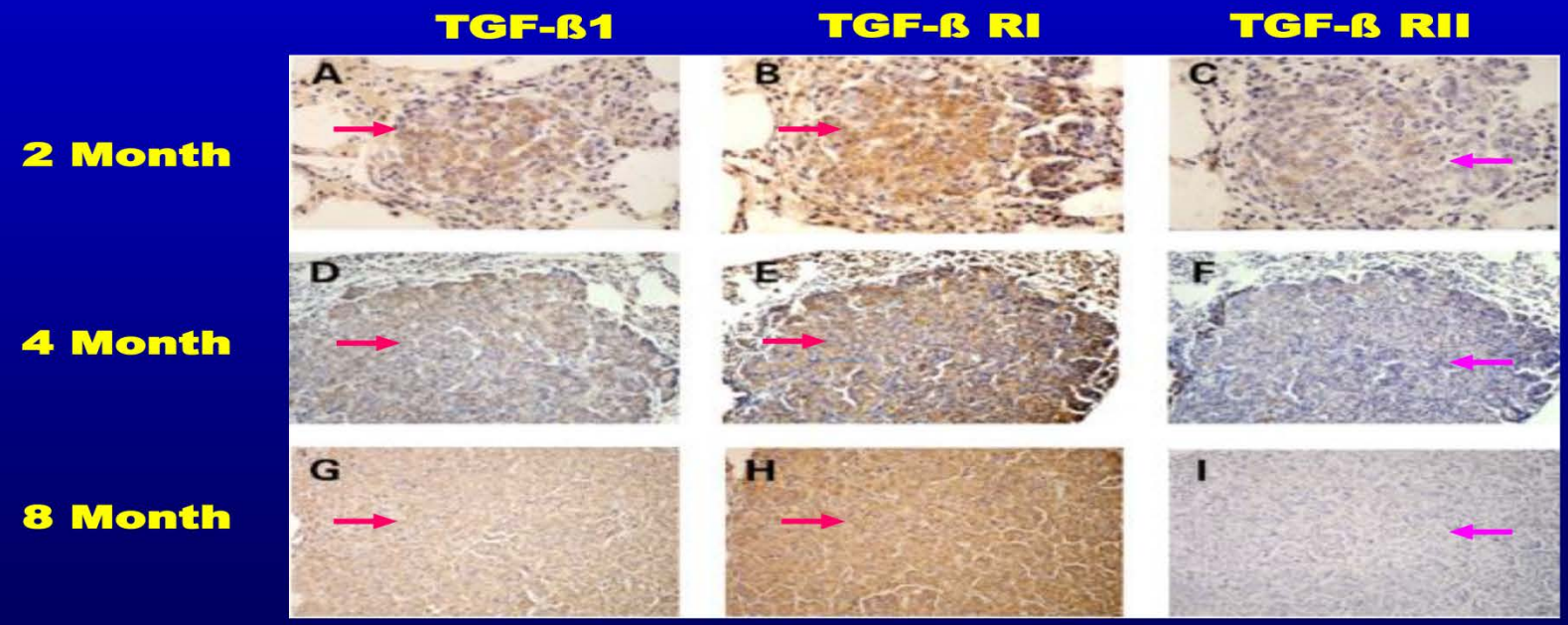
degraded by esterase

A/J mouse tumors

Production of Tumors in A/J Mice

TGF-beta1, RI and RII proteins

A/J Mouse Model
TGF-ß1, RI and RII Proteins in Lung Tumors

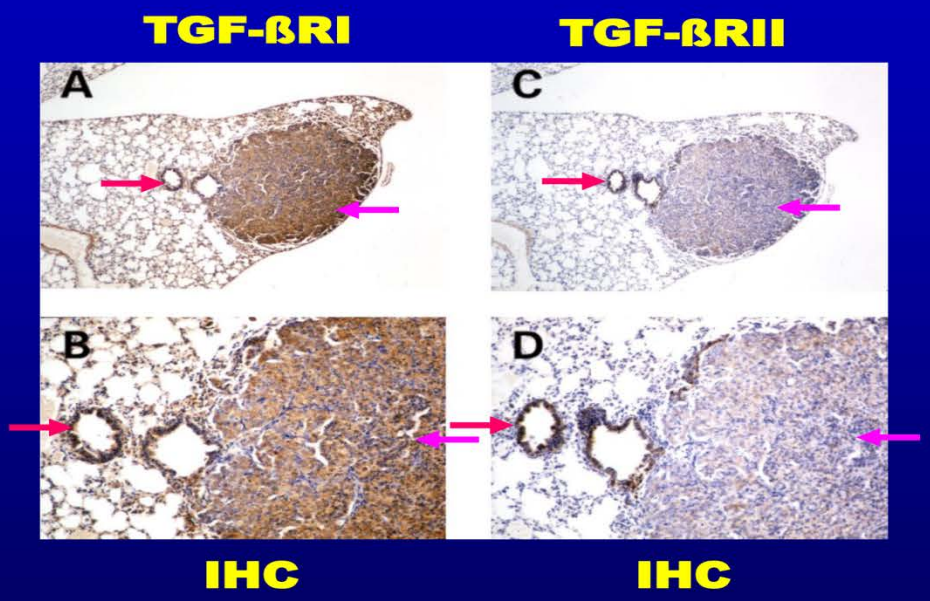


Decreased TGF-ß RII protein in tumors

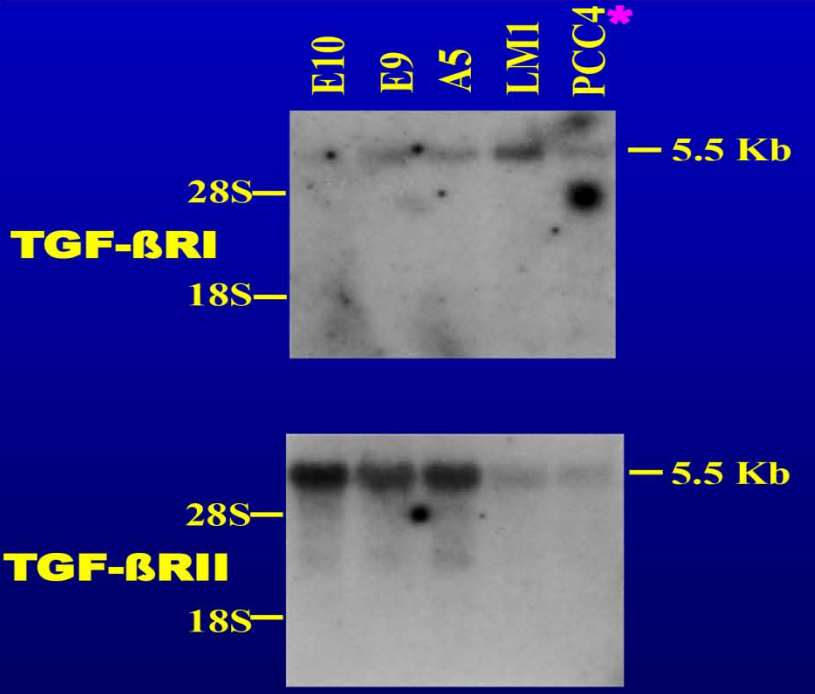
