Role of Metalloenzymes

PATHOMECHANISM OF TISSUE FIBROSIS

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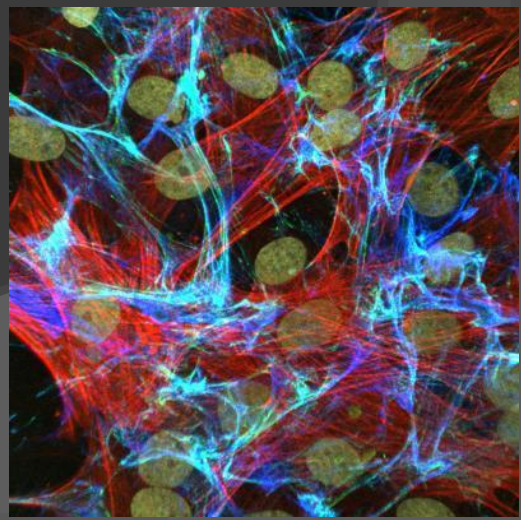
Fibrosis and fibrotic diseases

- 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 word-wide

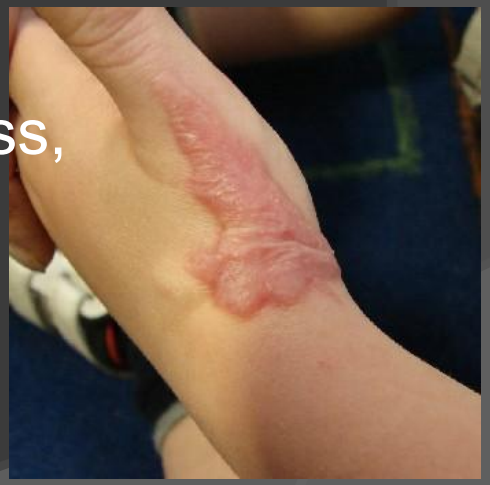


ScienceDaily 2007

Why do we have fibrosis?

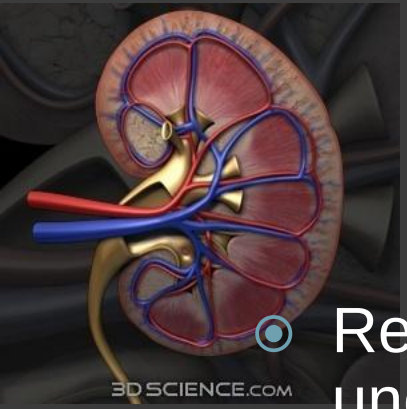
Fibrotic diseases

- 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

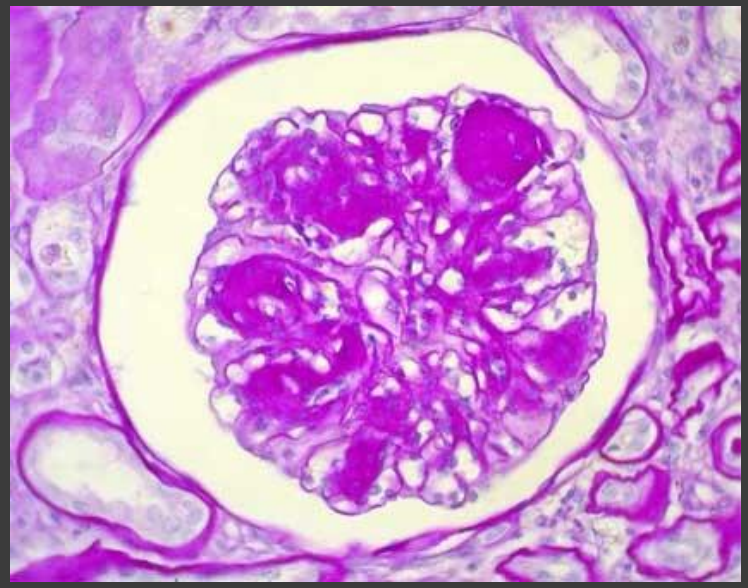
- pulmonary fibrosis, interstitial lung disease, liver fibrosis, cardiac fibrosis, macular degeneration, retinal and vitreal retinopathy, myocardial fibrosis, cardiovascular disease, atherosclerosis/restenosis, keloids and hypertrophic scars, cancer, Alzheimer's disease, scleroderma, glioblastoma, myleoid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



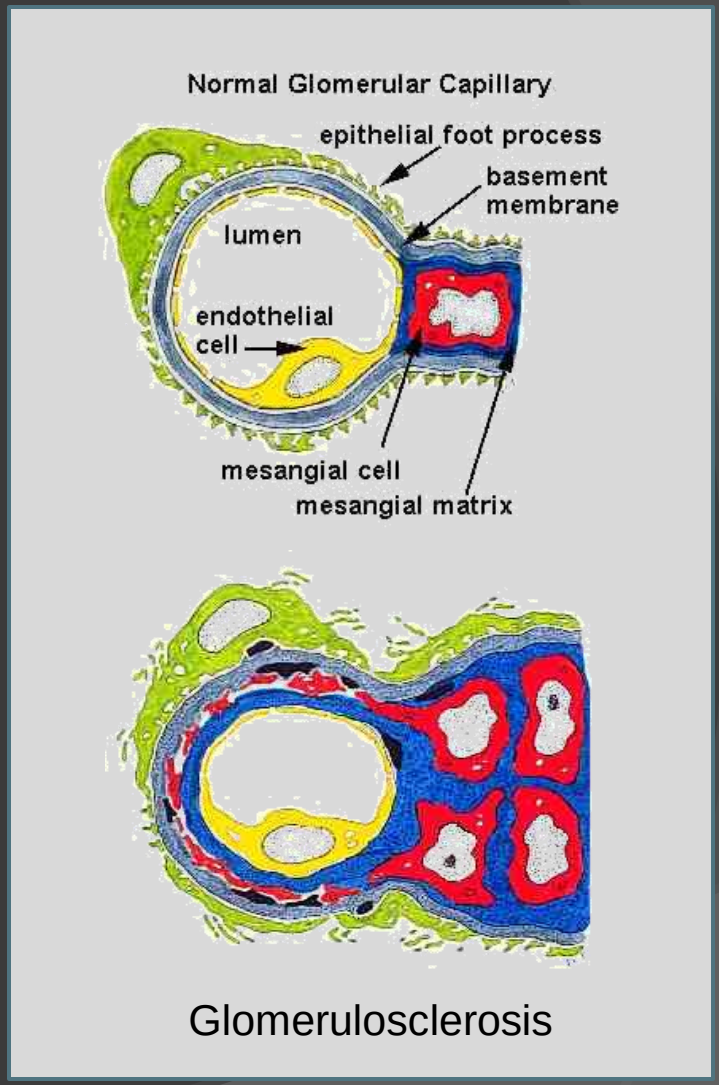
Kidney fibrosis

- 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



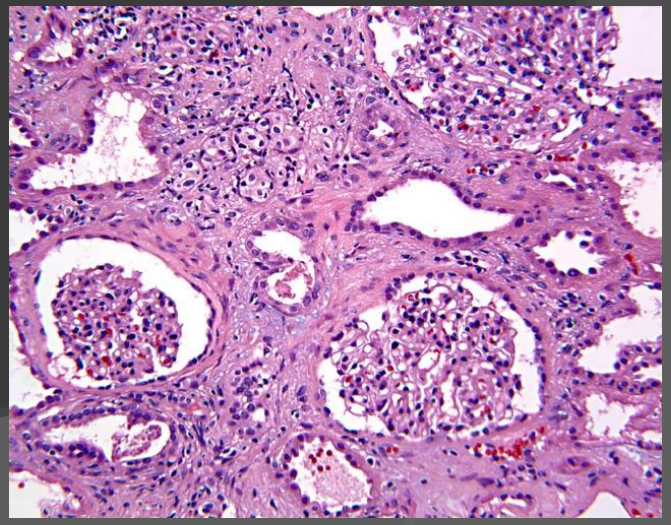
Glomerulosclerosis

Tubulointerstitial injury

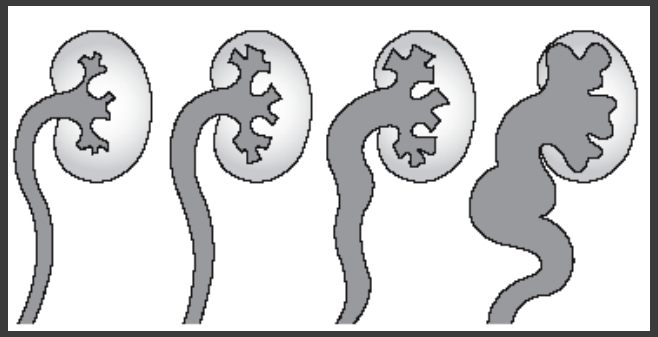
- Influx of inflammatory cells (lymphocytes, macrophages) and fibrocytes
- Apoptosis of tubular epithelial cells and tubular atrophy
- Epithelial-mesenchymal transformation (EMT):

