Lessons From Liver Fibrosis

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ESHRE Workshop Edinburgh 2013

Take Home Messages:

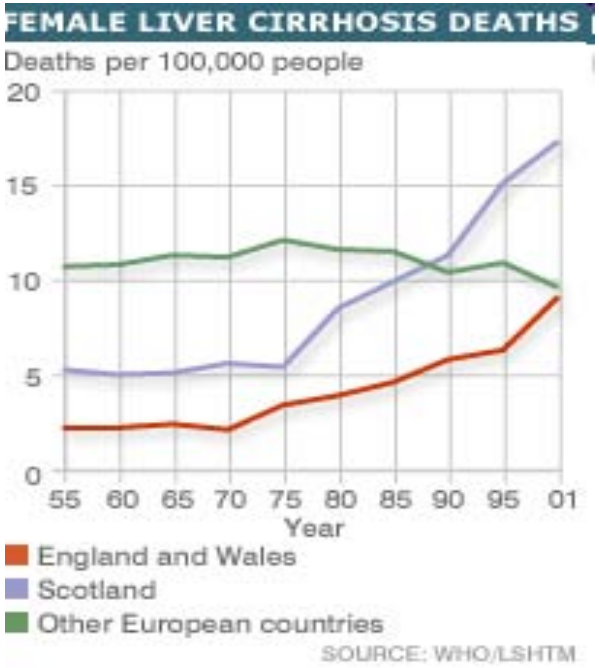
•Wound healing and fibrosis are generic mechanisms which demonstrate common attributes across a range of organs. Identified targets may well be valuable across organs

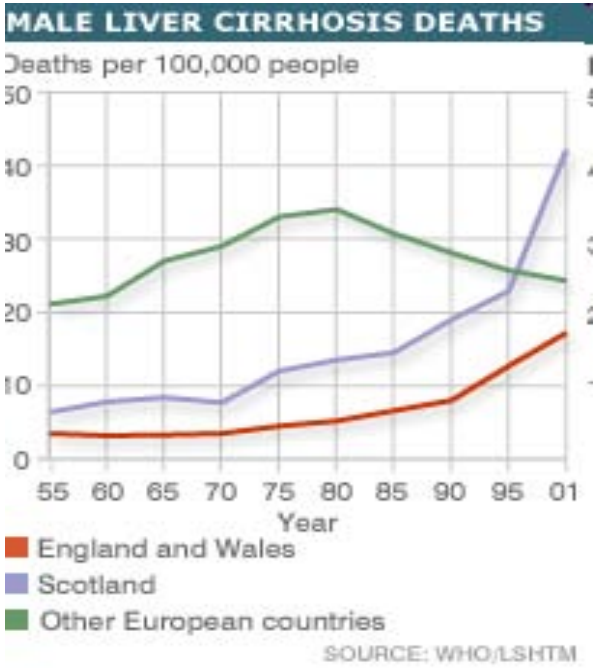
•The hepatic scar is dynamic with respect to both its cellular and matrix components and makes an excellent model to examine scar plasticity.

•The wound healing MFBs of the liver and the hepatic macrophages are key players in progressive and resolving fibrosis.

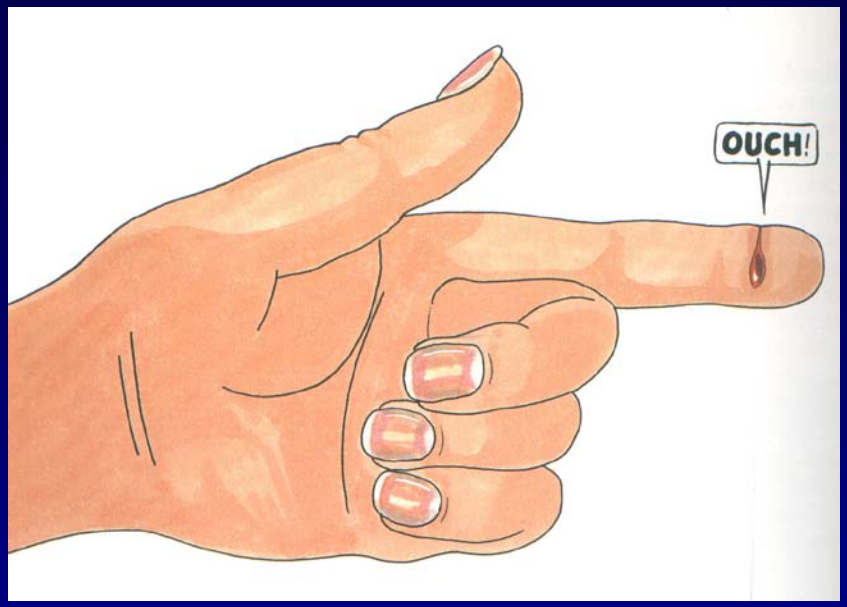
•Therapies targeting the TIMP/MMP balance and the dynamic functions of the wound healing MFB show promise not only in reducing fibrosis, but through alterations in contractility, portal pressure and other plastic attributes of scars.

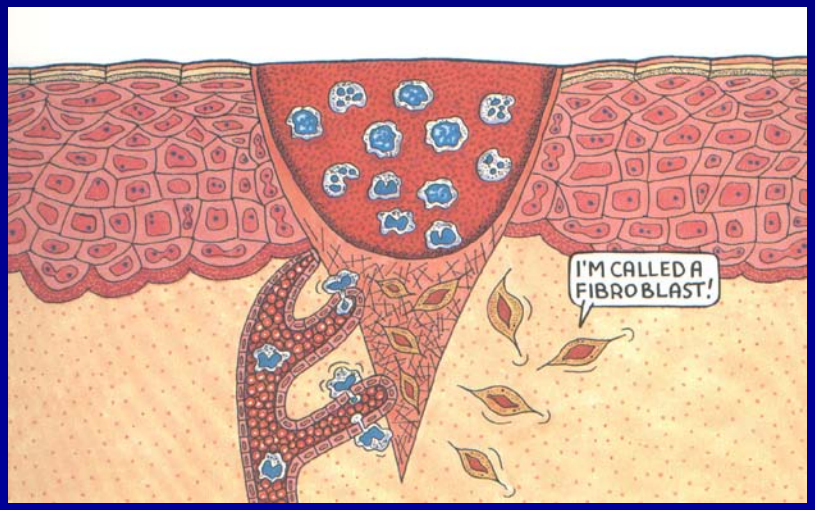
Cirrhosis in Scotland

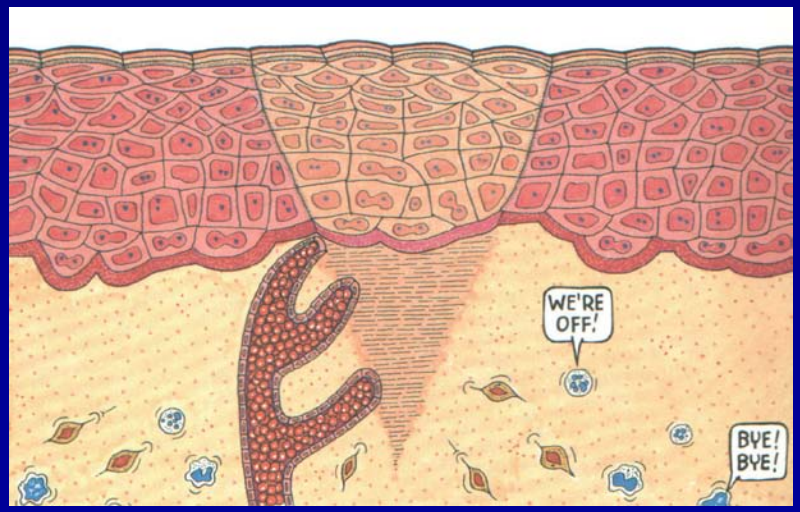




What do we mean by tissue fibrosis/repair?
Mammalian Wound Healing: A beginners guide