Decreased TGF-β RII in tumors

TGF-ß in A/J Mouse Model

EC-induced Lung Tumors

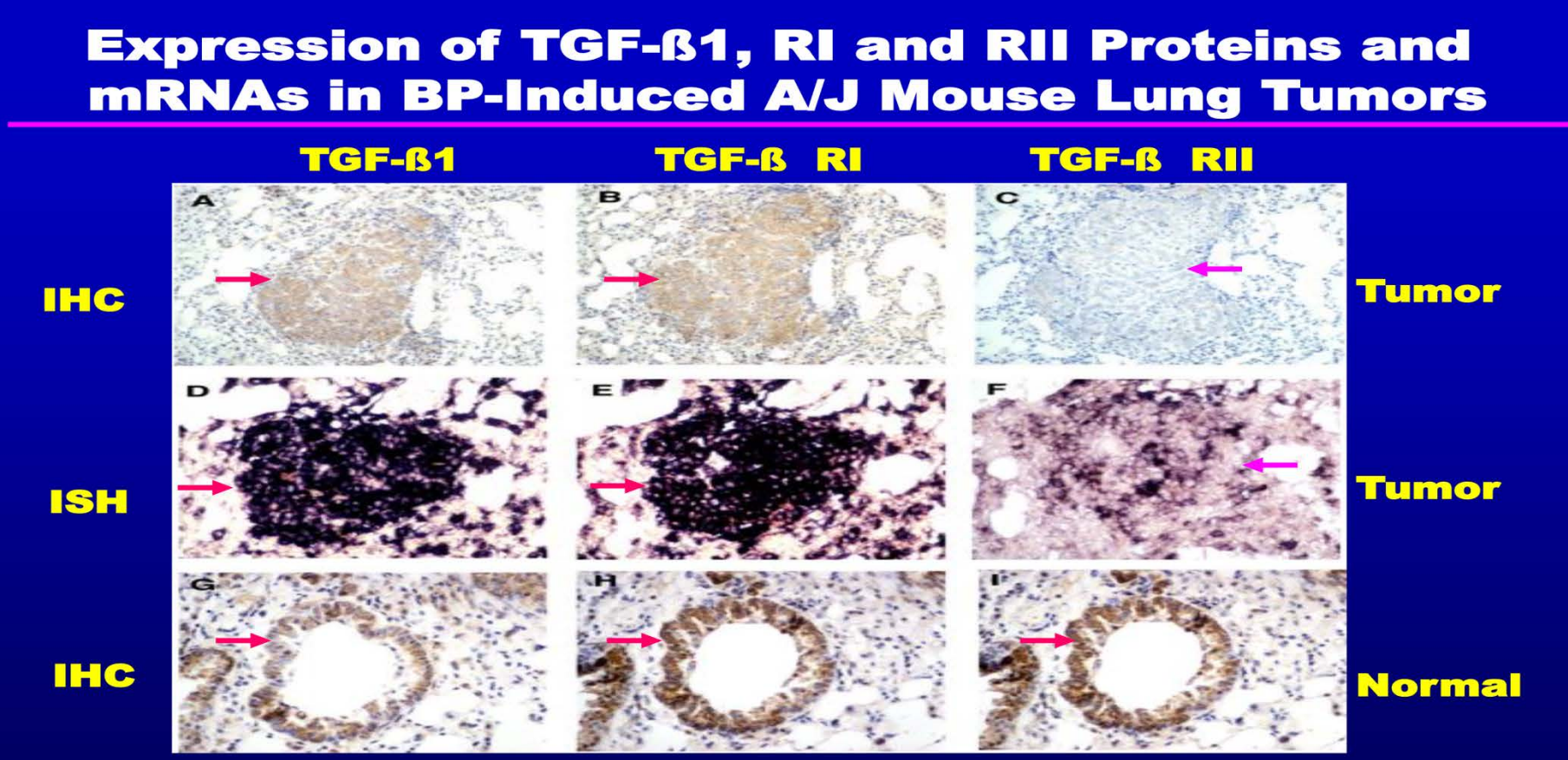


Lung Tumor Derived Cell Lines



Decreased TGF-ß RII protein and mRNA

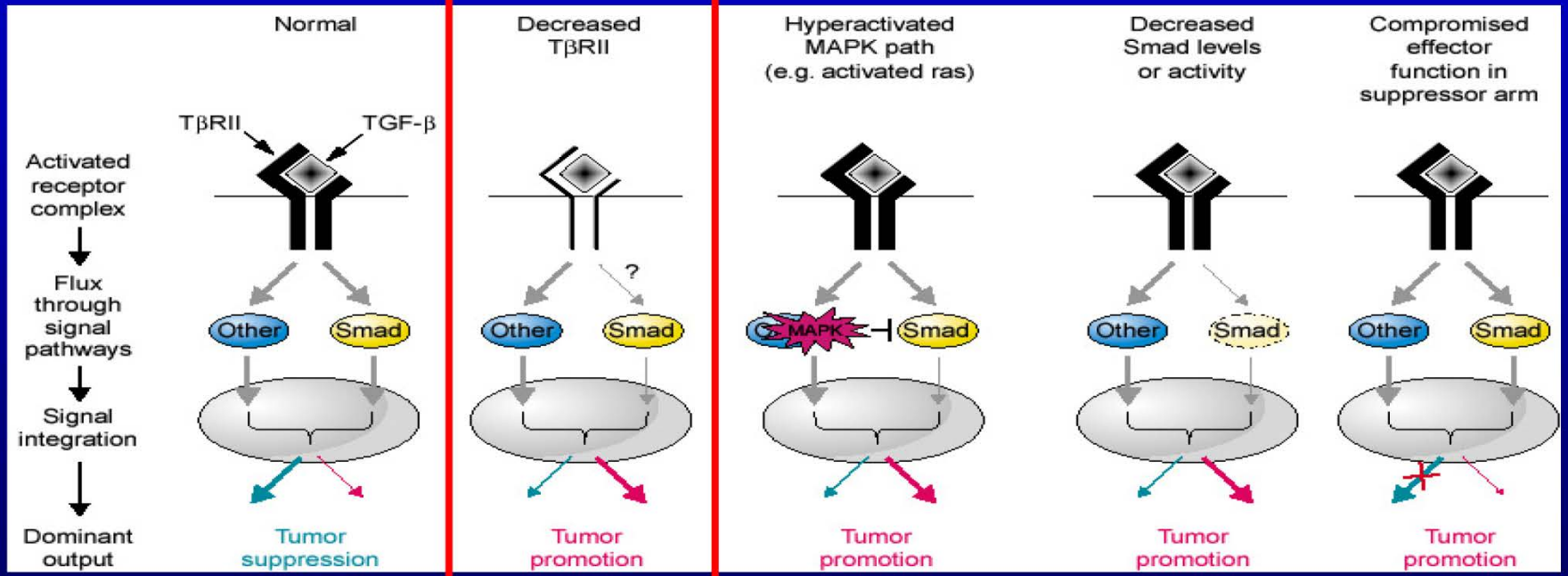
Expression of TGF-β1, RI and RII Proteins and mRNAs



