Role of Metalloenzymes

PATHOMECHANISM OF TISSUE FIBROSIS

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Fibrosis and fibrotic diseases

- Characterized by the excess production of a fibrous material within the extracellular matrix
- Changes tissue architecture
- Interferes with normal organ function
- Causes chronic disease to millions of people worldwide
Why do we have fibrosis?
Fibrotic diseases

- Human body responds to trauma and injury by scarring
- **Fibrosis = excessive scarring**
- Compared to normal “wound healing” the healing response continues causing excessive production and deposition of collagen
- “Injury”: infections, surgery, ischemic illness, environmental factors (alcohol, pollutant), radiation, chemotherapy
Fibrotic diseases

- pulmonary fibrosis, interstitial lung disease, liver fibrosis, cardiac fibrosis, macular degeneration, retinal and vitreal retinopathy, myocardial fibrosis, cardiovascular disease, atherosclerosis/restenosis, keloids and hypertrophic scars, cancer, Alzheimer's disease, scleroderma, glioblastoma, myeloid leukemia, acute myelogenous leukemia, myelodysplastic syndrome, myeloproliferative syndrome, inflammatory bowel disease, including collagenous colitis and ocular scarring and cataract.
Kidney fibrosis

- Renal (kidney) fibrosis is the principal process underlying the progression of chronic kidney disease (CKD) to end-stage renal disease (ESRD).

- Uniform response: distinct kidney diseases converge into this process - theoretically an excellent treatment target

- Involves:
  - glomerulosclerosis,
  - change in kidney vasculature,
  - **tubulointerstitial fibrosis** (predictor of irreversible loss of renal function)
Glomerular injury

Diabetic nephropathy, smoking

Glomerulosclerosis
Influx of inflammatory cells (lymphocytes, macrophages) and fibrocytes

Apoptosis of tubular epithelial cells and tubular atrophy

Epithelial-mesenchymal transformation (EMT): epithelial cell → myofibroblast

Accumulation of extracellular molecules (collagen)

Metabolic disease, drugs, atherosclerosis, Chronic kidney disease
Experimental model of interstitial fibrosis: Unilateral Ureteral Obstruction

Sham operated                     10 days of UUO
Masson’s trichrom staining

Sema Sivritas, Nicole Swavely
Quantification of fibrosis

Picrosirious red staining

Nicole Swavely
Therapy for chronic kidney disease and kidney fibrosis

- ACE inhibitors (in combination with ARBs) significantly increase life expectations, but do not cure the disease.
- Need new therapies and multidrug approaches in the future.

Problems: complicated regulatory mechanisms and redundancy in the system.

- Multidrug approaches are often not additive.
What to target in kidney fibrosis?
Keep looking into the RAAS

Renin-angiotensin-aldosterone system

- Angiotensinogen → Angiotensin I → Angiotensin II
- Renin
- Decrease in renal perfusion (juxtaglomerular apparatus)
- Lungs
- Kidney
- Surface of pulmonary and renal endothelium: ACE
- Tubular Na⁺ Cl⁻ reabsorption and K⁺ excretion. H₂O retention
- Adrenal gland: cortex
- Aldosterone secretion
- Arteriolar vasoconstriction. Increase in blood pressure
- ADH secretion (Pituitary gland: posterior lobe)
- Collecting duct: H₂O absorption

Legend:
- Blue: Secretion from an organ
- Green: Stimulatory signal
- Red: Inhibitory signal
- Black: Reaction
- Gray: Active transport
- Dashed: Passive transport

Water and salt retention. Effective circulating volume increases. Perfusion of the juxtaglomerular apparatus increases.
Potential new targets in RAAS

![Diagram of the RAAS system showing potential new targets](image)

- **Angiotensinogen**
  - **Renin**
    - **Angiotensin I**
      - **ACE, Chymase**
      - **Angiotensin II**
        - **ACE**
        - **ACE2**
        - **NEP**
      - **Angiotensin-(1-7)**
        - **ACE2**
      - **Angiotensin-(1-9)**
        - **ACE2**
      - **MLN-4760**
      - **losartan**
      - **AT1**
      - **AT2**
    - **Mas receptor**

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Vasopeptididase inhibition

- More pronounced blood pressure decrease, but
- Higher incidence of angioedema compared to ACEI
Targeting Epithelial-Mesenchymal Transition (EMT) through Smad

- TGF-beta is one of the best known profibrotic signaling molecules.
- Besides increasing secretion of collagen.
- It contributes to EMT.
- Smad-3 inhibitors are in clinical trials.
### Metalloenzyme involvement