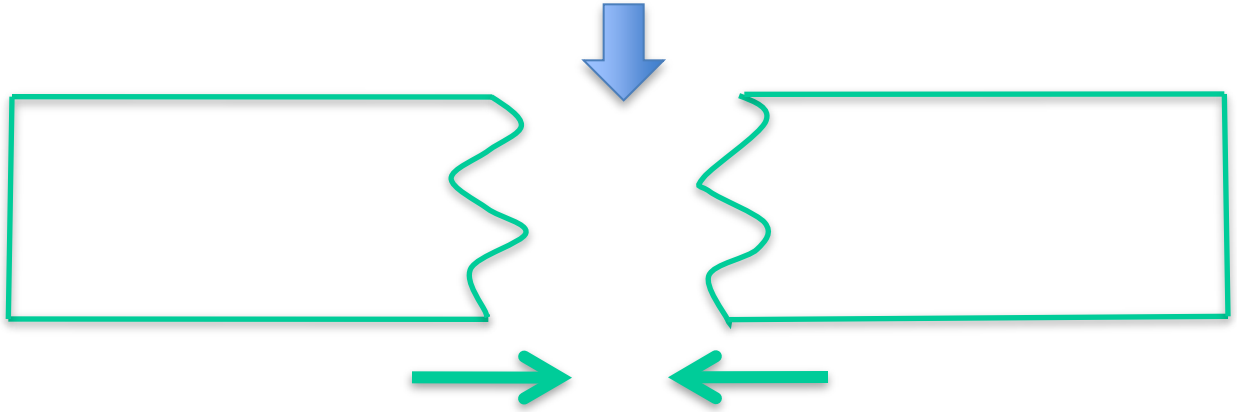
Collaborations underpinning Inflammation, fibrosis and regenerative medicine

Developmental Biology

Inflammation

Stem Cell Biology

Promote repair

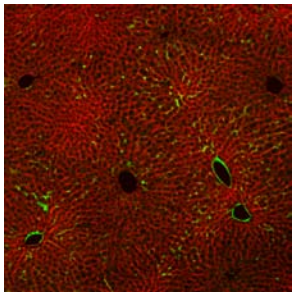


Tissue-specific Biology

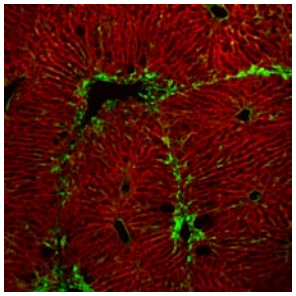
Tattooing the fibrogenic cell: mTmG;*PDGFRβ* Cre

