

# **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](mailto:jakowles@mail.nih.gov)**

# **Lung Cancer in 2008, USA**

- **Most common cause of cancer deaths in both men and women**
- **208,493 diagnosed new cases**
  - 111,886 men; 96,607 women**
- **158,592 deaths due to lung cancer**
  - 88,541 men; 70,051 women**
- **Most cases now occur in ex-smokers**
- **< 15% five year survival rate**

# Transforming Growth Factor- $\beta$ (TGF- $\beta$ )

**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- $\beta$  is an attractive candidate for new  
therapeutic intervention approaches**

# Transforming Growth Factors: The Beginning

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**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- $\alpha$** )
2. Does not compete for EGF binding, but colony forming activity is enhanced by EGF (**TGF- $\beta$** )

**Sarcoma growth factor = TGF- $\alpha$  + TGF- $\beta$**

*Roberts, Anzano, ... Sporn: Nature 295:417, 1982*

1983- Publication of the purification of TGF- $\beta$  from:

**Human platelets (*Rick Assoian*)**

**Human placenta (*Chuck Frolik*)**

**Bovine kidney (*Anita Roberts*)**

# Scale of TGF- $\beta$ 1 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

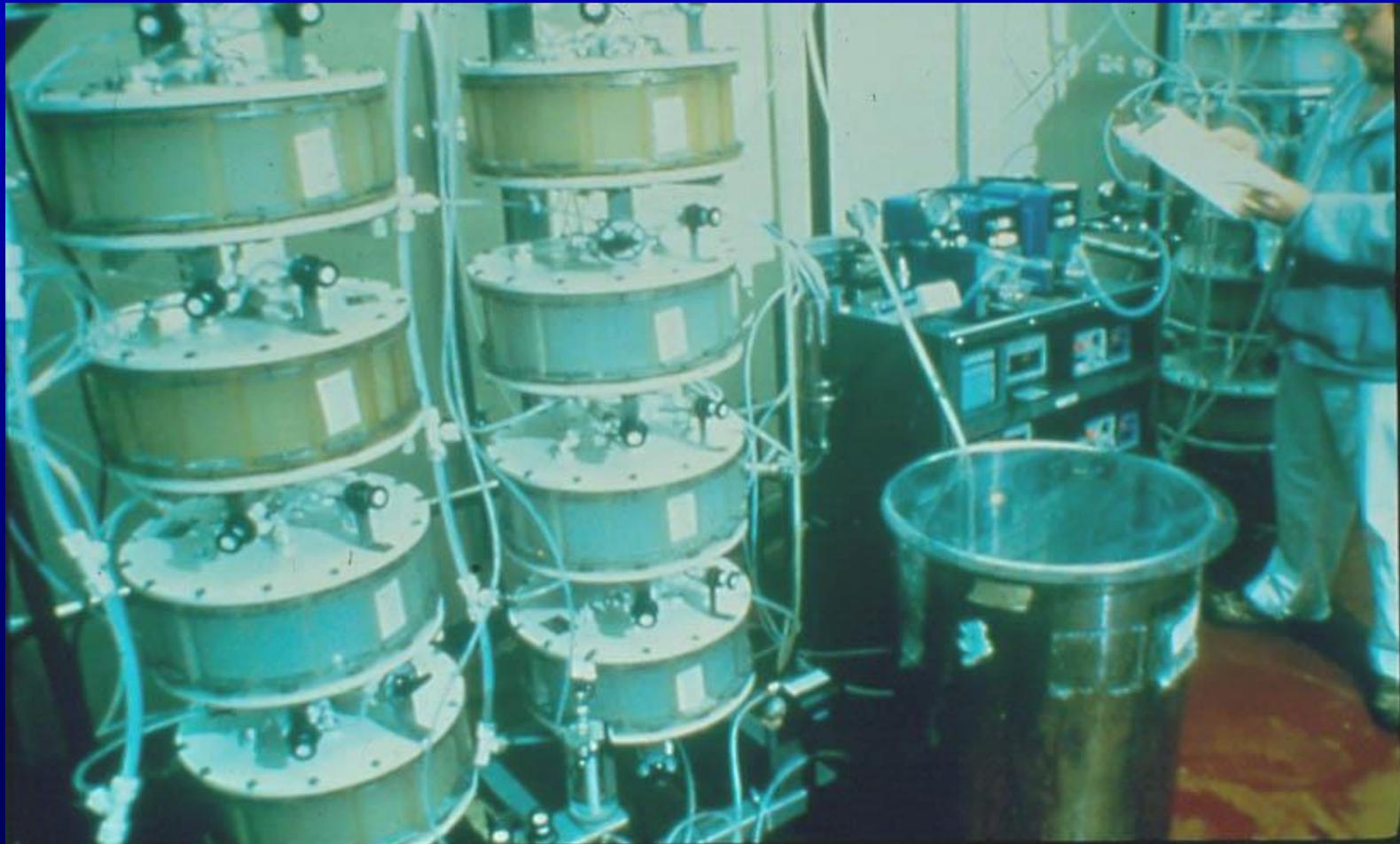
Collect 1 liter fractions

Lophilize and redissolve for further chromatography

Final Yield = 6  $\mu$ g TGF- $\beta$ 1

purification fold = 230,000; recovery = 10%

# The Columns for TGF-beta1 Purification



# Clonogenic Assay; Growth of NRK cells in soft agar

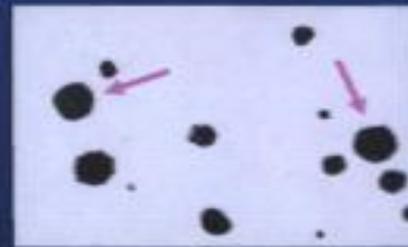
## The Assay: Growth of NRK Cells in Soft Agar



If no TGF- $\beta$  is present



- Plate agar base
- Add mix of media, serum, NRK cells, EGF, sample
- 1 wk/37°/5%CO<sub>2</sub>
- Stain
- Count colonies >3100  $\mu\text{m}^2$  with Omnicon Image Analysis System

