

Transforming Growth Factor- beta and Lung Tumorigenesis

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Lung Cancer in 2015, USA

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- **Most common cause of cancer deaths in both men and women**
- **221,200 diagnosed new cases**
115,610 men; 105,590 women
- **158,040 deaths due to lung cancer**
86,380 men; 71,660 women
- **Most cases now occur in ex-smokers**
- **< 15% five year survival rate**

Transforming Growth Factor- β (TGF- β)

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**

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- 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- β from:

Human platelets (Rick Assoian)

Human placenta (Chuck Frolik)

Bovine kidney (Anita Roberts)

Scale of TGF- β 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 μ g TGF- β 1

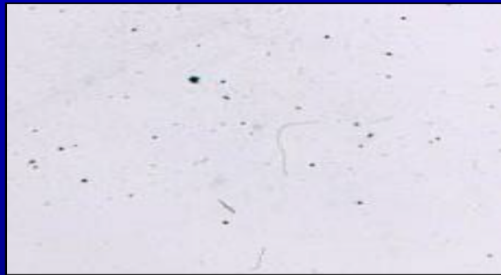
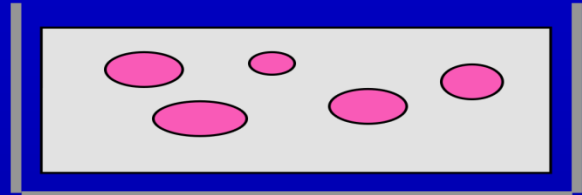
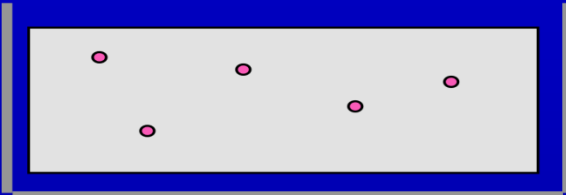
purification fold = 230,000; recovery = 10%

The Columns for TGF-beta1 Purification



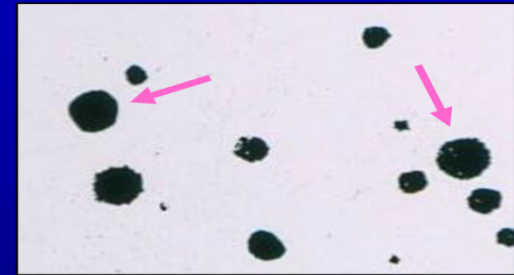
Clonogenic assay

The Assay: Growth of NRK Cells in Soft Agar



If no TGF- β is present

- Plate agar base
- Add mix of media, serum, NRK cells, EGF, sample
- 1 wk/37°/5%CO₂
- Stain
- Count colonies >3100 μm^2 with Omnicon Image Analysis System

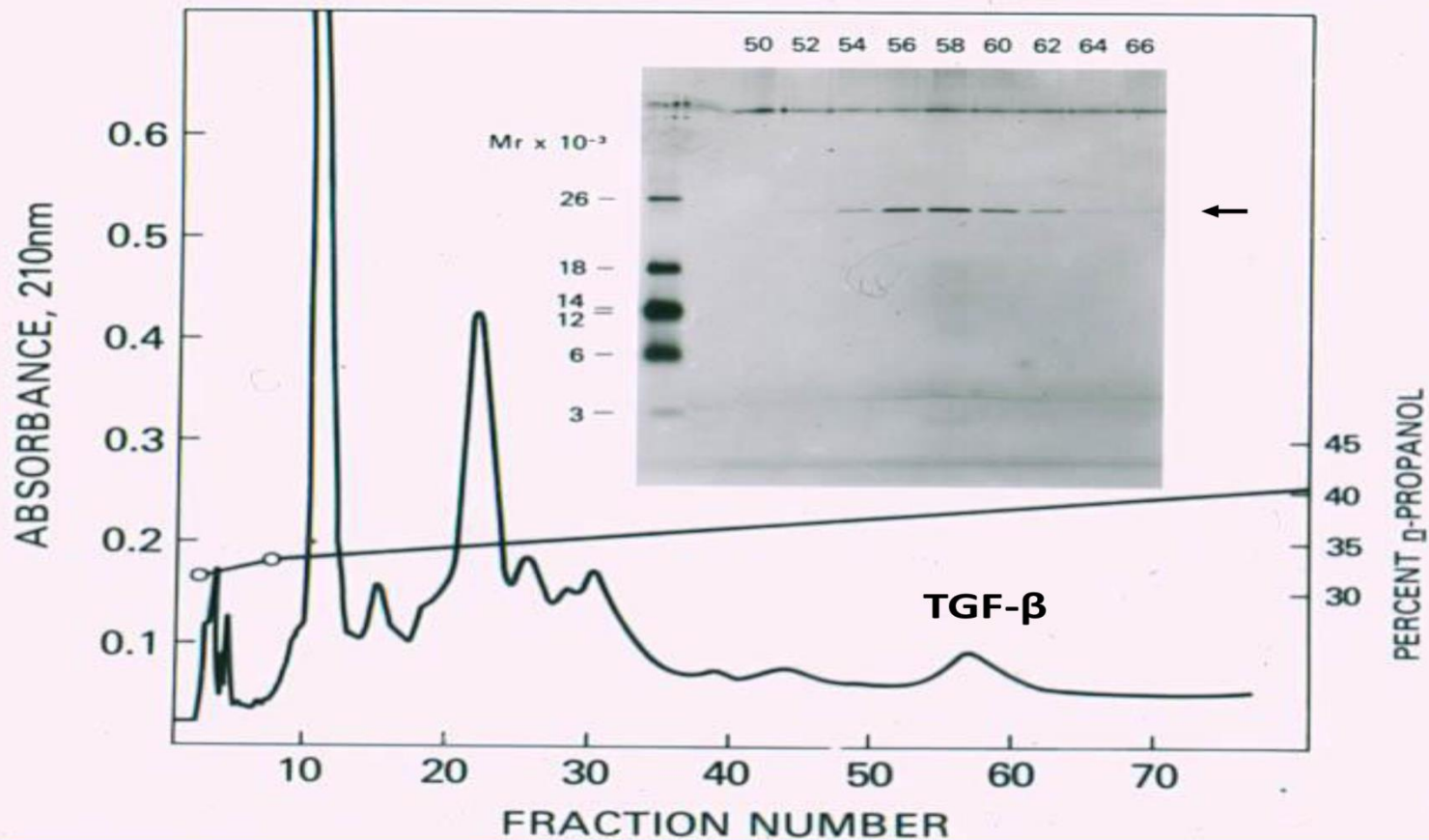


If TGF- β is present



HPLC Purification

The Final HPLC Purification



TGF beta

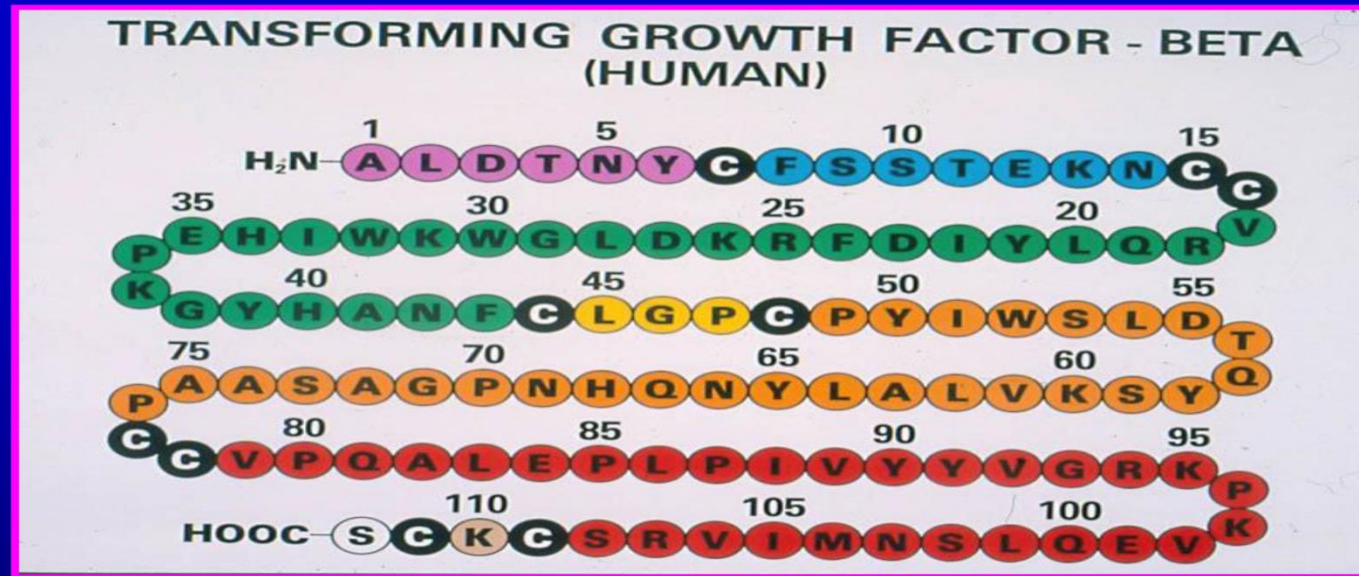
EUREKA!! TGF- β : Born at NCI

Michael Sporn & Anita Roberts



TGF beta structure

Sequence of
mature TGF-β1
monomer



Pre-pro TGF-β 391 amino acids



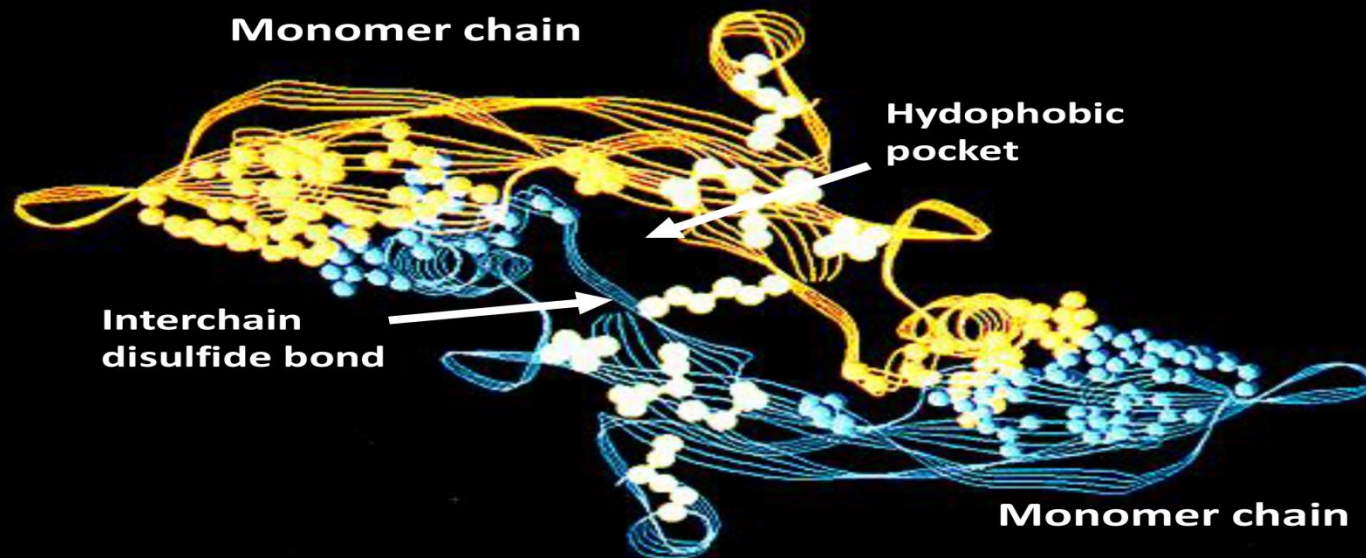
Signal
peptide

(latency associated peptide, LAP)