Decreased TGF-ß RII mRNA and protein in tumors

Tumor suppresion/promotion

TGF-ß in Tumor Suppression/Promotion



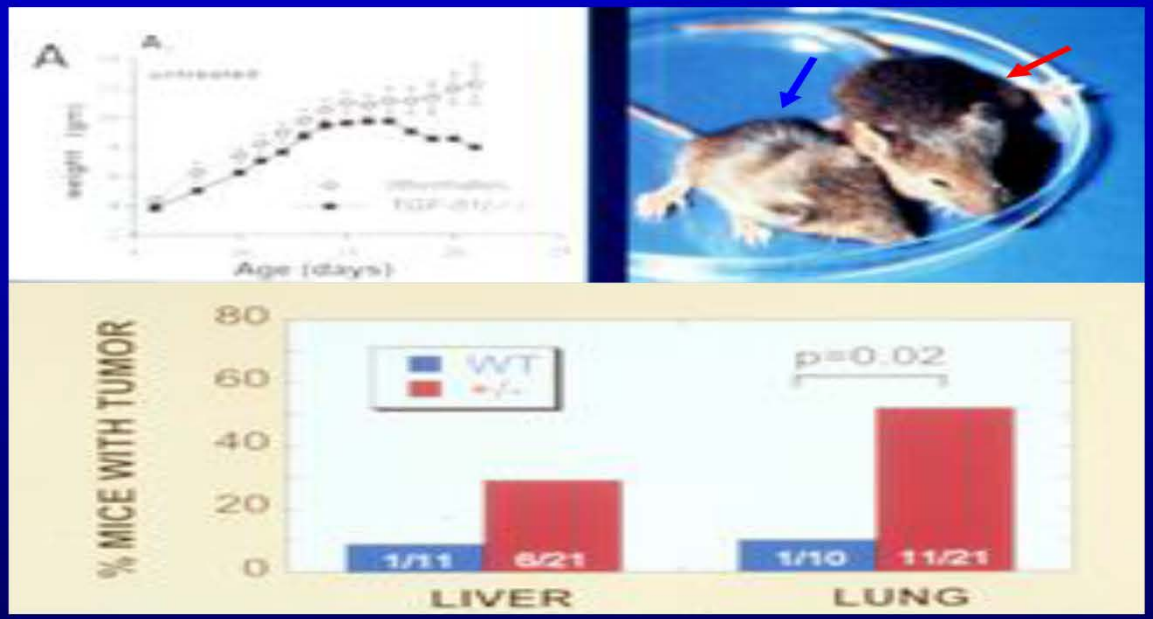
- **Reduced TGF-ß RII = Lung Tumor Promotion**