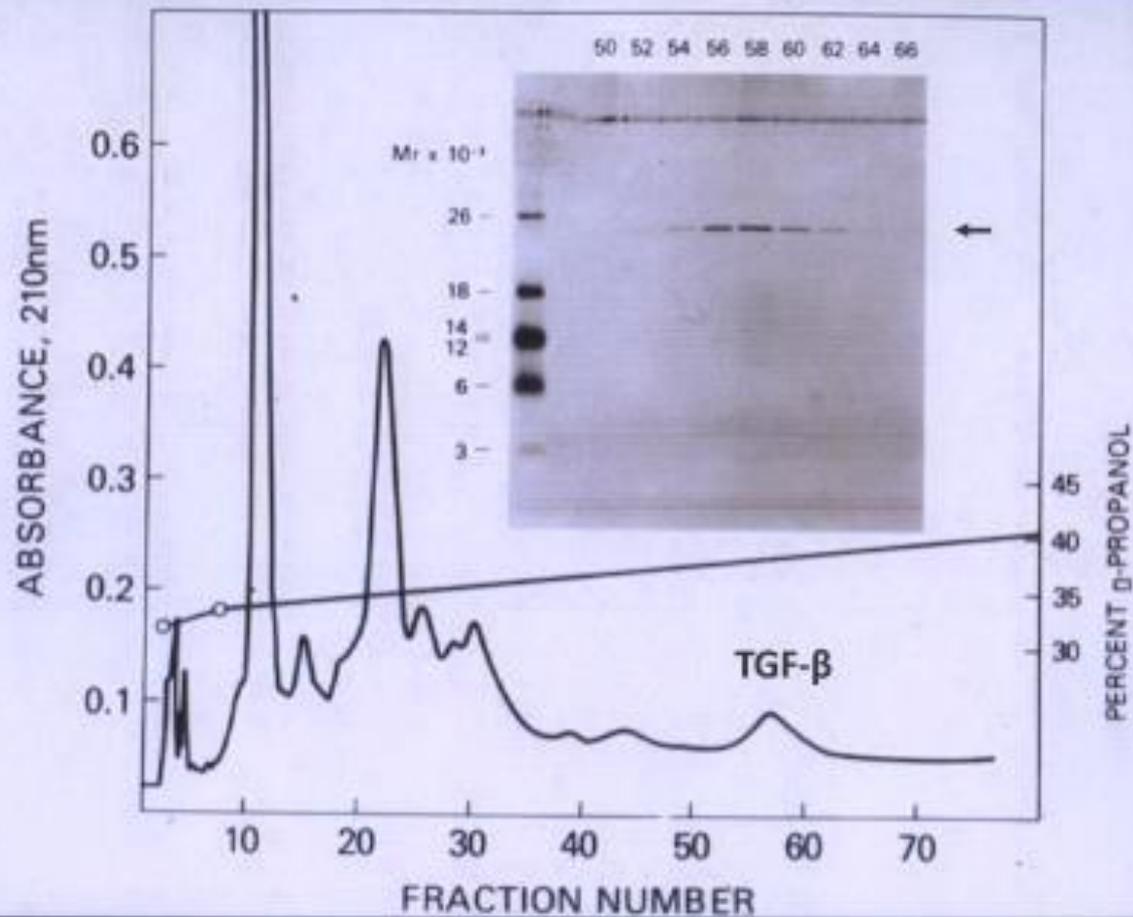


If TGF- $\beta$  is present



# Final HPLC Purification

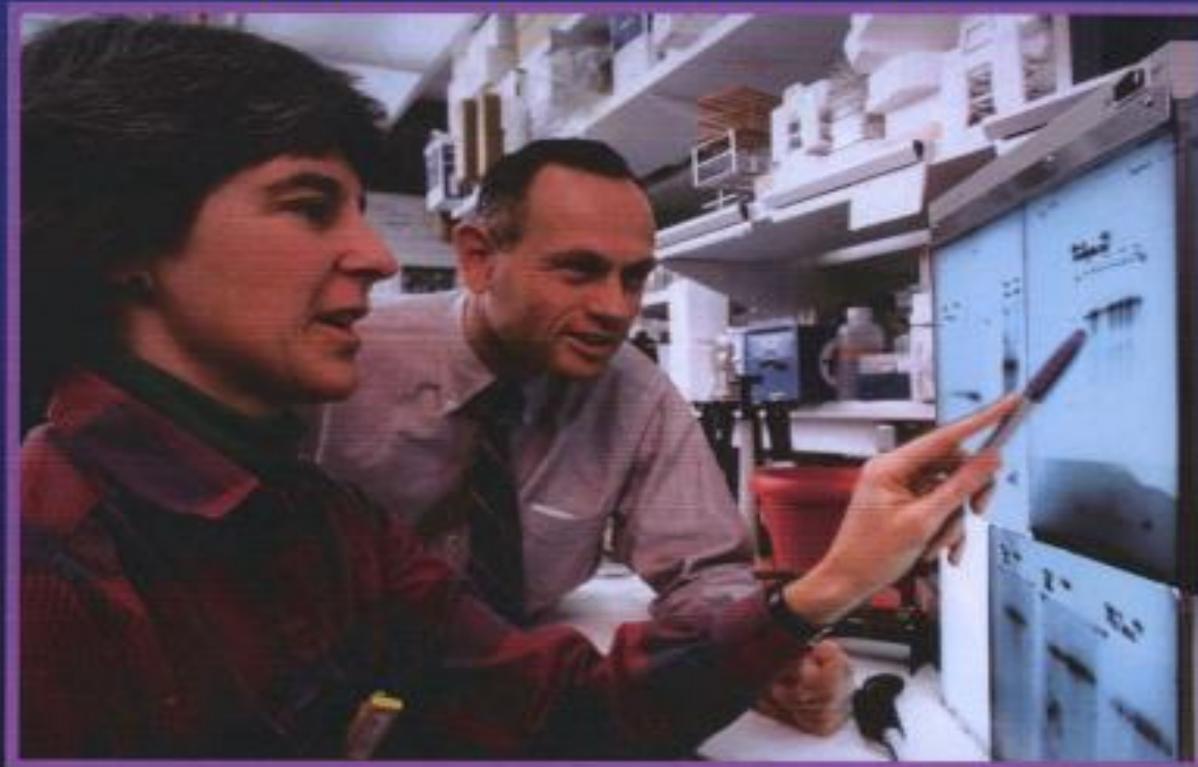
## The Final HPLC Purification



# TGF $\beta$ : Born at NCI

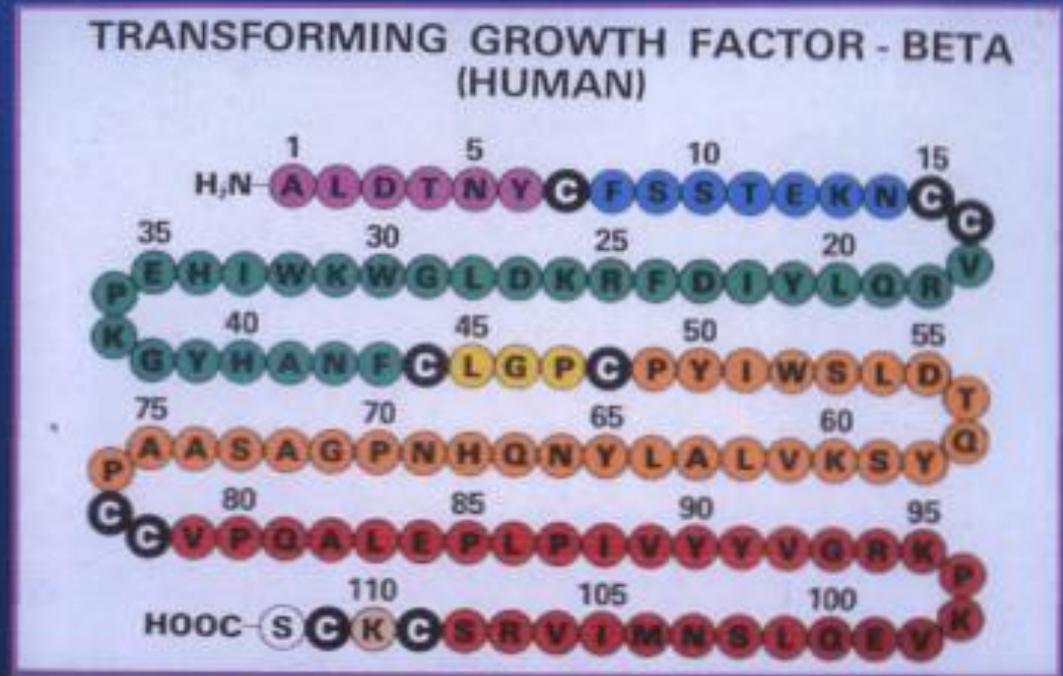
**EUREKA!! TGF- $\beta$ : Born at NCI**

Michael Sporn & Anita Roberts

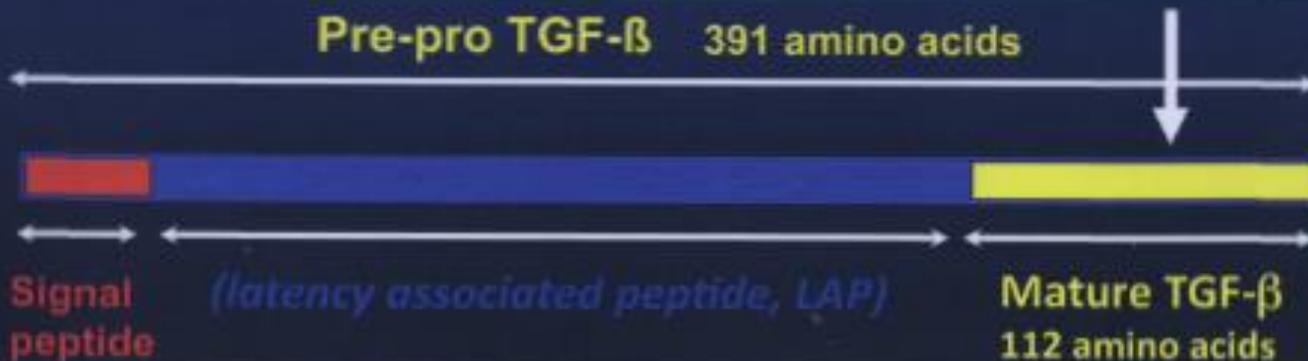


# Mature TGF $\beta$ 1

Sequence of  
mature TGF- $\beta$ 1  
monomer

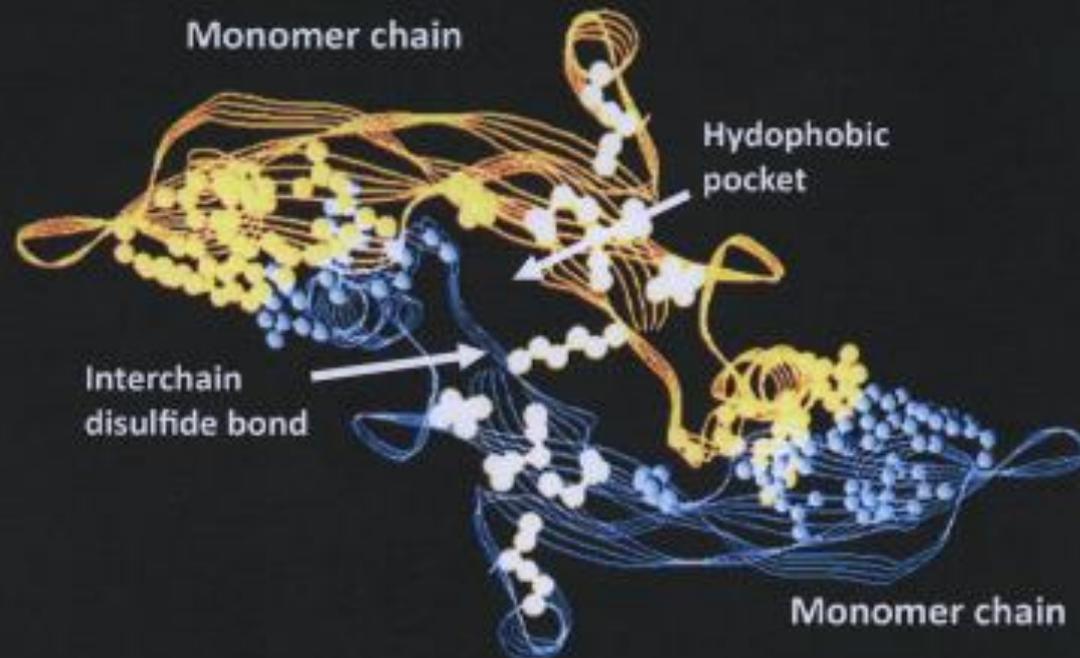


Pre-pro TGF- $\beta$  391 amino acids



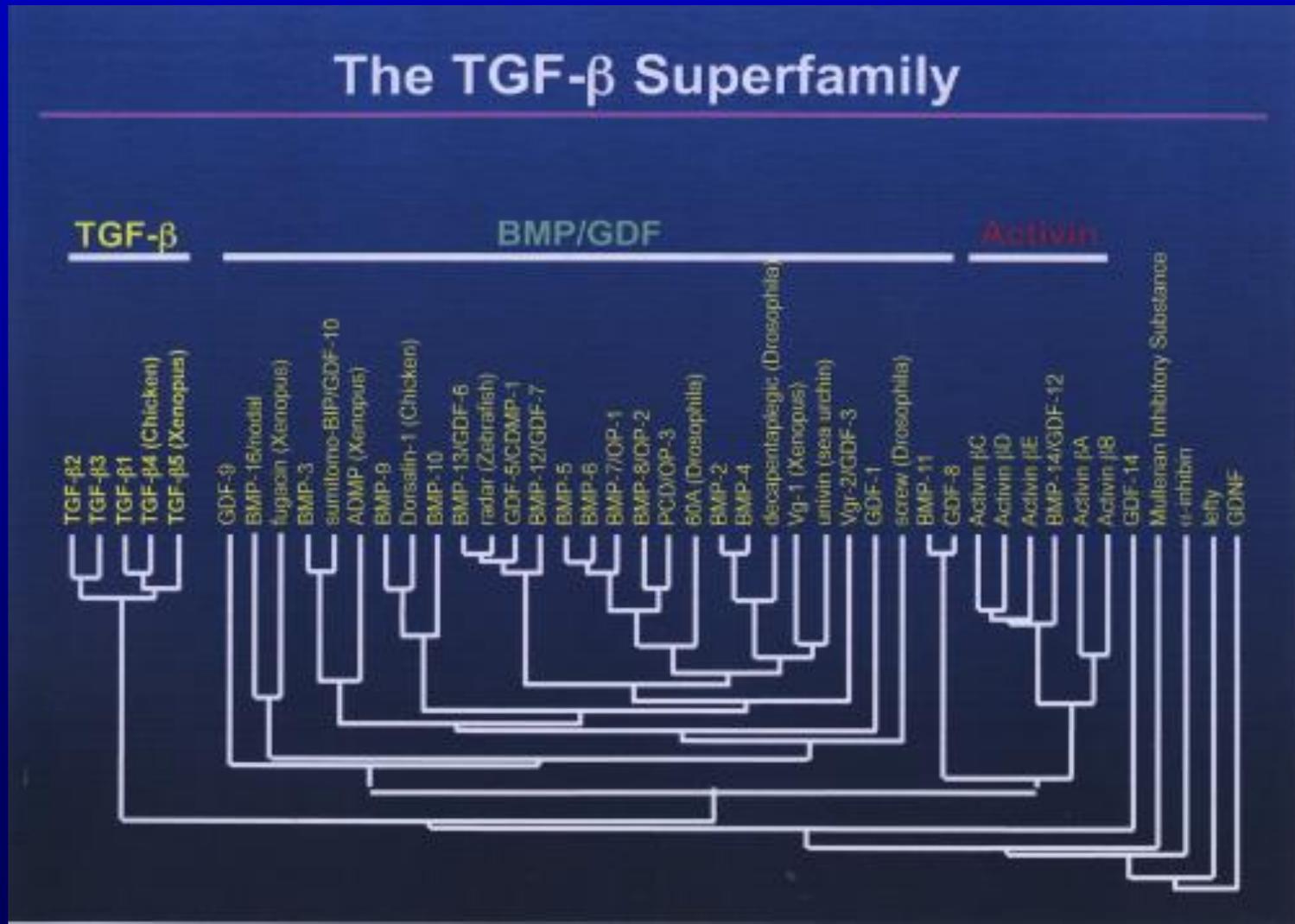
# TGF $\beta$ : 25 kDal homodimer

## TGF- $\beta$ : A Homodimer

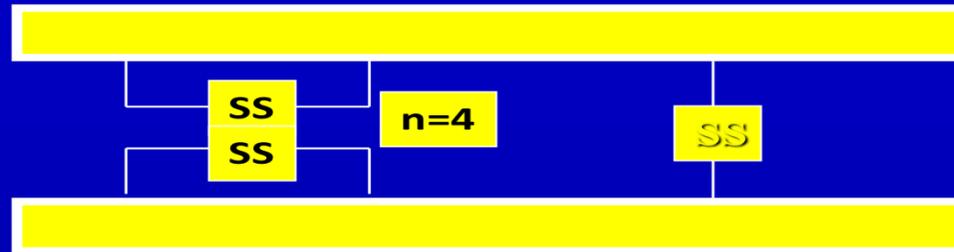


Daopin, S et al Science 257:369, 1992

# TGF $\beta$ superfamily



# Transforming growth factor beta



## Transforming Growth Factor- $\beta$

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- 25,000 MW disulfide-bonded homodimer
- 3 highly homologous isoforms (TGF- $\beta$  1, 2 and 3)
- Principal sources - platelets, bone, spleen
- Most cells express TGF- $\beta$  and its receptors
- Usually secreted in latent, inactive form
- Superfamily of TGF- $\beta$ s, 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- $\beta$ Superfamily: Central Control Modules

for Many Biological Processes

**TGF $\beta$  is associated with development, immune system function, reproduction, angiogenesis, aging, response to injury, metabolic regulation and proliferation.**