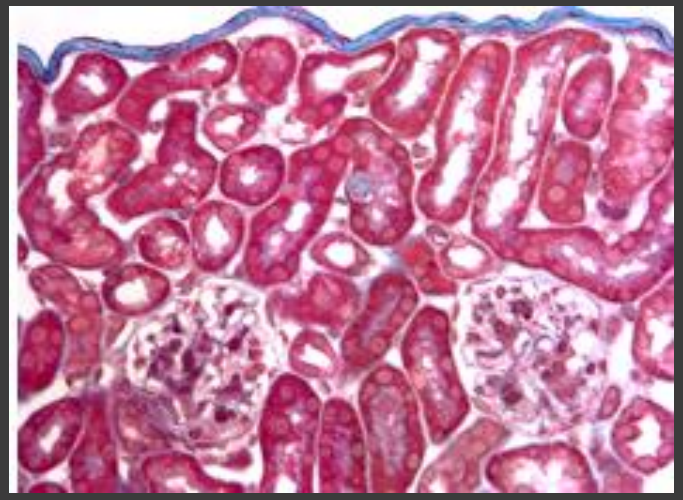
  epithelial cell $\rightarrow$ myofibroblast
- Accumulation of extracellular molecules (collagen)

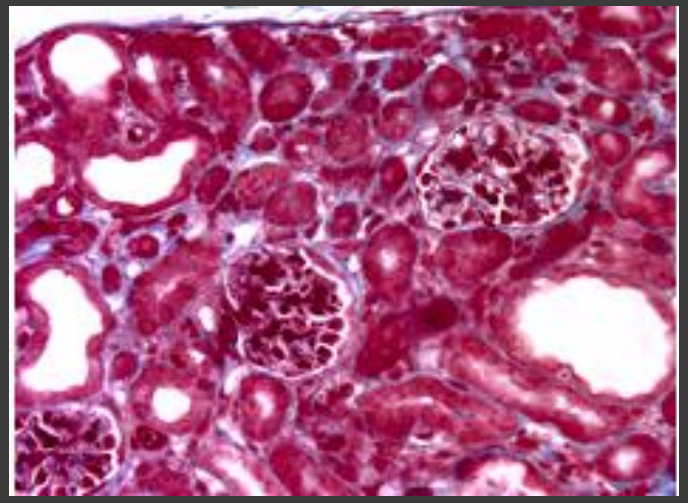
Metabolic disease, drugs, atherosclerosis,
Chronic kidney disease



Experimental model of interstitial fibrosis: Unilateral Ureteral Obstruction







Sham operated

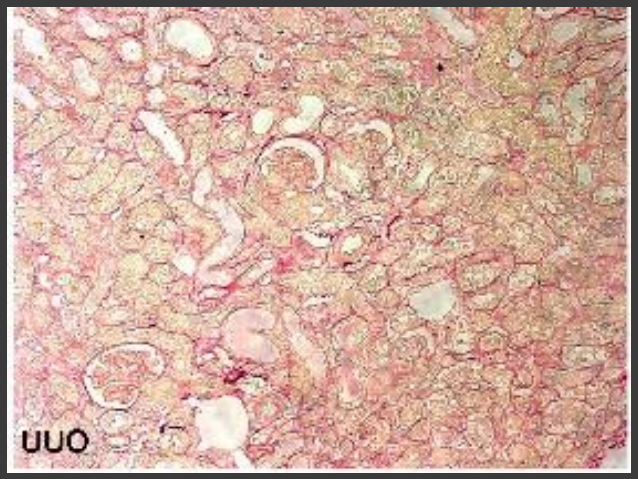
10 days of UUO

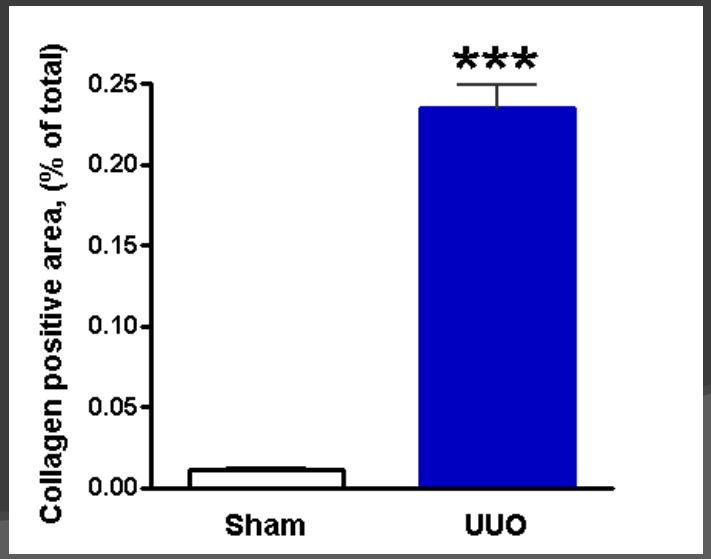
Masson's trichrom staining

Sema Sivritas, Nicole Swavely

Quantification of fibrosis

Picrosirious red staining





Nicole Swavely

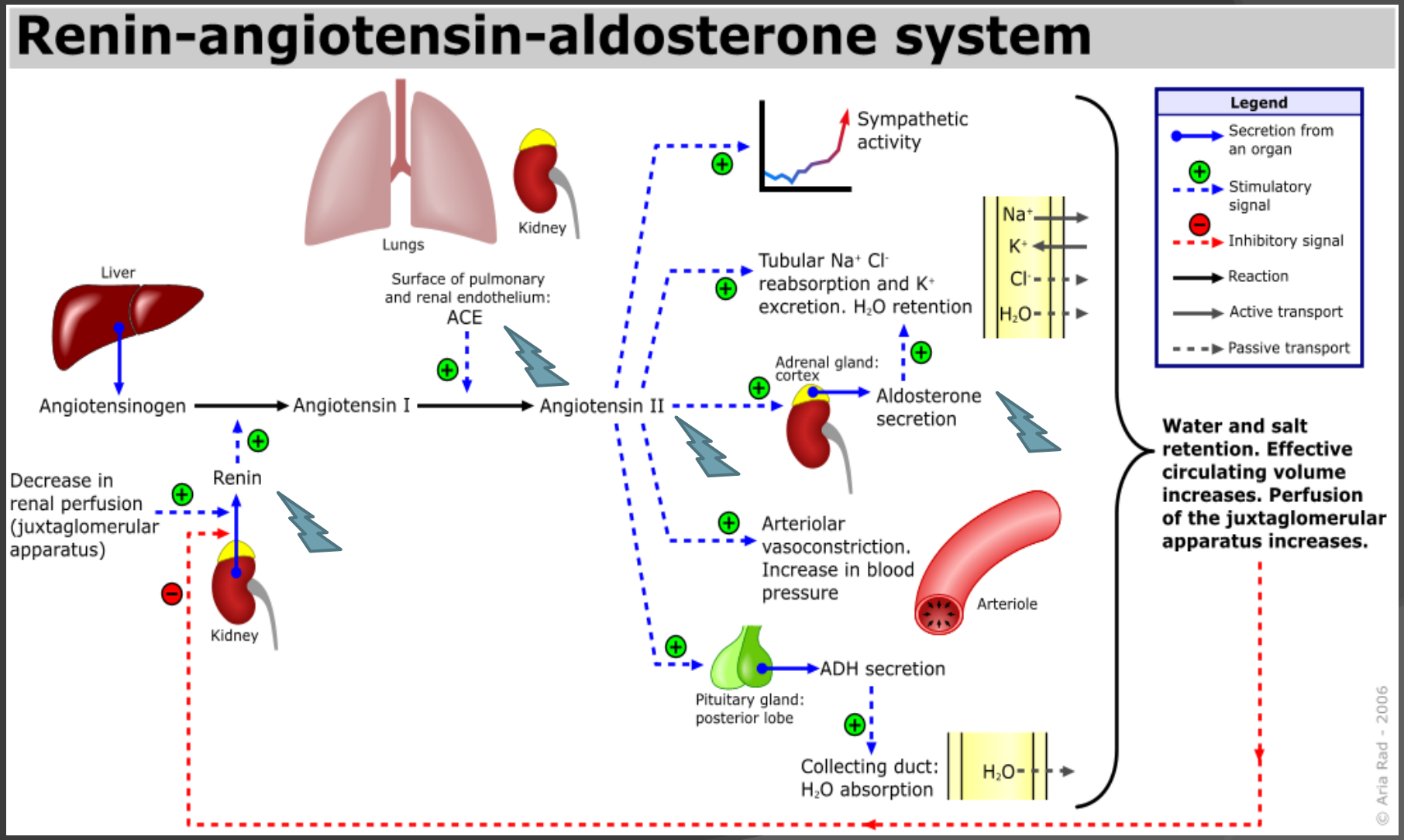
Therapy for chronic kidney disease and kidney fibrosis

