Connecting Tissues and Investigators: Fibrosis in Health and Disease

December 6, 2017
Can We Reduce Fibrosis by Increasing the Levels of the Klotho Hormone?

Carmela Abraham

Professor
Biochemistry and Pharmacology & Experimental Therapeutics, MED

Clotho, Zeus’ daughter who spins the thread of life. Klotho gene named after her by Dr. Kuro-o
Klotho suppresses fibrosis in an animal model of chronic kidney disease, unilateral ureter obstruction (UUO)

Fibrosis within 7 days
No renal failure

UUO
Day 3
Normal Rt Kidney
Vehicle
Klotho (0.01 mg/kg)
Klotho (0.02 mg/kg)

Day 7
Klotho (0.01 mg/kg)
Klotho (0.02 mg/kg)
Normal Lt Kidney
Vehicle

Day 3
Klotho (0.01 mg/kg)
Klotho (0.02 mg/kg)

Day 7
Klotho (0.01 mg/kg)
Klotho (0.02 mg/kg)

Slide from Dr. Makoto Kuro-o

Collagen-1

Relative mRNA level

qPCR

7 days

0 50 100 150
Irving J. Bigio

Professor
Biomedical Engineering and Electrical & Computer Engineering, ENG;
Physics, CAS; and Medicine, MED
Quantitative optical assessment of fibrosis:

1) Elastic-scattering spectroscopy (ESS): collagen in ECM (fibrosis) exhibits enhanced optical scattering, with short-wavelength bias.

2) Birefringence imaging: collagen is highly birefringent, can be quantitatively imaged with polarized microscopy.
Activation States of Perivascular Adventitial Fibroblasts

Jeff Browning

Research Professor
Microbiology and Rheumatology, MED
Adventitial fibroblasts are beginning to resemble the fibroblastic reticular networks that scaffold the lymphoid organs. Does the reticular network provide retention signals for lymphocytes?

Adventitial fibroblasts gain VCAM expression.

Adventitia, relatively non-inflammatory.

Lymphocytic Infiltrate
Collagen Matrix-Rich Niche
Mesenchymal Stem Cells
Monocytes (Resident?)
Well poised to orchestrate repair/remodeling

Artery
Vein
Matrix

Blood Vessels

Cutaneous lupus
Scleroderma
Normal


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Jacob Joseph

Adjunct Associate Professor
Medicine, MED
Reactive myocardial fibrosis


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Salivary Gland Repair, Regeneration and Fibrosis

*a model for patterned cell and matrix dynamics in branching morphogenesis*

Maria A. Kukuruzinska, PhD

*Molecular & Cell Biology, GSDM*
Fibrosis: a multifactorial dynamic disease

Fibroblast activation, Epigenetic changes, Atrophy, Metabolic alterations, EMT, Autophagy, ECM remodeling, Innate and adaptive immune responses

Salivary gland:
Development

Repair / Regeneration

Fibrosis: a multifactorial dynamic disease

Immune surveillance in the developing salivary gland

Lab Invest, 2013; Plos Comp Biol, 2016; Sci Reports, 2017 (in revision)
Regulation of Fibrosis Through Soluble & Mechanical Signals

Matthew Layne

Associate Professor
Biochemistry, MED
**Fibrosis Is Defective ECM Homeostasis**

- **Aortic carboxypeptidase-like protein (ACLP)**
- **Tissue Injury**
- **Inflammation**
- **Fibroproliferation**
- **Normal Wound Healing**

**Too Little:**
- **Humans:** Ehlers Danlos Syndrome
  - hypertrophic scars
  - wound healing
  - vascular disruption
- **Mice:** Delayed wound healing

**Too Much:**
- organ fibrosis
- CV disease

**What is mechanical signaling?**
- Cells sense the pillow (and not just the pillow case)
  - Pillow
  - Rock

**Collagen**
- SMA
- TGFβ
- Cell culture plastic

**TGFβ + Cell culture plastic**
- **pSmad3**
- **Smad2/3**

**4 kPa 25 kPa**
- ACLP
- SMA
- Collagen I
- Pan-actin

**Progenitor differentiation**
- Cont
- ACLP
- Cont
- ACLP

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Translational Research to Inform Tissue Fibrosis Mechanism and Drug Discovery