Mature TGF-β
112 amino acids

TGF beta dimer

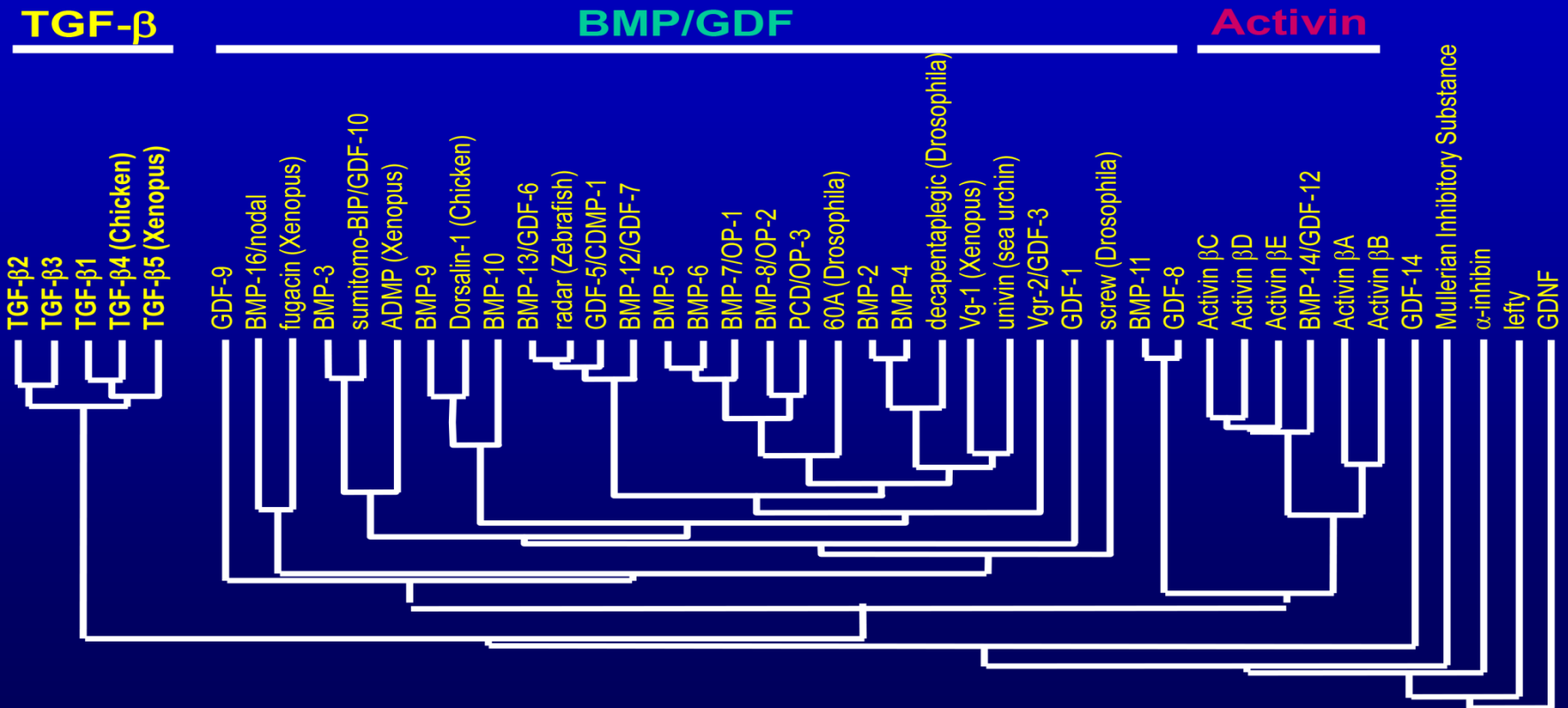
TGF- β : A Homodimer



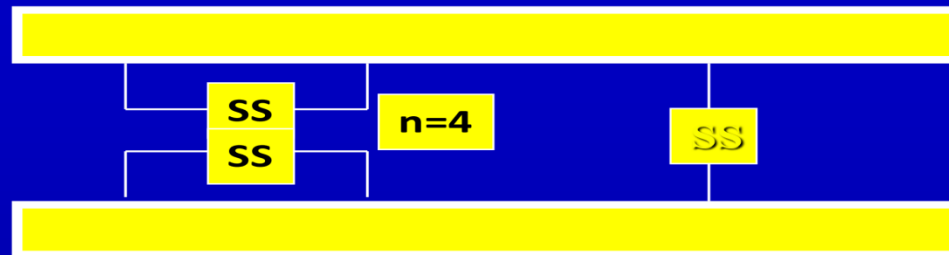
Daopin, S et al Science 257:369, 1992

TGF beta superfamily

The TGF- β 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

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

Model for TGF- β pathway

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

Clinical Observations

Clinical Observations

TGF- β is a tumor suppressor:

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

Clinical Observations

Clinical Observations

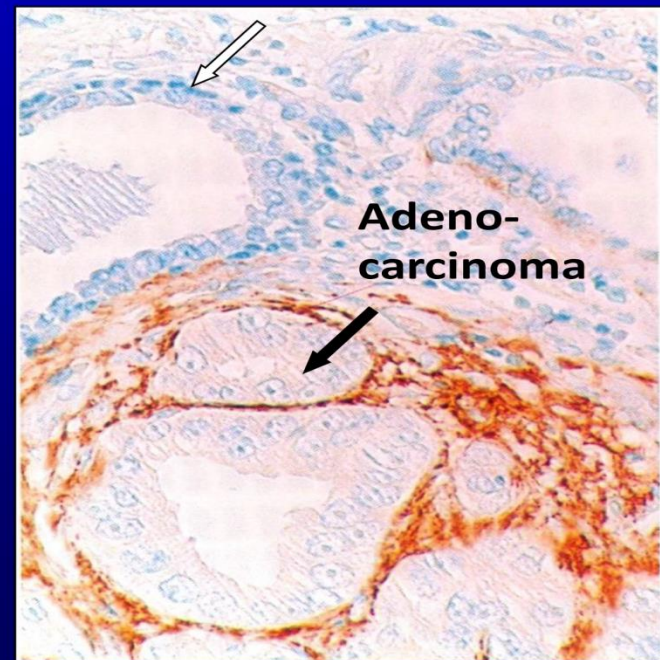
TGF- β is a tumor promoter:

- **TGF- β 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- β 1:
(Truong et al. Hum Pathol 1993)

TGF- β sits at the interface between tumor parenchyma and microenvironment



TGF beta in carcinogenesis

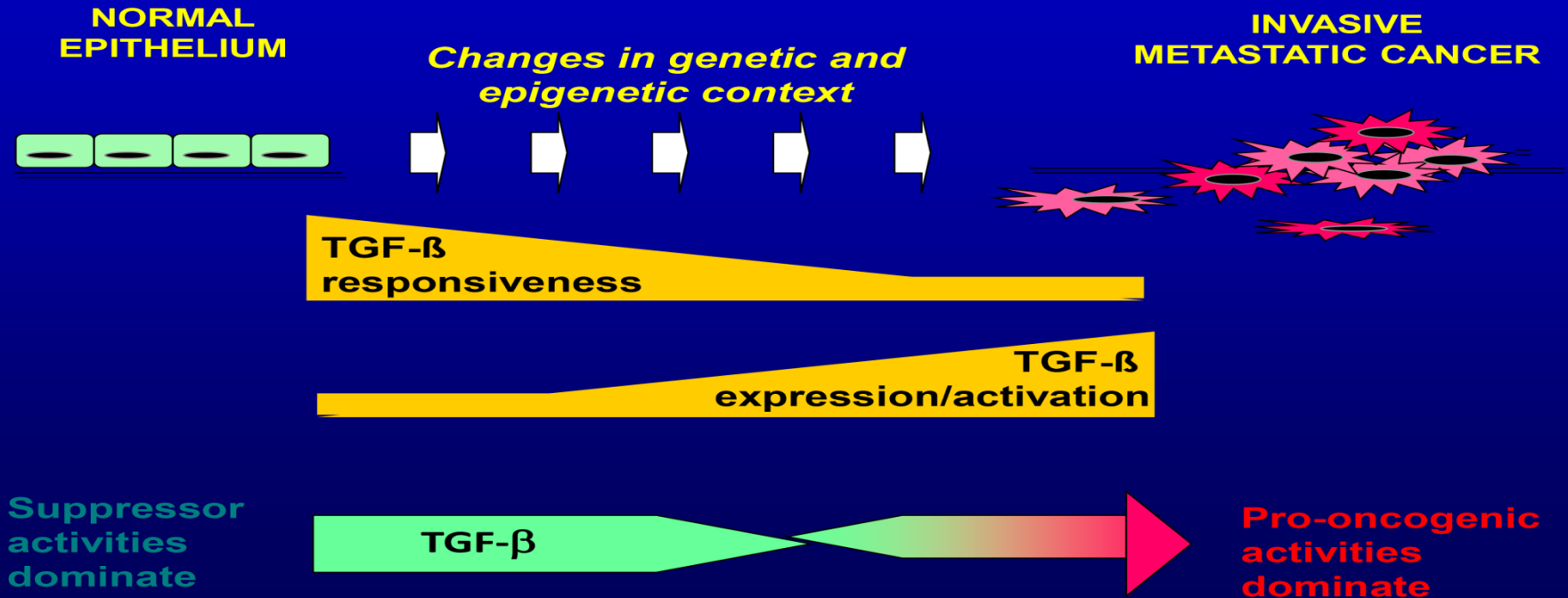
TGF- β in Carcinogenesis - Hero or villain?