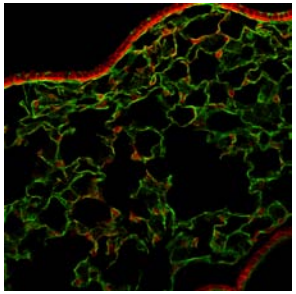
Uninjured

Fibrotic

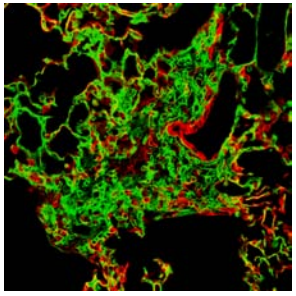


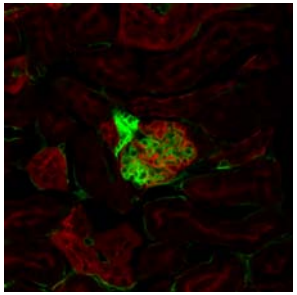
Liver



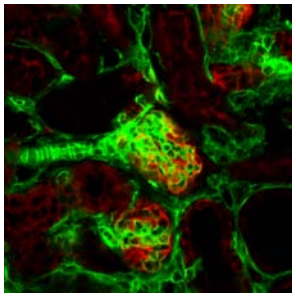


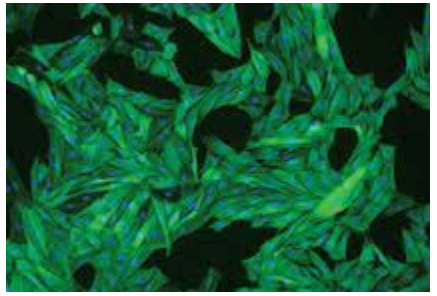
Lung



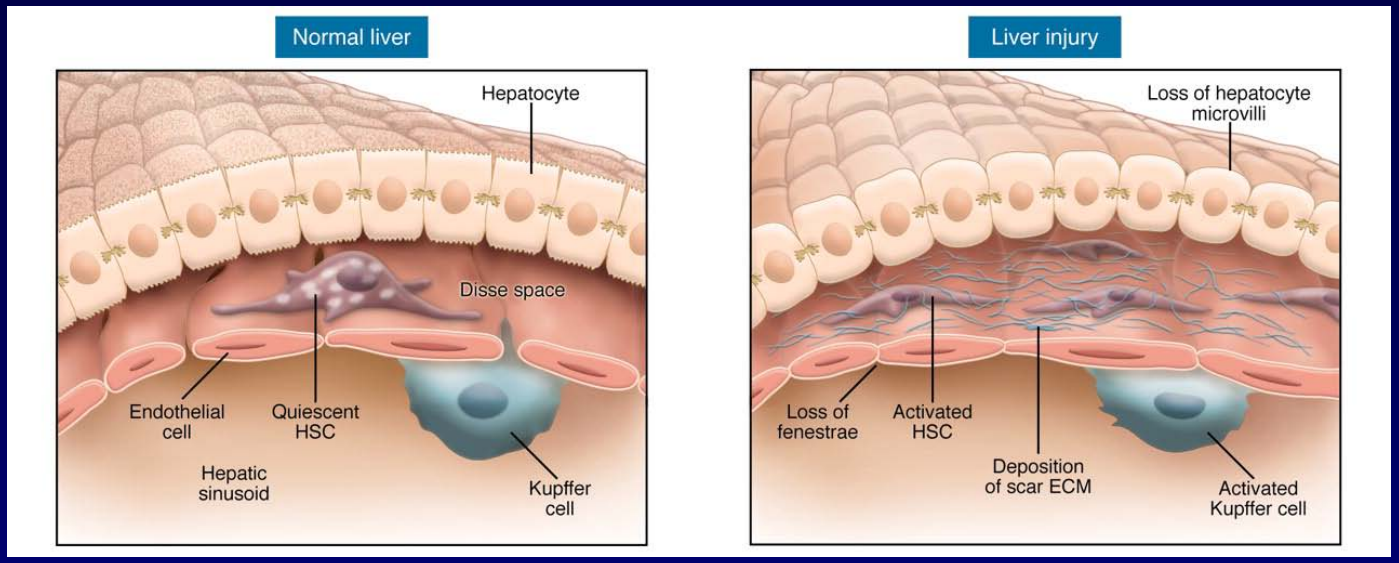


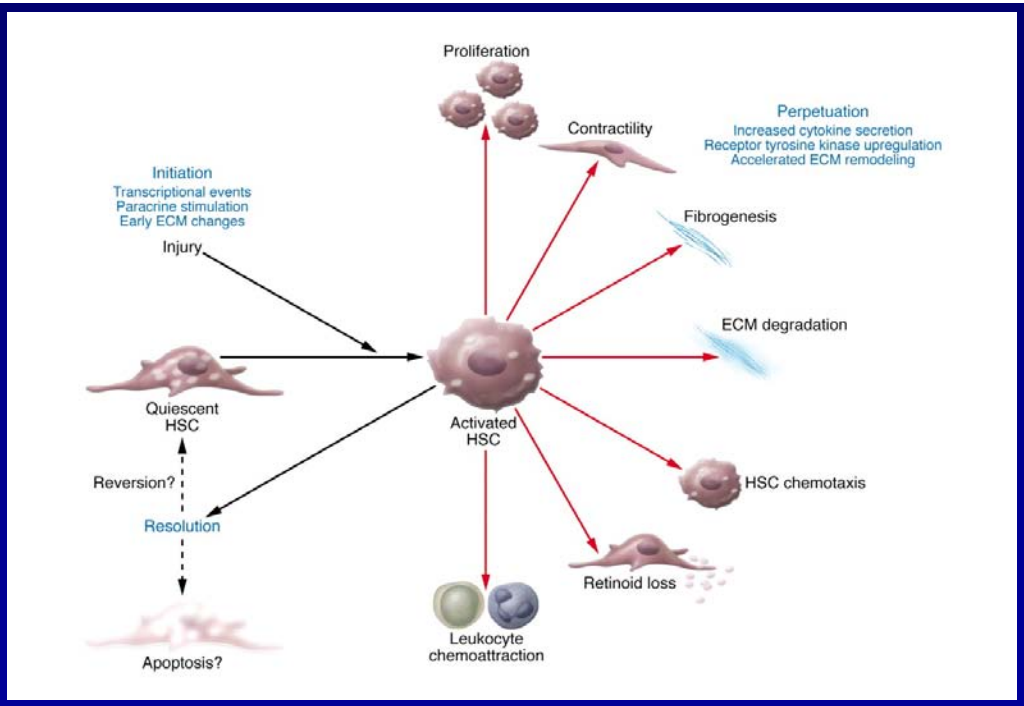
Kidney



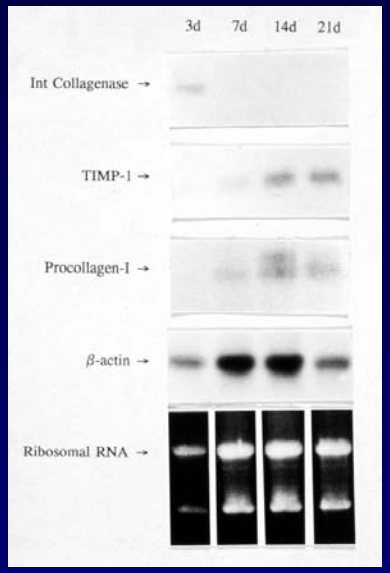


Henderson…Iredale and Shephard in revision





Friedman J Biol Chem 2000; Iredale, J. P. J. Clin. Invest. 2007



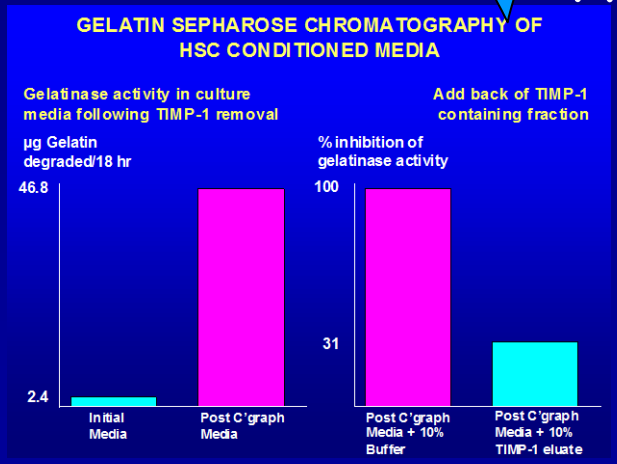
Matrix degrading metalloproteinase (MMP)

TIMP

MMP activity inhibite
No matrix degradatio
occurs

Activated HSC/MFB

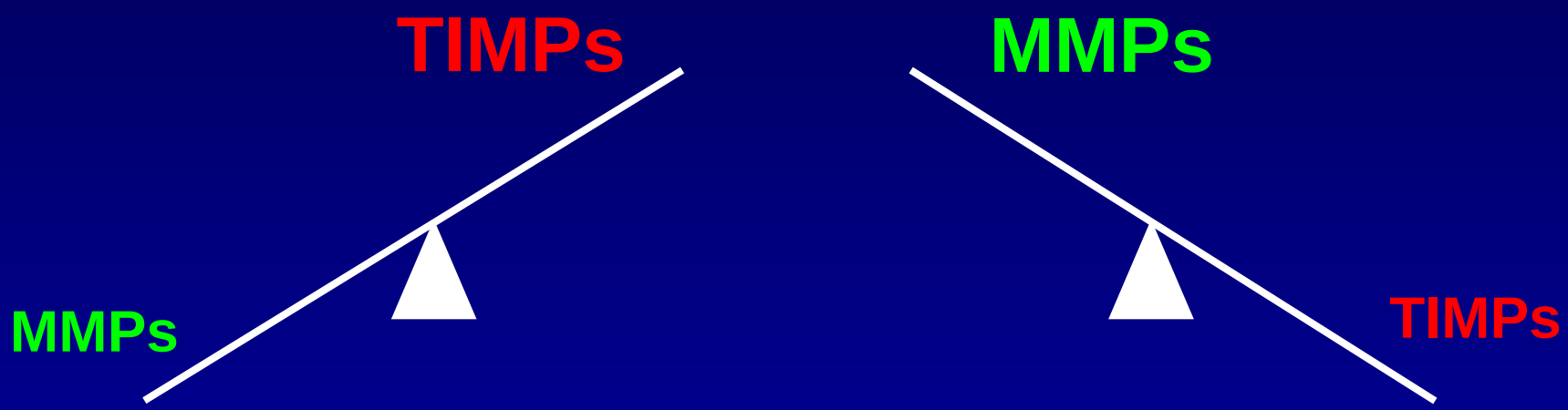
Collagen I-rich scar matrix



- HSC and Other NPCs/ICs express MMPs
- Latent capacity for degradation of fibrillar matrix is held in check

Iredale et al JCI 1992

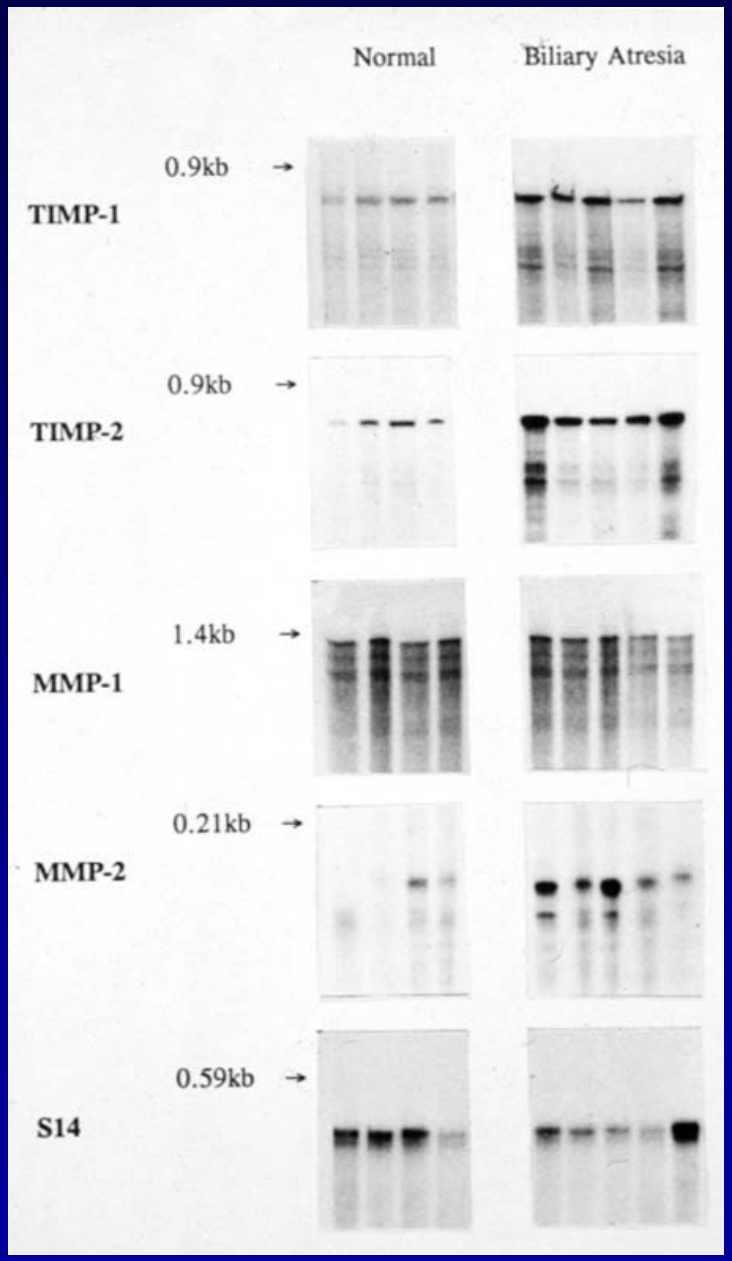
Fibrosis results from an imbalance between collagen synthesis and degradation