- 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

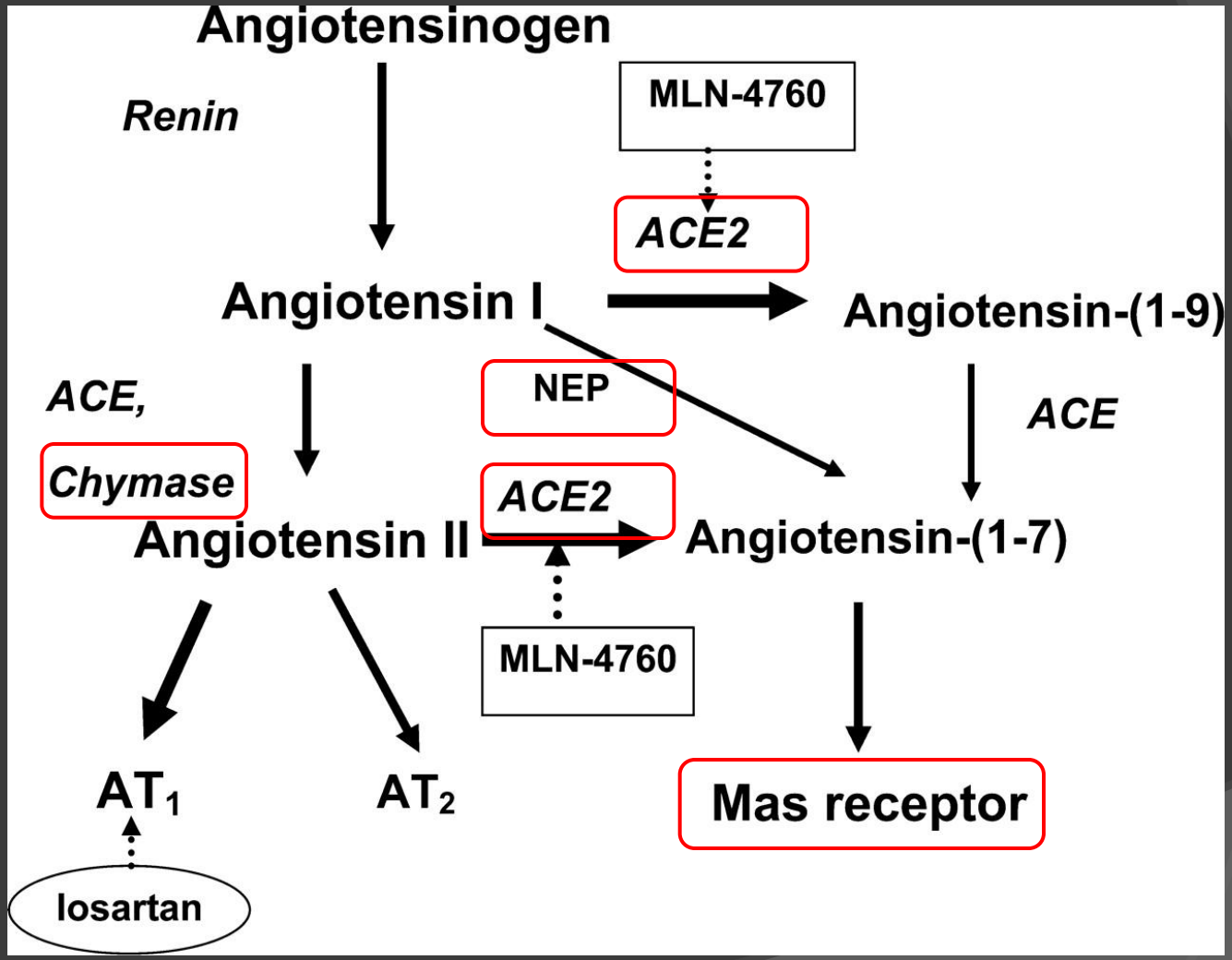
- Problems: complicated regulatory mechanisms and redundancy in the system
- Multidrug approaches are often not additive

What to target in kidney fibrosis?