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

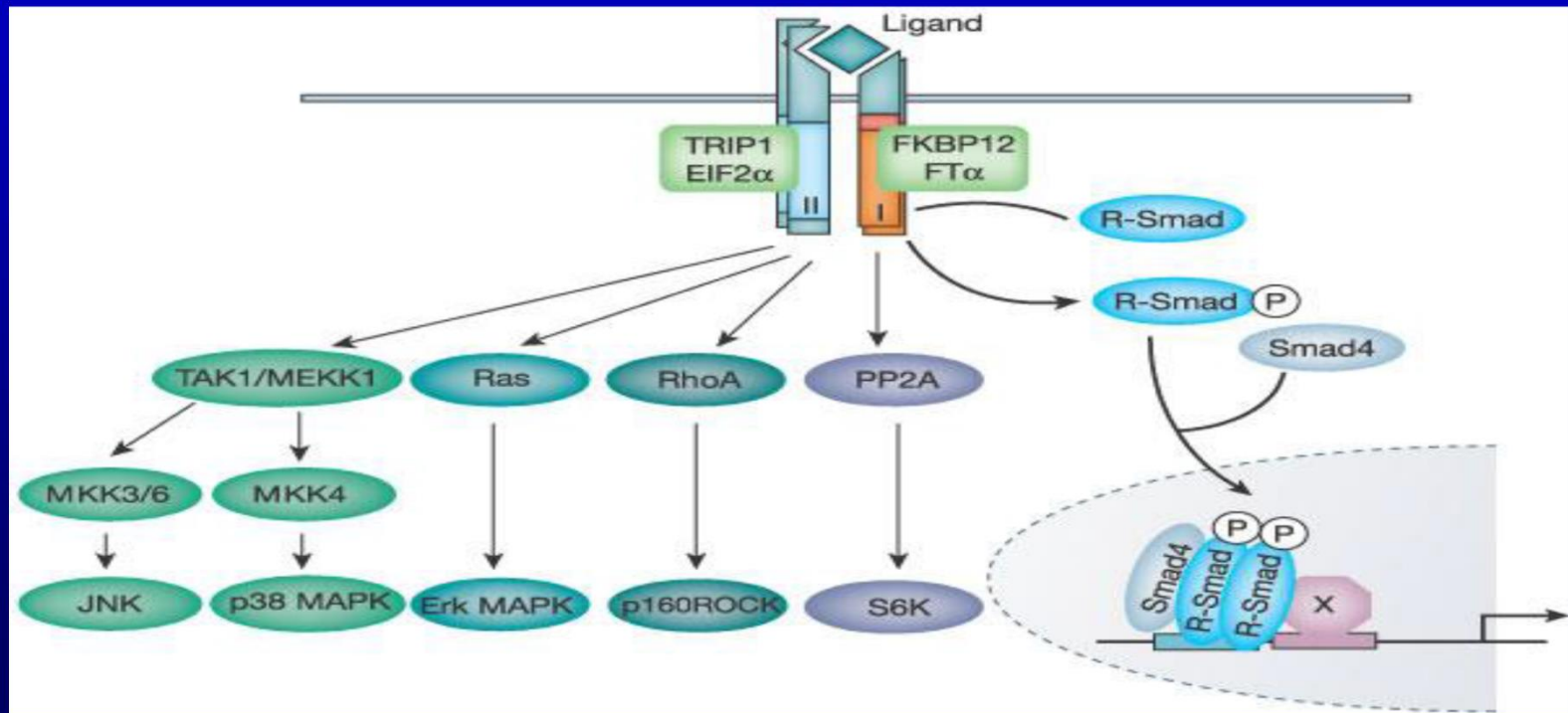
Major Biological Responses Regulated by TGF-beta

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



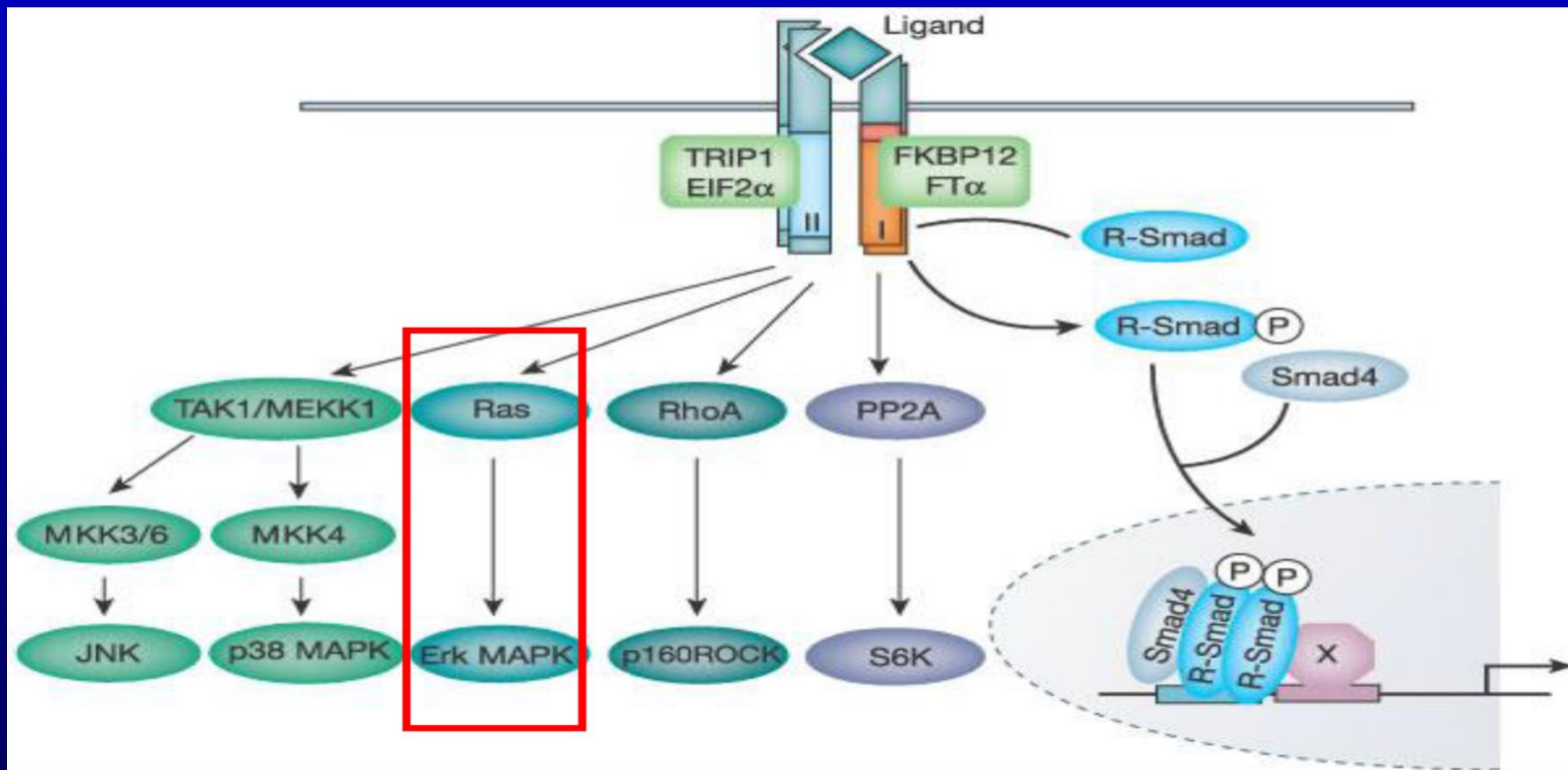
TGF-beta Smad-independent Pathways

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TGFbeta Smad-independent pathways

TGF- β Smad-independent Pathways



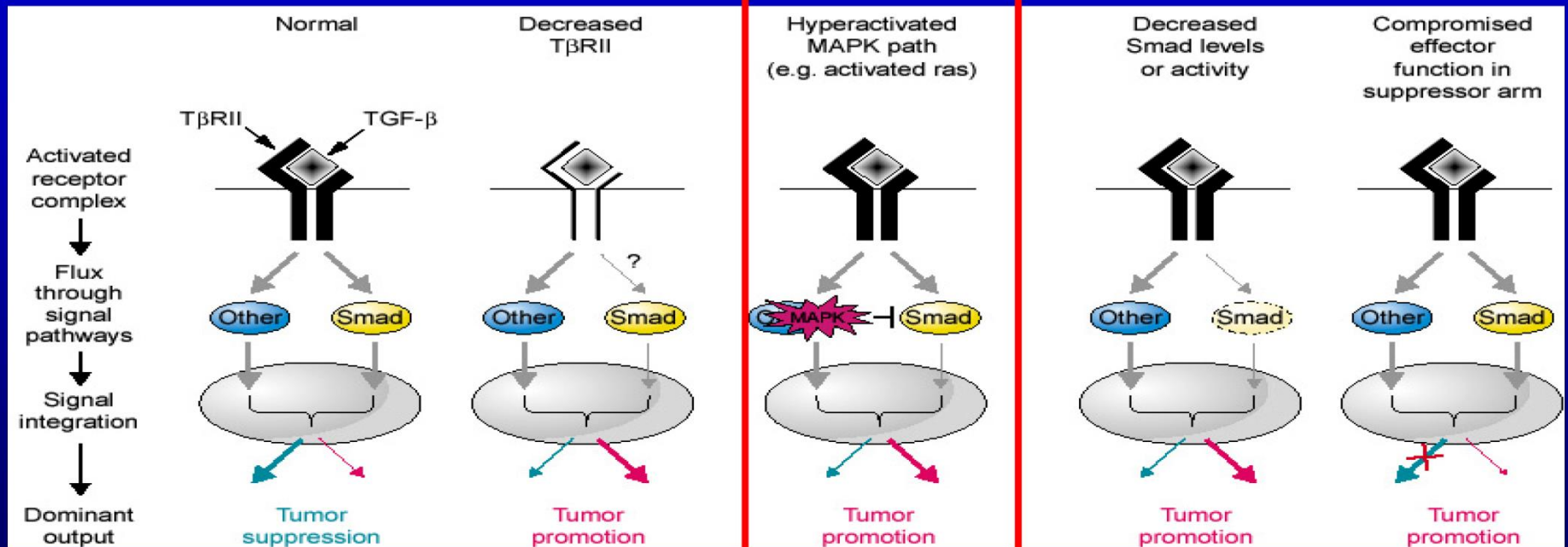
K-ras Protooncogene

K-ras Protooncogene

- **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- β 1 signaling through the Smads**
- **In-vitro studies show that TGF- β 1 dominates over mitogenic effects of ras, but activated ras overrides antiproliferative effect of TGF- β 1**

TGF β in Tumor Suppression/Promotion

TGF- β in Tumor Suppression/Promotion



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

Broad Goal

Broad Goal

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

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

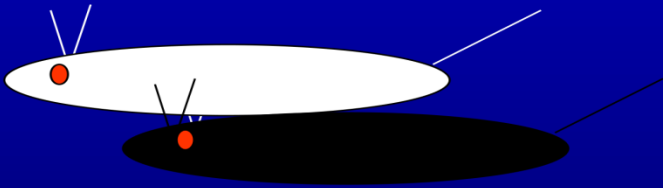
Objectives

Objectives

- **Examine the effect of TGF- β 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