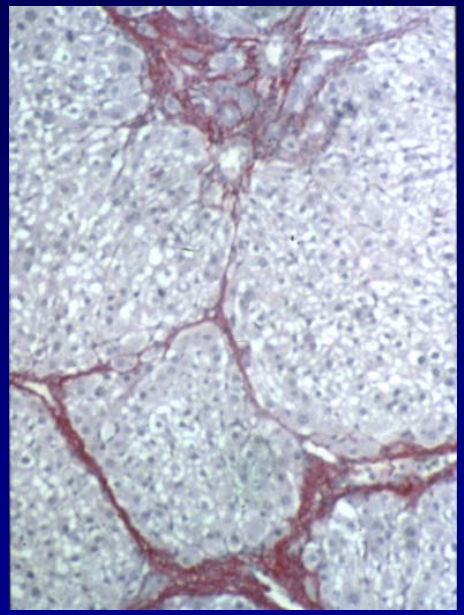
Favours matrix accumulation

Favours matrix degradation

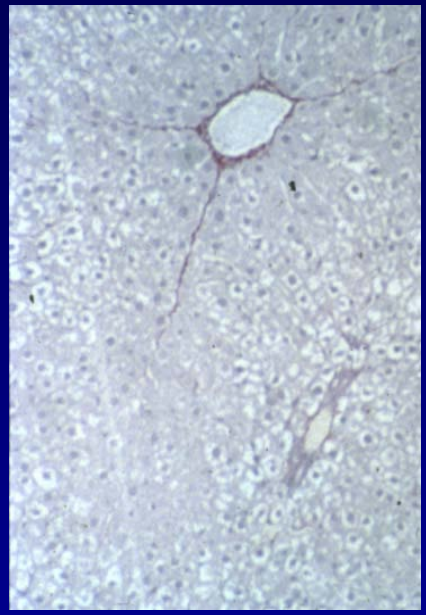
EXPRESSION OF TIMPS AND MMPS IN HUMAN LIVER



Central mechanisms mediating regression of liver fibrosis



4 weeks CCl_4 (rat)

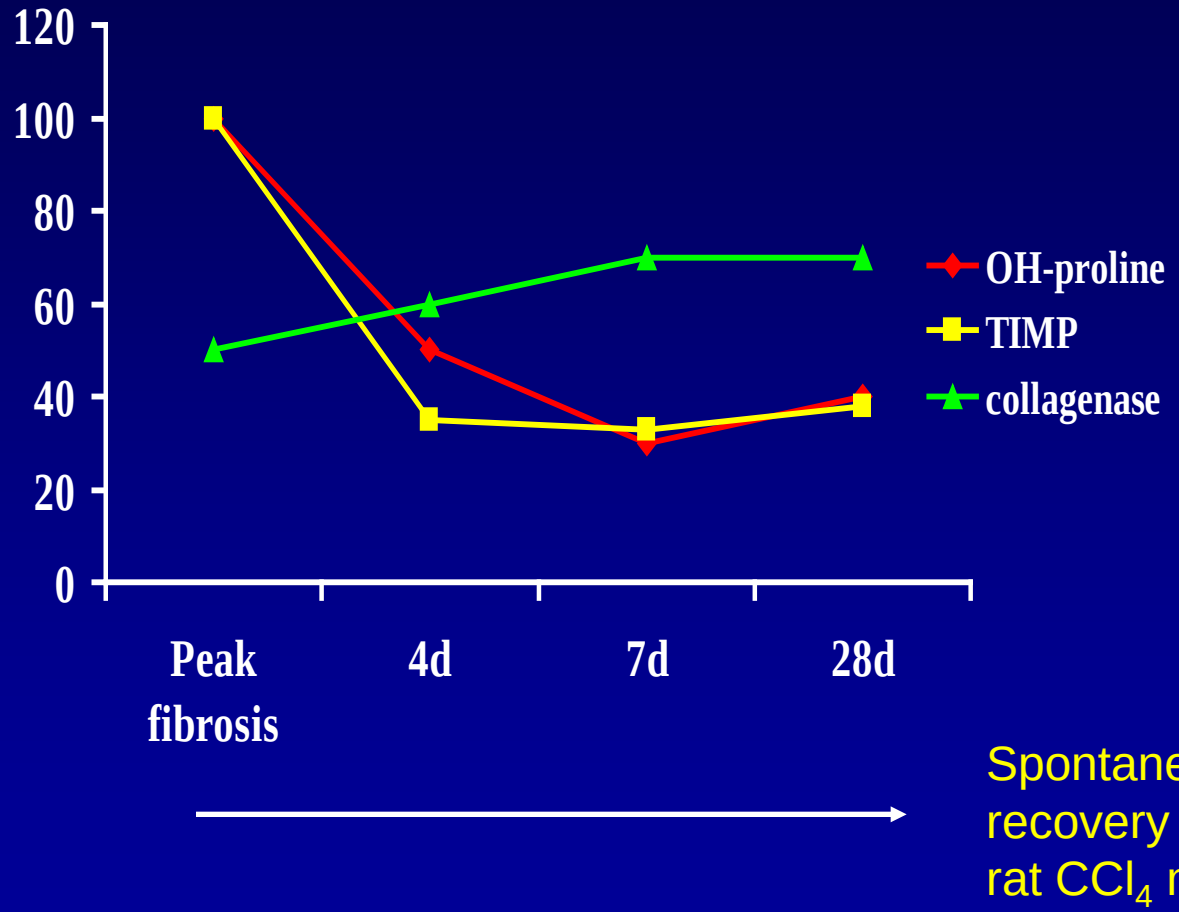


4 weeks CCl_4 + 10 days recovery

- Increased collagenolytic activity in the liver

Iredale et al JCI 2008

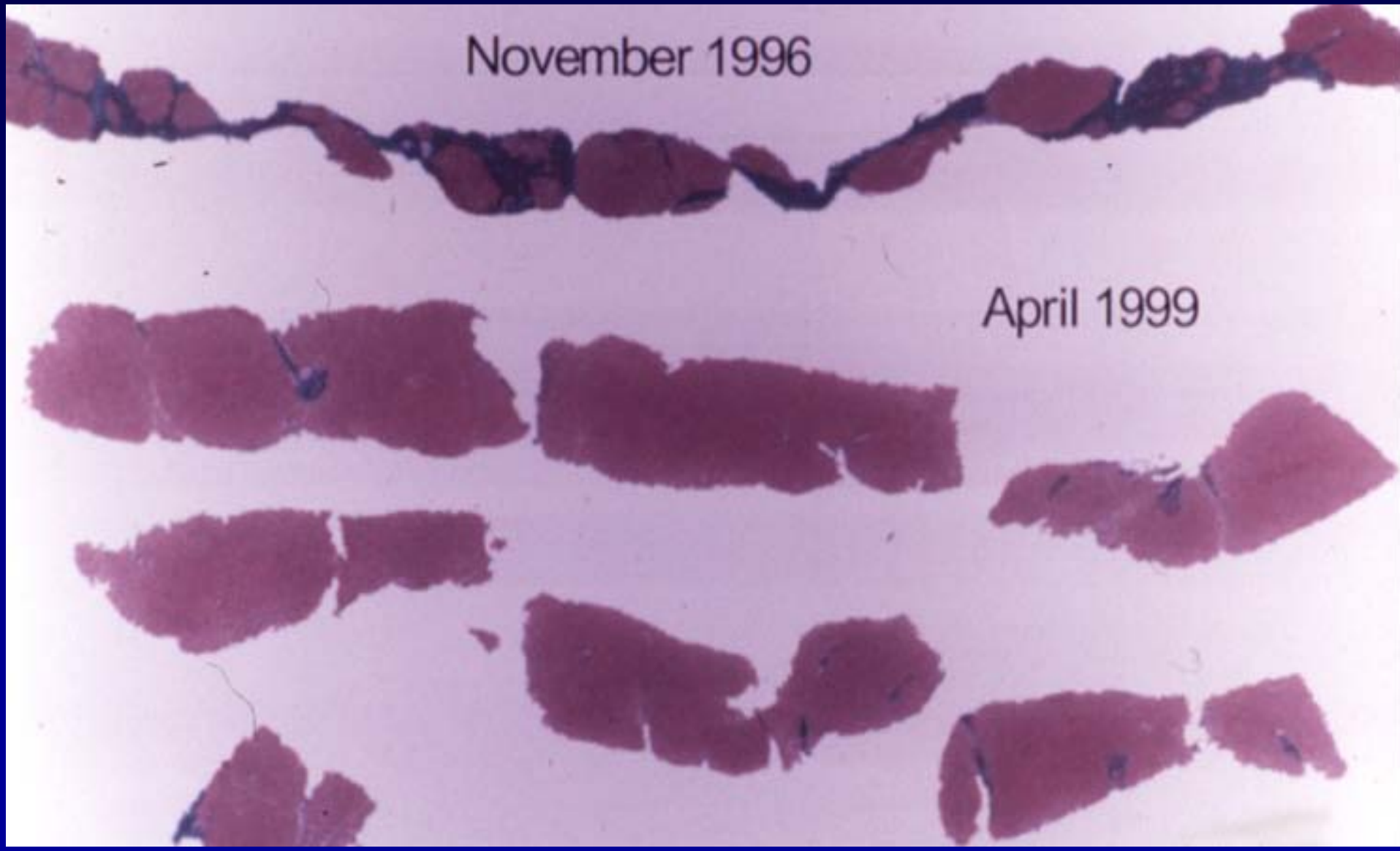
Apoptosis of HSCs removes the source of TIMP