## **Model for TGF- $\beta$ pathway**

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

# Clinical Observations

## Clinical Observations

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### **TGF- $\beta$ is a tumor suppressor:**

- **Germline mutations in TGF- $\beta$  pathway components cause familial predisposition to cancer**  
*(Smad4 in juvenile polyposis syndrome)*
- **TGF- $\beta$  pathway components are somatically mutated or deleted in some human cancers**  
*(T $\beta$ -RII in HNPCC, Smad4 in pancreatic cancer)*
- **Reduced expression of TGF- $\beta$ 1 signaling pathway components or overexpression of endogenous pathway inhibitors are associated with disease progression**  
*(T $\beta$ -RII, T $\beta$ -RI, Smad7, Ski)*

# Clinical Observations

## Clinical Observations

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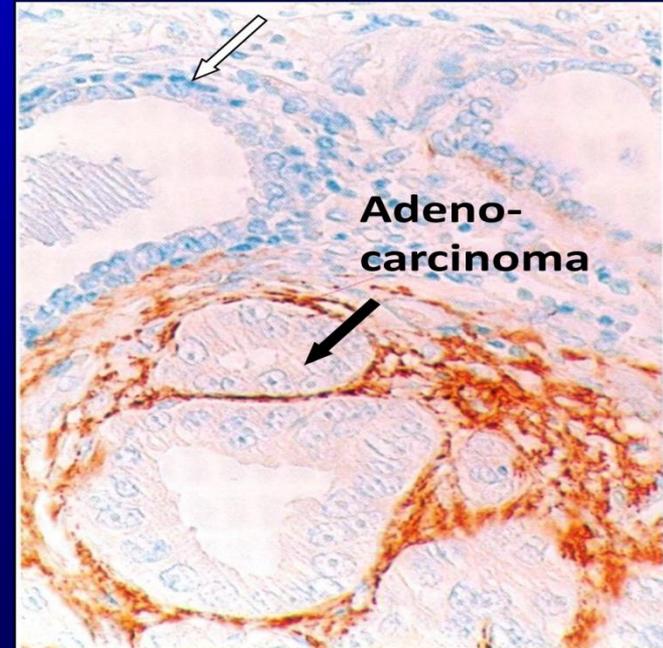
### **TGF- $\beta$ is a tumor promoter:**

- **TGF- $\beta$ 1 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- $\beta$ 1:**  
(Truong et al. Hum Pathol 1993)

**TGF- $\beta$  sits at the interface between tumor parenchyma and microenvironment**



# TGF- $\beta$ in Carcinogenesis - Hero or villain?

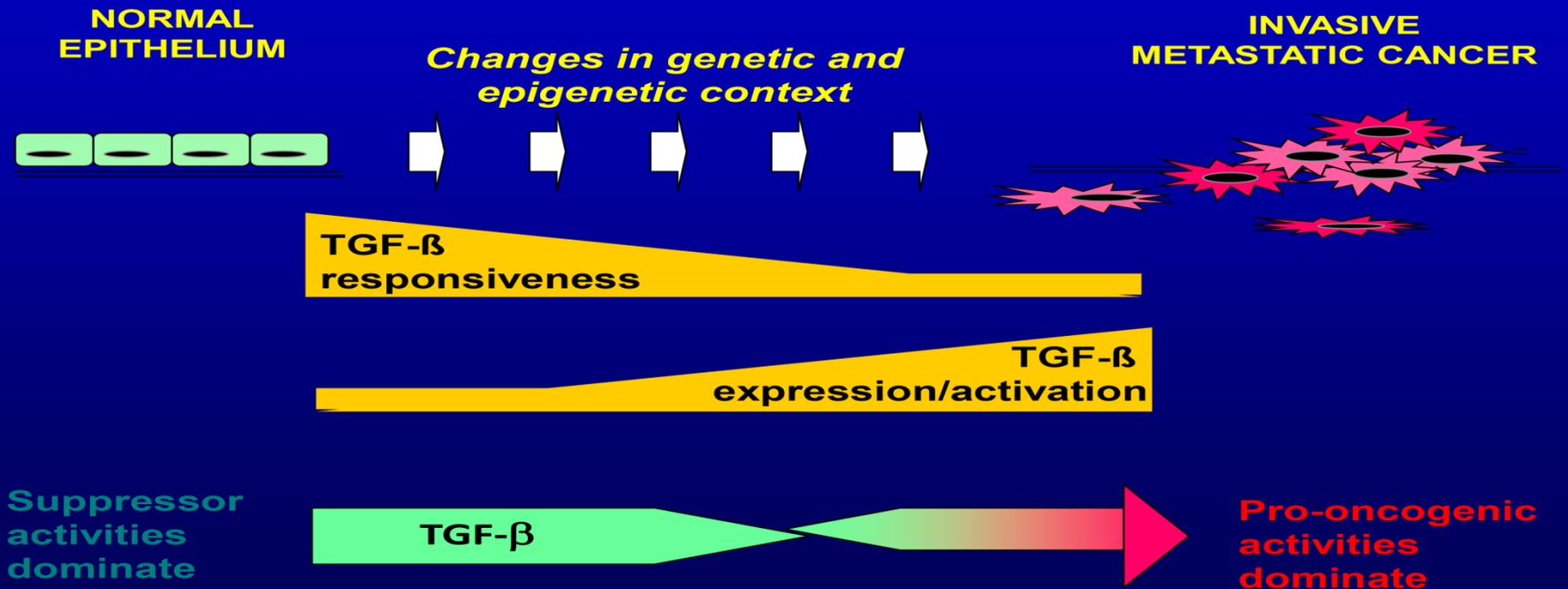
- TGF- $\beta$ , a proximal effector of the malignant phenotype.
- TGF- $\beta$ , potent growth inhibitor and tumor suppressor.
- TGF- $\beta$ , a pro-metastatic factor.



# Major Biological Responses Regulated by TGF-beta

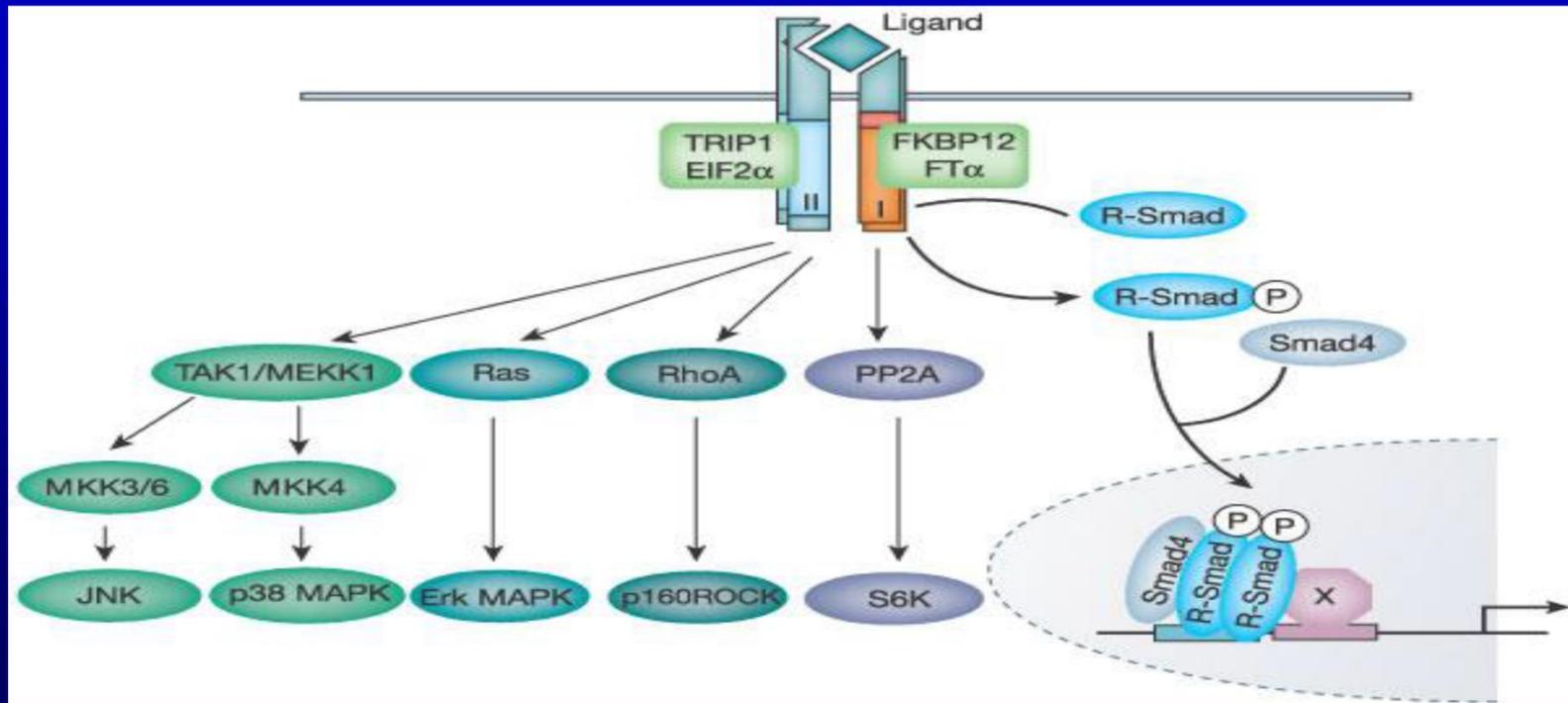
**Unifying Hypothesis:  
TGF- $\beta$  Switches from Tumor Suppressor to  
Pro-oncogenic Factor During Cancer Progression**

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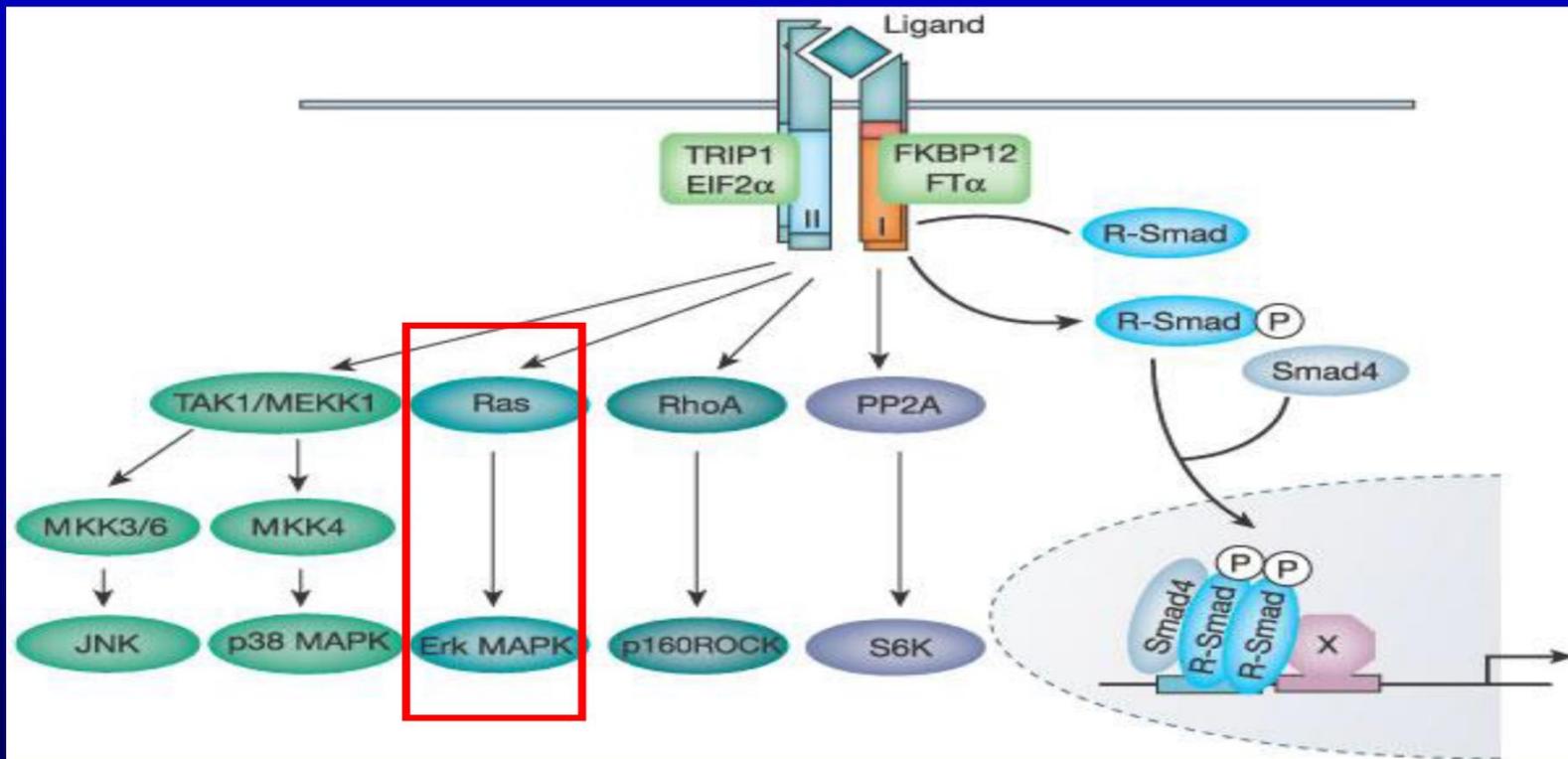
# TGF-beta Smad-independent Pathways