- **A/J**
- **C57BL6 TGF- β 1 HT**
- **AJBL6 TGF- β 1 HT**
- **TGF- β 1 HT/K-ras LA**

Question

Question

- **Does lung tumorigenesis affect the TGF- β signaling pathway?**
- **Does the TGF- β 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 CYP2E1 to vinyl
carbamate and vinyl carbamate
epoxide as well as
degraded by esterase**

A/J mouse tumors

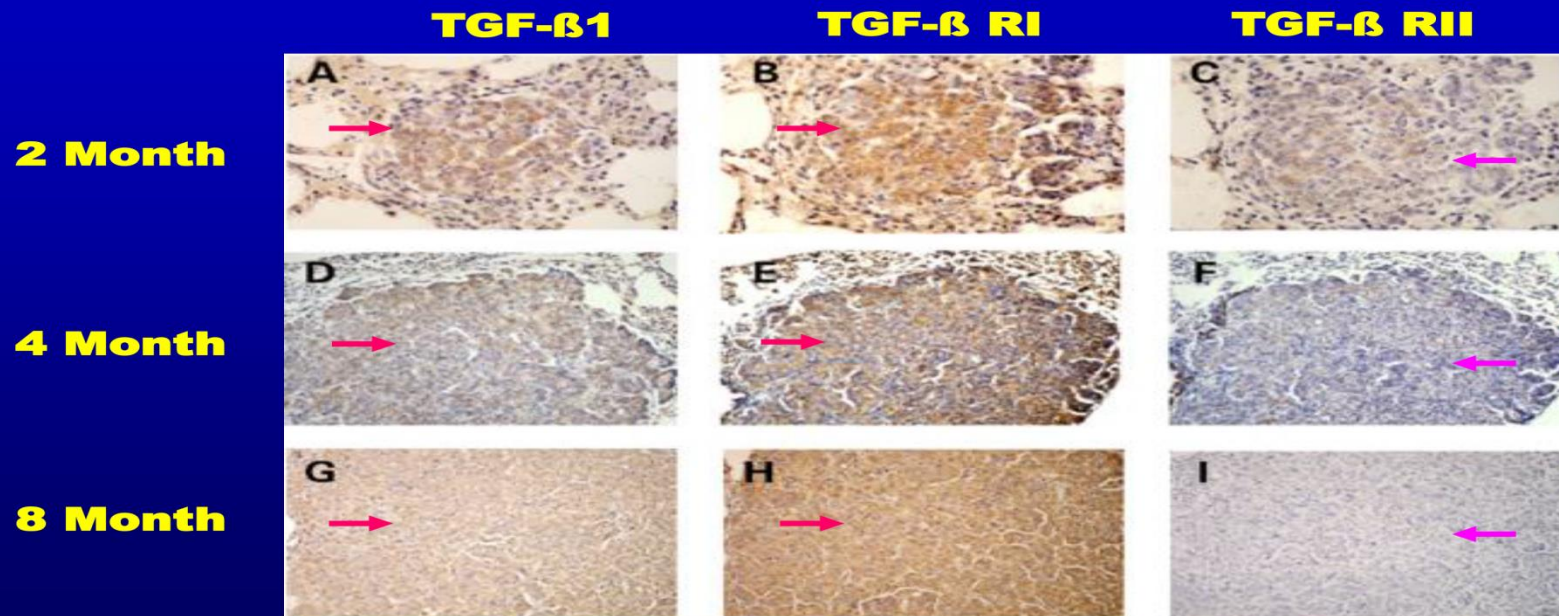
Production of Tumors in A/J Mice



TGF-beta1, RI and RII proteins

A/J Mouse Model

TGF- β 1, RI and RII Proteins in Lung Tumors



Decreased TGF- β RII protein in tumors

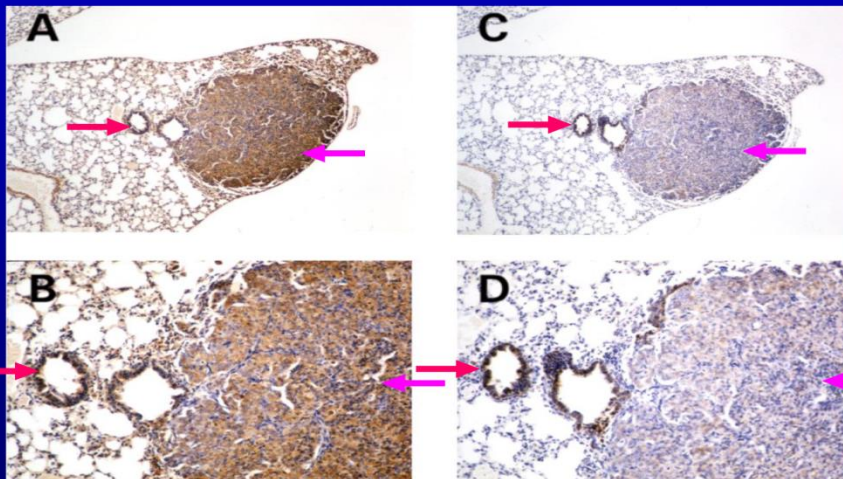
Decreased TGF- β RII in tumors

TGF- β in A/J Mouse Model

EC-induced Lung Tumors

TGF- β R1

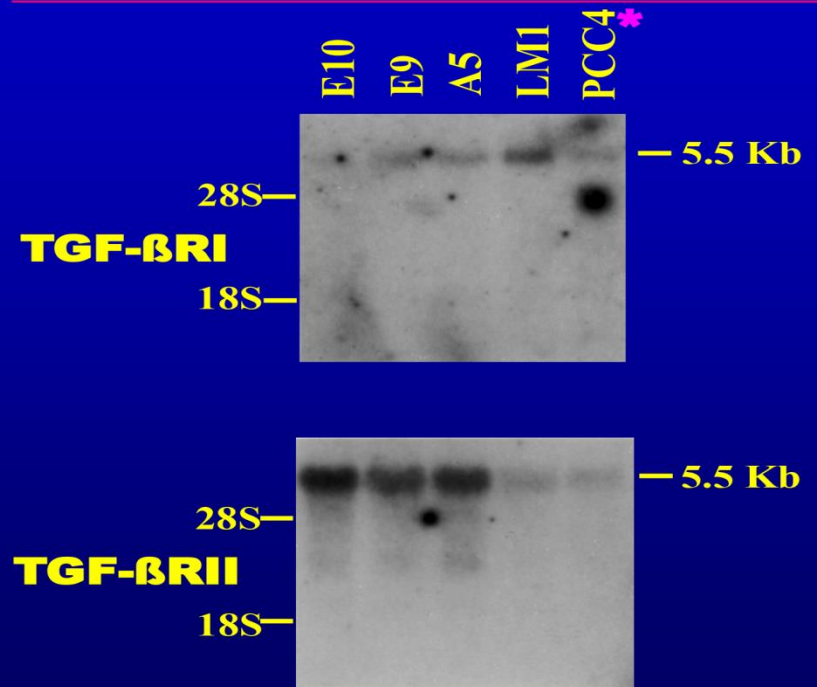
TGF- β R2



IHC

IHC

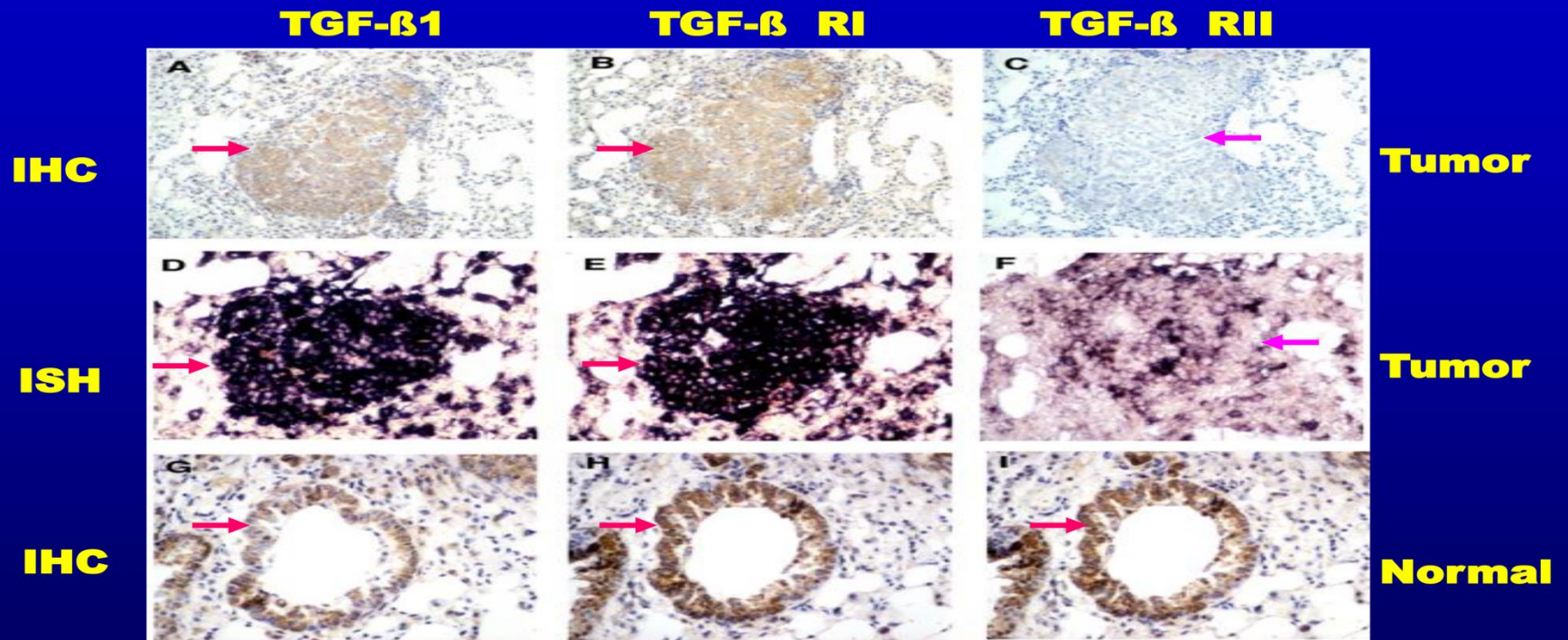
Lung Tumor Derived Cell Lines



Decreased TGF- β RII protein and mRNA

Expression of TGF- β 1, RI and RII Proteins and mRNAs

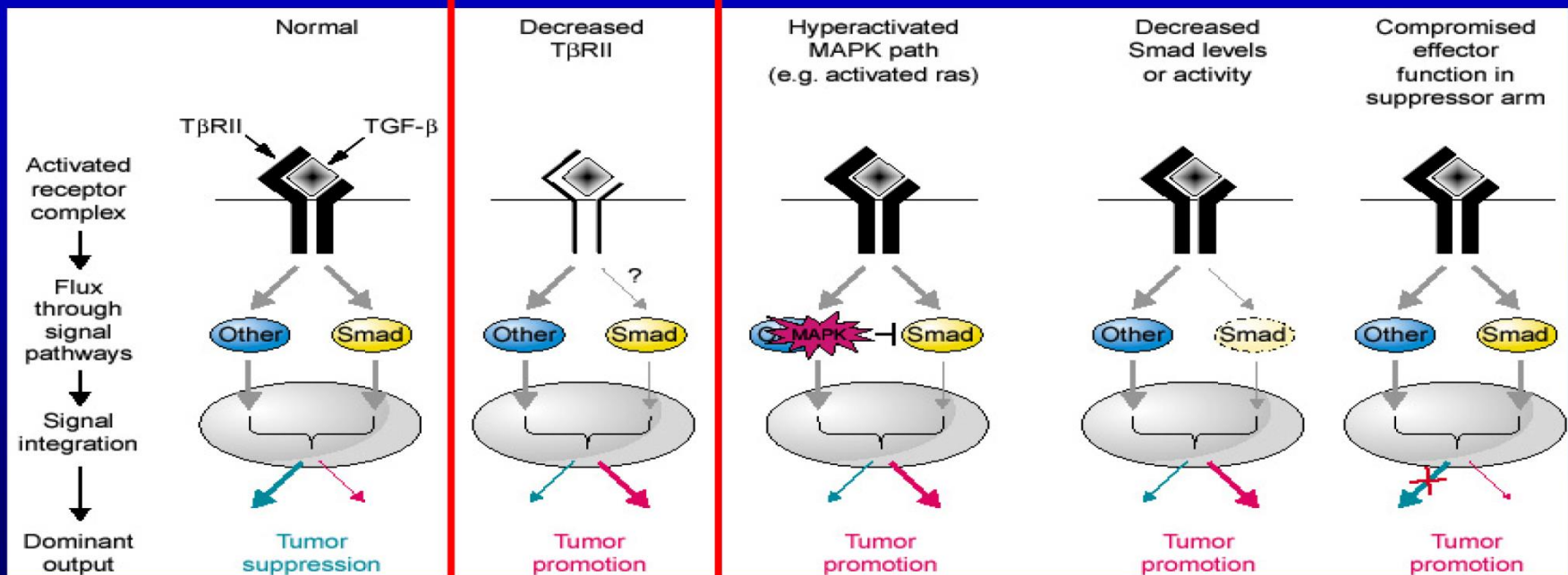
Expression of TGF- β 1, RI and RII Proteins and mRNAs in BP-Induced A/J Mouse Lung Tumors