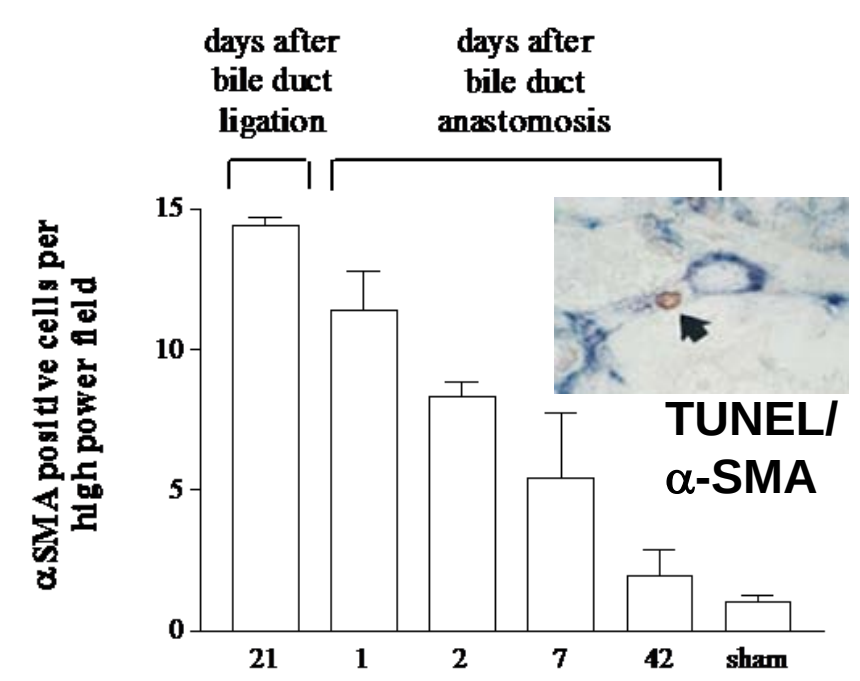
TIMP is reduced, scar is degraded

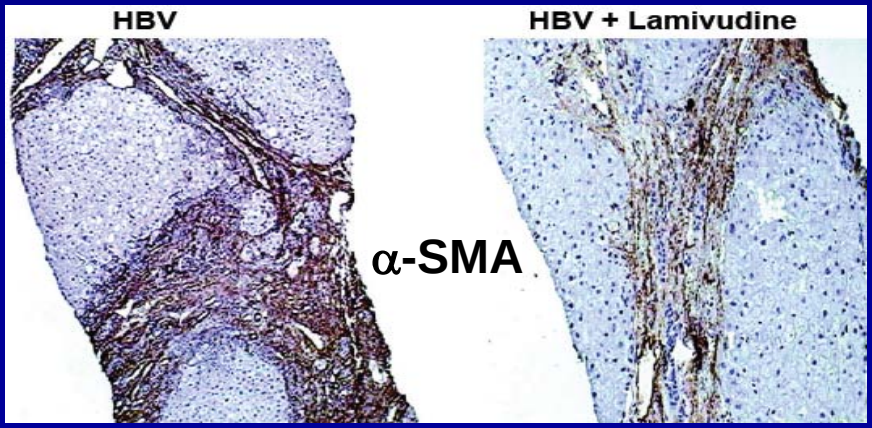
Iredale et al JCI 2008

AICAH



HSC apoptosis is a pivotal process in regression of liver fibrosis



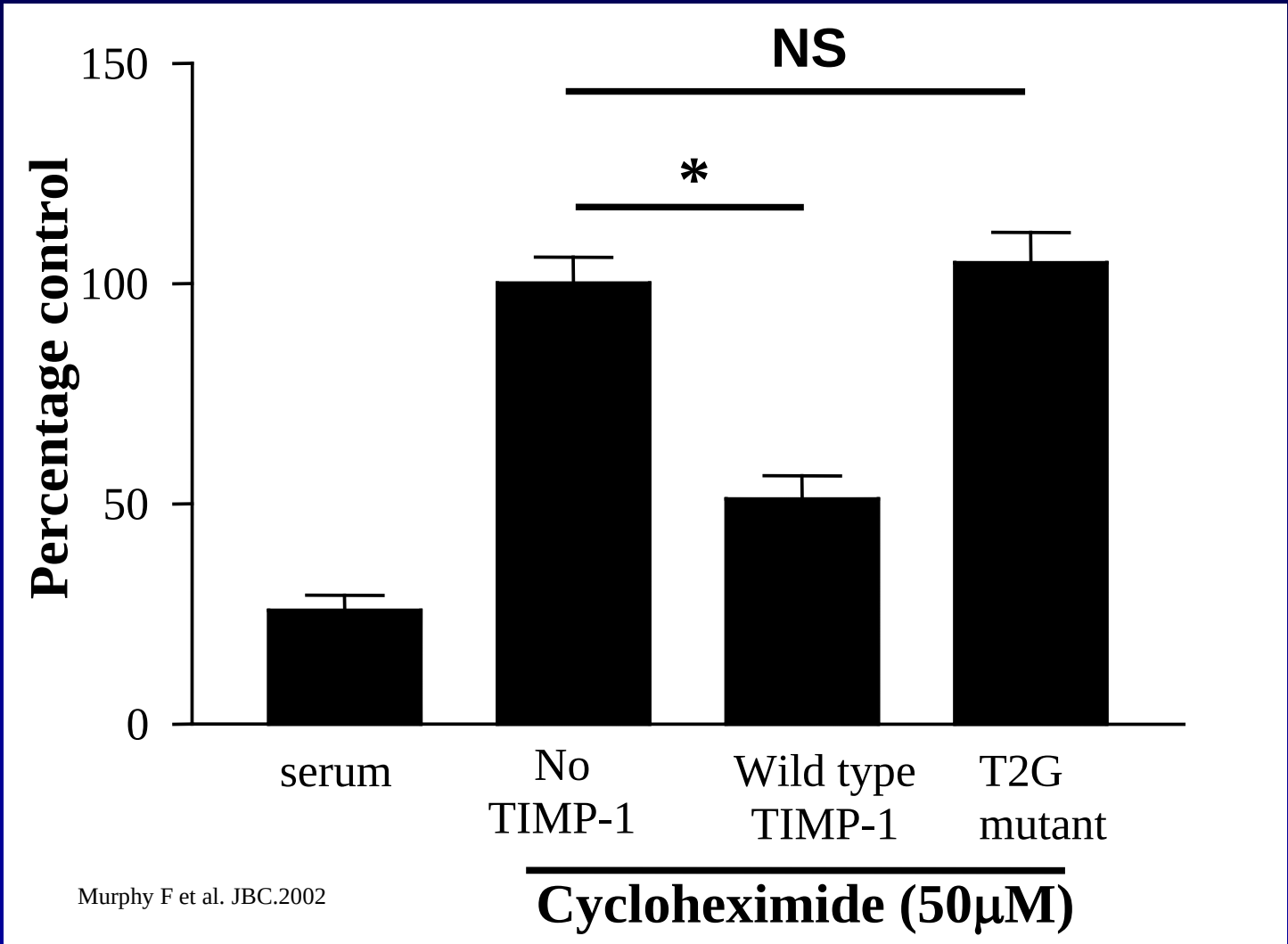


Possible mechanisms

- ↑ death receptors (e.g. Fas receptor, TNFR1) and ligands (e.g. FasL, TNFα, TRAIL, NGF)
- ↑ pro-apoptotic proteins (e.g. p53, Bax)
- ↓ survival factors (eg matrix, N-cadherin)
- induced e.g. gliotoxin, sulfasalazine
- *NK cells*
- *HSC senescence*

