Transforming Growth Factor-beta and Lung Tumorigenesis

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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-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 transformation
TGF-β is an attractive candidate for new therapeutic intervention approaches
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-β from:
Human platelets (*Rick Assoian*)
Human placenta (*Chuck Frolik*)
Bovine kidney (*Anita Roberts*)
Scale of TGF-β1 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-β1
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

- Plate agar base
- Add mix of media, serum, NRK cells, EGF, sample
- 1 wk/37°C/5%CO₂
- Stain
- Count colonies >3100 μm²

If no TGF-β is present

If TGF-β is present

Omnicon Image Analysis System
Final HPLC Purification
EUREKA!! TGF-β: Born at NCI

Michael Sporn & Anita Roberts
Mature TGF\(\beta\)1
TGFβ: 25 kDal homodimer
TGFβ superfamily
Transforming growth factor beta

- 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-\(\beta\) 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-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

**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
  
  *(Tβ-RII in HNPCC, Smad4 in pancreatic cancer)*

- Reduced expression of TGF-β1 signaling pathway components or overexpression of endogenous pathway inhibitors are associated with disease progression
  
  *(Tβ-RII, Tβ-RI, Smad7, Ski)*
Clinical Observations

TGF-β is a tumor promoter:

- TGF-β1 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-β1:
(Truong et al. Hum Pathol 1993)

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

NORMAL EPITHELIUM

INVASIVE METASTATIC CANCER

Changes in genetic and epigenetic context

TGF-β responsiveness

TGF-β expression/activation

Suppressor activities dominate

Pro-oncogenic activities dominate
TGF-beta Smad-independent Pathways
TGFbeta Smad-independent pathways

TGF-β Smad-independent Pathways
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-β in Tumor Suppression/Promotion

• Activated Ras/MAPK = Tumor Promotion
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

- 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-β1 HT
- AJBL6 TGF-β1 HT
- TGF-β1 HT/K-ras LA
Question

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

- Does the TGF-β signaling pathway affect lung tumorigenesis?
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 (i.e., 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
Production of tumors in A/J mice

Production of Tumors in A/J Mice

2 Month Old Mice

Months Sacrifice

20 Mice per Sacrifice

Inject Ethyl Carbamate
A/J Mouse model

A/J Mouse Model
TGF-β1, RI and RII Proteins in Lung Tumors

2 Month
TGF-β1 (A), TGF-β RI (B), TGF-β RII (C)

4 Month
TGF-β1 (D), TGF-β RI (E), TGF-β RII (F)

8 Month
TGF-β1 (G), TGF-β RI (H), TGF-β RII (I)

Decreased TGF-β RII protein in tumors
Decreased TGF-β RII in tumors
Expression of TGF-β1, RI and RII Proteins and mRNAs in BP-Induced A/J Mouse Lung Tumors

Decreased TGF-β RII mRNA and protein in tumors
TGF-β in Tumor Suppression/Promotion

• Reduced TGF-β RII = Lung Tumor Promotion
Question

Does deletion of TGF-β1 affect lung tumorigenesis?

C57BL/6 TGF-β1 Mouse
The C57BL/6 TGF-β1 Knockout Mouse

Increased tumor incidence in TGF-β1 HT mice
HT Mouse

AJBL6 TGF-β1 HT Mouse Derivation

A/J
TGF-β1 WT

X

C57BL/6
TGF-β1 HT

AJBL6
TGF-β1 HT + TGF-β1 WT
(F1)
Carcinogen

Lung Tumors
TGF-β1 reduced in HT

AJBL6 TGF-β1 HT and WT Mouse

IHC Staining & In Situ Hybridization

Ab
WT
Ab
HT
Ab + Ag
HT

IHC
Antisense
Antisense
Sense

In Situ

Northern Blotting & Competitive RT-PCR

TGF-β1 WT
TGF-β1 HT

Copies of Competitor Added

2.5 Kb
28S rRNA

2.5x10^6
1.25x10^6
6.25x10^5
3x10^5

Reduced expression of TGF-β1 in HT compared to WT
Production of Tumors

Production of Tumors

2 Month Old Mice

Months Sacrifice

1 2 3 4 6 8 10 12

20 Mice per Sacrifice

Inject Ethyl Carbamate

Groups

TGF-β1 HT

TGF-β1 WT
AJBL6 mice

Carcinogen-Induced Lung Tumorigenesis in AJBL6 TGF-β1 HT & WT Mice

A. Hyperplasia

B. Adenoma

C. Carcinoma

Increased tumor incidence and multiplicity and decreased tumor latency in TGF-β1 HT mouse.
Carcinogen-Induced Lung Tumorigenesis in AJBL6 TGF-β1 HT & WT Mice

A. Hyperplasia
- TGF-β1 WT
- TGF-β1 HT

B. Adenoma

C. Carcinoma

Increased tumor incidence and multiplicity and decreased tumor latency in TGF-β1 HT mouse
TGF-β RII Protein in Lung Lesions from AJBL6 TGF-β1 WT and HT Mice

Hyperplasia

Adenoma

Carcinoma

Decreased TGF-β RII in tumors of TGF-β1 HT mice
Relative TGF-β RII mRNA Levels
Lesions from AJBL6 TGF-β1 HT Mouse Lungs Treated with Ethyl Carbamate
Decreasing TGF-β 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
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
Mouse survival

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

- **A**: TGF-B1 HT, K-ras LA
- **B**: TGFβ-1 WT, K-ras LA
- **C**: TGF-B1 HT, K-ras WT
- **D**: TGFβ-1 WT, K-ras WT

Graph showing mortality (% vs. age in days) for different conditions.
Lifespan

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

A: TGF-B1 HT, K-ras LA
B: TGFB-1 WT, K-ras LA
C: TGFB-1 HT, K-ras WT
D: TGFB-1 WT, K-ras WT

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

Pathology of Lung Lesions

Hyperplasia

Numbers

Adenomas

Numbers

Adenocarcinoma

Numbers

WT/LA

HT/LA

Increased hyperplasia & adenoma in WT/LA
Increased carcinoma in HT/LA
TGF-β1 and TGF-β RII

TGF-β 1 and TGF-β RII in Lung Lesions

Reduced TGF-β1 & RII in HT/LA adenocarcinomas
Smad3

TGFβ RII and Smad3 in Lung Tumorigenesis

<table>
<thead>
<tr>
<th>Months</th>
<th>WT/LA</th>
<th>HT/LA</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
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WT/WT vs. HT/WT:

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

## TGFβ Pathway in HT/LA Lung Tumorigenesis

<table>
<thead>
<tr>
<th>Western Blot:</th>
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<tbody>
<tr>
<td><strong>TGF β RII</strong></td>
<td>Expedited TGF-β RII reduction</td>
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<tr>
<td><strong>Smad3</strong></td>
<td>Expedited Smad3 production</td>
</tr>
<tr>
<td><strong>Smad4</strong></td>
<td>Reduced Smad4 production</td>
</tr>
<tr>
<td><strong>Smad7</strong></td>
<td>Reduced Smad7 production</td>
</tr>
<tr>
<td><strong>K-ras</strong></td>
<td>Expedited K-ras production</td>
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<tr>
<td><strong>Raf-1</strong></td>
<td>Expedited Raf-1 production</td>
</tr>
</tbody>
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## 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
Acknowledgements

Epithelial Carcinogenesis Group

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Sarah Umphress

MIT
Tyler Jacks
Kim Mercer
TGFβ 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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