Decreased TGF- β RII mRNA and protein in tumors

Tumor suppression/promotion

TGF- β in Tumor Suppression/Promotion



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

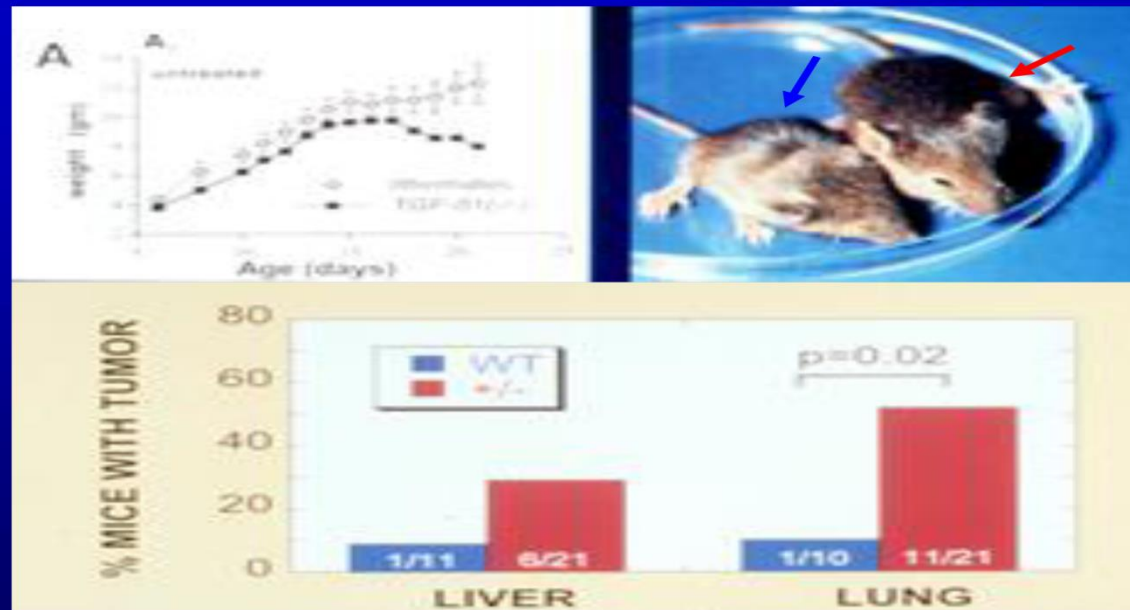
Question

**Does deletion of TGF- β 1
affect lung tumorigenesis?**

C57BL/6 TGF- β 1 Mouse

TGF-beta1 knockout mice

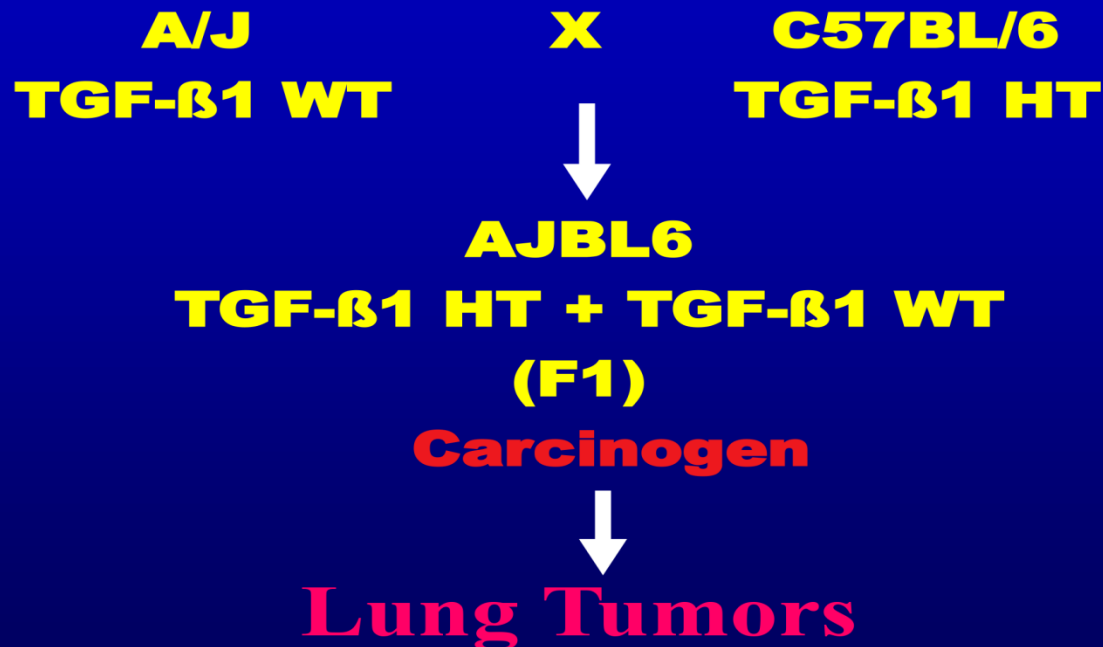
The C57BL/6 TGF- β 1 Knockout Mouse



Increased tumor incidence in TGF- β 1 HT mice

Mouse models

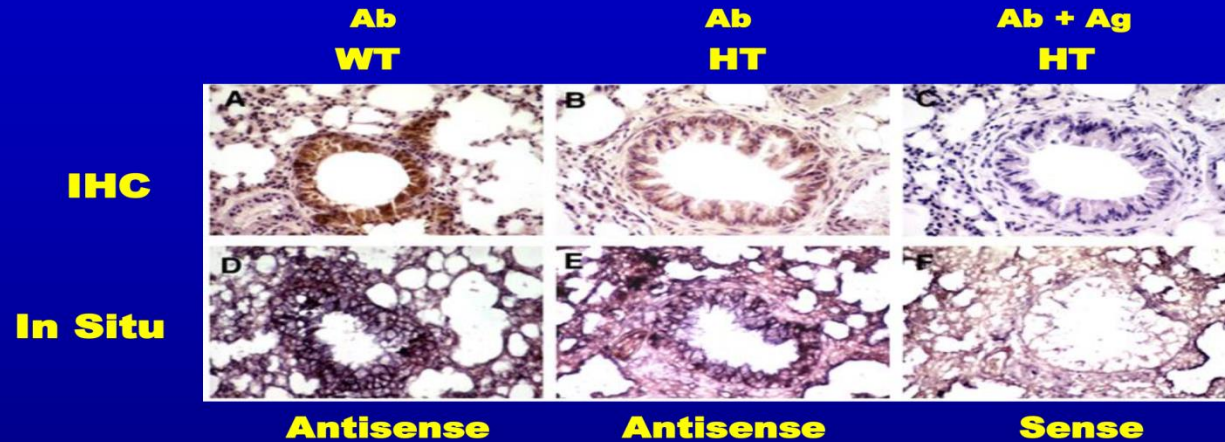
AJBL6 TGF- β 1 HT Mouse Derivation



TGF-beta1 in HT and WT mice

AJBL6 TGF-β1 HT and WT Mouse

IHC Staining & In Situ Hybridization



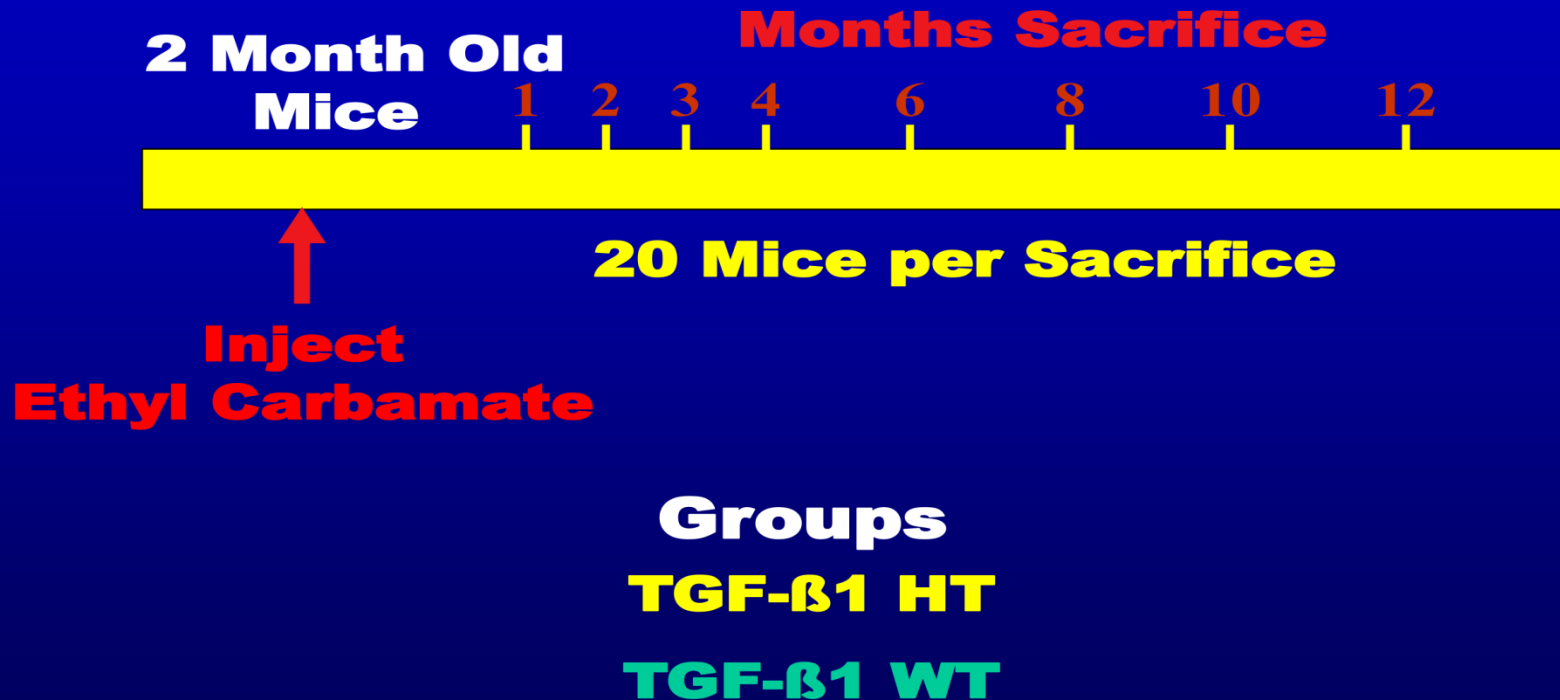
Northern Blotting & Competitive RT-PCR



Reduced expression of TGF-β1 in HT compared to WT

