Transforming Growth Factor-beta and Lung Tumorigenesis

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Lung Cancer in 2015, USA

- 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

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%
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 Omnicom Image Analysis System

If no TGF-β is present

If TGF-β is present
HPLC Purification

The Final HPLC Purification

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, a 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

Changes in genetic and epigenetic context

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
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

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

Production of Tumors in A/J Mice

2 Month Old Mice

Inject Ethyl Carbamate

Months Sacrifice

20 Mice per Sacrifice
TGF-beta1, RI and RII proteins

A/J Mouse Model
TGF-β1, 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-βRI

TGF-βRII

IHC

Lung Tumor Derived Cell Lines

E10  E9  A5  LM1  PCC4*

TGF-βRI

TGF-βRII

28S

18S

5.5 Kb

5.5 Kb

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

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
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 TGF-β1 WT  X  C57BL/6 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

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<th>Ab</th>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>HT</td>
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In Situ

Antisense  | Antisense  | Sense

Northern Blotting & Competitive RT-PCR

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<thead>
<tr>
<th></th>
<th>2.5 Kb</th>
<th>28S rRNA</th>
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<tr>
<td>TGF-β1 WT</td>
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<tr>
<td>TGF-β1 HT</td>
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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

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
TGF-beta1 and K-ras

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

TGF-β1 HT (C57BL/6) \( \times \) 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 WT/K-ras WT - WT/WT Wild Type
TGF-beta1 and K-ras

TGF-β1 and K-ras Mouse Lungs

A. TGF-β1 HT, K-ras LA (HT/LA)
B. TGF-β1 WT, K-ras LA (WT/LA)
C. TGF-β1 HT, K-ras WT (HT/WT)
D. TGF-β1 WT, K-ras WT (WT/WT)
Mouse Survival

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

Mortality (%) vs Age (Days)
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

ADENOMAS

ADENOCARCINOMA

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 pathway

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<tbody>
<tr>
<td>TGFβ RII</td>
<td>Expedited TGF-β RII reduction</td>
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<tr>
<td>Smad3</td>
<td>Expedited Smad3 production</td>
</tr>
<tr>
<td>Smad4</td>
<td>Reduced Smad4 production</td>
</tr>
<tr>
<td>Smad7</td>
<td>Reduced Smad7 production</td>
</tr>
<tr>
<td>K-ras</td>
<td>Expedited K-ras production</td>
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<tr>
<td>Raf-1</td>
<td>Expedited Raf-1 production</td>
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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
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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