## TGF- $\beta$ Smad-independent Pathways



# TGFbeta Smad-independent pathways

## TGF- $\beta$ Smad-independent Pathways



# K-ras Protooncogene

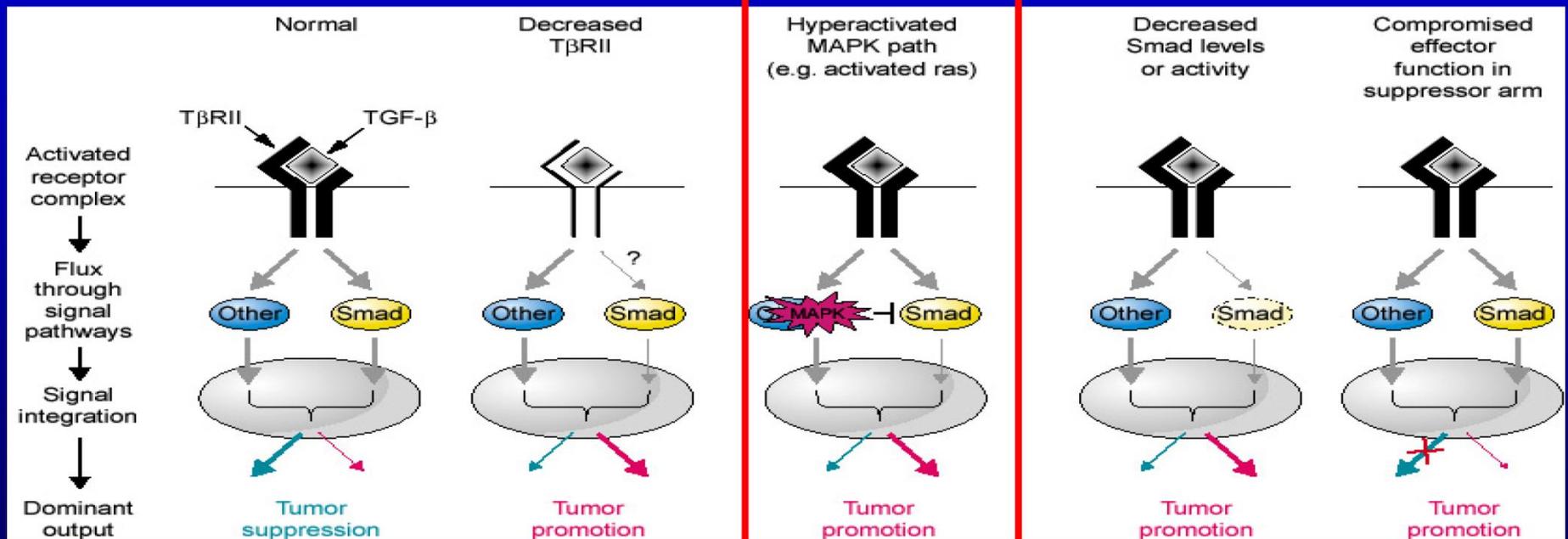
## K-ras Protooncogene

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- **K-ras shows an activating 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- $\beta$ 1 signaling through the Smads**
- **In-vitro studies show that TGF- $\beta$ 1 dominates over mitogenic effects of ras, but activated ras overrides antiproliferative effect of TGF- $\beta$ 1**

# TGF $\beta$ in Tumor Suppression/Promotion

## TGF- $\beta$ in Tumor Suppression/Promotion



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

# Broad Goal

## Broad Goal

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- **Determine the role of Transforming Growth Factor- $\beta$  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

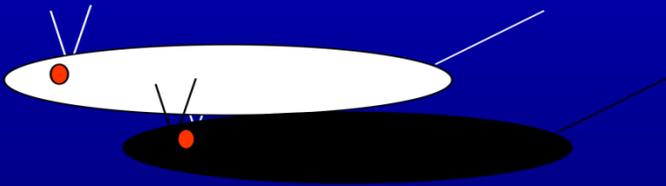
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- **Examine the effect of TGF- $\beta$ 1 deletion and K-ras mutation alone and in combination on lung tumor incidence and pathology**
- **Determine early events in the development of lung lesions and their progression**
- **Identify potential signal transduction pathway changes with tumorigenesis**

# Mouse models

## Mouse Models

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- **A/J**
- **C57BL6 TGF- $\beta$ 1 HT**
- **AJBL6 TGF- $\beta$ 1 HT**
- **TGF- $\beta$ 1 HT/K-ras LA**

# Question

## Question

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- **Does lung tumorigenesis affect the TGF- $\beta$  signaling pathway?**
- **Does the TGF- $\beta$  signaling pathway affect lung tumorigenesis?**

# A/J Mouse Model

## A/J Mouse Model

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- **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 CYP2E1 to vinyl  
carbamate and vinyl carbamate  
epoxide as well as  
degraded by esterase**

# Production of tumors in A/J mice

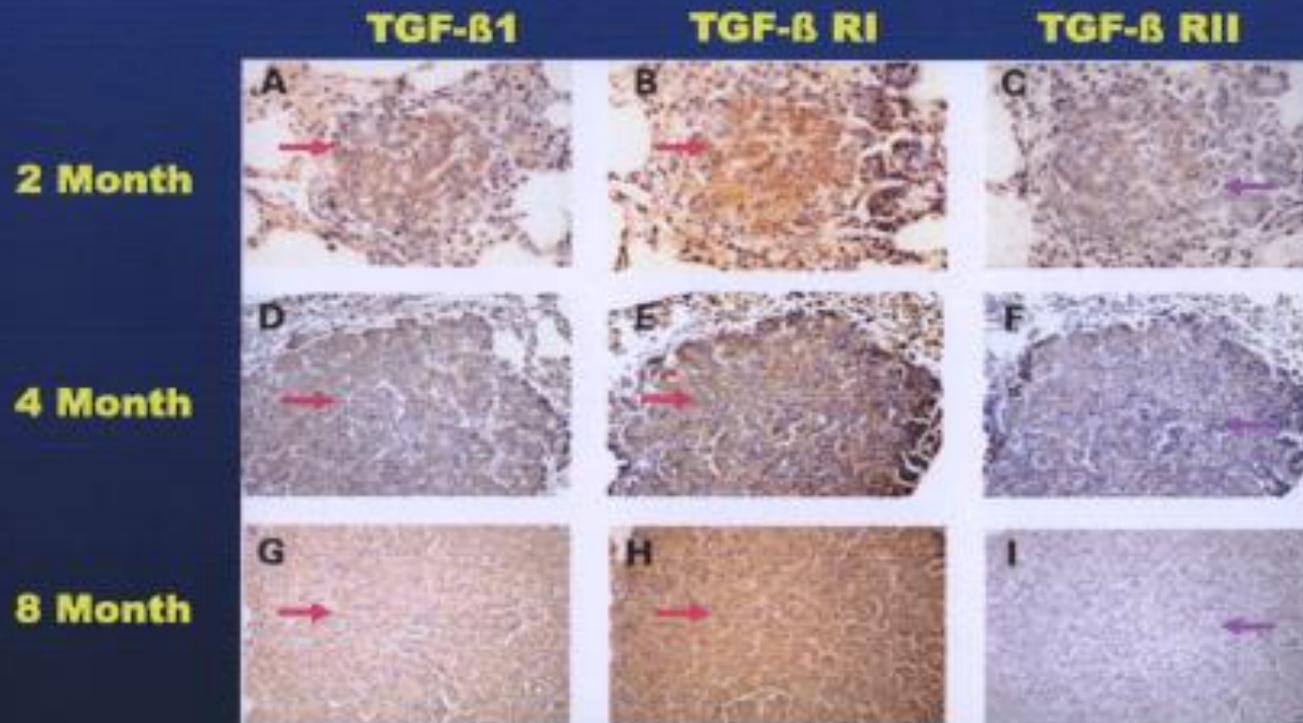
## Production of Tumors in A/J Mice

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# A/J Mouse model

## A/J Mouse Model TGF- $\beta$ 1, RI and RII Proteins in Lung Tumors



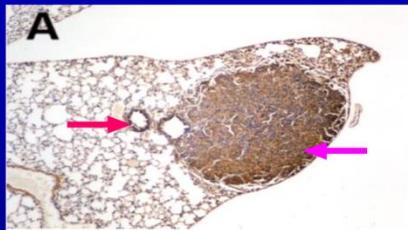
**Decreased TGF- $\beta$  RII protein in tumors**