Question

Does deletion of TGF-β1 affect lung tumorigenesis?
C57BL/6 TGF-β1 Mouse

TGF-beta1 knockout mice

The C57BL/6 TGF-ß1 Knockout Mouse



Increased tumor incidence in TGF-ß1 HT mice

Mouse models

AJBL6 TGF-ß1 HT Mouse Derivation

A/J TGF-ß1 WT X C57BL/6 TGF-ß1 HT

↓

AJBL6
TGF-ß1 HT + TGF-ß1 WT
(F1)
Carcinogen

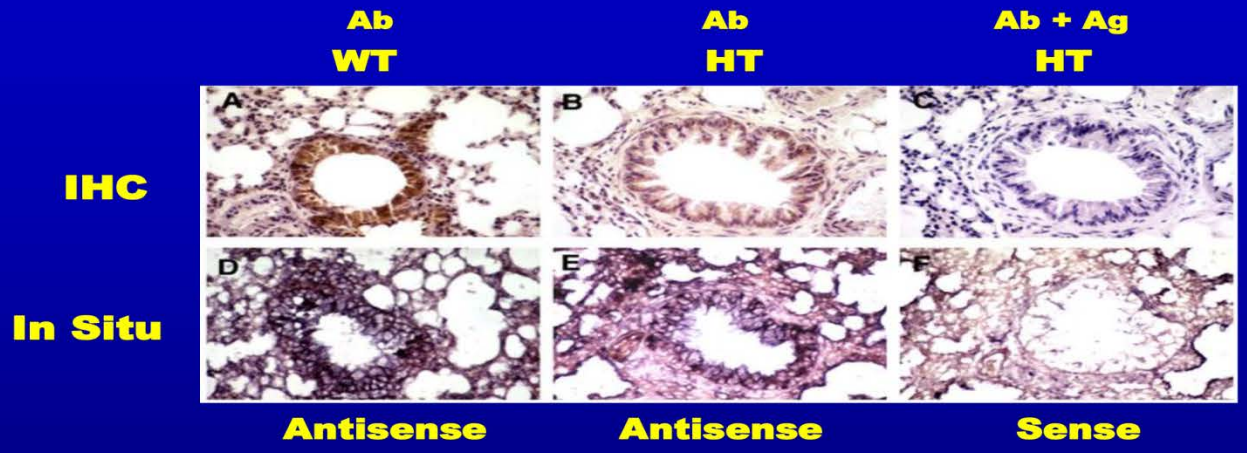
↓

Lung Tumors

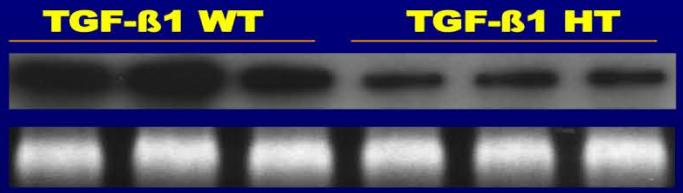
TGF-beta1 in HT and WT mice

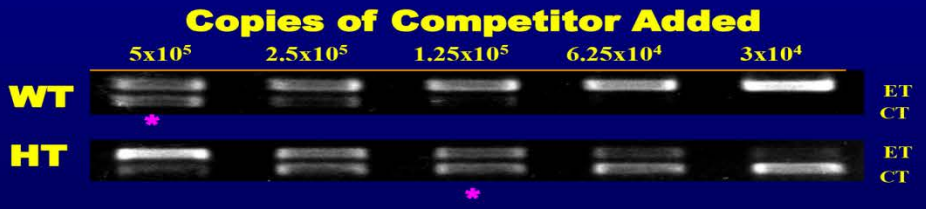
AJBL6 TGF-ß1 HT and WT Mouse

IHC Staining & In Situ Hybridization



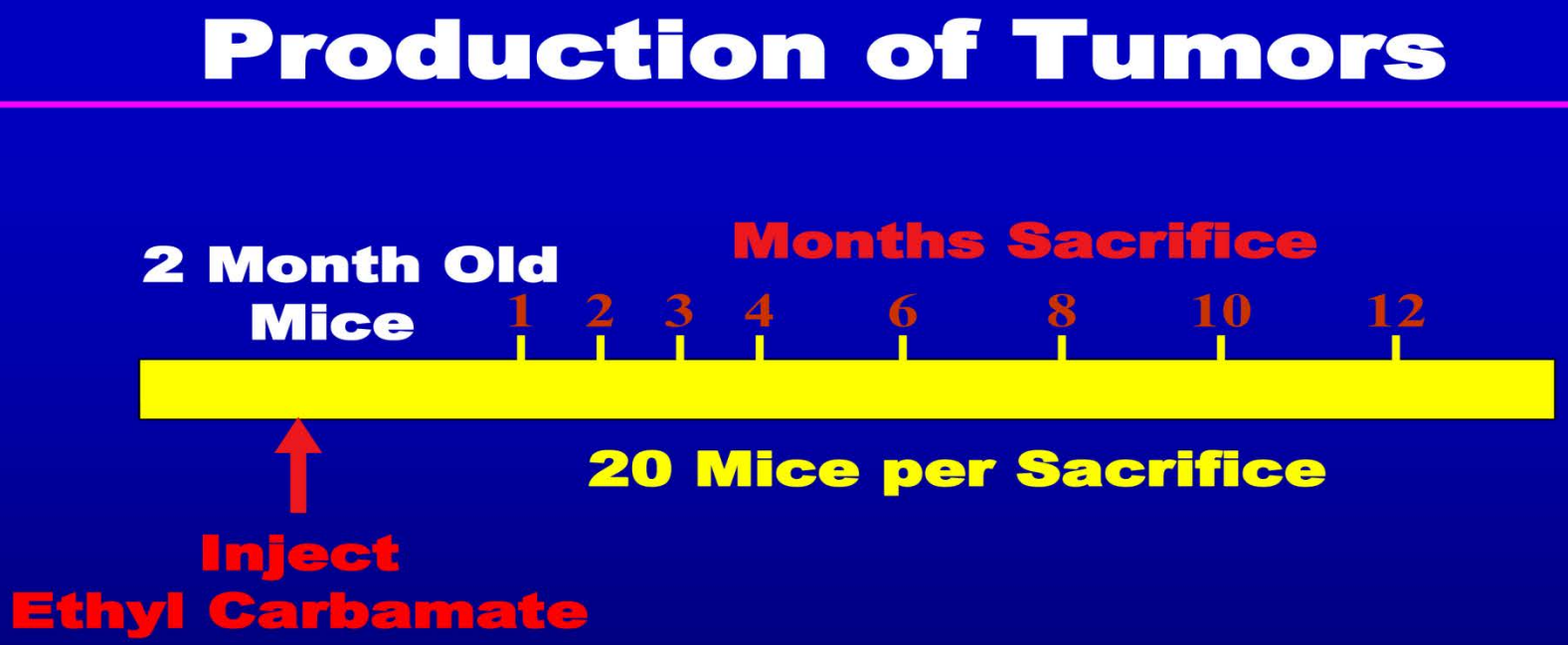
Northern Blotting & Competitive RT-PCR



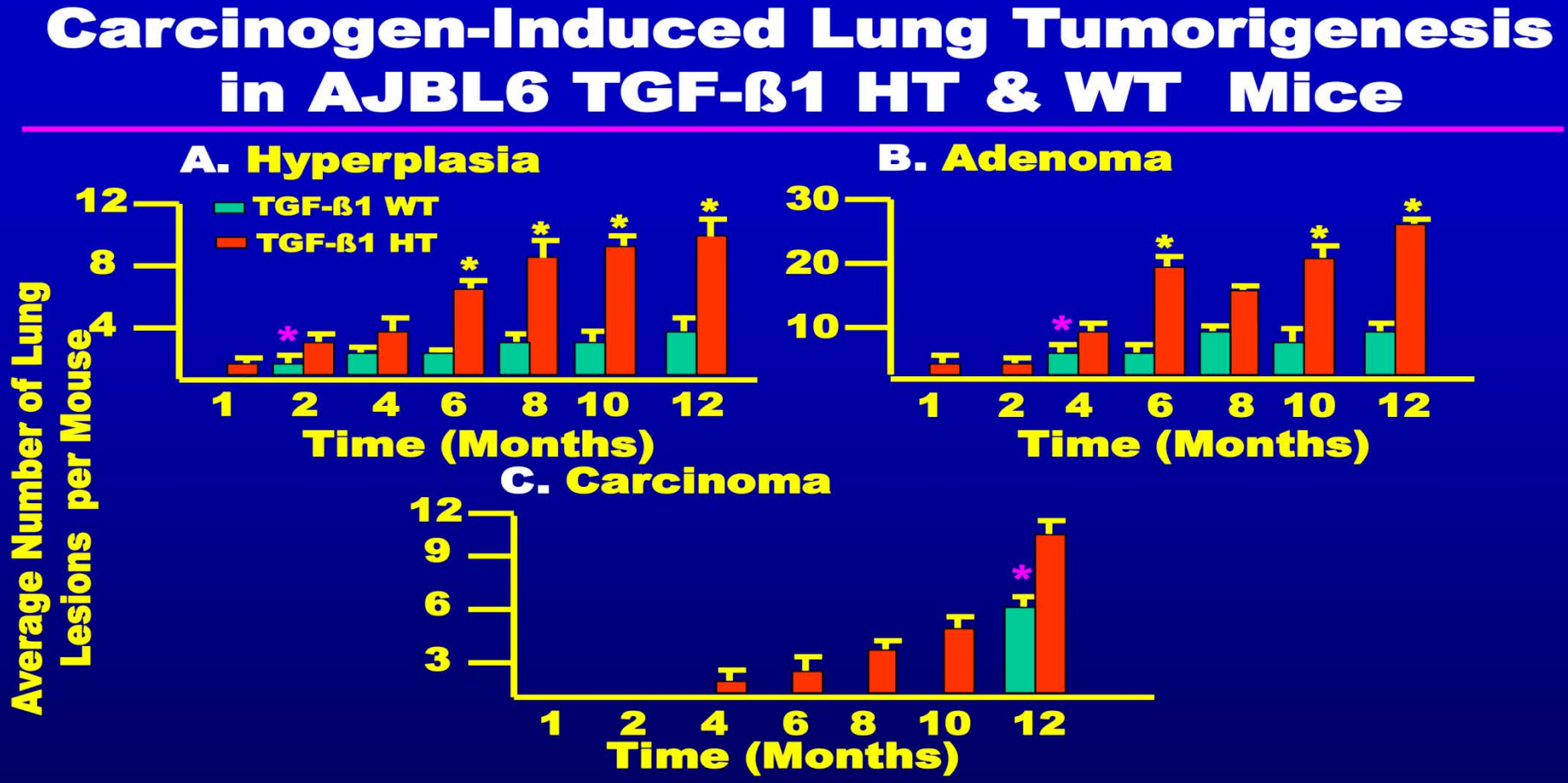


Reduced expression of TGF-ß1 in HT compared to WT

Production of Tumors



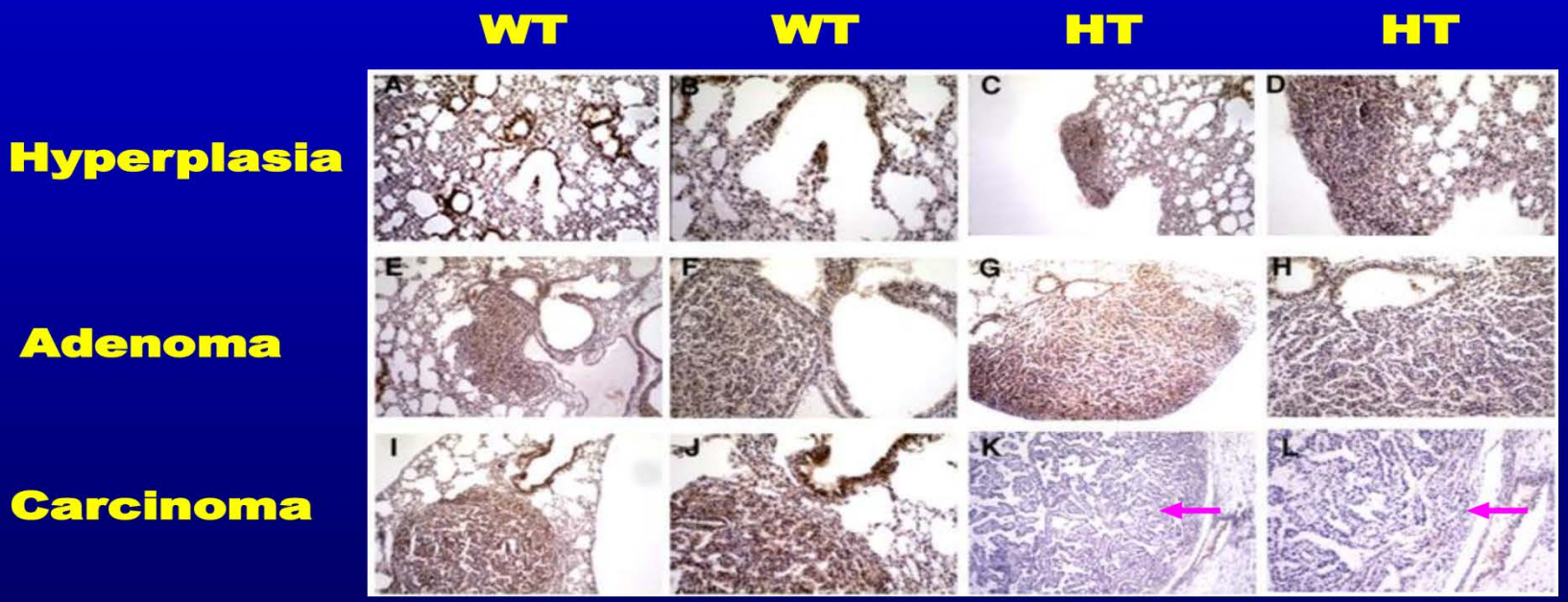