Keep looking into the RAAS

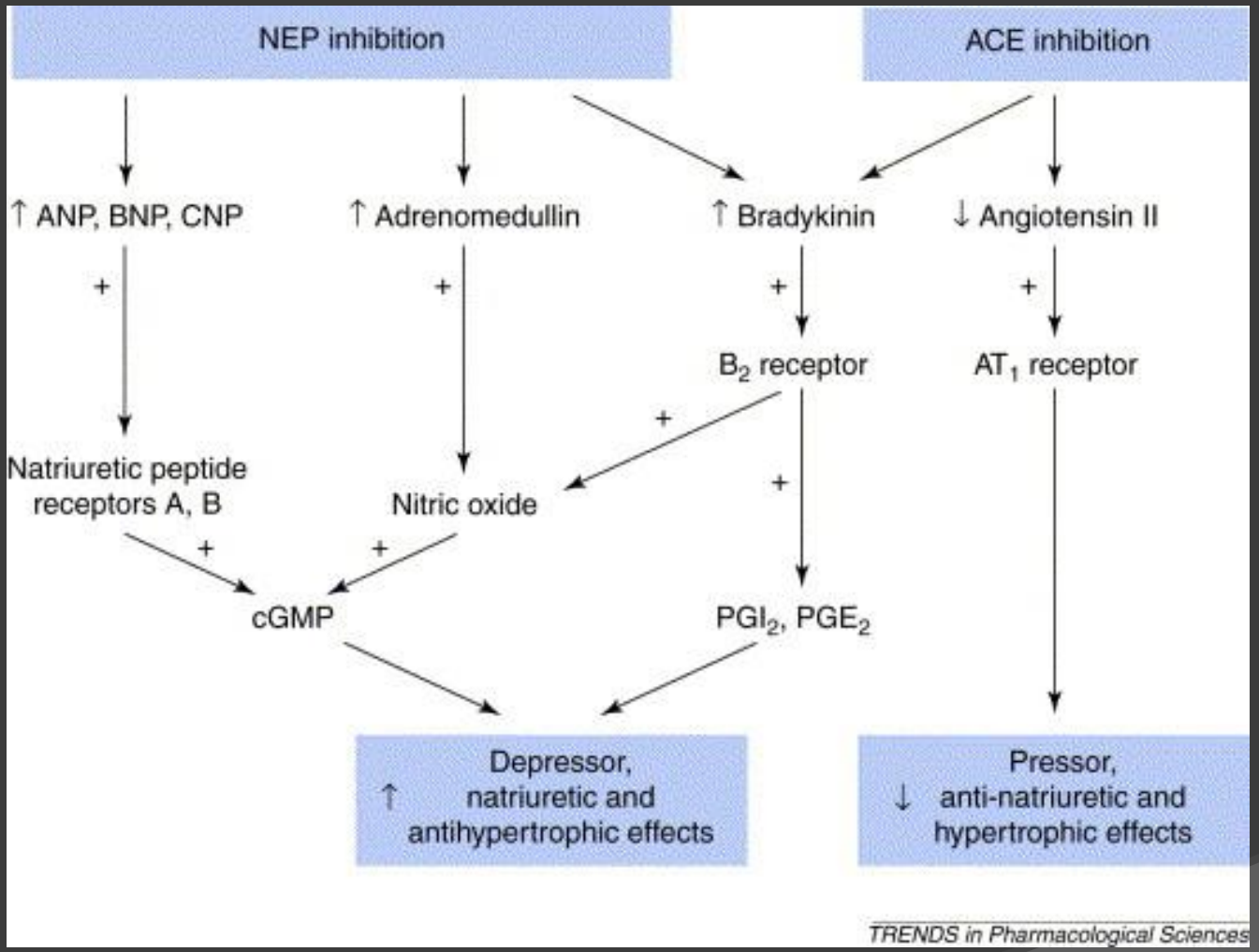


Potential new targets in RAAS



Dilauro M et al. Am J Physiol Renal Physiol 2010;298:F1523-F1532

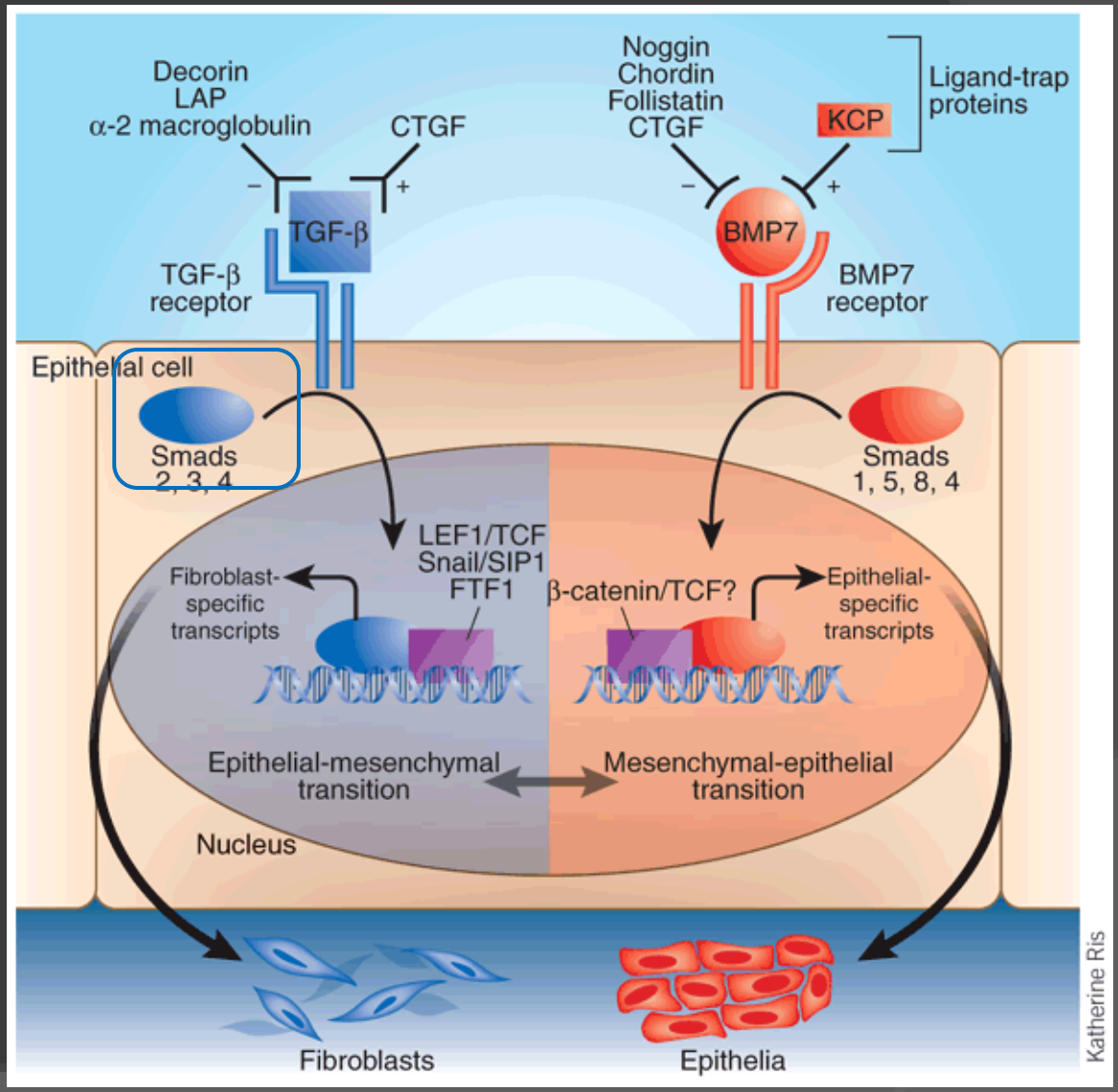
Vasopeptidase inhibition



- 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 know profibrotic signaling molecule
- Besides increasing secretion of collagen
- It contributes to EMT
- Smad-3 inhibitors are in clinical trials



Treatment targets in renal fibrosis

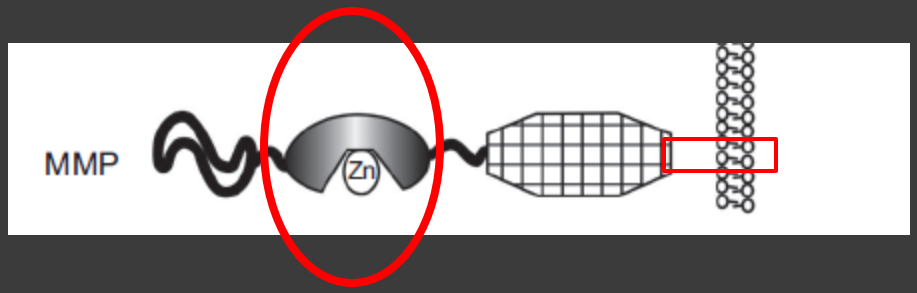
Metalloenzyme involvement

Already available / in trials

Target group	Therapeutic target and/or approach
Renin–angiotensin–aldosterone system	ACE *(ACEI)*, Ang II R 1 *(ARB)*, aldosterone *(aldosterone antagonists)*, ACE + neutral endopeptidase *(vasopeptidase inhibitors)*, renin *(renin inhibitors, (P)RR antagonist)*
Kallikrein–kinin system	kallikrein–kinin–B2 receptors; kininase II
Endothelin	ET_A and ET_B *(dual inhibitor bosentan)*
Sympathic nerve system	*α and β blockers, moxonidine*
Environmental factors	*cessation of smoking, stress, caffeine intake, infections, heavy metals*
Obesity, hypercholesterolemia	*diet, statins*
Immunosupressants	*MMF, rapamycin, FTY720, dexamethasone*
ECM turnover and composition	MMP-1, MMP-2, TIMPs-1, ADAM-19, ADAM-17, ADAMTS-1, tissue transglutaminase, integrins (α1β1, $\alpha_v\beta6$), ILK, relaxin *(relaxin), trypsin+bromelain+rutosid, pirfenidone*
Fibrinolytic system	PAI-1, tPA, plasminogen, uPAR
Complement system	C5 *(anti-C5 Ab)*, C5b-9 *(CD59)*, C5a *(C5aR antagonist)*, Crry
Cytokines	IL-1 *(IL-1 receptor antagonist)*, IL-4, IL-8, IL-10 *(anti-IL-10 Ab)*, IFN-γ *(IFN-γ)*, IFN-α *(IFN-α)*, TNF-α *(anti-TNF-α-Ab)*
Chemokines	MCP-1/CCR2, RANTES/CCR1, M-CSF, osteopontin, CX3CR1, SLC/CCR7 *(chemokine receptor antagonists)*
TGF-β signalling	TGF-β, Smad-7, Smad-3 (*halofuginone)*, Snail, Ski, SnoN, ALK5, decorin
TGF-β antagonists	BMP-7, USAG-1, KCP
Other growth factors	PDGF (see Table 3), HGF, CTGF *(Anti-CTGF Ab)*, FGF-1, FGF-2, VEGF *(anti-VEGF-Ab)*, EGFR *(anti-EGFR-Ab)*
Nitric oxide	L-arginine, iNOS, eNOS, sGC, PDE *(PDE antagonist pentoxifiline)*, PDE-4, PDE-5 *(PDE-5 antagonist sildenafil), NO-donors*
Intracellular transduction cascades	NF-κB *(curcumin)*, Rho/ROCK *(Rho inhibitors)*, p38 MAPK *(p38 MAPK inhibitors)*, JNK, PKC-β, PI3Kγ *(PI3Kγ inhibitors)*, transcription factor Sp1, *various tyrosin kinase inhibitors*
Various	stem cells, mast cells, B-cells, selectins, AGEs, AOPPs, PPARγ *(glitazones)*, ADMA *(DDAH), tranilast, 1,25-dihydroxyvitamin D, paracalcitol, retinoid receptor agonist isotretinoin, erythropoietin, polyunsaturated fatty acids, thromboxane receptor antagonist terutroban, N-acetyl-cysteine*