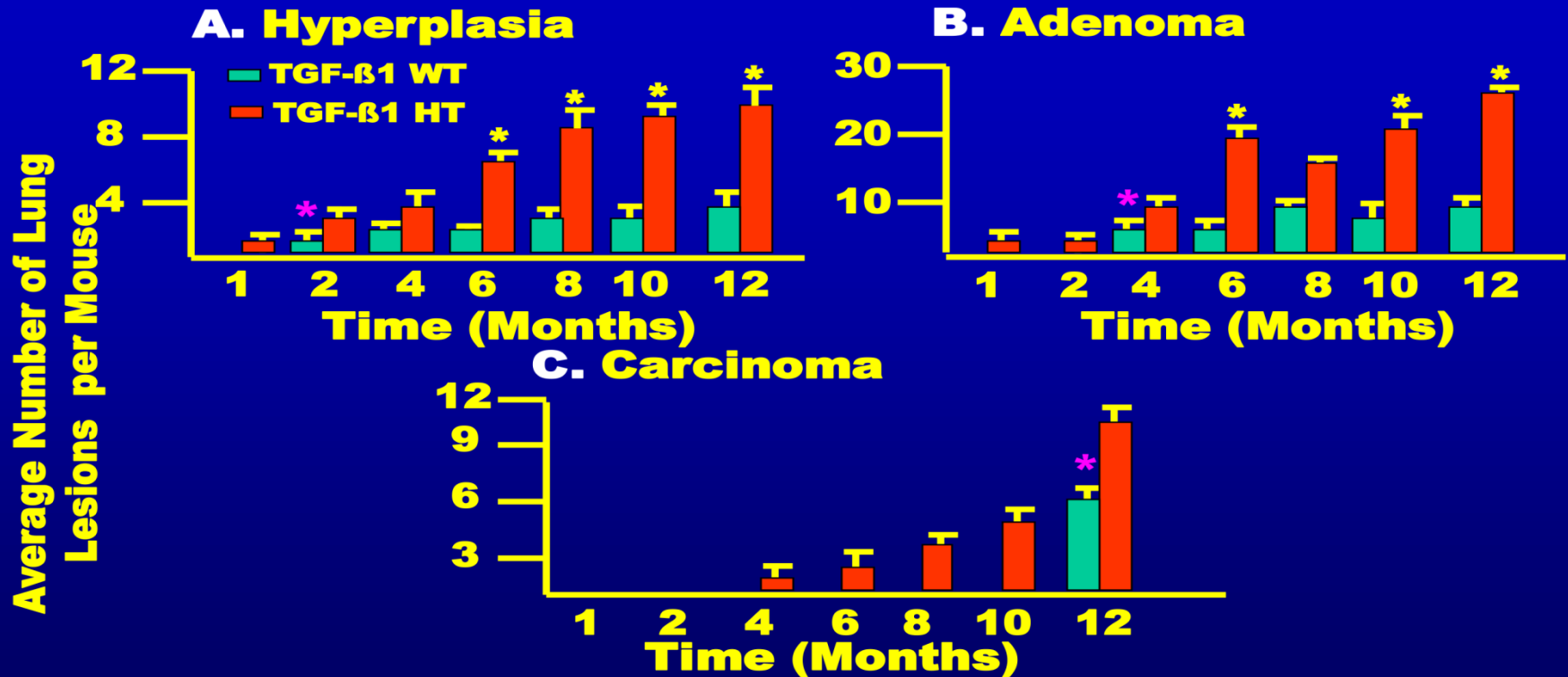
Production of Tumors

Production of Tumors



AJBL6 TGF- β 1 HT & WT Mice

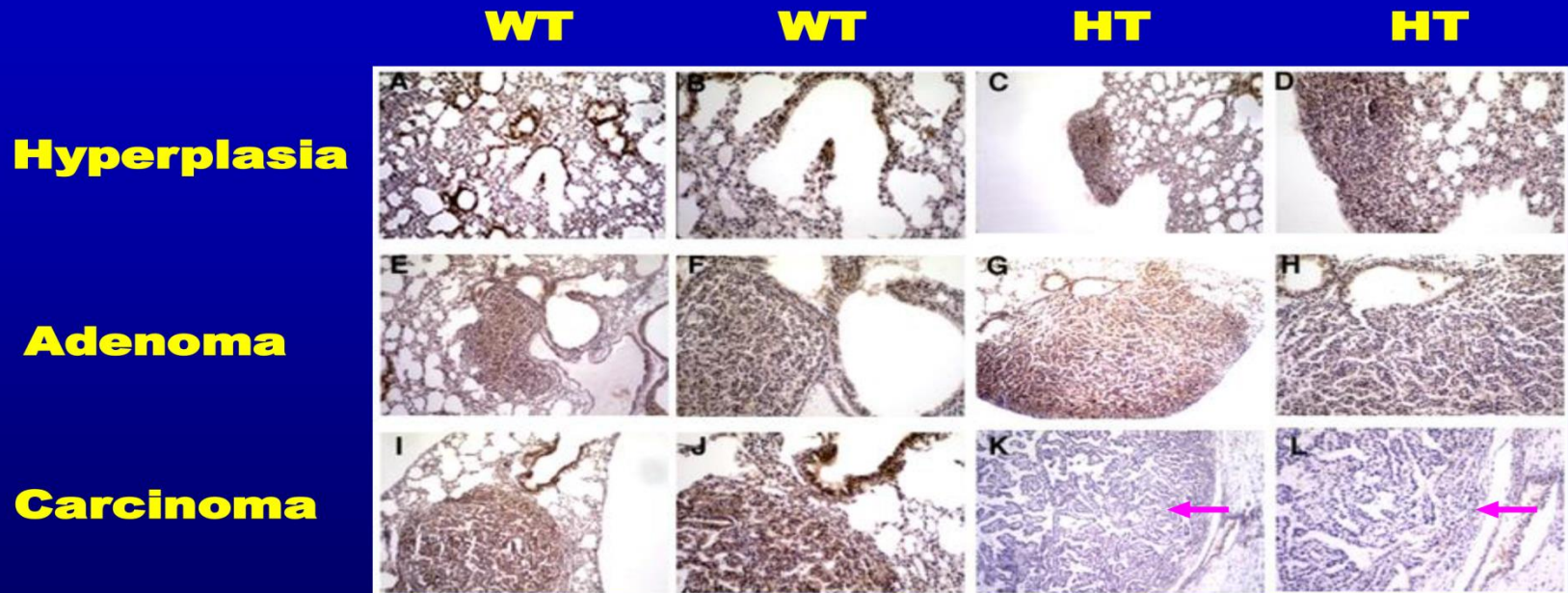
Carcinogen-Induced Lung Tumorigenesis in AJBL6 TGF- β 1 HT & WT Mice



Increased tumor incidence and multiplicity and decreased tumor latency in TGF- β 1 HT mouse

TGF-beta RII

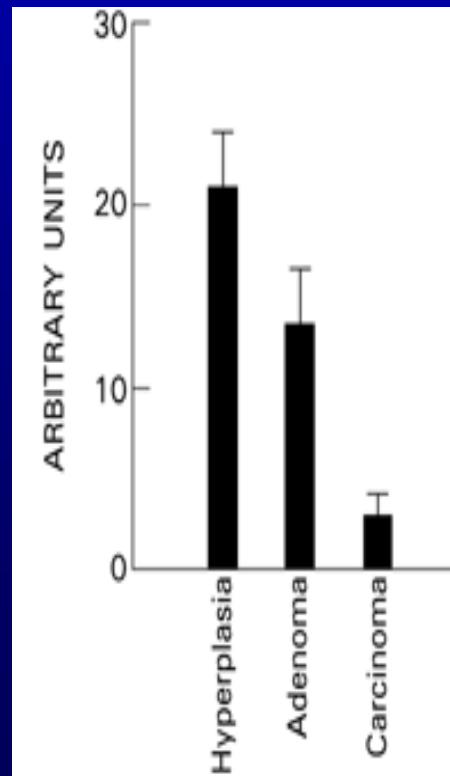
TGF- β RII Protein in Lung Lesions from AJBL6 TGF- β 1 WT and HT Mice



Decreased TGF- β RII in tumors of TGF- β 1 HT mice

Relative TGF- β RII mRNA Levels Lesions from AJBL6 TGF- β 1 HT Mouse Lungs Treated with Ethyl Carbamate

Decreasing TGF- β RII mRNA with increasing
lung tumorigenesis



Question

Does deletion of TGF- β 1 and mutation of K-ras affect lung tumorigenesis? TGF- β 1 HT/K-ras LA mouse