AJBL6 TGF-β1 HT & WT Mice



Increased tumor incidence and multiplicity and decreased tumor latency in TGF-ß1 HT mouse

TGF-beta RII

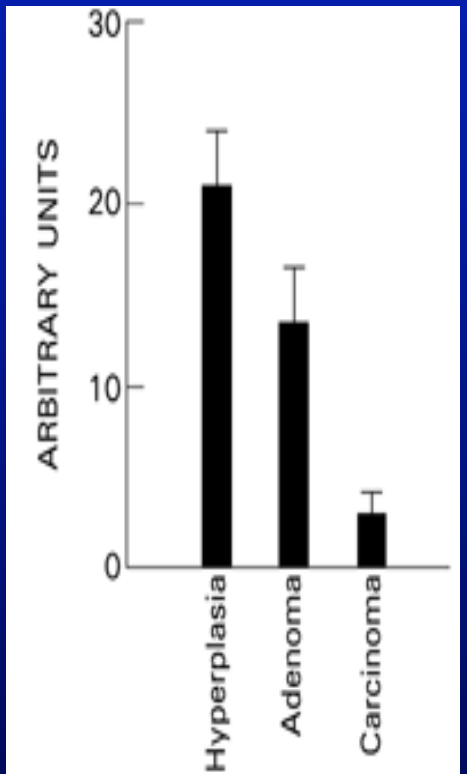
TGF-ß RII Protein in Lung Lesions from AJBL6 TGF-ß1 WT and HT Mice



Decreased TGF-ß RII in tumors of TGF-ß1 HT mice

Relative TGF-ß RII mRNA Levels Lesions from AJBL6 TGF-ß1 HT Mouse Lungs Treated with Ethyl Carbamate

Decreasing TGF-ß RII mRNA with increasing lung tumorigenesis



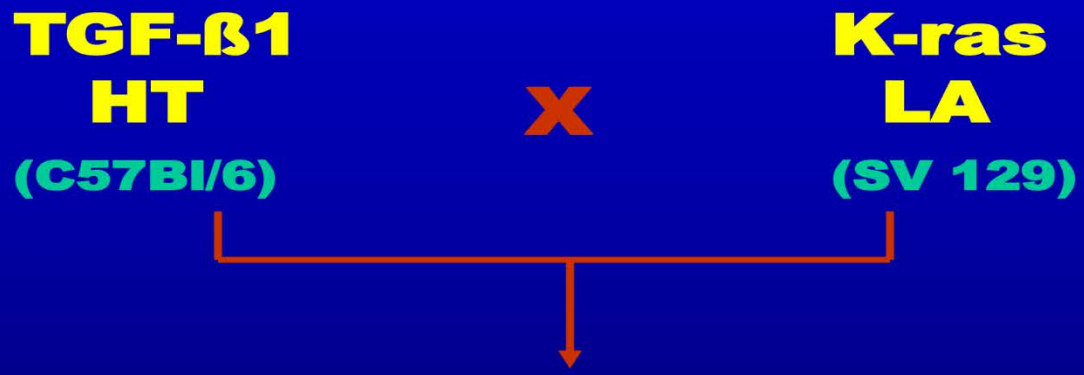
Question

Does deletion of TGF-β1 and mutation of K-ras affect lung tumorigenesis?

TGF-β1 HT/K-ras LA mouse

TGF-beta1 and K-ras

To Study the Interplay of TGF-β1 and K-ras: Generation of TGF-β1/ K-ras LA Mice



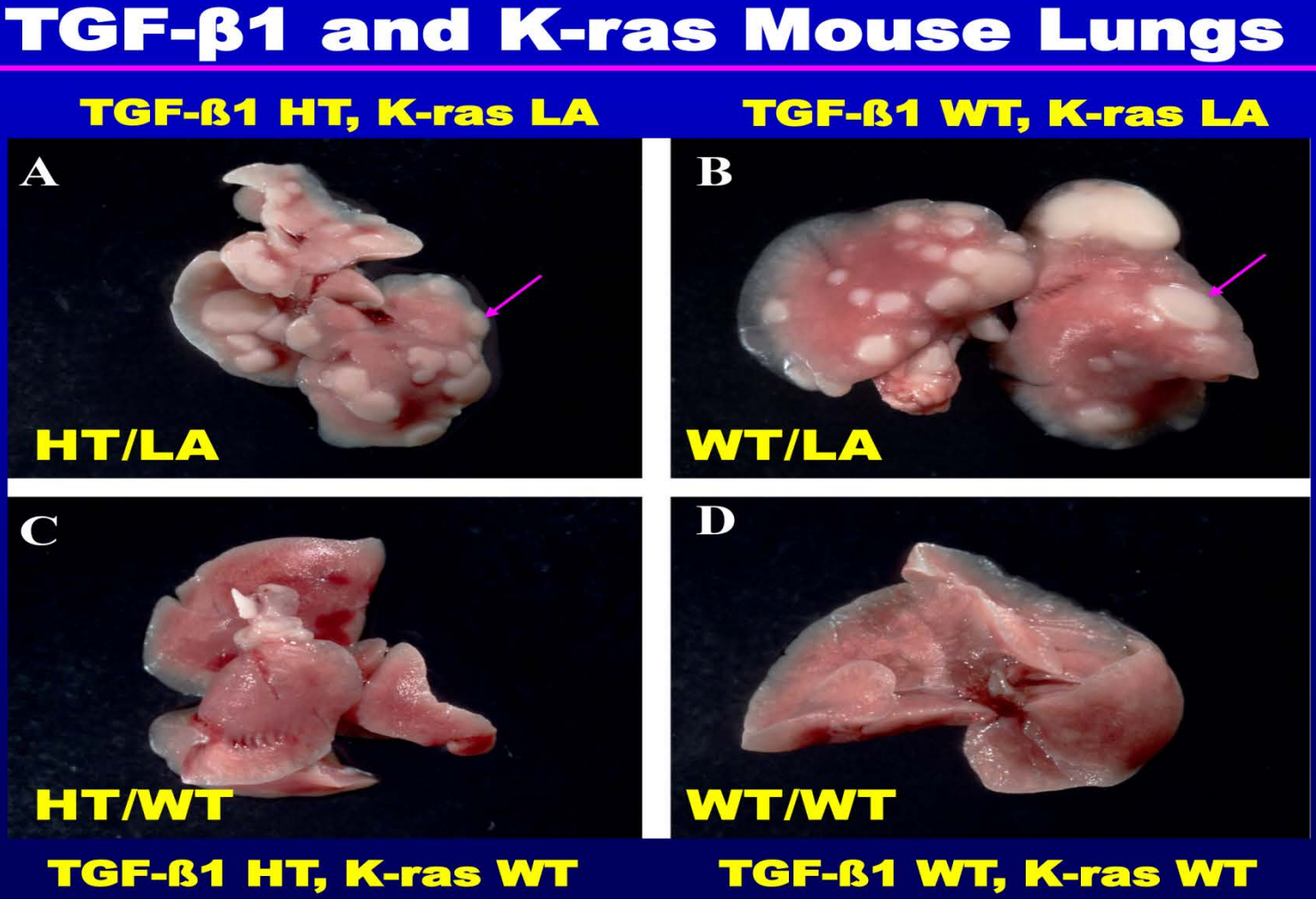
TGF-ß1 HT/K-ras LA - HT/LA Double Mutant