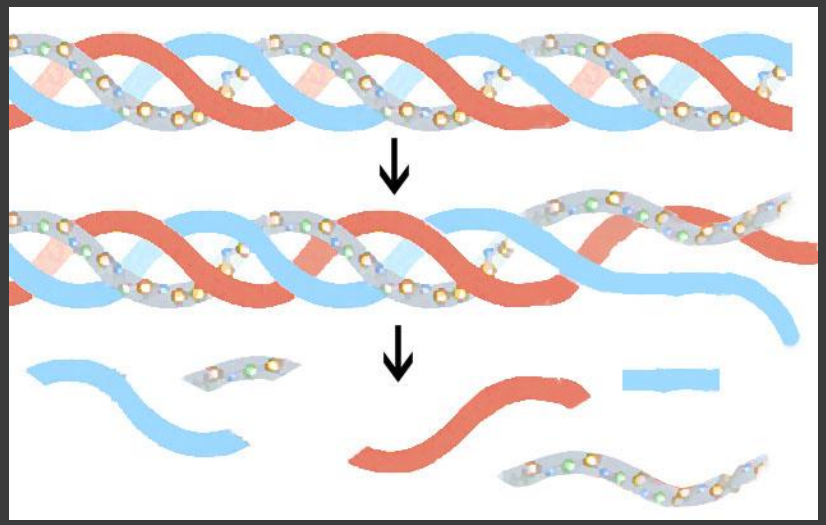
Let’s talk about Metalloenzymes!

Metalloenzyme domain structure



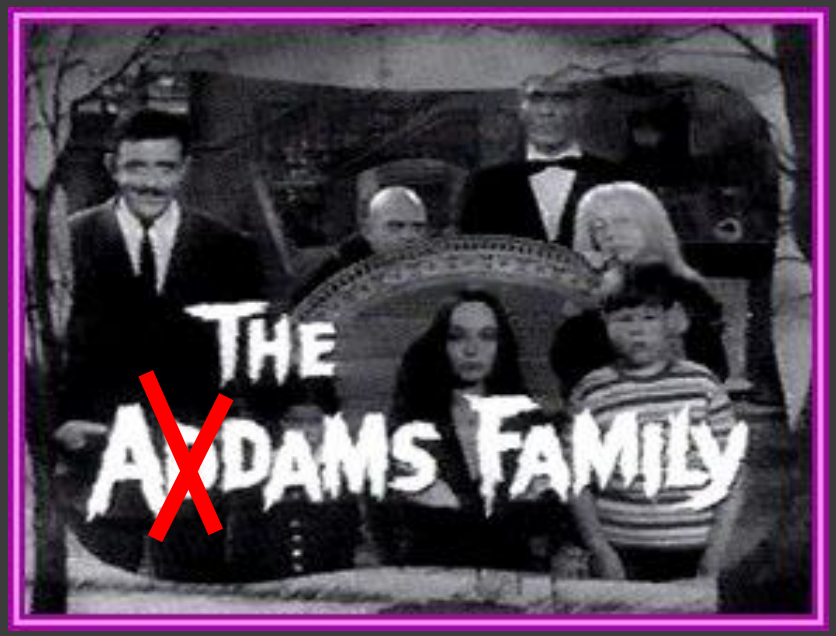
- Zn-dependent endopeptidases
- Produced as pre-pro-enzymes
- Activated by other MMPs, matrix molecules
- Secreted: MMP-2, -9
- Membrane-bound: MT-MMP

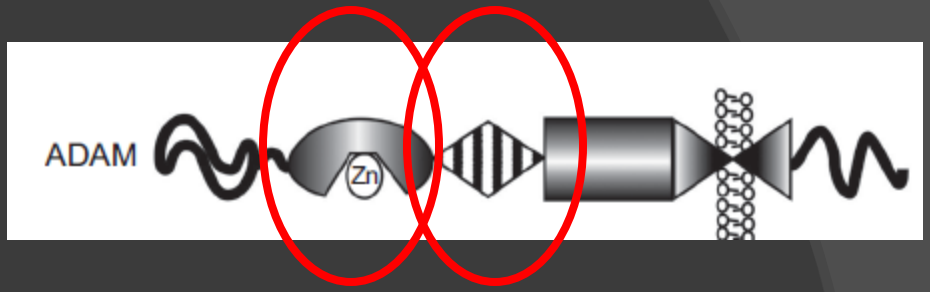
Matrix metalloenzymes in kidney fibrosis



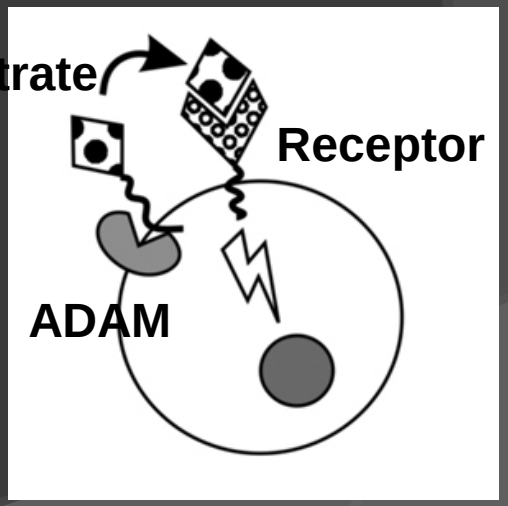
- Matrix MetalloProteases (MMPs) regulate matrix turnover (degradation)
- Tissue Inhibitors of MetalloProteinases (TIMPs) are naturally ocurring inhibitors of MMPs

Fibrosis=Imbalance: MMP activity<TIMP



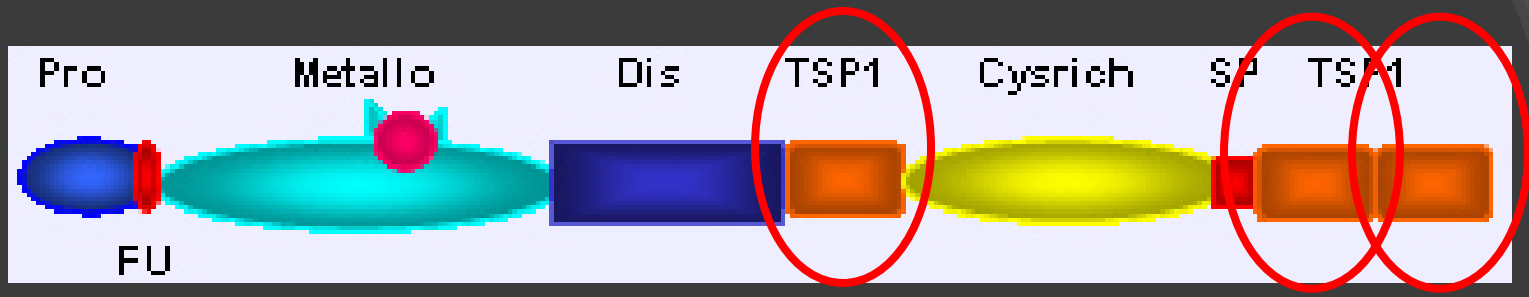


- ADAMs are metalloproteinase disintegrins, transmembrane proteins.
- They cleave cell surface proteins: growth factors, inflammatory cytokines and receptors.