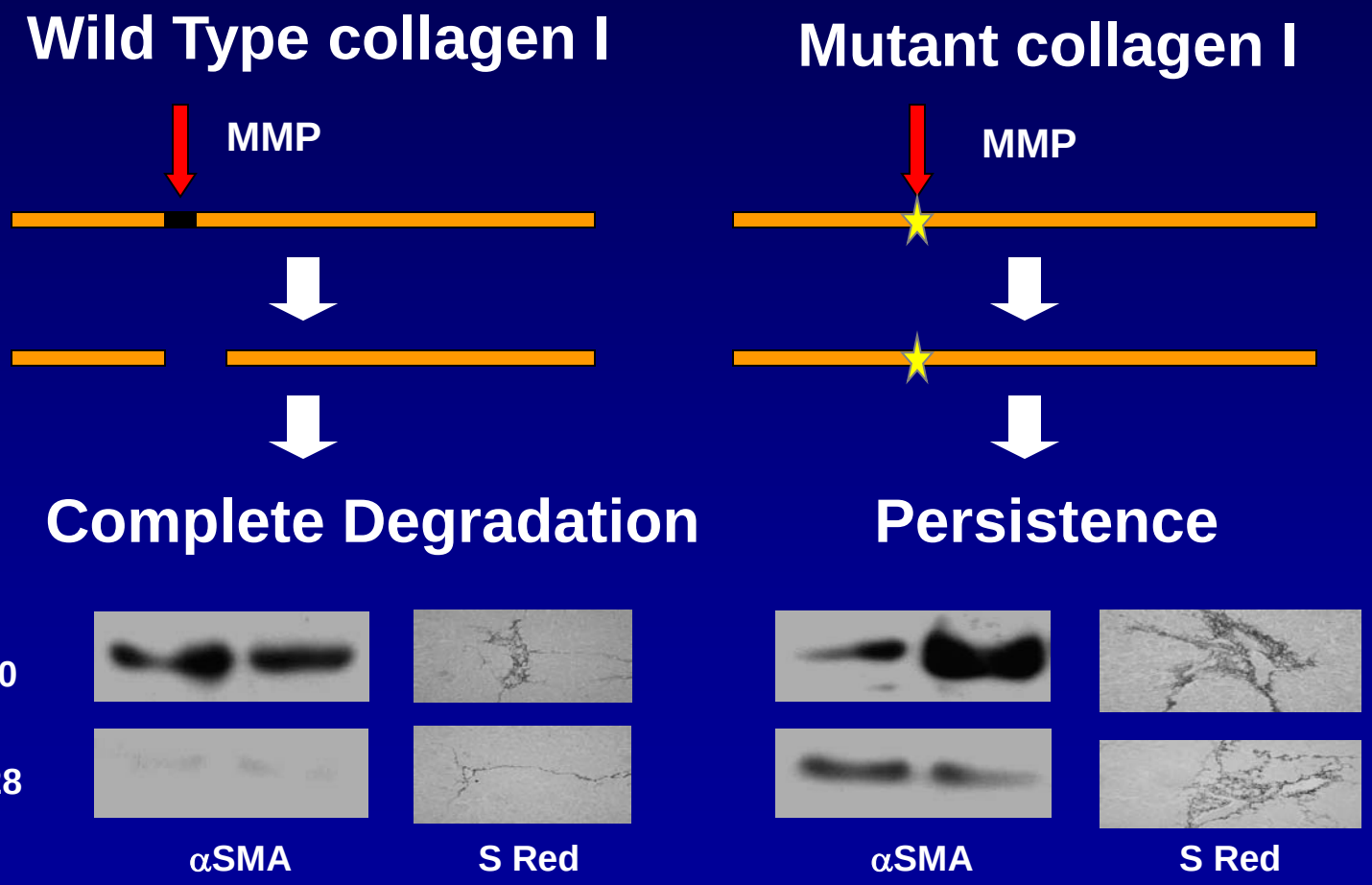
Issa R et al, Gut 2001 Kweon Y et al, J Hepatol 2001

TIMP-1 reduces apoptosis by MMP inhibition



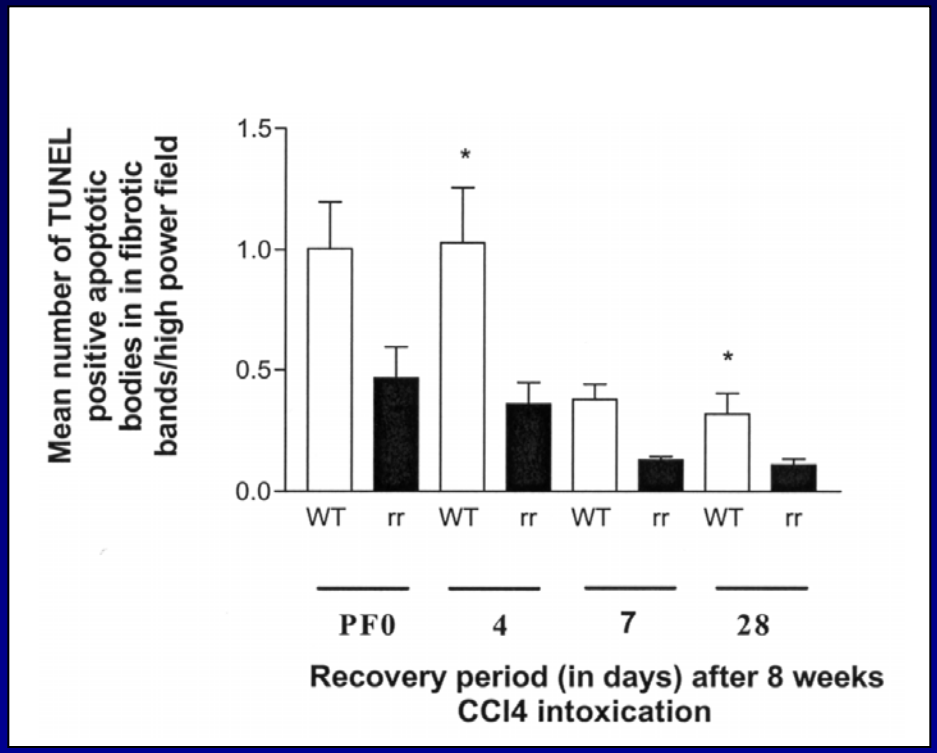
Murphy F et al. JBC.2002

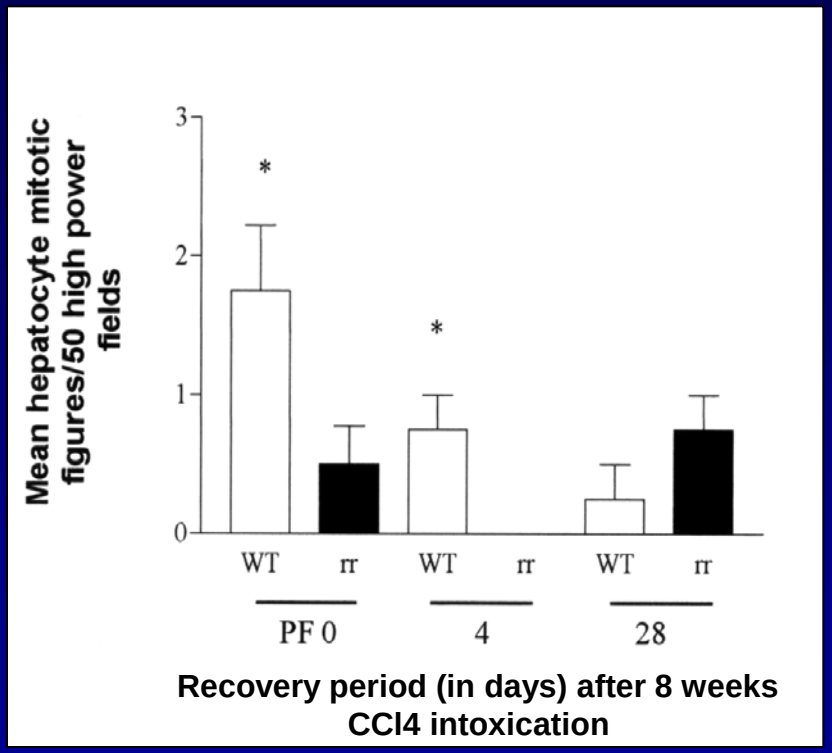
Assessing the role of collagen-I in mediating HSC survival in rr mice