TGF-ß1 WT/K-ras LA - WT/LA Single Mutant

TGF-ß1 HT/K-ras WT - HT/WT Single Mutant

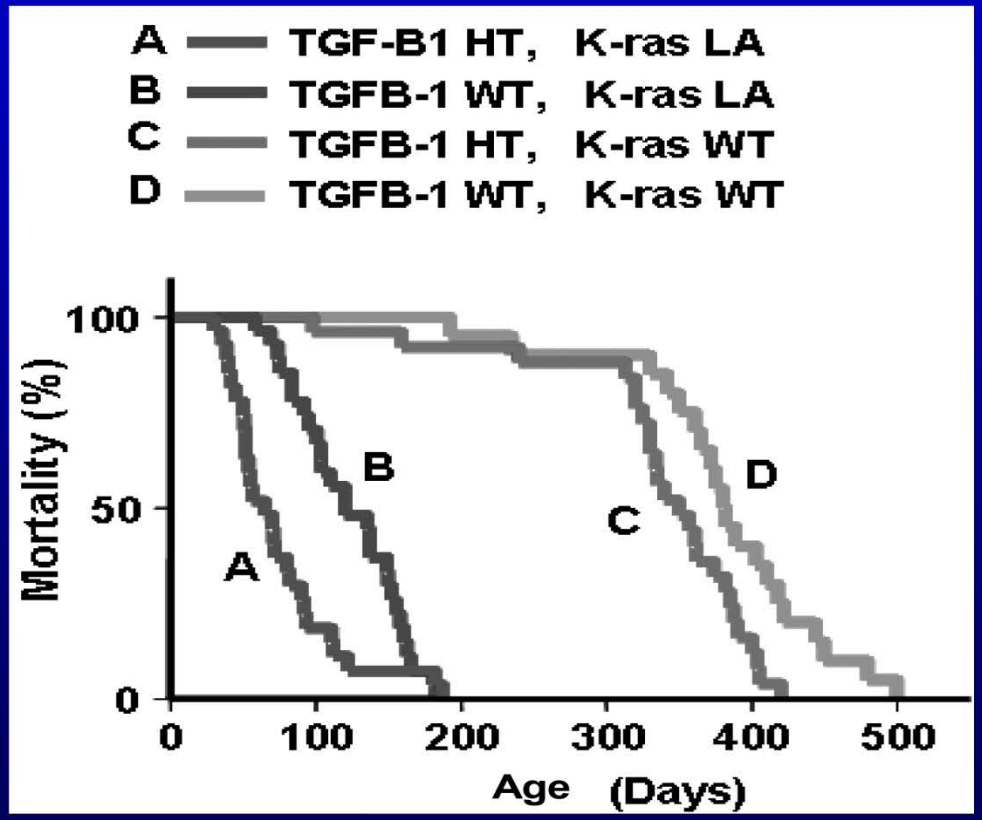
TGF-ß1 WT/K-ras WT - WT/WT Wild Type

TGF-beta1 and K-ras



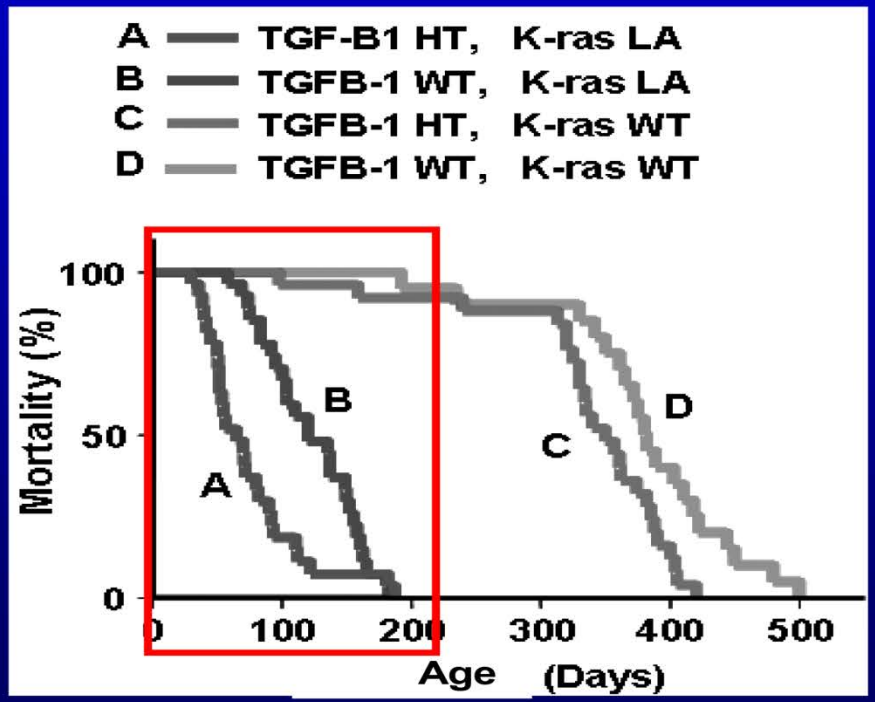
Mouse Survival

Effect of TGF-β1 Gene Deletion and K-ras Mutation on Mouse Survival



Mouse survival

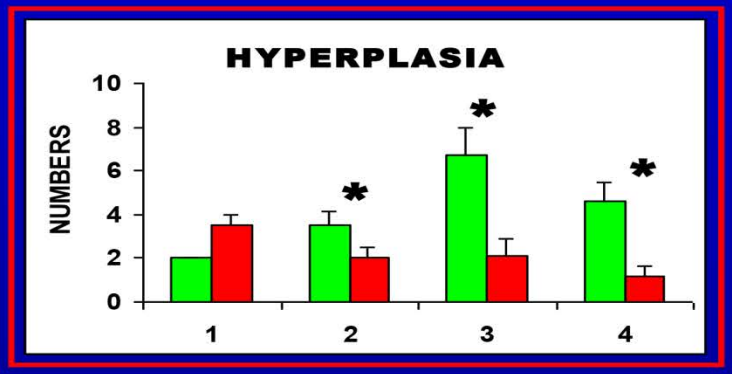
Effect of TGF-β1 Gene Deletion and K-ras Mutation on Mouse Survival