Gooz, M. Crit Rev Biochem Mol Biol 2010, 45;147

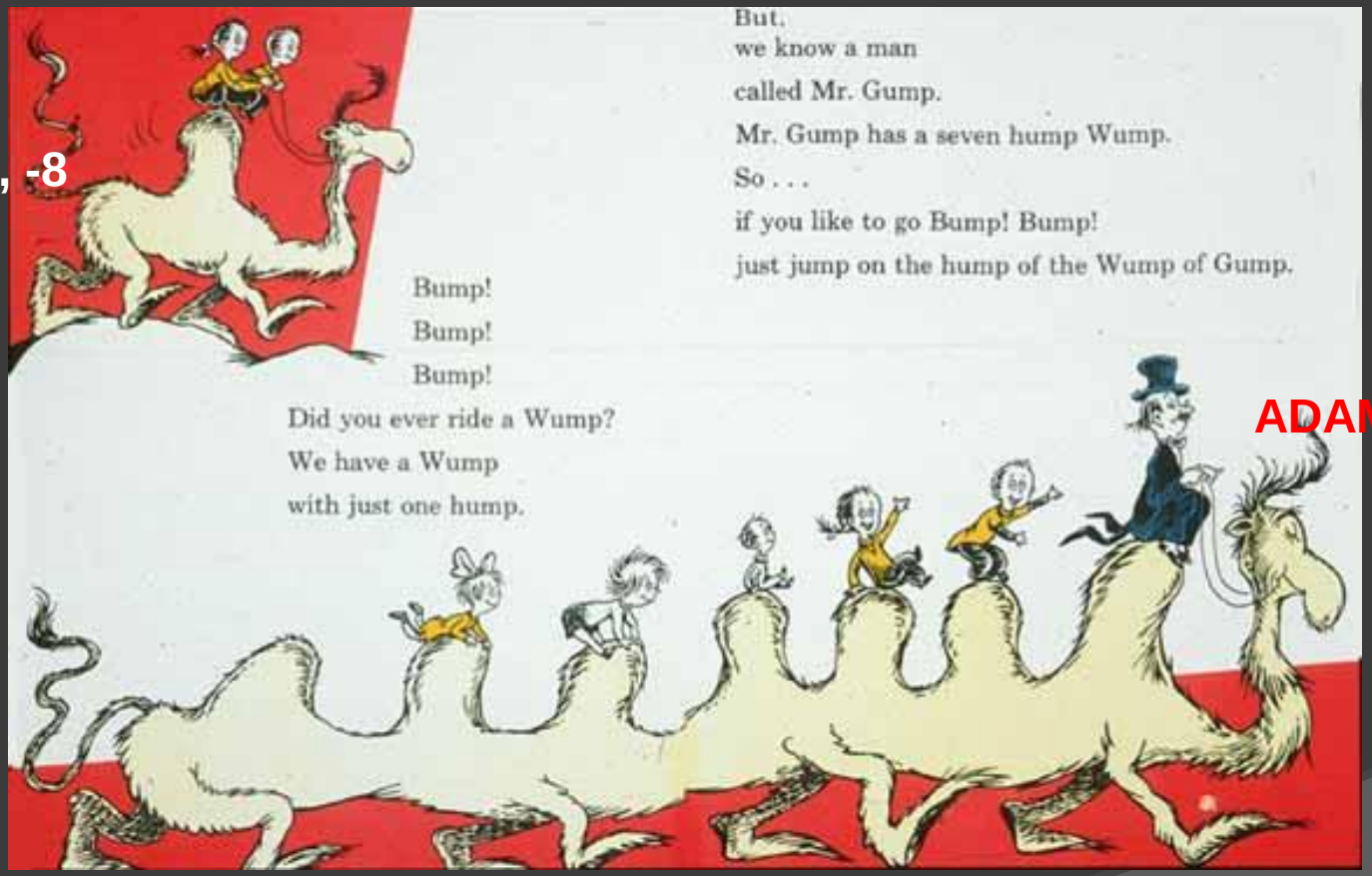
ADAMTS (ADAM-TS)



- 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

ADAMTS5, -8



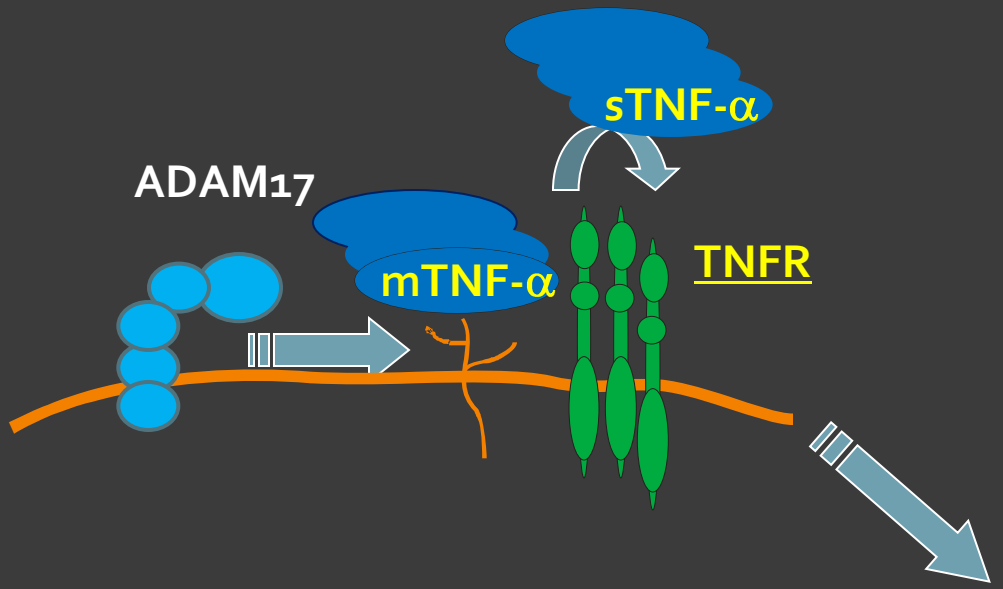
ADAMTS9,-20

From: "One Fish, Two Fish" by Dr. Seuss.

ADAMs in kidney physiology and pathophysiology

Enzyme	Role	Role in kidney
ADAMTS-1	Inflammation, inhibition of vasculogenesis	urogenital system development
ADAMTS-9		Expressed in developing kidney
ADAMTS-10		Expressed in developing kidney
ADAMTS-13	Cleavage of von Willebrand factor	Thrombotic microangiopathy (TMA)
ADAM9	Present in renal epithelial cells	
ADAM15		Mesangial cell migration Kidney carcinoma
ADAM17	Vasculogenesis, lung development	Kidney fibrosis induced by 5-HT or A-II Polycystic kidney disease Increased level during allograph rejection Kidney carcinoma
ADAM19		Nephrogenesis Increased expression in deteriorating kidney and renal allograph rejection
ADAM31		Present in convoluted tubules of the kidney
All ADAMs		Renal cell carcinoma (biomarkers?)

ADAM17/TACE (Tumor Necrosis Factor Converting Enzyme)



Interstitial fibrosis has an inflammatory component

- Inflammation (Leukocyte recruitment)
- Insulin resistance

A Disintegrin And Metalloenzyme-17

Black *et al*; Moss *et al* Nature 1997

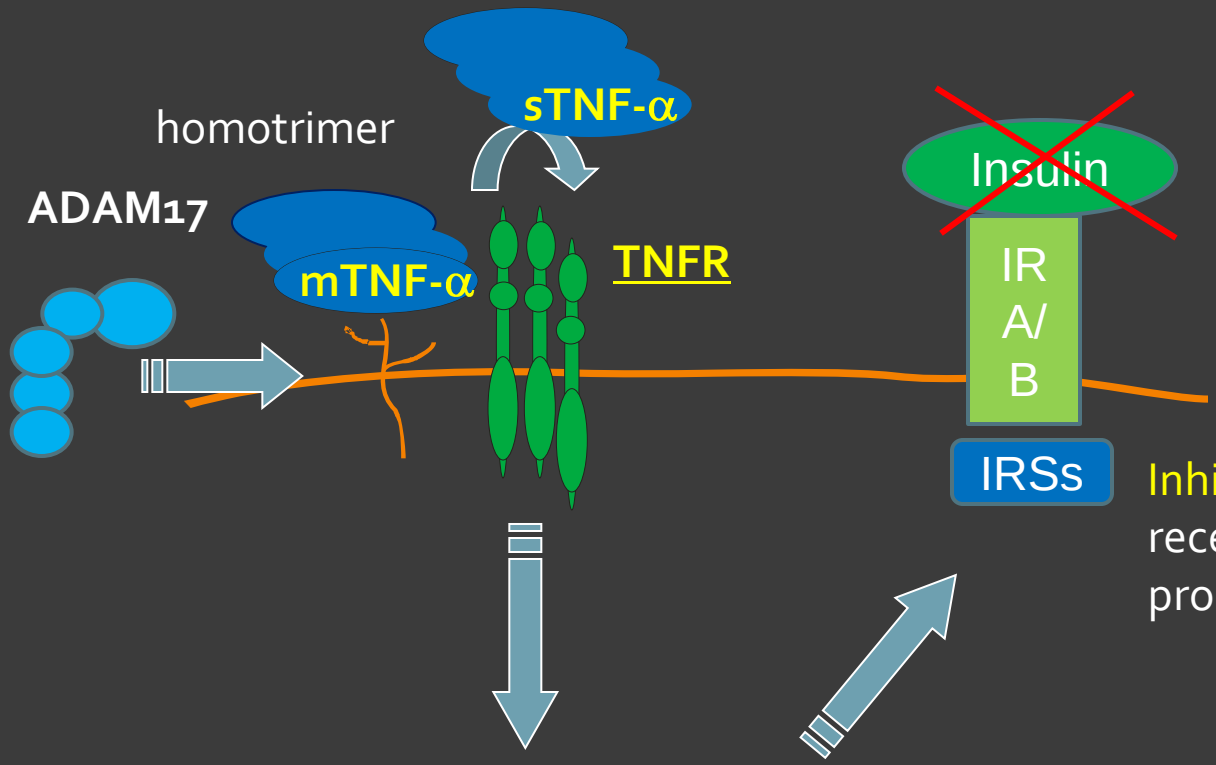
Role of ADAM17 in Insulin Resistance and Diabetes