Issa et al FASEB J 2002

Assessing the role of collagen-I in mediating HSC survival



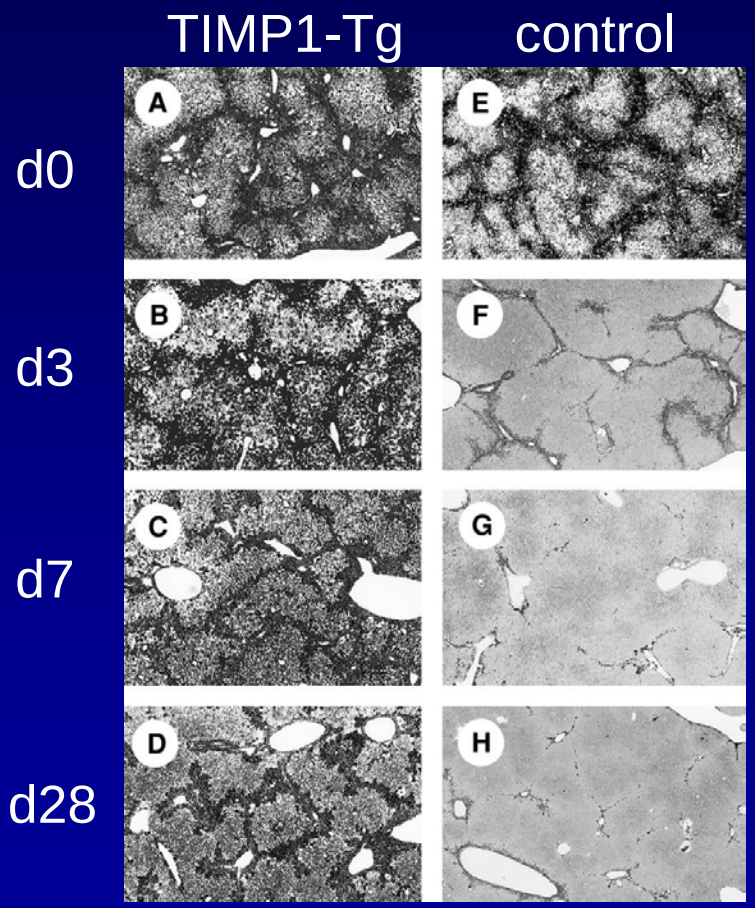


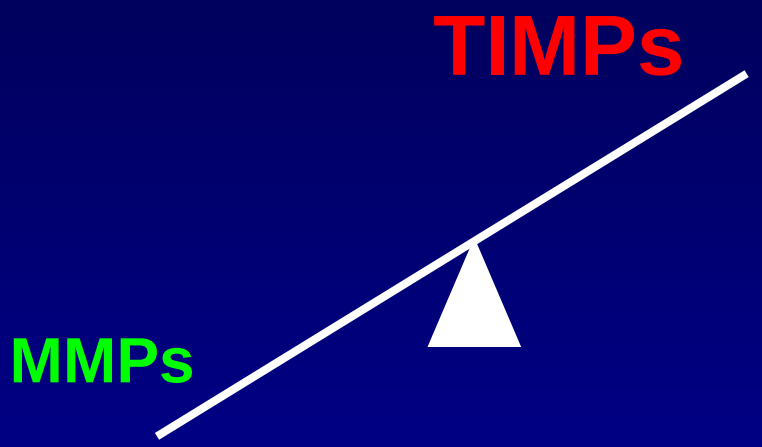
Persistant Col-I:
Inhibits HSC/MFB apoptosis

Inhibits Hepatocyte and ?OC prolif

Issa et al FASEB J 2002

Overexpression of TIMP-1 attenuates regression of experimental hepatic fibrosis





- ↓ matrix remodeling
- ↓ HSC apoptosis

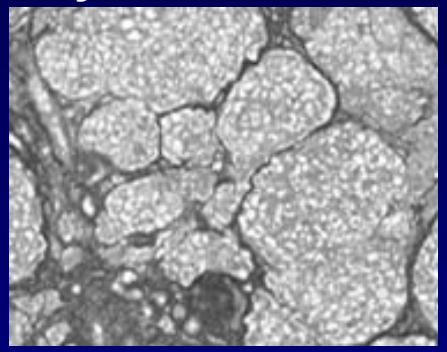
Yoshiji H et al, Hepatology 2002

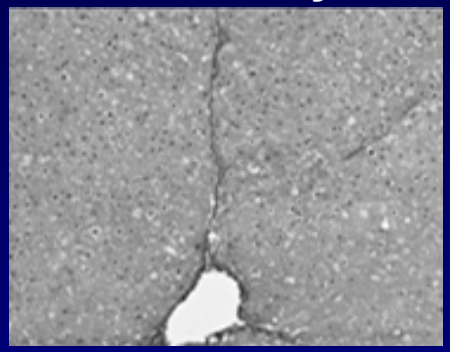
Advanced rat cirrhosis does not completely regress

Day 0 **Day 366**

collagen

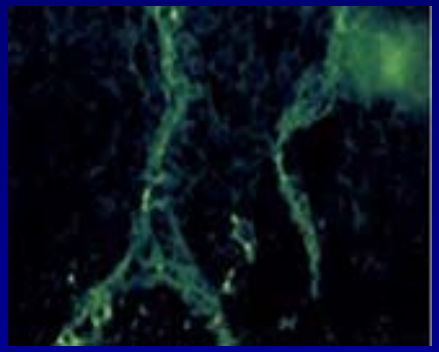
sirius red

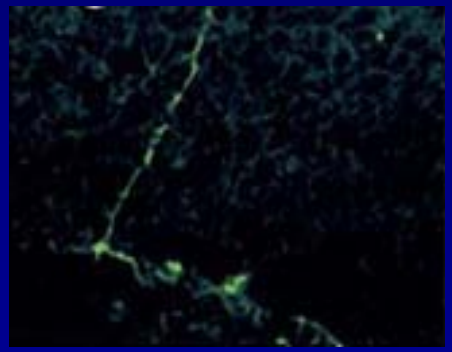




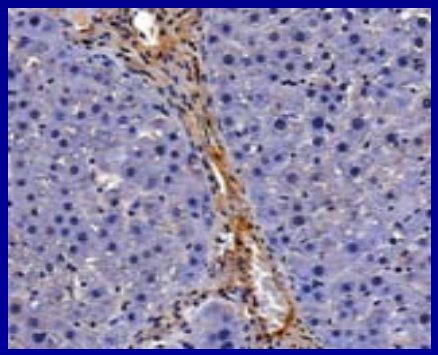
cross links

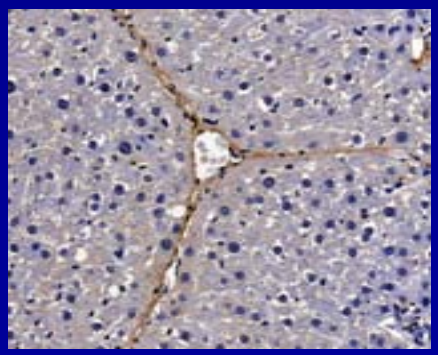
Lysine-(N-ε γ glutamyl)



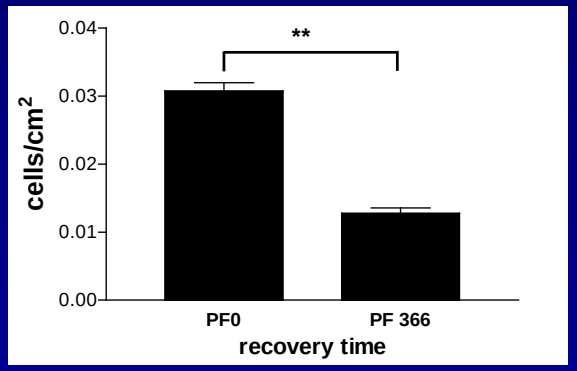


elastin





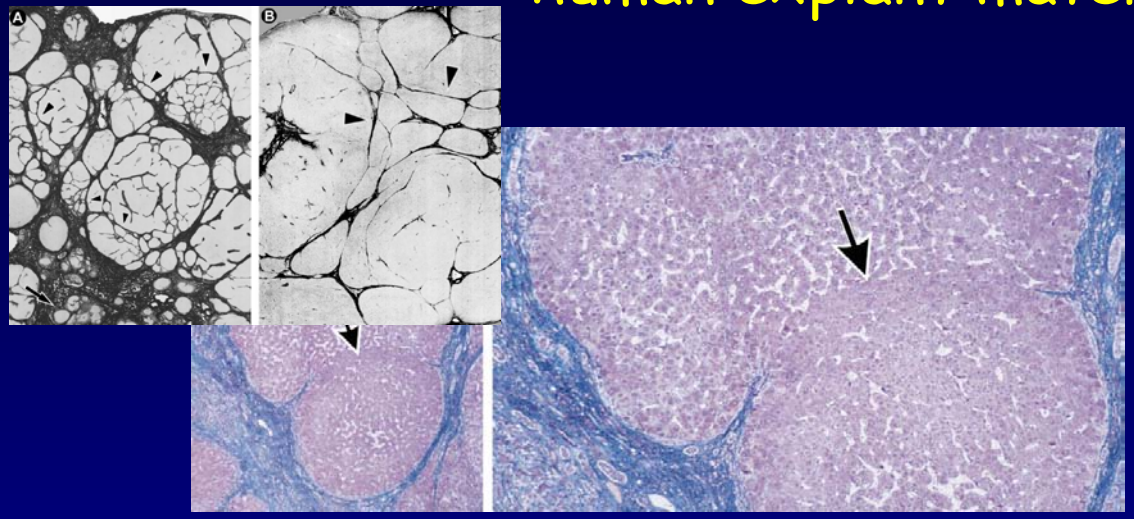
Spontaneous recovery in 12 week rat CCl_4 model