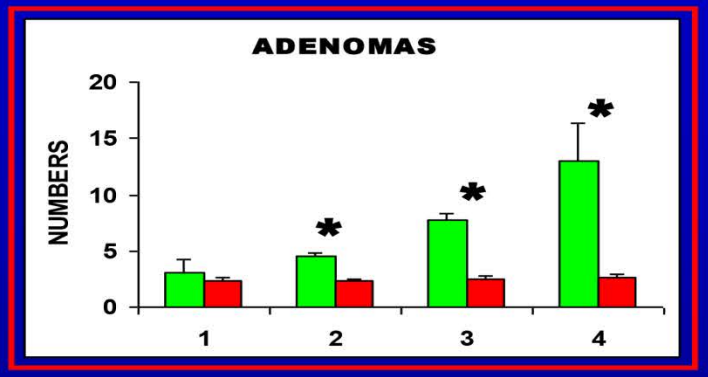


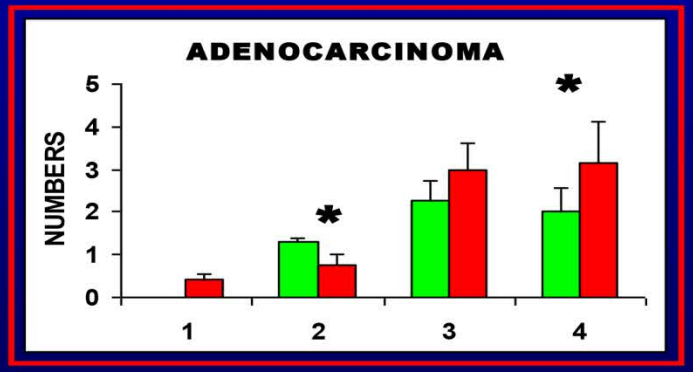
Decreased lifespans in HT/LA and WT/LA mice

Lung Pathology

Pathology of Lung Lesions







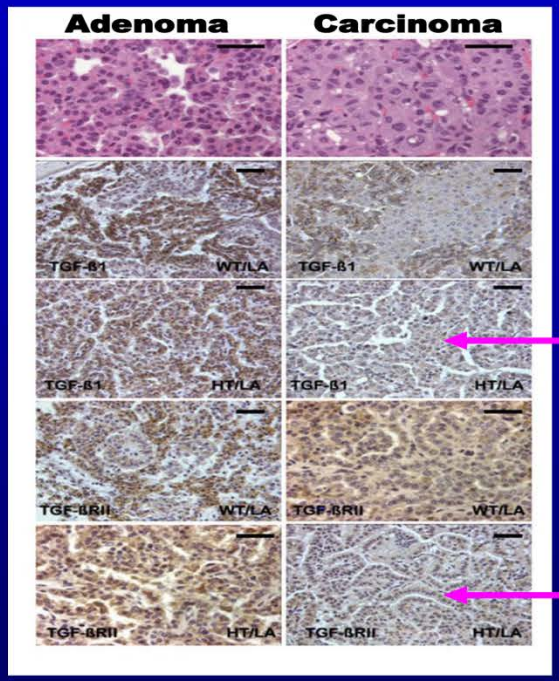
WT/LA

HT/LA

Increased hyperplasia & adenoma in WT/LA
Increased carcinoma in HT/LA

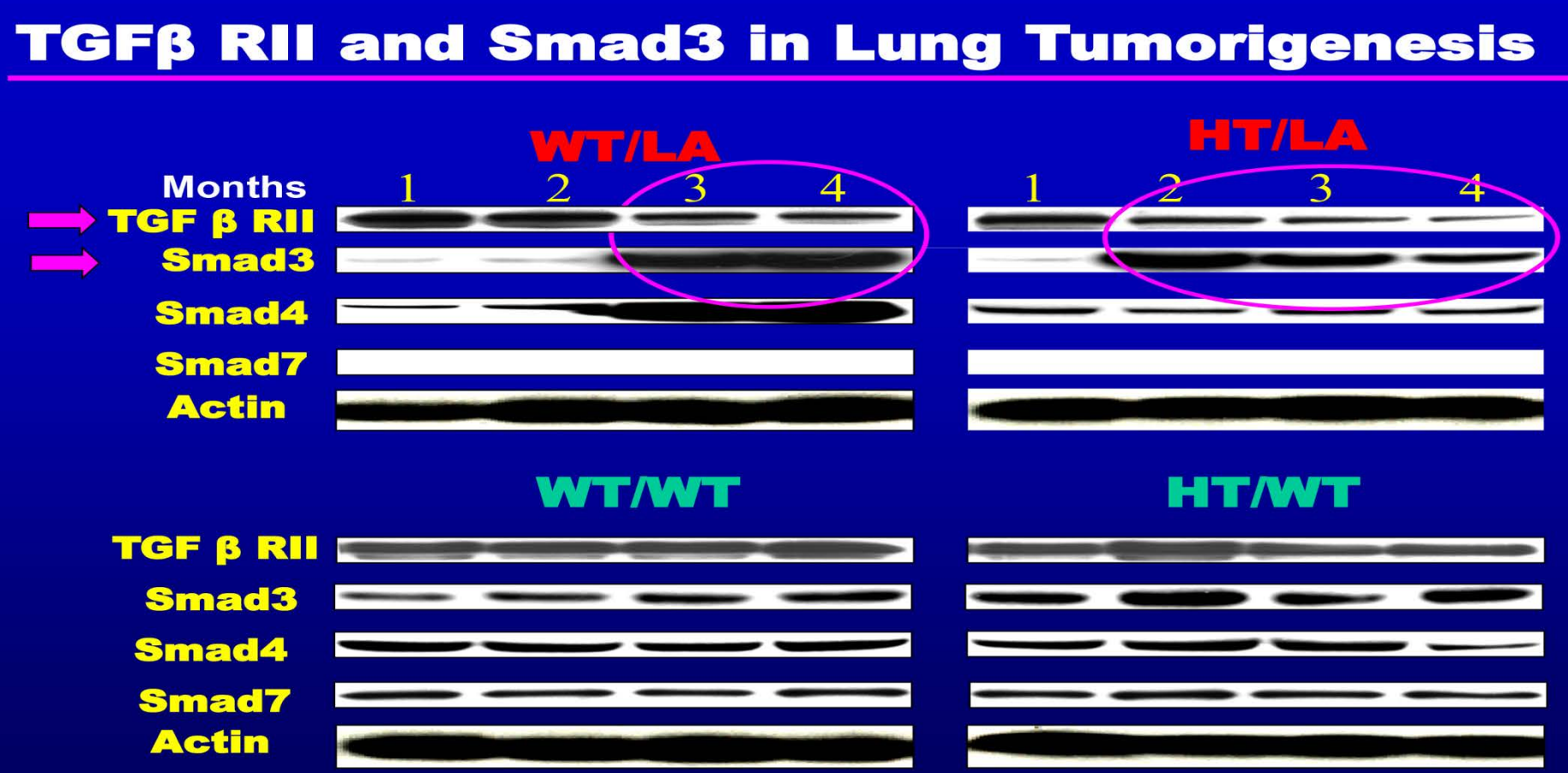
TGF-beta1 and RII

TGF-β1 and TGF-β RII in Lung Lesions



Reduced TGF-ß1 & RII in HT/LA adenocarcinomas

TGF-beta RII and Smad 3



HT/LA: Expedited TGF-ß RII reduction & Smad3 production

TGF-beta pathway

TGFβ Pathway in HT/LA Lung Tumorigenesis

Western Blot:

