Transforming Growth Factor-beta and Lung Tumorigenesis

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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-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
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*


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

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 with Omnicon Image Analysis System

If no TGF-β is present

If TGF-β is present
HPLC Purification

The Final HPLC Purification

ABSORBANCE, 210nm

FRACTION NUMBER

PERCENT D-PROPAOL

TGF-β
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

- 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, 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 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

Changes in genetic and epigenetic context

NORMAL EPITHELIUM

TGF-β responsiveness

TGF-β expression/activation

INVASIVE METASTATIC CANCER

Suppressor activities dominate

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

TGF-β Smad-independent Pathways
TGF-beta 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

- 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

- 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 carbamate epoxide as well as degraded by esterase
A/J mouse tumors

Production of Tumors in A/J Mice

2 Month Old Mice

1  2  3  4  6  8  10  12

Months Sacrifice

20 Mice per Sacrifice

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

<table>
<thead>
<tr>
<th>2 Month</th>
<th>4 Month</th>
<th>8 Month</th>
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<tbody>
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<td>TGF-β1</td>
<td>TGF-β RI</td>
<td>TGF-β RII</td>
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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-βRI**

**TGF-βRII**

Lung Tumor Derived Cell Lines

**TGF-βRI**

**TGF-βRII**

Decreased TGF-β RII protein and mRNA
Expression of TGF-β1, RI and RII Proteins and mRNAs

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

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<th>TGF-β1</th>
<th>TGF-β RI</th>
<th>TGF-β RII</th>
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</table>

Tumor

Normal

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-β1 affect lung tumorigenesis?
C57BL/6 TGF-β1 Mouse
TGF-beta1 knockout mice

The C57BL/6 TGF-β1 Knockout Mouse

Increased tumor incidence in TGF-β1 HT mice
Mouse models

AJBL6 TGF-β1 HT Mouse Derivation

A/J  X  C57BL/6
TGF-β1 WT  TGF-β1 HT

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

Lung Tumors
TGF-beta1 in HT and WT mice

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

2.5 Kb

28S rRNA

Copies of Competitor Added

5x10^5

2.5x10^5

1.25x10^5

6.25x10^4

3x10^4

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

2 Month Old Mice

Inject Ethyl Carbamate

Months Sacrifice

20 Mice per Sacrifice

Groups
TGF-β1 HT
TGF-β1 WT
AJBL6 TGF-β1 HT & WT 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
TGF-beta RII

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-β1 and K-ras: Generation of TGF-β1/ K-ras LA Mice

TGF-β1 HT (C57Bl/6) × K-ras LA (SV 129)

- TGF-β1 HT/K-ras LA - HT/LA Double Mutant
- TGF-β1 WT/K-ras LA - WT/LA Single Mutant
- TGF-β1 HT/K-ras WT - HT/WT Single Mutant
- TGF-β1 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

A

HT/LA

B

WT/LA

C

HT/WT

D

WT/WT

TGF-β1 HT, K-ras WT

TGF-β1 WT, K-ras WT
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

- **Hyperplasia**
  - WT/LA (green)
  - HT/LA (red)

- **Adenomas**
  - WT/LA (green)
  - HT/LA (red)

- **Adenocarcinoma**
  - WT/LA (green)
  - HT/LA (red)

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-β RII and Smad 3 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
## Anti TGFB compounds currently in Clinical Trials

<table>
<thead>
<tr>
<th>Class</th>
<th>Compound</th>
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<td>Fresolumimab</td>
<td>TGF-ß 1-3</td>
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Acknowledgements

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