TGF-beta1 and K-ras

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

TGF- β 1
HT
(C57Bl/6)

X

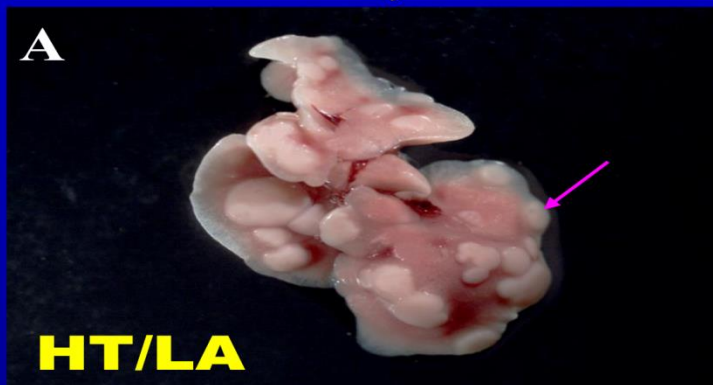
K-ras
LA
(SV 129)

TGF- β 1 HT/K-ras LA - HT/LA Double Mutant
TGF- β 1 WT/K-ras LA - WT/LA Single Mutant
TGF- β 1 HT/K-ras WT - HT/WT Single Mutant
TGF- β 1 WT/K-ras WT - WT/WT Wild Type

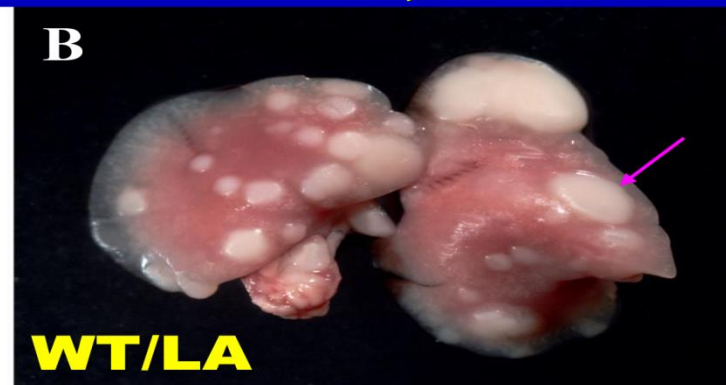
TGF-beta1 and K-ras

TGF-β1 and K-ras Mouse Lungs

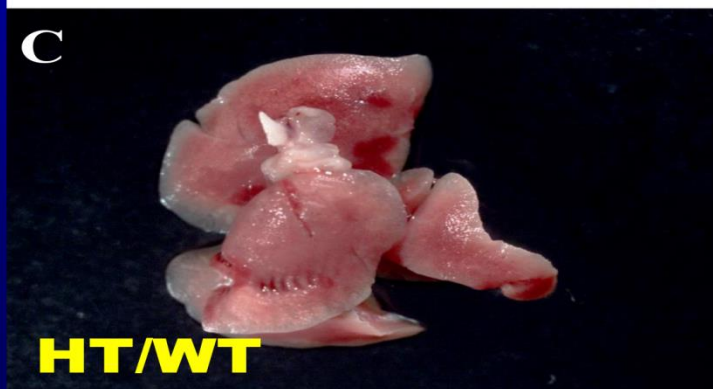
TGF-β1 HT, K-ras LA



TGF-β1 WT, K-ras LA



C



TGF-β1 HT, K-ras WT

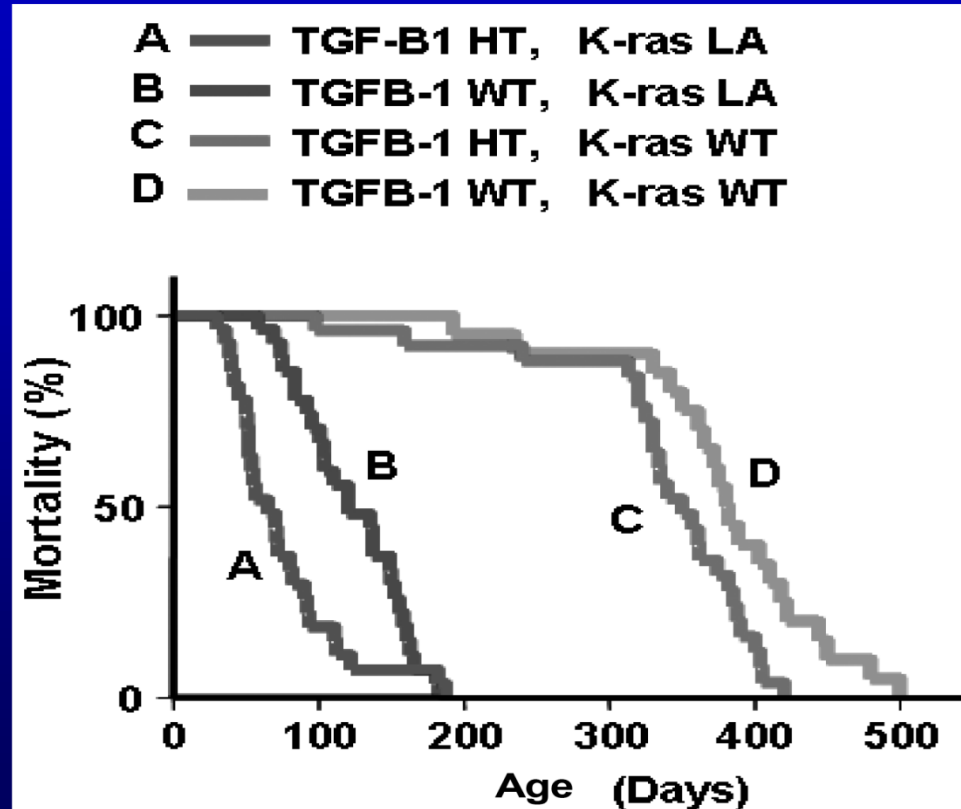
D



TGF-β1 WT, K-ras WT

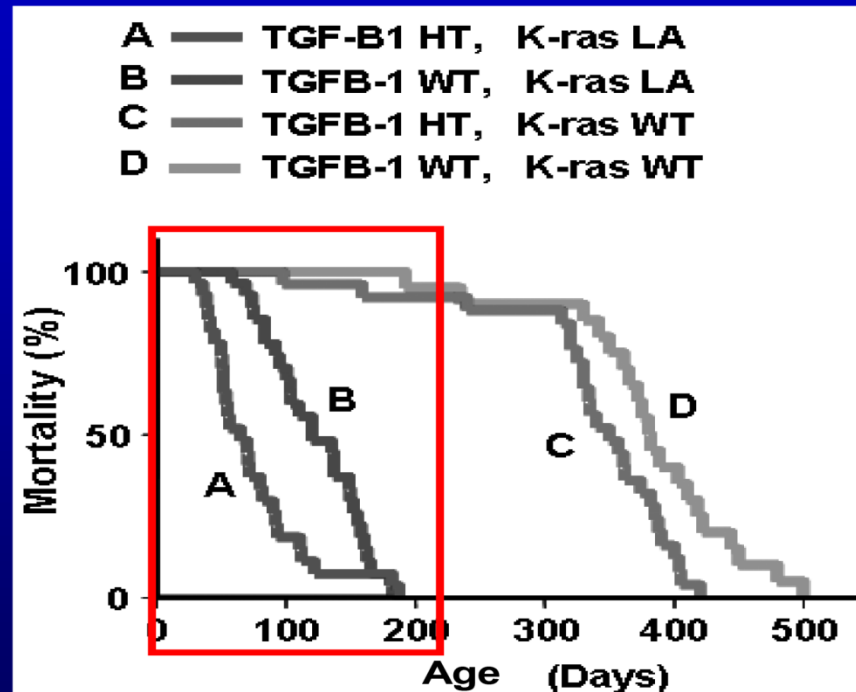
Mouse Survival

Effect of TGF- β 1 Gene Deletion and K-ras Mutation on Mouse Survival



Mouse survival

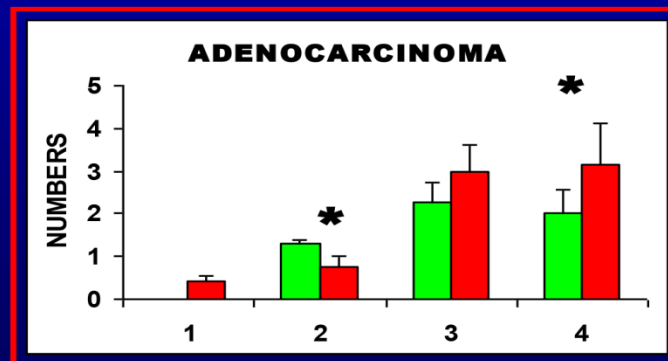
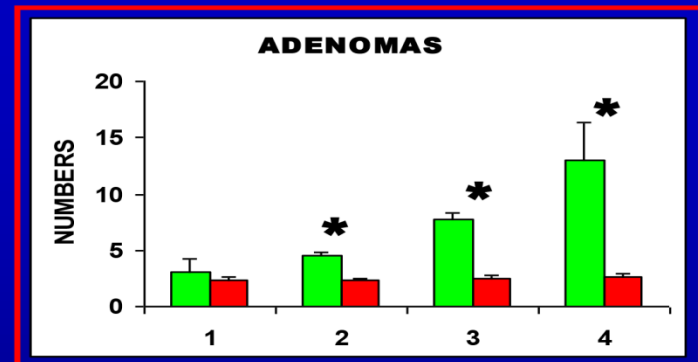
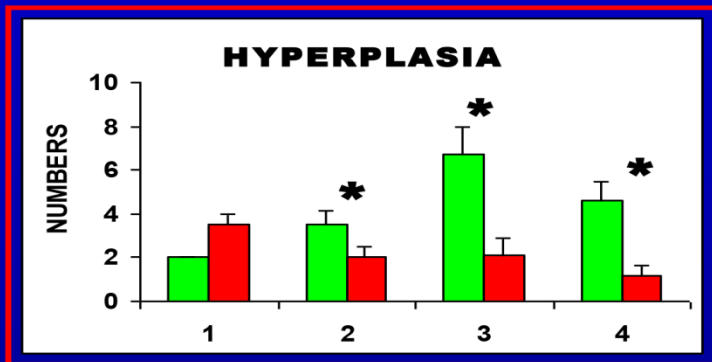
Effect of TGF- β 1 Gene Deletion and K-ras Mutation on Mouse Survival