# Decreased TGF- $\beta$ RII in tumors

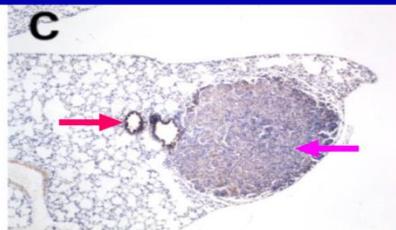
## TGF- $\beta$ in A/J Mouse Model

### EC-induced Lung Tumors

#### TGF- $\beta$ RI



#### TGF- $\beta$ RII



IHC

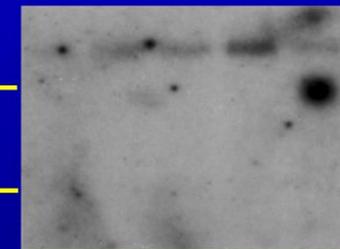
IHC

### Lung Tumor Derived Cell Lines

E10 E9 A5 LM1 PCC4\*

#### TGF- $\beta$ RI

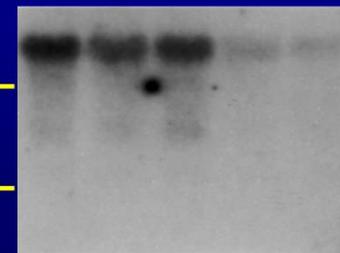
28S—  
18S—



— 5.5 Kb

#### TGF- $\beta$ RII

28S—  
18S—

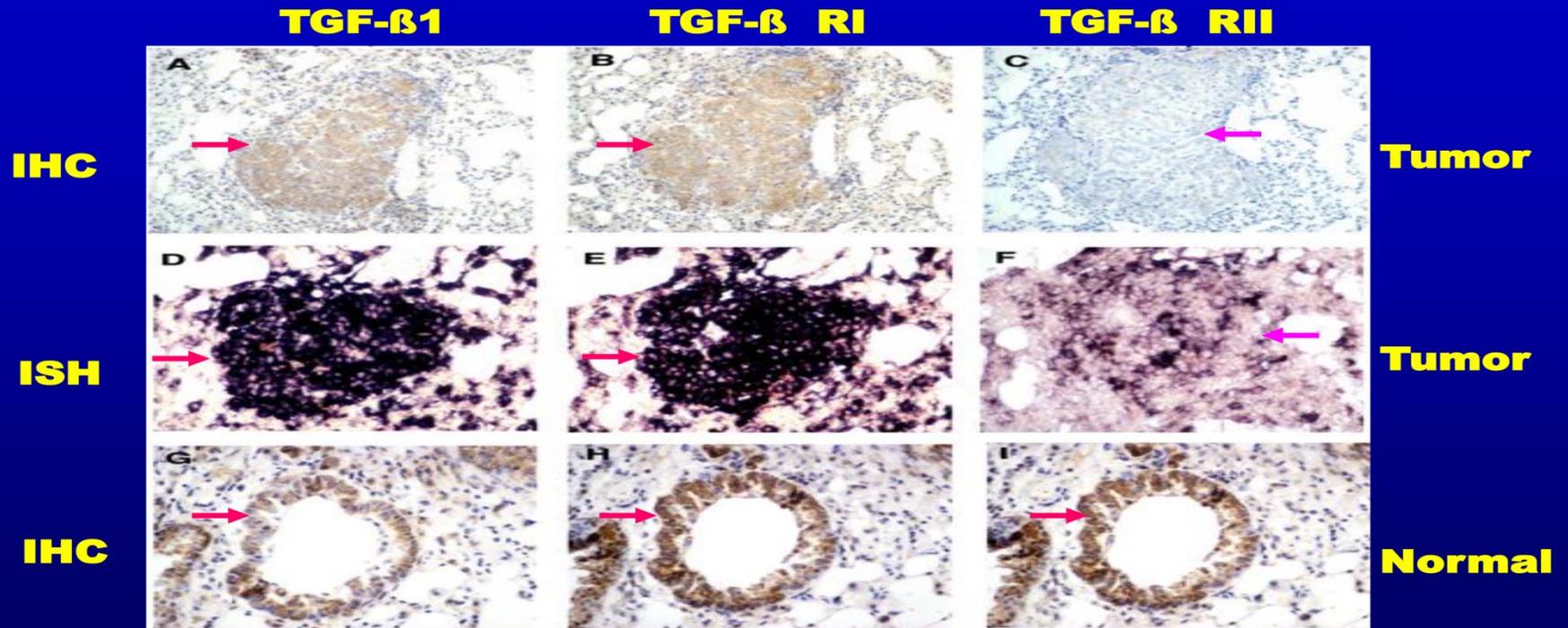


— 5.5 Kb

Decreased TGF- $\beta$  RII protein and mRNA

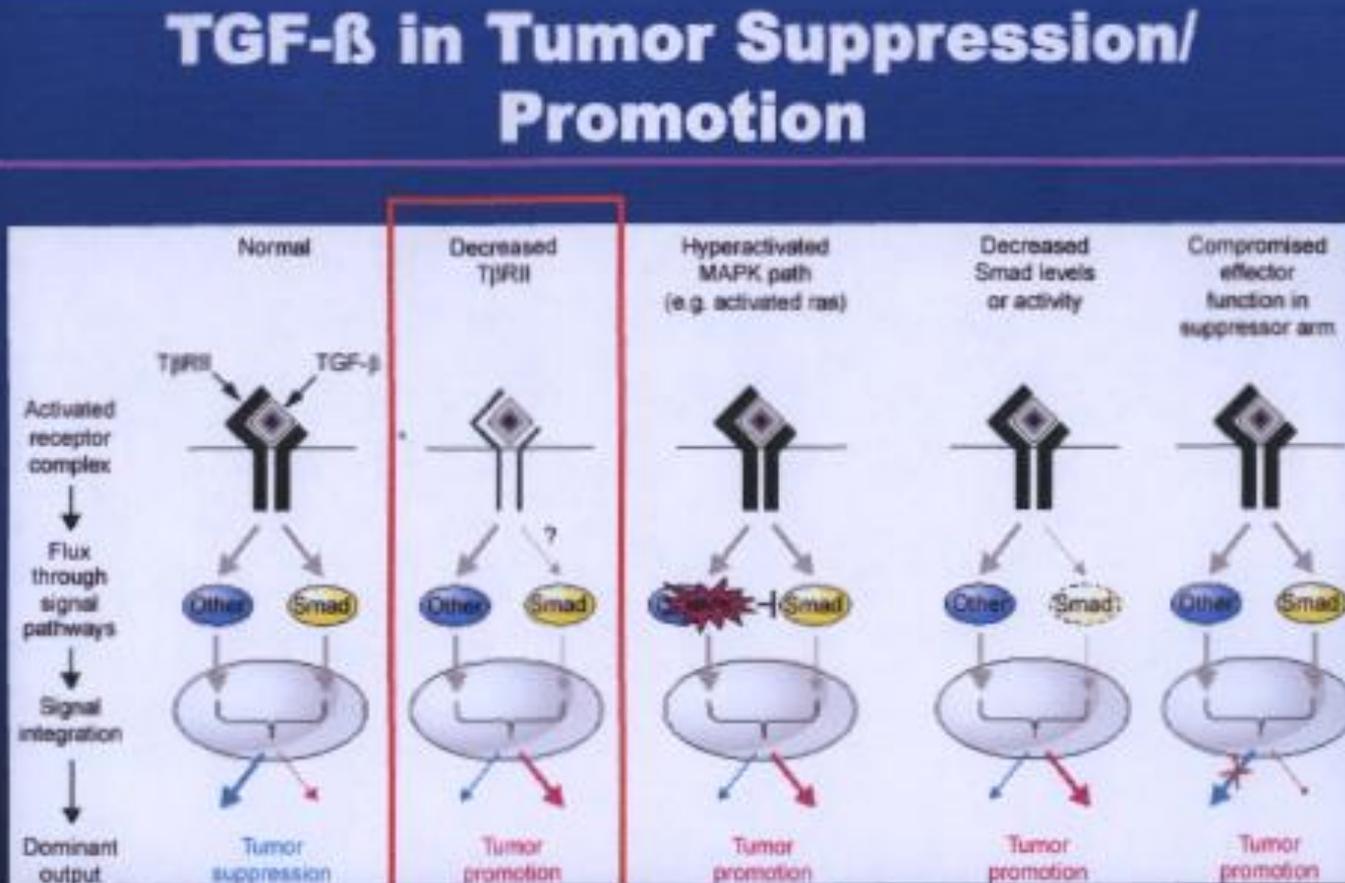
# Expression of TGF- $\beta$ 1, RI and RII Proteins and mRNAs

**Expression of TGF- $\beta$ 1, RI and RII Proteins and mRNAs in BP-Induced A/J Mouse Lung Tumors**



**Decreased TGF- $\beta$  RII mRNA and protein in tumors**

# TGF- $\beta$ in Tumor Suppression/Promotion



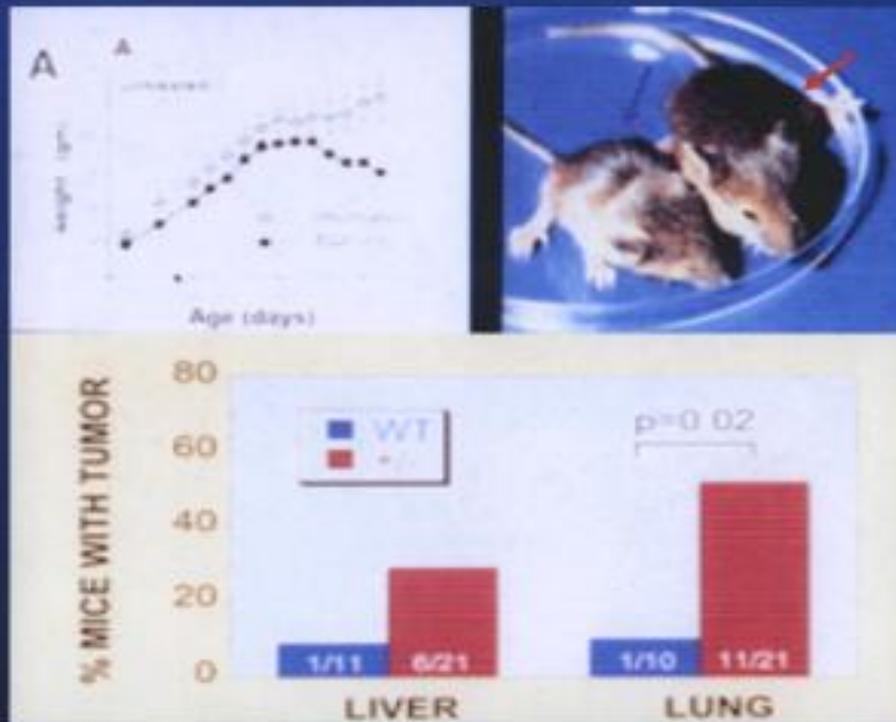
• **Reduced TGF- $\beta$  RII = Lung Tumor Promotion**

# Question

**Does deletion of TGF- $\beta$ 1  
affect lung tumorigenesis?  
C57BL/6 TGF- $\beta$ 1 Mouse**

# The C57BL/6 TGF- $\beta$ 1 Knockout Mouse

## The C57BL/6 TGF- $\beta$ 1 Knockout Mouse



**Increased tumor incidence in TGF- $\beta$ 1 HT mice**

# HT Mouse

## AJBL6 TGF- $\beta$ 1 HT Mouse Derivation

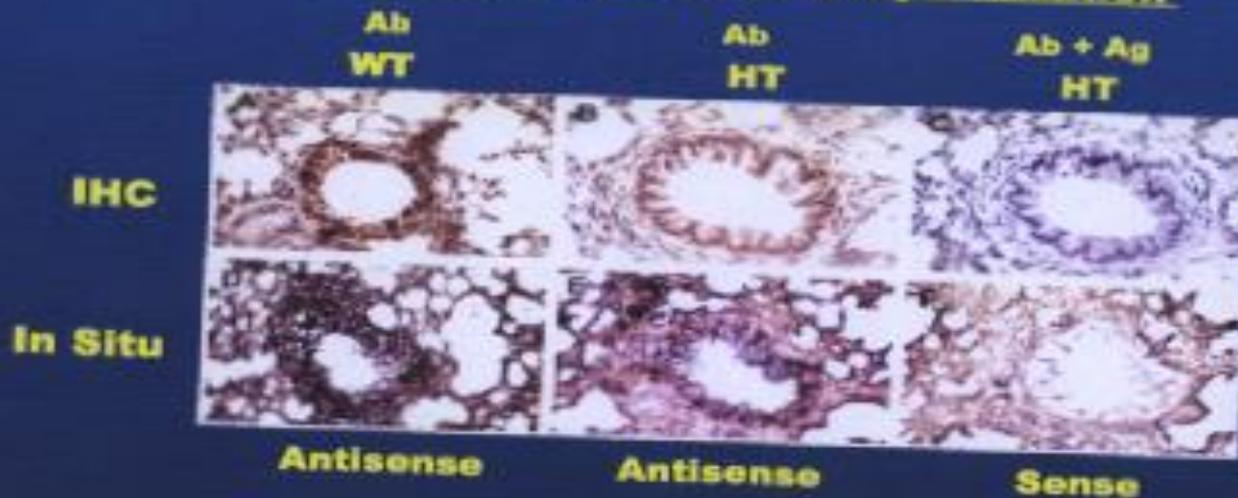
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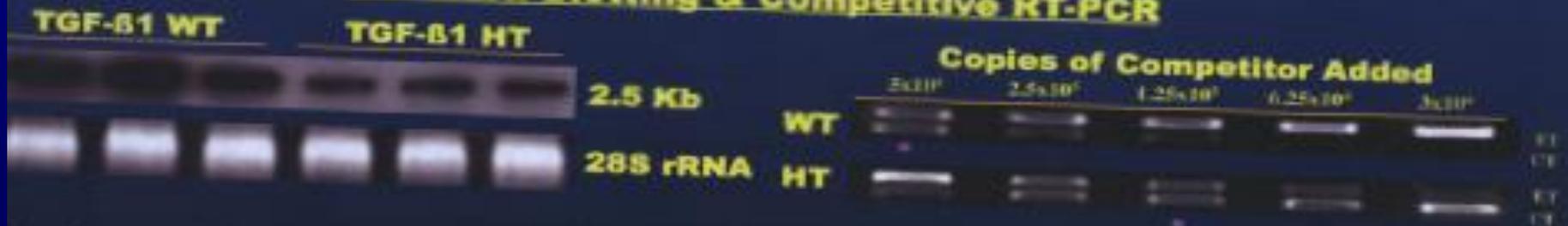
# TGF- $\beta$ 1 reduced in HT