- 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



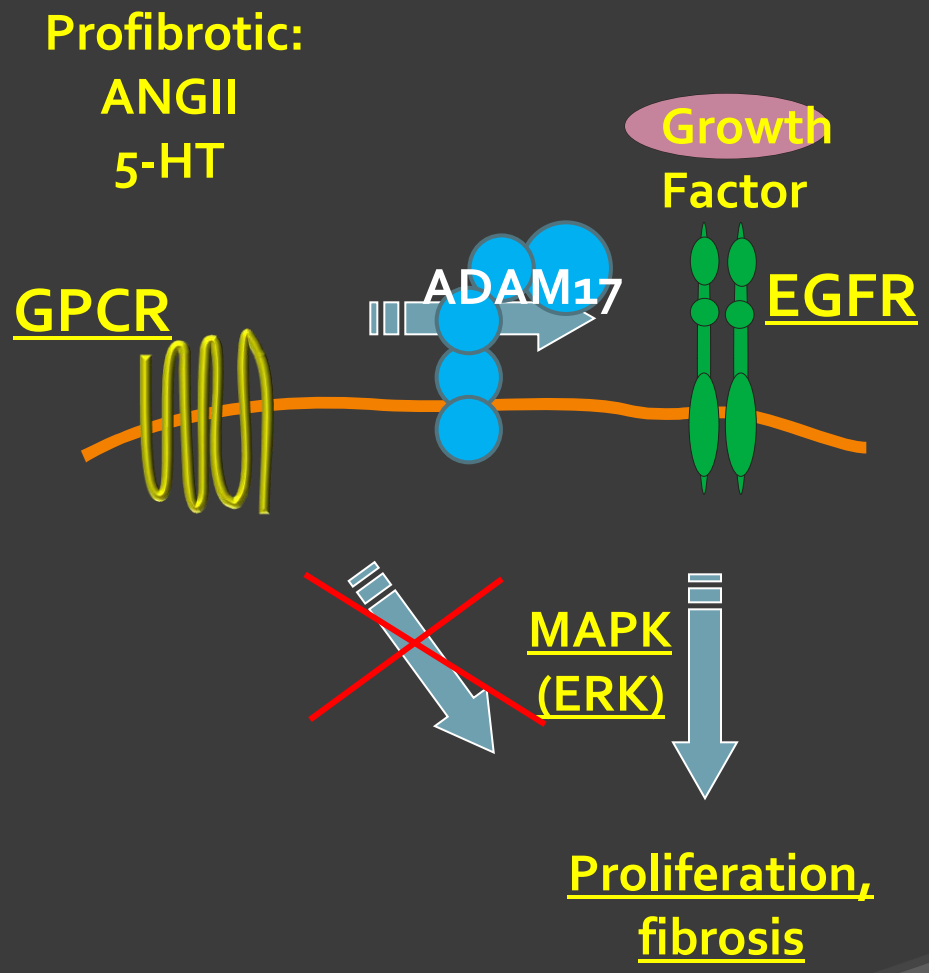
Inhibits Tyr-P of insulin receptor substrate (IRS) by promoting Ser-P

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

Apoptosis: Caspases

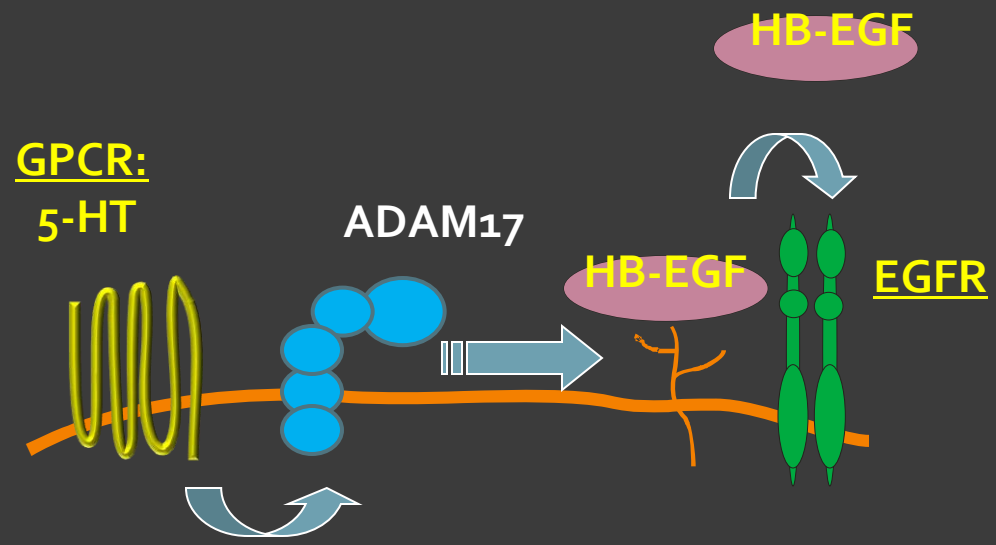
Reviewed by Taniguchi *et al.* Nat Rev Mol Biol 2006