Weining Lu

Associate Professor
Medicine and Pathology & Laboratory Medicine, MED
LU LAB: Apply translational developmental genetics to study fibrotic disease mechanism

Normal kidney development

Congenital anomalies of the kidney and urinary tract (CAKUT)

Molecular genetics approach to study fibrotic disease mechanism

Podocyte

Pericyte

Human genetics

Mouse genetics

ZEB and SLIT-ROBO signaling pathways
LU LAB: Translational research approach to novel drug discovery in fibrotic disease

A The roadblocks
Valley of death
1st Mountain of despair
2nd Mountain of despair

Basic research
Basic researchers
Clinical research
Clinical researchers
Clinical practice
Clinicians
Improved outcomes
Access (policy makers, guideline, patients)

B The connections
Bench-to-Bedside Bridge
Bed-to-Bench Bridge

Clinical research to clinical practice tunnel
Clinical practice to improved outcomes tunnel (Implementation)

TRADITIONAL APPROACH TO TARGET SELECTION
Forward drug discovery

Gene/protein
Target
Drug
Disease
Mechanism?

Reverse drug discovery

TRANSLATIONAL APPROACH TO TARGET SELECTION

Gene/protein
Target
Mechanism
Drug

Modified from Sanofi

Translational approach to drug discovery

Academic-industry collaboration accelerates novel drug discovery

Novel drugs for fibrotic disease

Eye
Strabismus

Skin
Scleroderma
Keratoacanthosis
Nephrogenic systemic fibrosis

Cardiac fibrosis
Diastolic dysfunction
Heart failure, with reduced or preserved ejection fraction
Atrial fibrillation

Cirrhosis
Portal hypertension
Ascites
Gastroesophageal varices
Hepatocellular carcinoma
Hepatocellular carcinoma
Post-pneumocystis pneumonia
Hepatic encephalopathy
Hepatocellular carcinoma

Pulmonary fibrosis
Restrictive lung disease
Pulmonary hypertension
Right-sided heart failure

Pancreatic fibrosis
Chronic pain
Diabetes mellitus
Malabsorption
Cancer

Renal fibrosis
Chronic kidney disease
Hypertension
Anemia
Electrolyte disturbances
Rockey NEJM 2015
The Impact of a Bone Marrow Fibrotic Niche on Blood Cell Development

Katya Ravid
Professor
Medicine and Biochemistry, MED
Whitaker Cardiovascular Institute
The impact of a bone marrow fibrotic niche on blood cell development

Normal bone marrow (BM)

Fibrotic BM

Malignant clones

Fibrotic, Stiffer ECM

Proplatelet formation

Type IV Collagen (less stiffer than Type I collagen)

Signaling from the ECM to the cell


We identified the following sensors of a stiffer ECM towards regulation of platelet production:

- Reticulin fibers made of secreted type III collagen
- Secreted Lysyl Oxidase is associated with a cross-linked stiffer collagen
- Transient receptor potential cation channel subfamily V member 4 (TRPV4)
- Piezo 1/2 mechanosensetive receptors

Inquiry: How do these mechanosensors control BM cells and platelet development?

ECM->Mechanosensitive ion channels ->, <- ? YAP/TAZ
Darren Roblyer

Assistant Professor
Biomedical Engineering, MED
Multiphoton label-free imaging of fibrosis

**Second Harmonic Generation Imaging**

- MDA-MB-231 (tumor) cells
- Intravital tumor collagen
- Mouse skin slide

**Ex** = 880 nm
**Em** = 440 nm

**Label free imaging of type I and II collagen**
- fixed or fresh samples
- In vivo (skin, window chamber)
- layer thicknesses, orientation, 3-D volumes