## AJBL6 TGF- $\beta$ 1 HT and WT Mouse

### IHC Staining & In Situ Hybridization



### Northern Blotting & Competitive RT-PCR

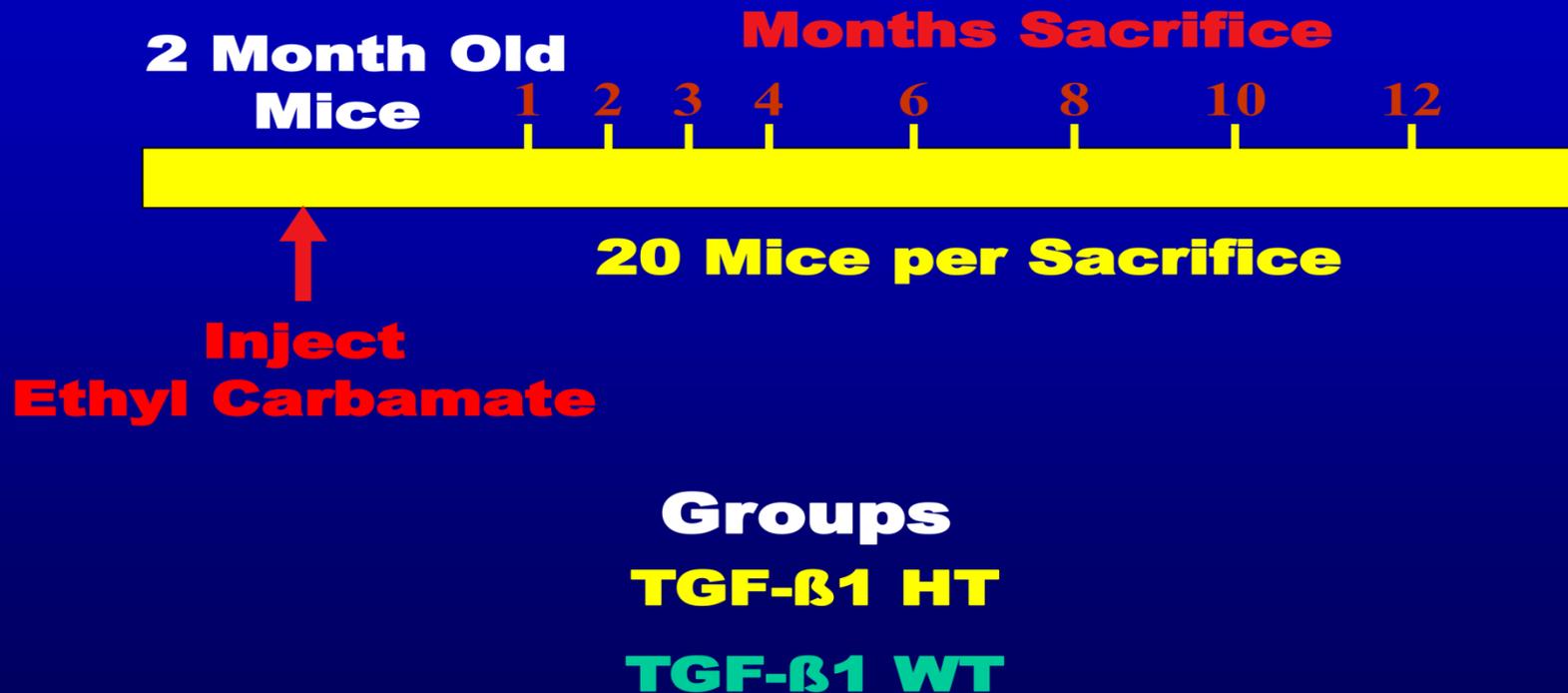


reduced expression of TGF- $\beta$ 1 in HT compared to WT

# Production of Tumors

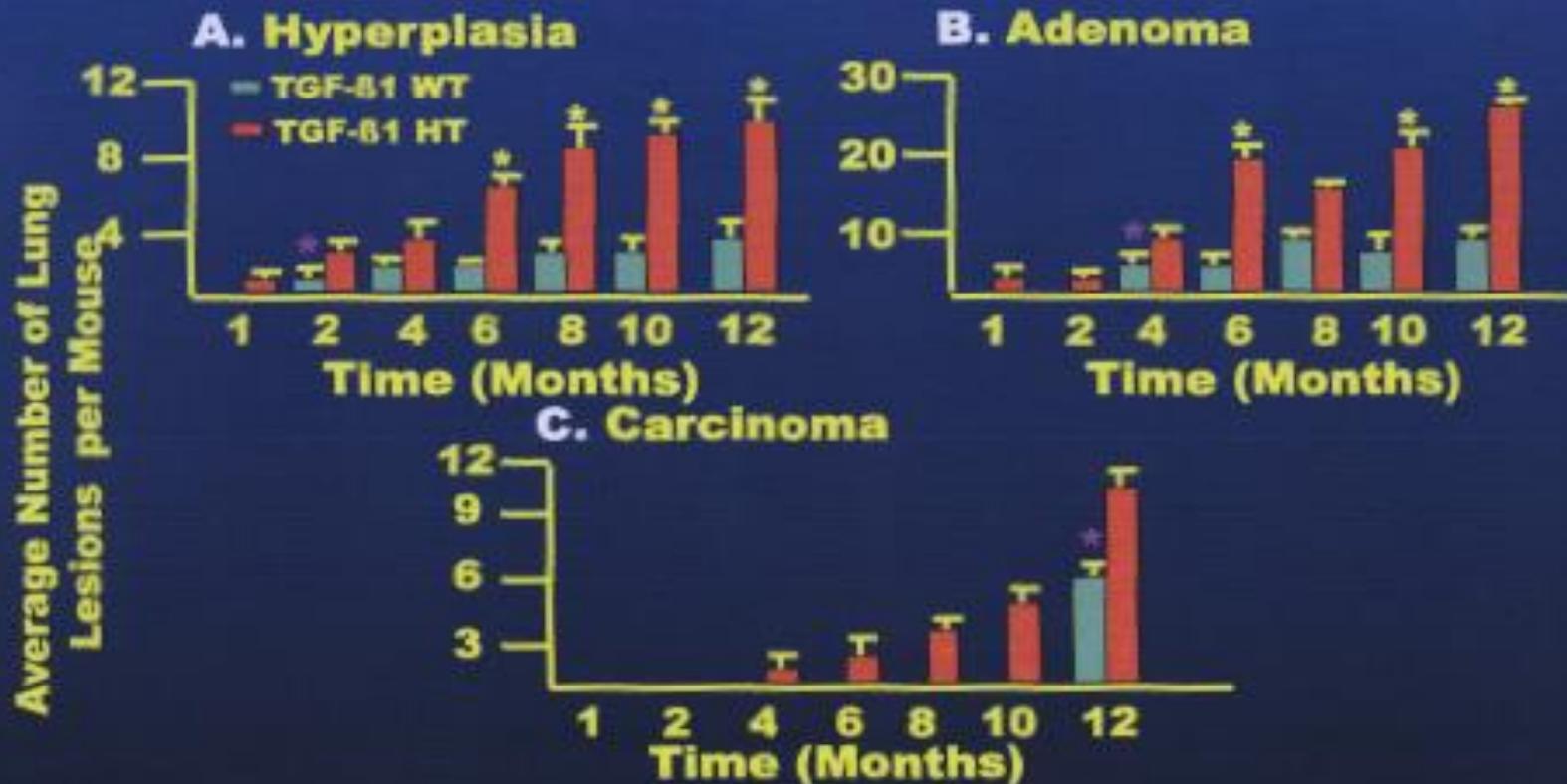
## Production of Tumors

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# AJBL6 mice

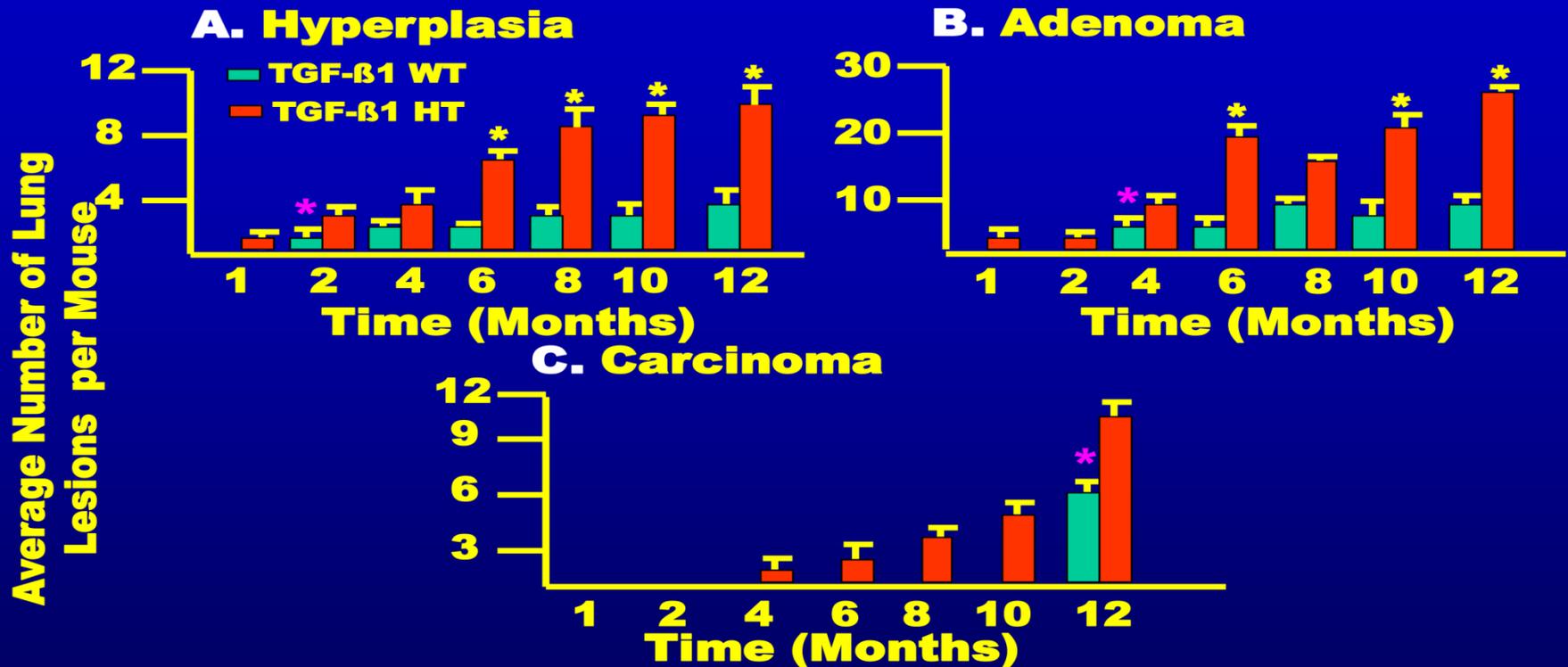
## Carcinogen-Induced Lung Tumorigenesis in AJBL6 TGF- $\beta$ 1 HT & WT Mice