<table>
<thead>
<tr>
<th>Target group</th>
<th>Therapeutic target and/or approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin–angiotensin–aldosterone system</td>
<td>ACE (ACEI), Ang II R 1 (ARB), aldosterone (aldosterone antagonists), ACE + neutral endopeptidase (vasopeptidase inhibitors), renin (renin inhibitors, (P)RR antagonist)</td>
</tr>
<tr>
<td>Kallikrein–kinin system</td>
<td>kallikrein–kinin–B2 receptors; kininase II</td>
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<tr>
<td>Endothelin</td>
<td>ET\textsubscript{a} and ET\textsubscript{b} (dual inhibitor bosentan)</td>
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<tr>
<td>Sympathic nerve system</td>
<td>(\alpha) and (\beta) blockers, moxonidine</td>
</tr>
<tr>
<td>Environmental factors</td>
<td>cessation of smoking, stress, caffeine intake, infections, heavy metals</td>
</tr>
<tr>
<td>Obesity, hypercholesterolemia</td>
<td>diet, statins</td>
</tr>
<tr>
<td>Immunosuppressants</td>
<td>MMF, rapamycin, FTY720, dexamethasone</td>
</tr>
<tr>
<td>ECM turnover and composition</td>
<td>MMP-1, MMP-2, TIMPs-1, ADAM-19, ADAM-17, ADAMTS-1, tissue transglutaminase, integrins (a1(\beta)1, a(\beta)6), ILK, relaxin (relaxin), trypsin+bromelain+rutosid, pirfenidone</td>
</tr>
<tr>
<td>Fibrinolytic system</td>
<td>PAI-1, tPA, plasminogen, uPAR</td>
</tr>
<tr>
<td>Complement system</td>
<td>C5 (anti-C5 Ab), C5b-9 (CD59), C5a (C5aR antagonist), Cr Gry</td>
</tr>
<tr>
<td>Cytokines</td>
<td>IL-1 (IL-1 receptor antagonist), IL-4, IL-8, IL-10 (anti-IL-10 Ab), IFN-(\gamma) (IFN-(\gamma)), IFN-(\alpha) (IFN-(\alpha)), TNF-(\alpha) (anti-TNF-(\alpha)-Ab)</td>
</tr>
<tr>
<td>Chemokines</td>
<td>MCP-1/CCR2, RANTES/CCR1, M-CSF, osteopontin, CX3CR1, SLC/CCR7 (chemokine receptor antagonists)</td>
</tr>
<tr>
<td>TGF-(\beta) signalling</td>
<td>TGF-(\beta), Smad-7, Smad-3 (halofuginone), Snail, Ski, SnoN, ALK5, decorin</td>
</tr>
<tr>
<td>TGF-(\beta) antagonists</td>
<td>BMP-7, USAG-1, KCP</td>
</tr>
<tr>
<td>Other growth factors</td>
<td>PDGF (see Table 3), HGF, CTGF (Anti-CTGF Ab), FGF-1, FGF-2, VEGF (anti-VEGF-Ab), EGFR (anti-EGFR-Ab)</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>L-arginine, iNOS, eNOS, sGC, PDE (PDE antagonist pentoxifiline), PDE-4, PDE-5 (PDE-5 antagonist sildenafil), NO-donors</td>
</tr>
<tr>
<td>Intracellular transduction cascades</td>
<td>NF-(\kappa)B (curcumin), Rho/ROCK (Rho inhibitors), p38 MAPK (p38 MAPK inhibitors), JNK, PKC-(\beta), PI3K(\gamma) (PI3K(\gamma) inhibitors), transcription factor Sp1, various tyrosin kinase inhibitors</td>
</tr>
<tr>
<td>Various</td>
<td>stem cells, mast cells, B-cells, selectins, AGES, AOPPs, PPAR(\gamma) (glitazones), ADMA (DDAH), tranilast, 1,25-dihydroxyvitamin D, paracalcitol, retinoid receptor agonist isotretinoin, erythropoietin, polyunsaturated fatty acids, thromboxane receptor antagonist terutroban, N-acetyl-cysteine</td>
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Let’s talk about Metalloenzymes!
Metalloenzyme domain structure

- Zn-dependent endopeptidases
- Produced as pre-pro-enzymes
- Activated by other MMPs, matrix molecules
- Secreted: MMP-2, -9
- Membrane-bound: MT-MMP
Matrix metalloenzymes in kidney fibrosis

- Matrix MetalloProteases (MMPs) regulate matrix turnover (degradation)
- Tissue Inhibitors of MetalloProteinases (TIMPs) are naturally occurring inhibitors of MMPs

Fibrosis=Imbalance: MMP activity<TIMP
ADAMs are metalloproteinase disintegrins, transmembrane proteins.