**New! <5 pubs on fibrosis (mostly liver fibrosis)**

**Green** = collagen
**Red** = FAD
An Agent-Based Network Model of Pulmonary Fibrosis Development

Béla Suki

Professor

Tyler J. Wellman

Biomedical Engineering, ENG
**Motivation**

- Idiopathic Pulmonary Fibrosis (IPF) lungs exhibit distinct structural changes as a result of pathologic changes in cell behavior:
  - Subpleural Honeycombing / Cyst formation
  - Traction Bronchiectasis
  - Reduction in Lung Volume, Compliance

**Rationale**

- The known cell behaviors should lead to the observed changes in lung structure and associated mechanical dysfunction:
  - Resistance to Apoptosis
  - Collagen Expression / Deposition
  - Cell Motility / Invasiveness

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**Conclusion:** 1) Cell activation and migration on stiff tissue and 2) Mechanical failure can explain deterioration in lung structure and function in pulmonary fibrosis.

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Multifunctional Lysyl Oxidases and Fibrosis

Philip Trackman

Professor
Molecular & Cell Biology, GSDM
Lysyl Oxidases

- Gene family made up of five genes: LOX, LOXL1 – LOXL4
- Critically required for ECM biosynthesis:
  Extracellular maturation of collagens & elastin via oxidation of lysine residues.
- The Three Bears and Goldi-LOX:
  Too little: poor connective tissue structure: osteolathyrism, aneurisms
  Too much: excess collagen accumulation and fibrosis, metastasis
- Novel substrates and functions relevant to fibrosis:
  PDGFR-β (increased cell proliferation, chemotaxis?)
  αV-integrin (increased fibroblast adhesion, unpublished)
- Propeptide regions direct proenzymes to ECM molecules for activation by procollagen C-proteinases and function: fibulins, fibrillin, tropoelastin, periostin, fibronectin, others? *Implies importance of functional extracellular protein complexes.*
- LOX-PP is released, has independent functions and binding partners: tumor growth inhibitor, has no enzyme activity. *Some functions are independent of enzyme activity.*
- Novel small pharmacologic inhibitors are being developed, and some are available from companies.
- Genetic models: We are creating a Floxed LOX mouse by CRISPR/Cas9 technology for tissue-specific knockout studies.
Beyond Fibrosis: The Challenges of Scleroderma

Maria Trojanowska

Professor
Medicine, MED;
Director, Arthritis Center
Organ complications associated with systemic sclerosis (scleroderma)

- Interstitial lung disease (40%)
- PAH (15%)
- Musculoskeletal problems (65%)
- Renal crisis (5–10%)
- Gastrointestinal complications (10%)

- Skin fibrosis (>90%)
- Raynaud phenomenon (90%)
- Telangiectasia (75%)
- Ulcers (40%)
- Calcinosis (25%)

FUTURE CHALLENGES:
- To better understand the interactions among different disease components
- To find a cure!
Epithelial Fli1 deficiency drives autoimmunity and fibrosis

Disease models

Transcription factor Fli1, member of the Ets family (Friend murine leukemia integration-1)

Fli1 plays a central role in ECM regulation

Endothelial Fli1 deficiency phenocopy SSc vasculopathy

Simultaneous downregulation of Fli1 and KLF5 reproduces key features of scleroderma

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YAP/TAZ Signaling in Fibrosis

Bob Varelas

Associate Professor
Biochemistry, MED
Aberrant YAP/TAZ signaling drives pro-fibrotic disease phenotypes

- Test how altering YAP/TAZ activity affects fibrotic processes in vivo
- Define transcriptional events regulated by YAP/TAZ
- Characterize secreted factors regulated by YAP/TAZ
- Identify downstream YAP/TAZ-regulated effectors important for promoting fibrosis (biomarkers/therapy)
The Role of ECM in Fibrosis: Collaborative Studies

Joyce Y. Wong

Professor
Biomedical Engineering and Materials Science & Engineering, ENG
Research on Tap: Connecting Tissues and Investigators

Can we ‘see’ fibrosis (ECM)?

Detection Modality
- ultrasound
- MRI
- CT
- fluorescence

We can make ECM alloy fibers:

Theranostics ARC (Herrera, Ruiz-Opazo) (Zhong)

MRI contrast agent

tether

ligand

contrast agent

drug

Inflammation

(Holt, Kasotakis, Nagy)

(Smith)

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