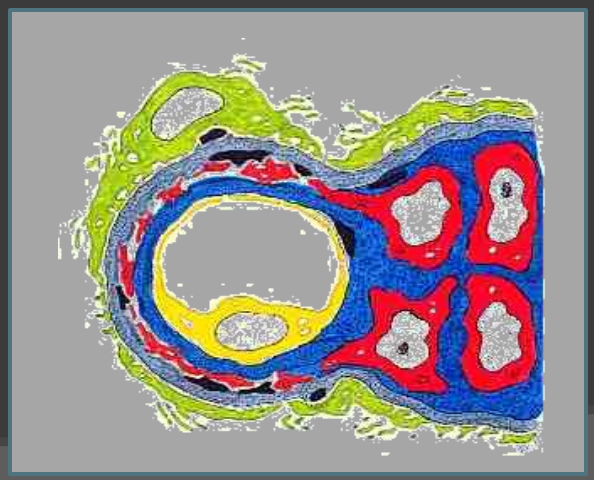
Crosstalk between GPCRs and the EGFR



Prenzel *et al* Science1999

Role of ADAM17 in mesangial cell proliferation

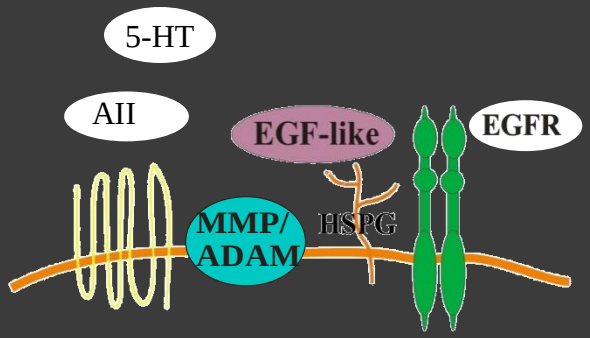


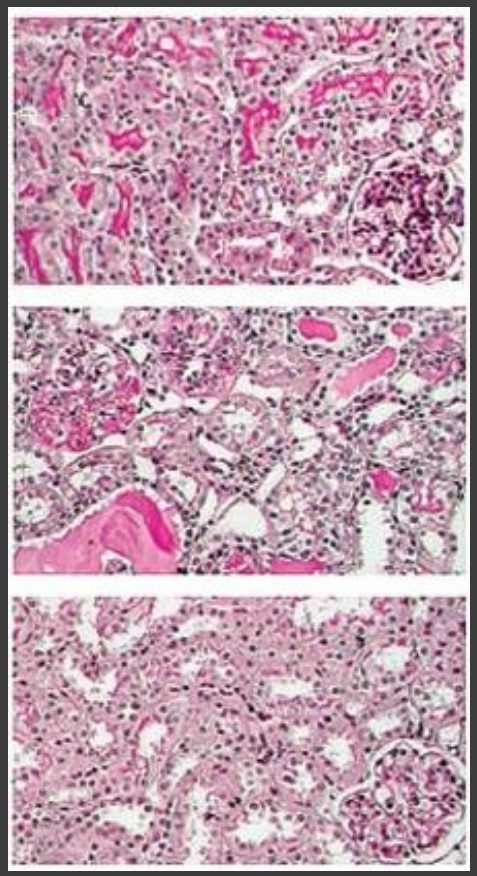


Proliferation of mesangial cells, Fibrosis

Gooz et al. JBC 2006

ADAM17 and chronic kidney disease





Control

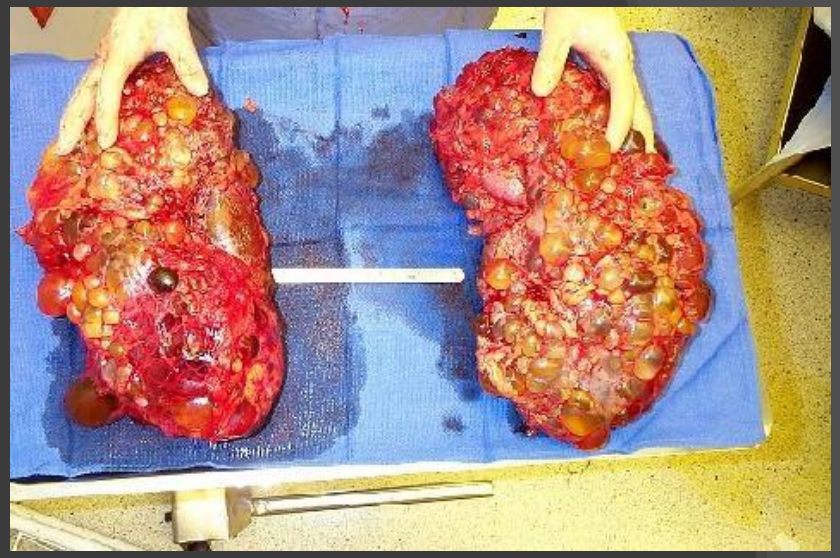
Ang-II

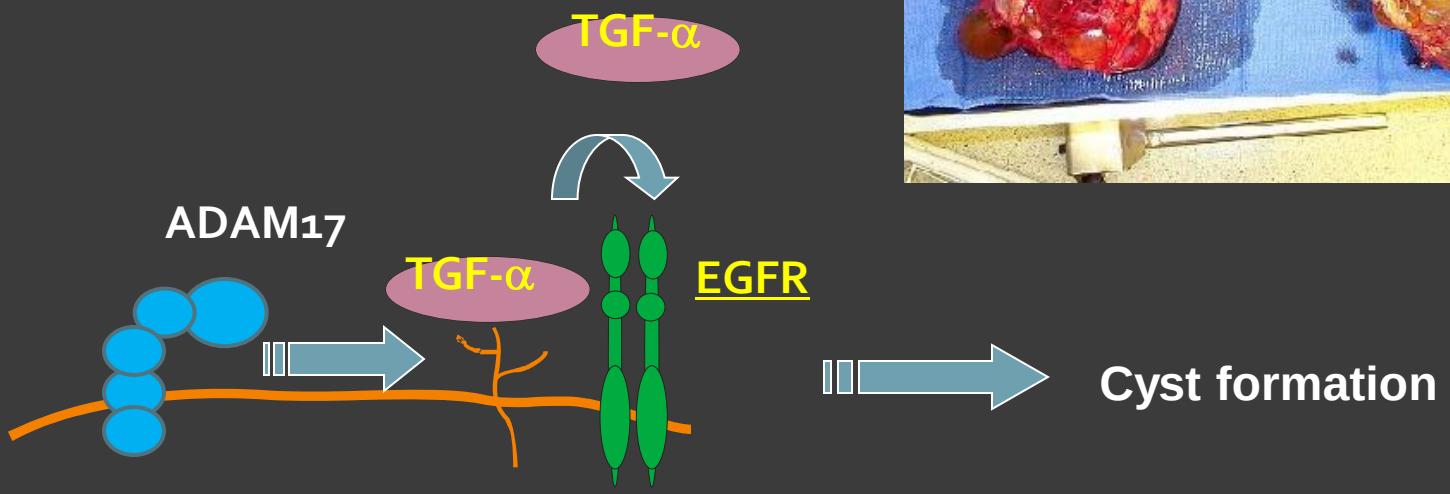
Ang-II +
ADAM17 inhibitor

Mononuclear cell infiltration
Interstitial fibrosis

Lautrette *et al.*, Nature Med 11(8):867-874, 2005.

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

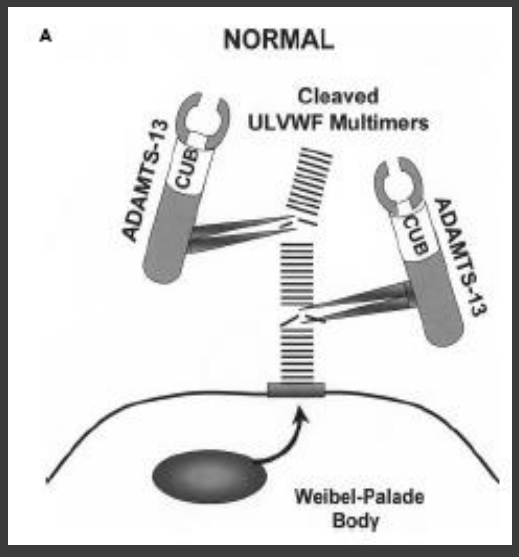
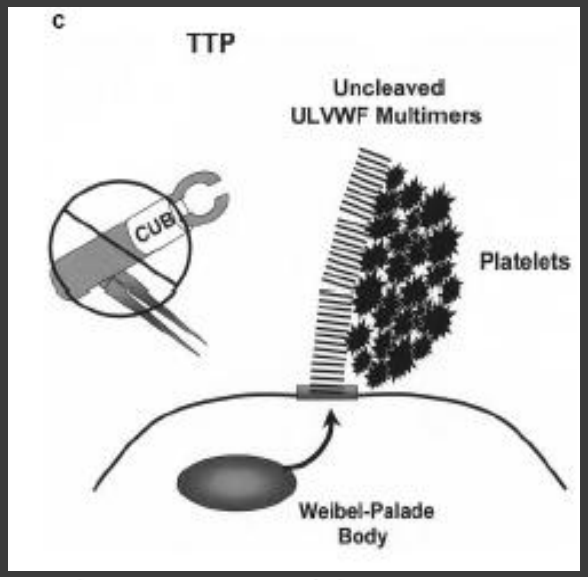


Figure 2. ADAMTS13 activity in normal and thrombotic thrombocytopenia purpura (TTP) plasma.

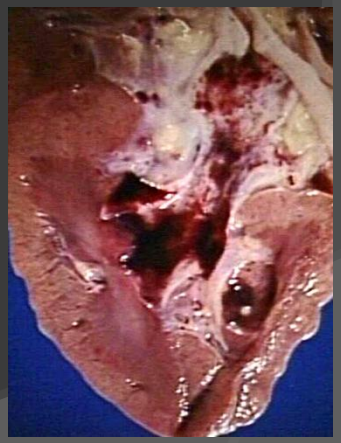
(A) In normal individuals, ADAMTS13 enzyme molecules from the plasma attach to, and then cleave, unusually large von Willebrand factor (ULVWF) multimers that are secreted in long "strings" from stimulated endothelial cells.

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



(C) Absent or severely reduced activity of ADAMTS13 in patients with TTP prevents the timely cleavage of ULVWF multimers secreted by endothelial cells. Uncleaved ULVWF multimers induce the adhesion and subsequent aggregation of platelets in flowing blood. Congenital deficiencies of ADAMTS13 activity caused by gene mutations or acquired defects of ADAMTS13 caused by autoantibodies result in TTP.

- 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.