**Increased tumor incidence and multiplicity and decreased tumor latency in TGF- $\beta$ 1 HT mouse**

# AJBL6 TGF- $\beta$ 1 HT & WT Mice

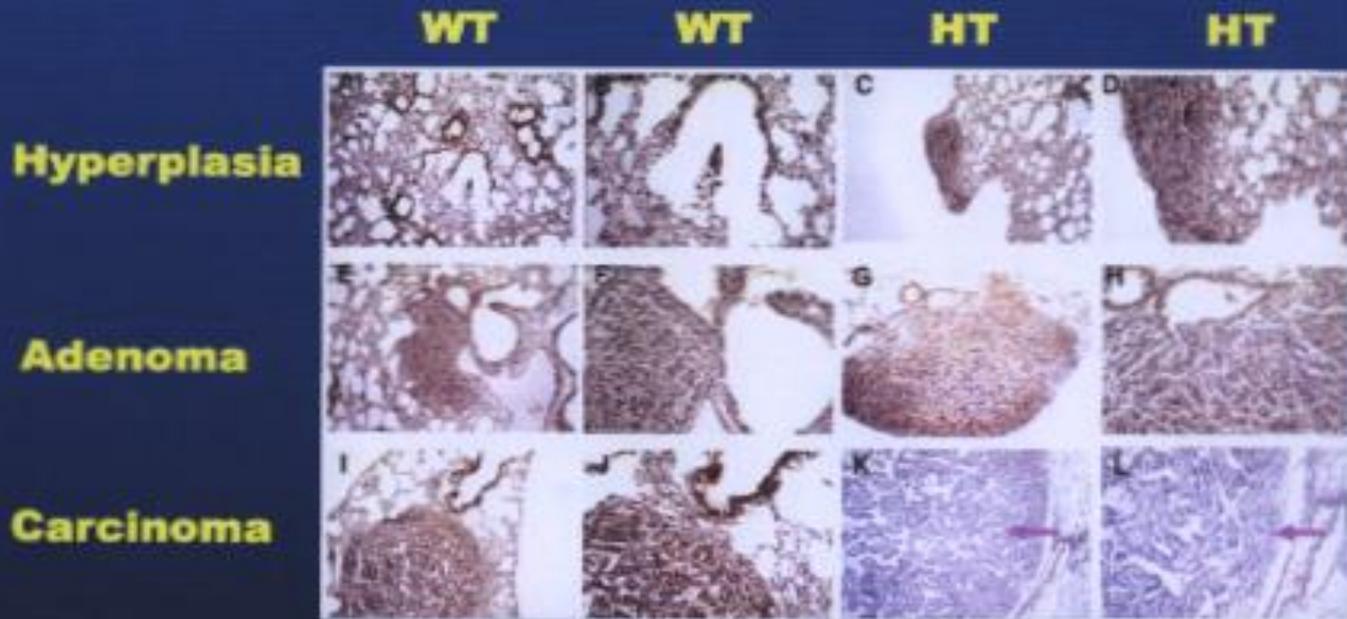
## Carcinogen-Induced Lung Tumorigenesis in AJBL6 TGF- $\beta$ 1 HT & WT Mice



**Increased tumor incidence and multiplicity and decreased tumor latency in TGF- $\beta$ 1 HT mouse**

# TGF- $\beta$ RII protein

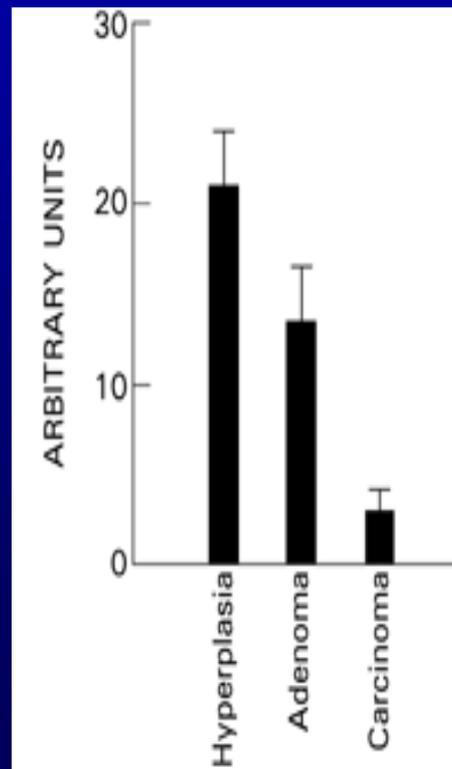
## TGF- $\beta$ RII Protein in Lung Lesions from AJBL6 TGF- $\beta$ 1 WT and HT Mice



Decreased TGF- $\beta$  RII in tumors of TGF- $\beta$ 1 HT mice

# Relative TGF- $\beta$ RII mRNA Levels Lesions from AJBL6 TGF- $\beta$ 1 HT Mouse Lungs Treated with Ethyl Carbamate

Decreasing TGF- $\beta$  RII mRNA with increasing  
lung tumorigenesis



# Question

**Does deletion of TGF- $\beta$ 1 and mutation of K-ras affect lung tumorigenesis? TGF- $\beta$ 1 HT/K-ras LA mouse**

# Mouse mating

**To Study the Interplay of TGF- $\beta$  1 and K-ras:  
Generation of TGF- $\beta$  1/ K-ras LA Mice**

**TGF- $\beta$ 1**  
**HT**  
(C57Bl/6)

**X**

**K-ras**  
**LA**  
(SV 129)

**TGF- $\beta$ 1 HT/K-ras LA - HT/LA Double Mutant**  
**TGF- $\beta$ 1 WT/K-ras LA - WT/LA Single Mutant**  
**TGF- $\beta$ 1 HT/K-ras WT - HT/WT Single Mutant**  
**TGF- $\beta$ 1 WT/K-ras WT - WT/WT Wild Type**

# Mouse lungs

## TGF- $\beta$ 1 and K-ras Mouse Lungs

TGF- $\beta$ 1 HT, K-ras LA



TGF- $\beta$ 1 WT, K-ras LA



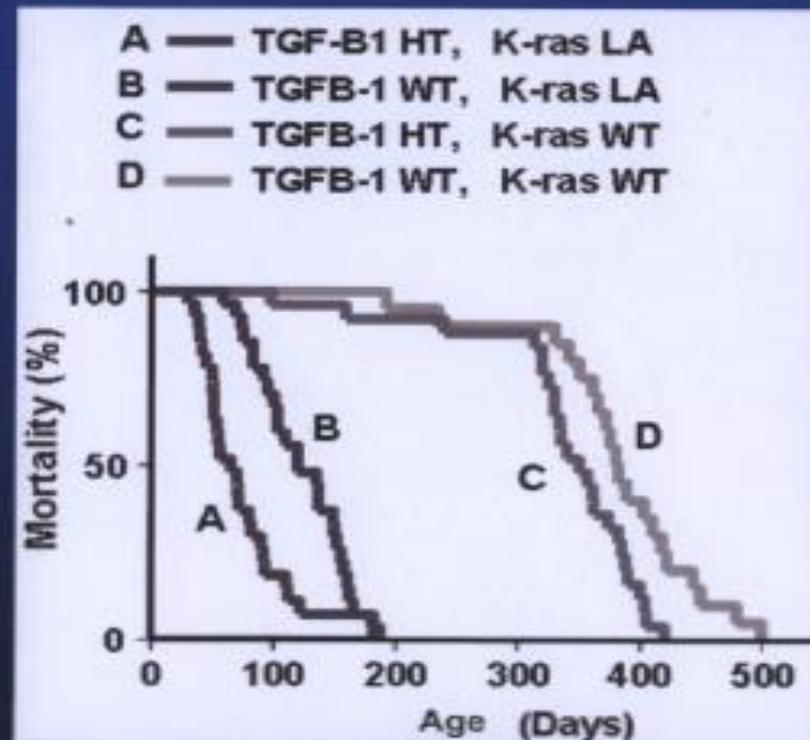
TGF- $\beta$ 1 HT, K-ras WT



TGF- $\beta$ 1 WT, K-ras WT

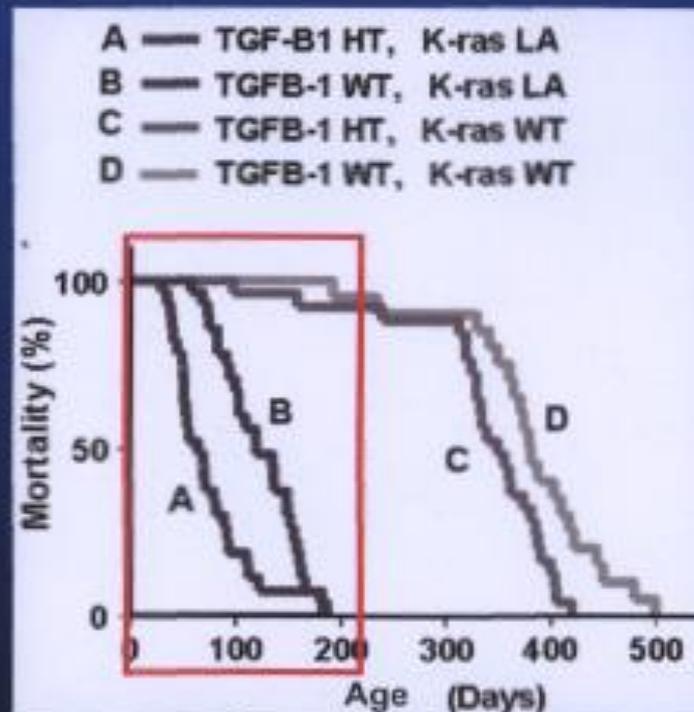
# Mouse survival

## Effect of TGF- $\beta$ 1 Gene Deletion and K-ras Mutation on Mouse Survival



# Lifespans

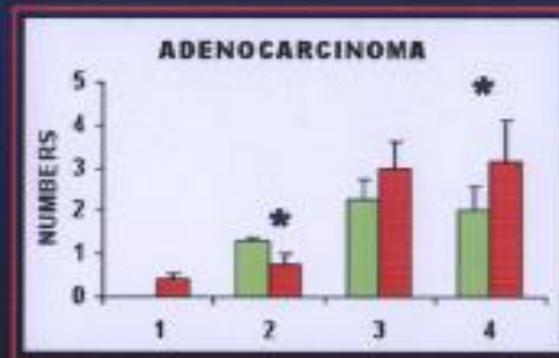
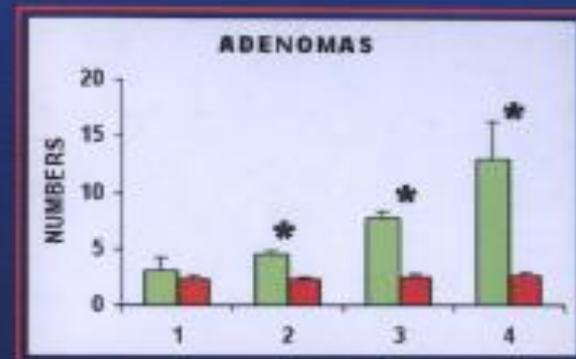
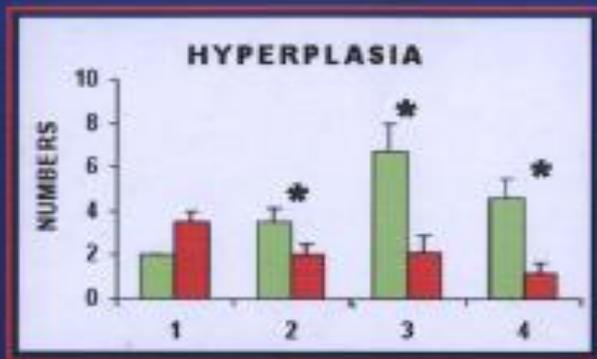
## Effect of TGF- $\beta$ 1 Gene Deletion and K-ras Mutation on Mouse Survival