Decreased lifespans in HT/LA and WT/LA mice

Lung Pathology

Pathology of Lung Lesions



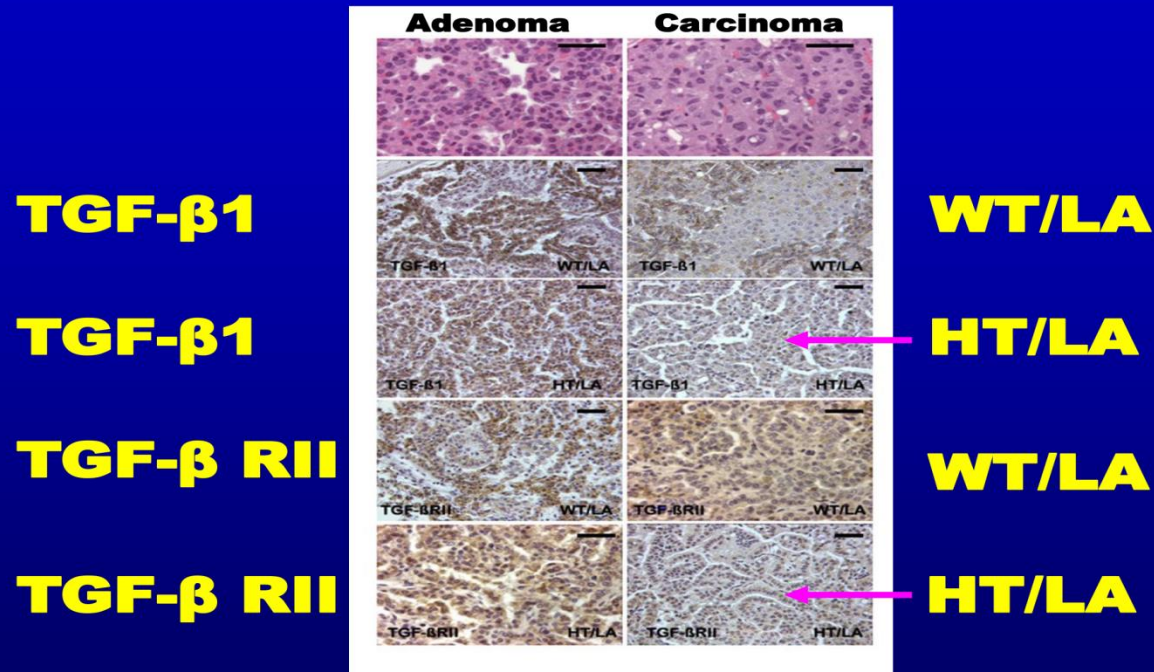
WT/LA ■

HT/LA ■

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

TGF-beta1 and RII

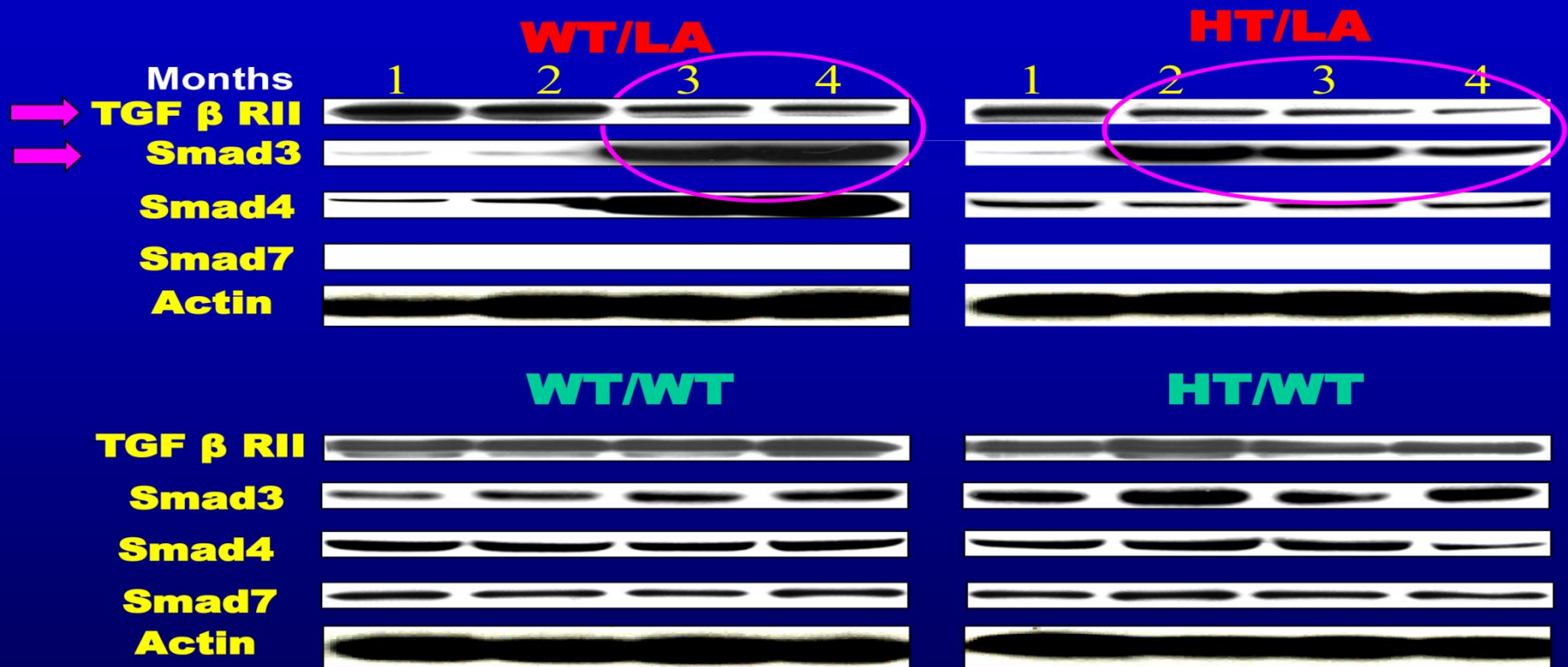
TGF-β1 and TGF-β RII in Lung Lesions



Reduced TGF-β1 & RII in HT/LA adenocarcinomas

TGF-beta RII and Smad 3

TGFβ RII and Smad3 in Lung Tumorigenesis



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

TGF-beta pathway

TGFβ Pathway in HT/LA Lung Tumorigenesis

Western Blot:

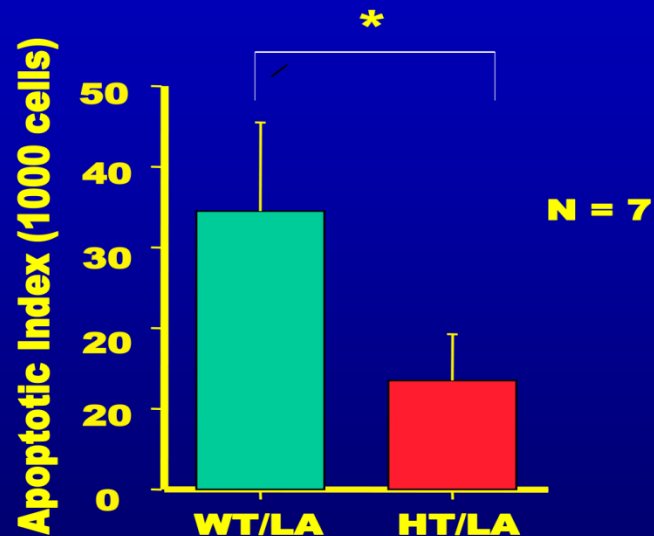
TGF β RII	Expedited TGF-β RII reduction
Smad3	Expedited Smad3 production
Smad4	Reduced Smad4 production
Smad7	Reduced Smad7 production
K-ras	Expedited K-ras production
Raf-1	Expedited Raf-1 production

Real Time RT-PCR:

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

Apoptotic index

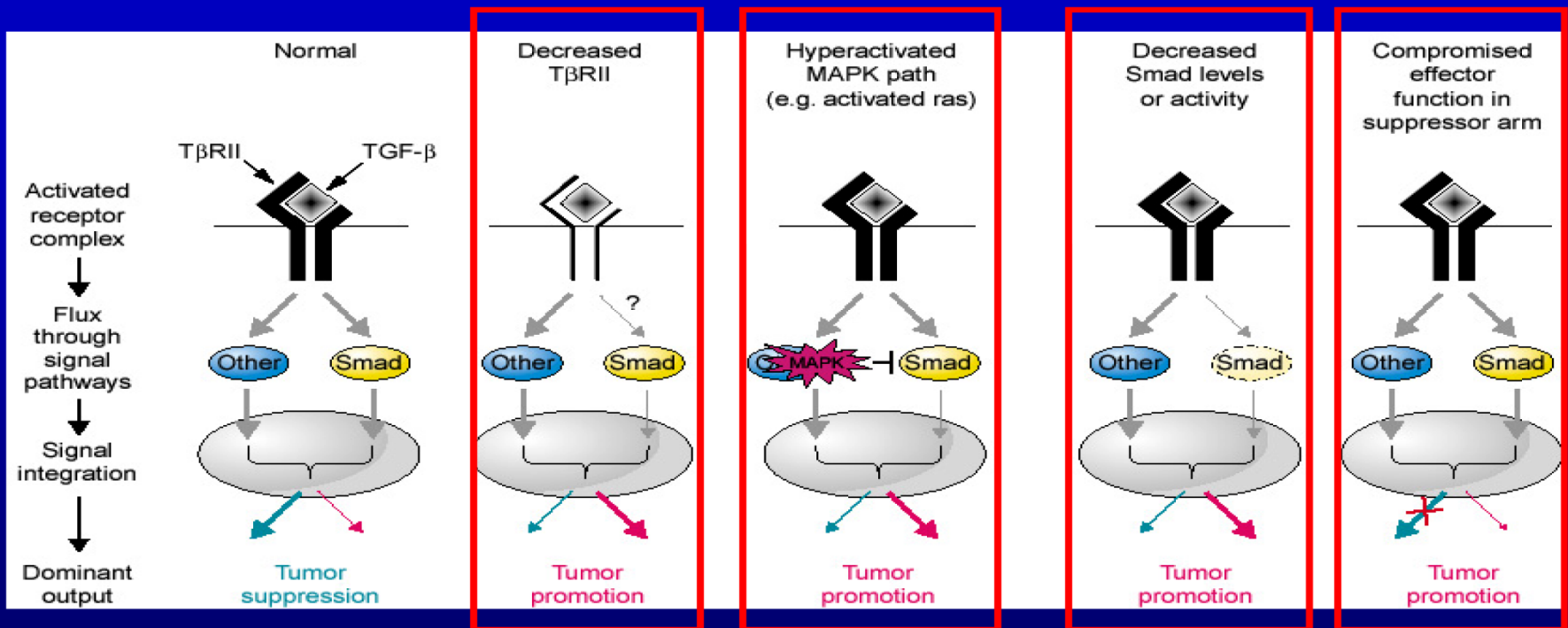
Apoptotic Index in WT/LA & HT/LA Mouse Lung Adenomas



Reduced apoptosis in HT/LA adenomas

Tumor suppression/promotion

TGF- β in Tumor Suppression/Promotion



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

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