They cleave cell surface proteins: growth factors, inflammatory cytokines and receptors.

Gooz, M. Crit Rev Biochem Mol Biol 2010, 45;147
ADAMTS (ADAM-TS)

- ADAMTSs are metalloproteinase disintegrins with ThromboSpondin motifs, secreted proteins.
- They cleave matrix molecules (procollagen, versican, aggrecan), and the von Willebrand factor
ADAMTS variable modules range between 1-14 TS module.

From: "One Fish, Two Fish" by Dr. Seuss.
## ADAMs in kidney physiology and pathophysiology

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<tr>
<th>Enzyme</th>
<th>Role</th>
<th>Role in kidney</th>
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<tr>
<td>ADAMTS-1</td>
<td>Inflammation, inhibition of vasculogenesis</td>
<td>urogenital system development</td>
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<td>ADAMTS-9</td>
<td></td>
<td>Expressed in developing kidney</td>
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<td>Expressed in developing kidney</td>
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<td>ADAMTS-13</td>
<td>Cleavage of von Willebrand factor</td>
<td>Thrombotic microangiopathy (TMA)</td>
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<td>Present in renal epithelial cells</td>
<td>Mesangial cell migration</td>
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<td></td>
<td>Kidney carcinoma</td>
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<td>ADAM17</td>
<td>Vasculogenesis, lung development</td>
<td>Kidney fibrosis induced by 5-HT or A-II Polycystic kidney disease</td>
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<td>Present in convoluted tubules of the kidney</td>
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<td>All ADAMs</td>
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<td>Renal cell carcinoma (biomarkers?)</td>
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ADAM17/TACE (Tumor Necrosis Factor Converting Enzyme)

Interstitial fibrosis has an inflammatory component

- Inflammation (Leukocyte recruitment)
- Insulin resistance

Black et al; Moss et al  Nature 1997
Role of ADAM17 in Insulin Resistance and Diabetes

- **TNFα is over-expressed** in the adipose tissue and skeletal muscle of obese humans.
- **ADAM17 activity is upregulated** in skeletal muscle of obese type 2 DM patients (TIMP-3↓).
- **Anti-TNF drugs improve insulin resistance** in patients with rheumatoid arthritis.
ADAM17 in Insulin Resistance and Diabetes - Animal models

- Mice heterozygous for ADAM17 are partially protected from obesity-induced insulin resistance and diabetes (Serino et al Diabetes 2007)
- ADAM17 inhibitor improves insulin resistance in fructose-fed rats: ADAM17 plays important role in non-obese insulin-resistant model (Togashi et al Hypertension 2002)
TNFα-induced Insulin Resistance

Cell survival: MAPK cascade activation (JNK, ERK p38) and NF-kB pathway
Apoptosis: Caspases

Crosstalk between GPCRs and the EGFR

Profibrotic:
- ANGII
- 5-HT

GPCR

ADAM17

Growth Factor

EGFR

MAPK (ERK)

Proliferation, fibrosis

Prenzel et al. Science 1999
Role of ADAM17 in mesangial cell proliferation

Proliferation of mesangial cells, Fibrosis

Gooz et al. JBC 2006
ADAM17 and chronic kidney disease

5-HT

AII

EGF-like

EGFR

MMP/ADAM


Control

Ang-II

Ang-II + ADAM17 inhibitor

Mononuclear cell infiltration
Interstitial fibrosis
ADAM17 in Polycystic Kidney Disease (PKD)

Bpk mice (AR model) ADAM17 inhibitor decreased cyst formation
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ADAMTS-13: von Willebrand factor cleaving enzyme

These smaller vWF molecules do not cause platelet aggregation in the circulation.

- microangiopathic hemolytic anemia
- thrombocytopenia
- microvascular thrombosis:
- fever
- neurological abnormalities
- renal failure

Semin in Hematol. 2004; 41(1)
Summary / Drug development

- **Metalloenzymes** are important therapeutic targets in renal fibrosis and CKD
- **ADAM inhibitors** are developed and are in clinical study
- **Combinational** therapy
- First treatment approved and now available for atypical HUS is Soliris (Eculizumab), made by Alexion.