**Decreased lifespans in HT/LA and WT/LA mice**

# Pathology

## Pathology of Lung Lesions



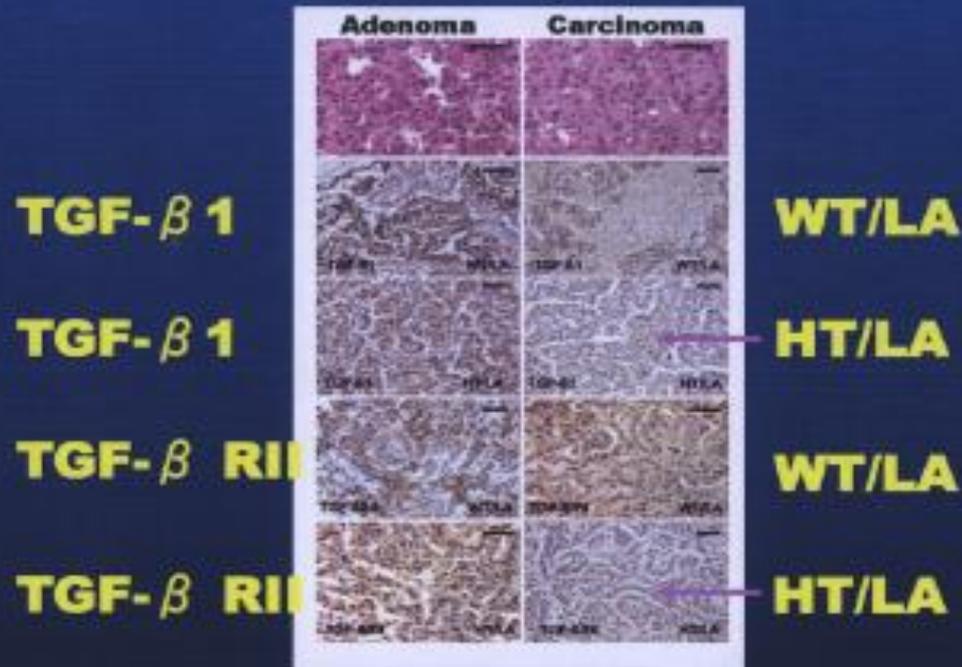
WT/LA ■

HT/LA ■

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

# TGF- $\beta$ 1 and TGF- $\beta$ RII

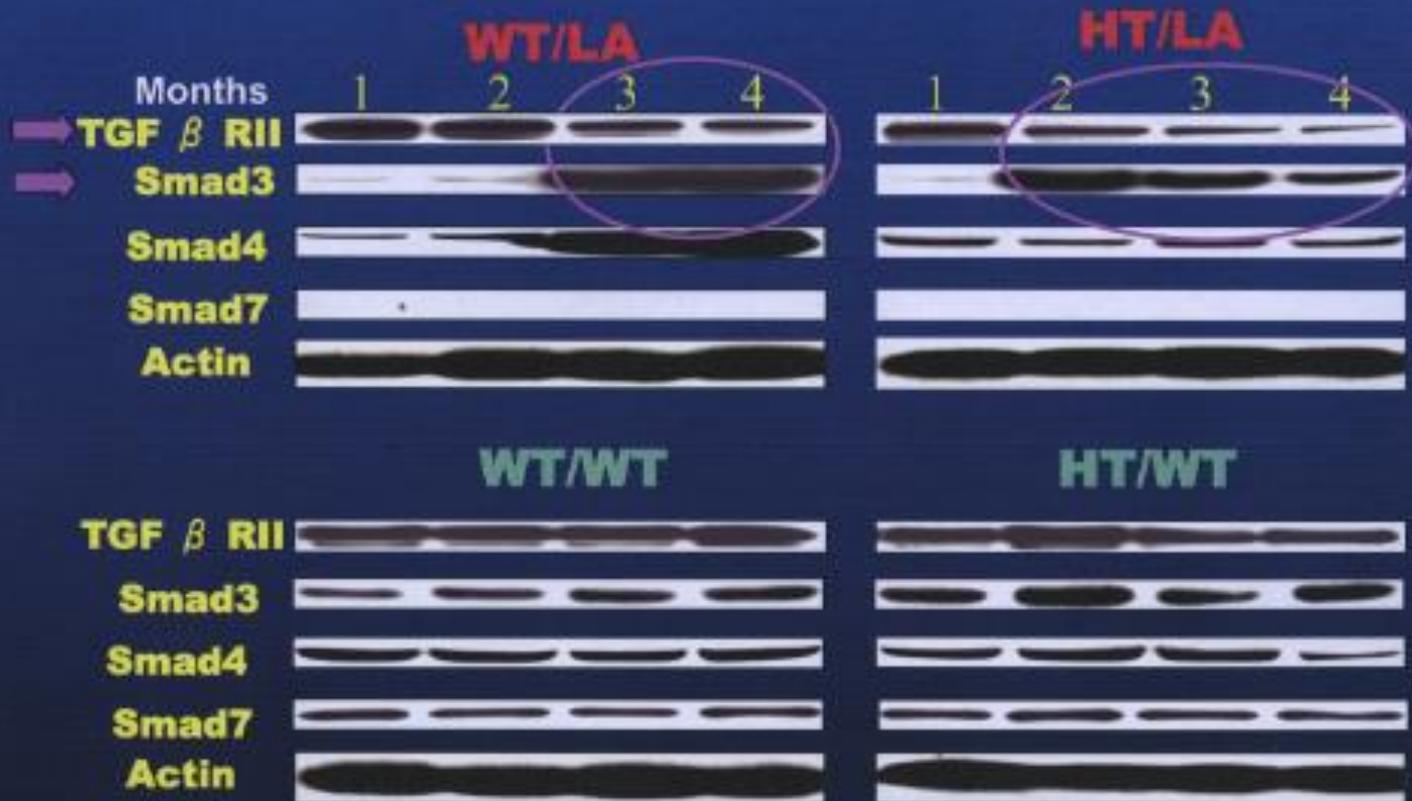
## TGF- $\beta$ 1 and TGF- $\beta$ RII in Lung Lesions



**Reduced TGF- $\beta$ 1 & RII in HT/LA adenocarcinomas**

# Smad3

## TGF $\beta$ RII and Smad3 in Lung Tumorigenesis



HT/LA: Expedited TGF- $\beta$  RII reduction & Smad3 production

# TGF $\beta$ pathway

## TGF $\beta$ Pathway in HT/LA Lung Tumorigenesis

### Western Blot:

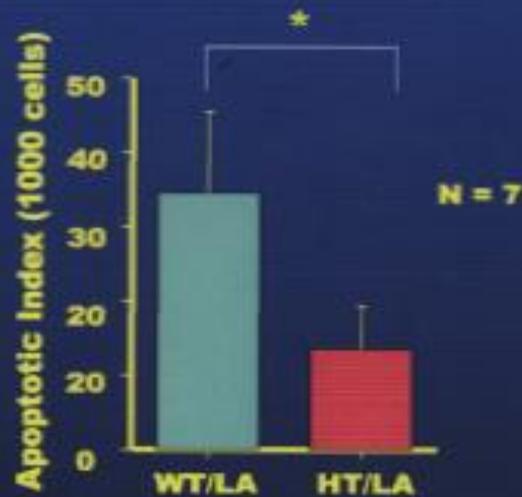
<b>TGF <math>\beta</math> RII</b>	<b>Expedited TGF-<math>\beta</math> RII reduction</b>
<b>Smad3</b>	<b>Expedited Smad3 production</b>
<b>Smad4</b>	<b>Reduced Smad4 production</b>
<b>Smad7</b>	<b>Reduced Smad7 production</b>
<b>K-ras</b>	<b>Expedited K-ras production</b>
<b>Raf-1</b>	<b>Expedited Raf-1 production</b>

### Real Time RT-PCR:

**Reduced Smads 2, 3, 4 & 7 in adenomas**  
**Reduced TGF- $\beta$  RII & Smads in carcinomas**

# Apoptotic Index

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



**Reduced apoptosis in HT/LA adenomas**

# Acknowledgements

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### Epithelial Carcinogenesis Group

**Sonia B. Jakowlew**

**Jerry Angdisen**

**Yang Kang**

**Alena Naumova**

**Jyotsna Pandey**

**Sarah Umphress**

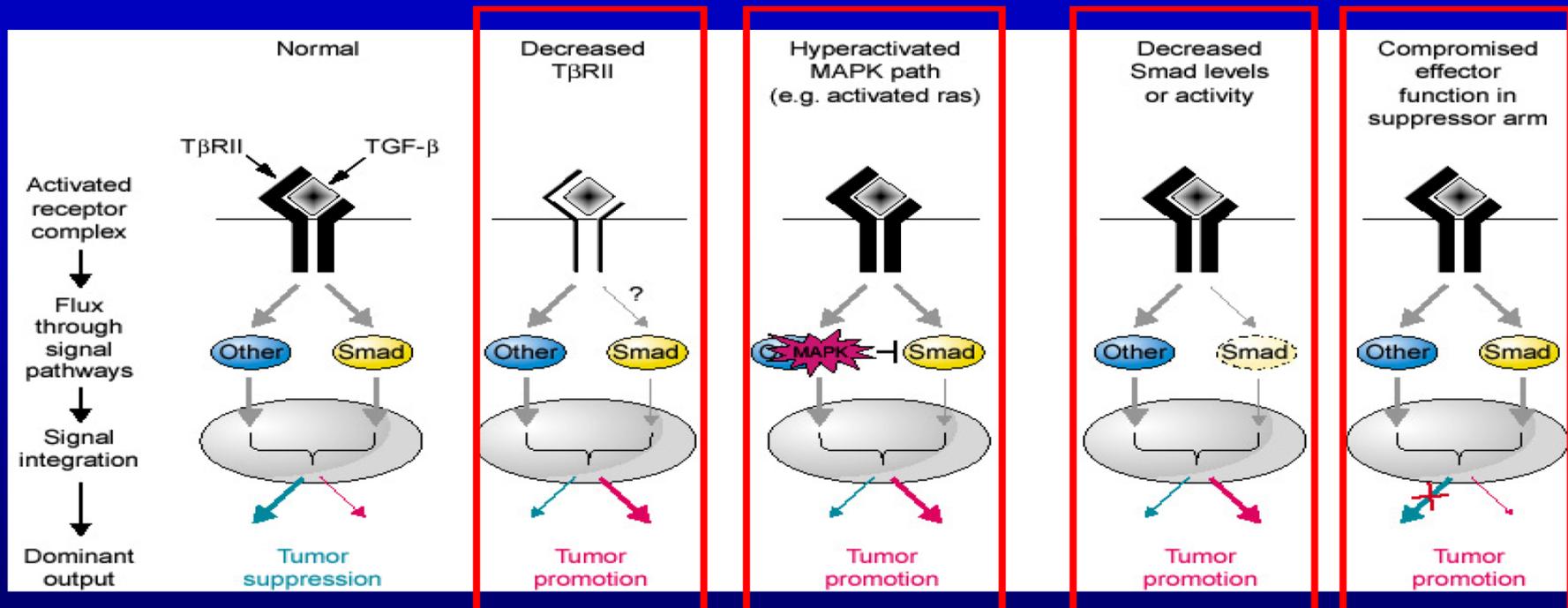
**MIT**

**Tyler Jacks**

**Kim Mercer**

# TGF $\beta$ in Tumor Suppression/Promotion

## TGF- $\beta$ in Tumor Suppression/Promotion



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

# Acknowledgments

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