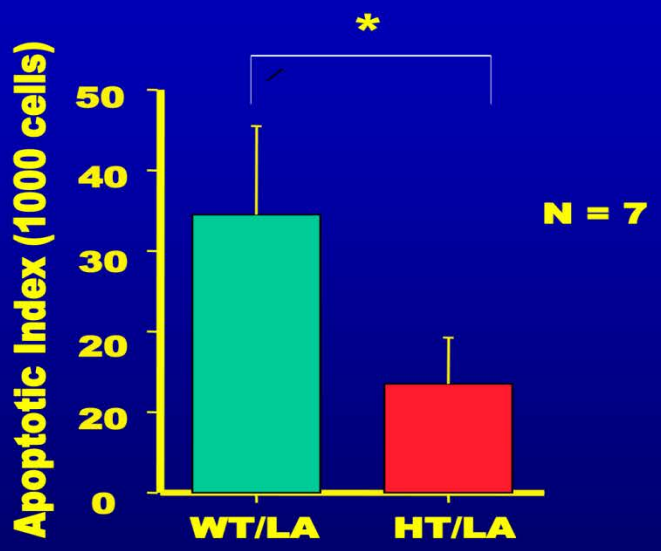
TGF β RII	Expedited TGF-ß RII reduction
Smad3	Expedited Smad3 production
Smad4	Reduced Smad4 production
Smad7	Reduced Smad7 production
K-ras	Expedited K-ras production
Raf-1	Expedited Raf-1 production

Real Time RT-PCR:

Reduced Smads 2, 3, 4 & 7 in adenomas
Reduced TGF-ß RII & Smads in carcinomas

Apoptotic index

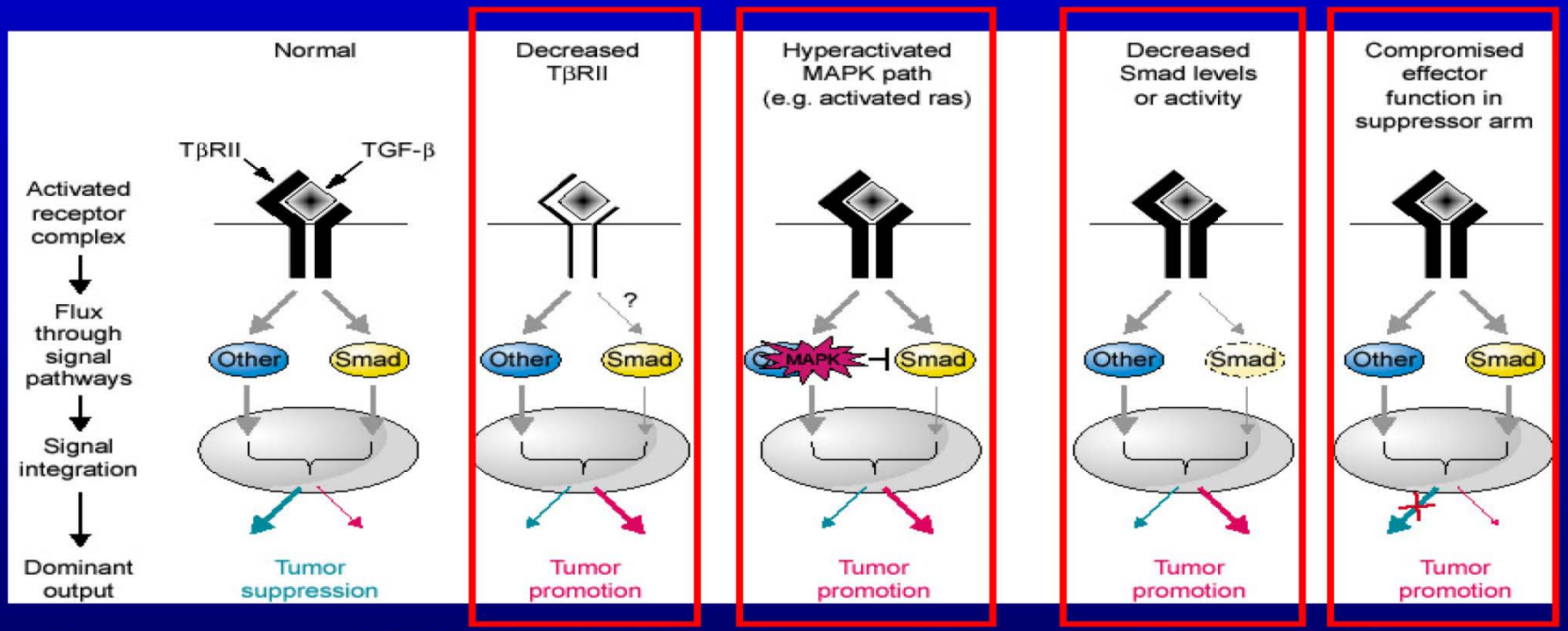
Apoptotic Index in WT/LA & HT/LA Mouse Lung Adenomas



Reduced apoptosis in HT/LA adenomas

Tumor suppression/promotion

TGF-ß in Tumor Suppression/Promotion



- **Decreased TGF-ß RII = Lung Tumor Promotion**
- **Activated Ras/MAPK = Lung Tumor Promotion**
- **Decreased Smad4 = Lung Tumor Promotion**
- **Compromised Apoptosis = Lung Tumor Promotion**

Anti TGFB compounds currently in Clinical Trials

Class	Compound	Target	Company	Phase	Disease
Antibodies	Fresolumimab	TGF-ß 1-3	Genzyme	Ph I	RCC & Melanoma
				Ph I - r	Met Breast Cancer
				Ph I - r	Advanced Melanoma
Antibody Targeting: ALK1	PF-03446962	ALK1	Pfizer	Ph I - r	Advanced Solid Tumors
Antisense Oligonucleotides	Trabedersen	TGF-ß2 mRNA	Antisense Pharma	Ph I	Pancreatic & Colon
				Ph IIb	Melanoma & Glioma
				Ph III - r	Glioblastoma
Receptor Kinase Inhibitors	LY2157299	ALK5	Eli Lilly	Ph Ib/Iia - r	Glioma, Hepatocellular
				Ph II - r	Pancreatic Cancer
				Ph Ib/II - r	Pancreatic Cancer
Combined TGF-ß + Vaccine	Lucanix	TGF-ß2	NovaRx	Ph II, Ph II - r	NSCLC, NSCLC
	TGF-ß2 + GMCSF Exp Vector	TGF-ß2 + GMCSF	MCMRC	Ph I - r	Advanced Cancer

Anti-TGFB Compounds currently in Clinical Trials

Class	Compound	Target	Company	Phase	Disease
Antibodies	Fresolumimab	TGF-ß 1-3	Genzyme	Ph I	RCC & Melanoma
				Ph I - r	Met Breast Cancer
				Ph I - r	Advanced Melanoma
Antibody Targeting: ALK1	PF-03446962	ALK1	Pfizer	Ph I - r	Advanced Solid Tumors
Antisense Oligonucleotides	Trabedersen	TGF-ß2 mRNA	Antisense Pharma	Ph I	Pancreatic & Colon
				Ph IIb	Melanoma & Glioma
				Ph III - r	Glioblastoma
Receptor Kinase Inhibitors	LY2157299	ALK5	Eli Lilly	Ph Ib/Iia - r	Glioma, Hepatocellular
				Ph II - r	Pancreatic Cancer
				Ph Ib/II - r	Pancreatic Cancer
Combined TGF-ß + Vaccine	Lucanix	TGF-ß2	NovaRx	Ph II, Ph II - r	NSCLC, NSCLC
	TGF-ß2 + GMCSF Exp Vector	TGF-ß2 + GMCSF	MCMRC	Ph I - r	Advanced Cancer

Acknowledgements

Acknowledgements

Epithelial Carcinogenesis Group

Sonia B. Jakowlew
Jerry Angdisen
Yang Kang
Alena Naumova
Jyotsna Pandey
Sarah Umphress

MIT

Tyler Jacks
Kim Mercer