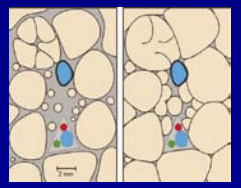


cellularity

Issa R et al, Gastroenterol 2004
Popov Y et al Gastroenterol 2011

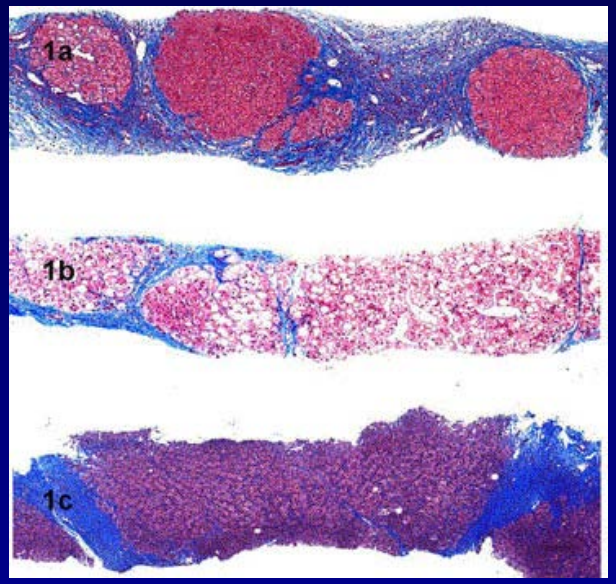
Evidence for limited matrix degradation in human explant material

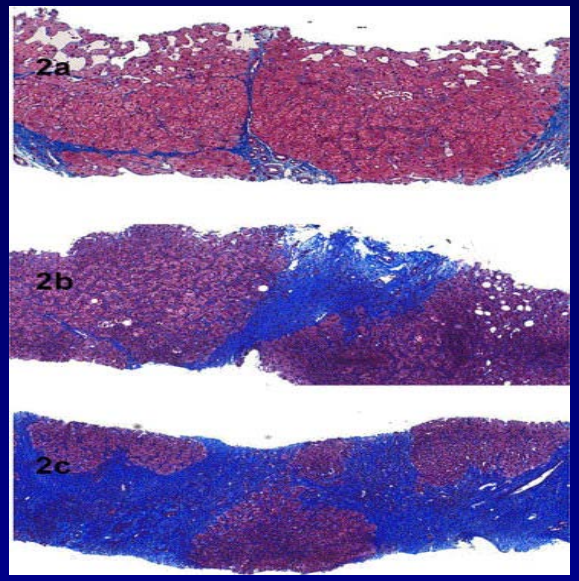


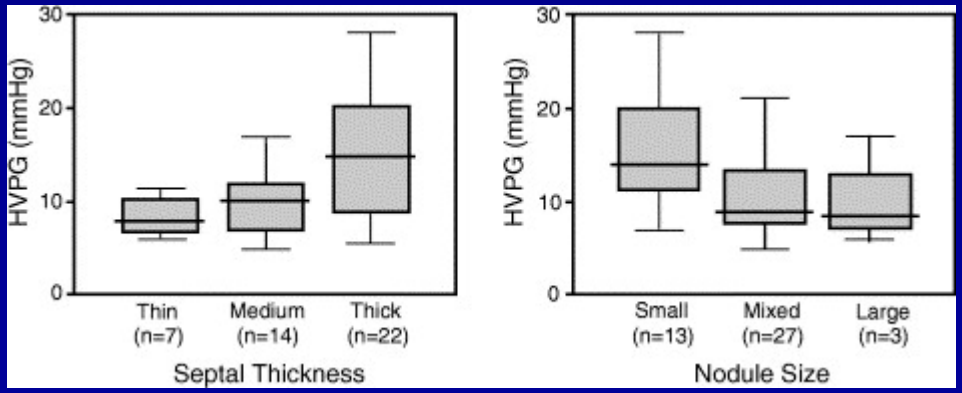


Wanless I et al Arch Path Lab Med 2000

Correlation of Histological Parameters of Cirrhosis with Portal Hypertension



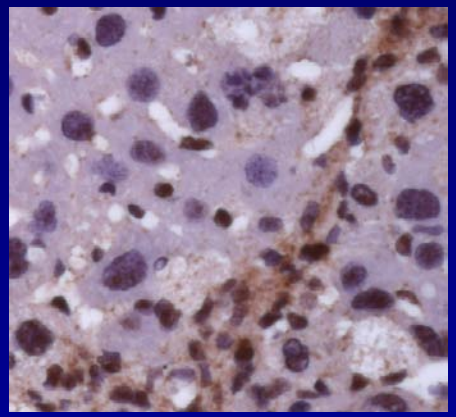




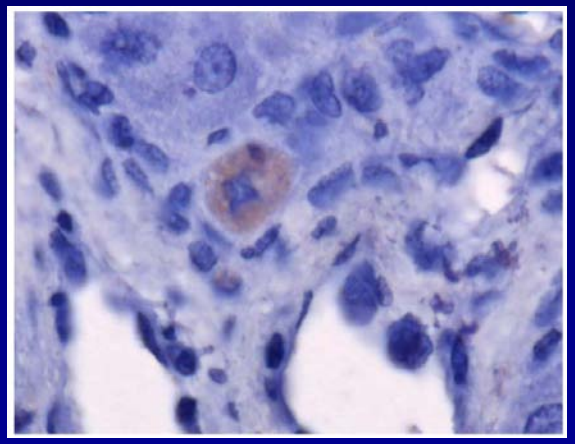
Nagula J Hedatol 2006

HSC/MFBs express abundant TIMP-1 mRNA, Mono/Macrophages express MMPs 12 and 13: *in situ*

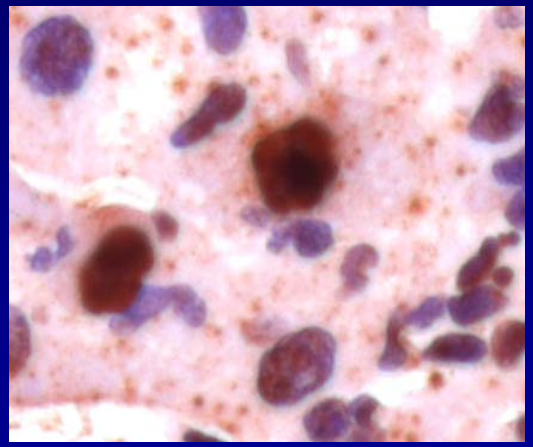
TIMP-1

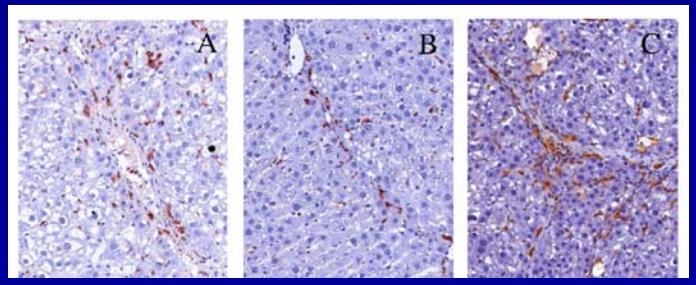


MMP-12

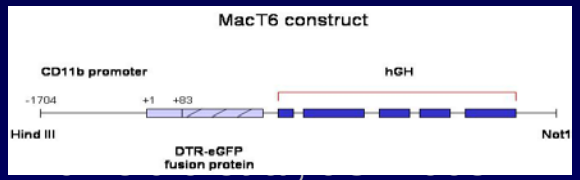


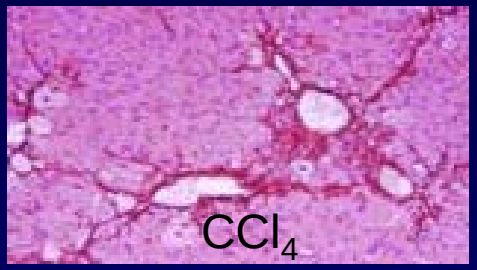
MMP-13



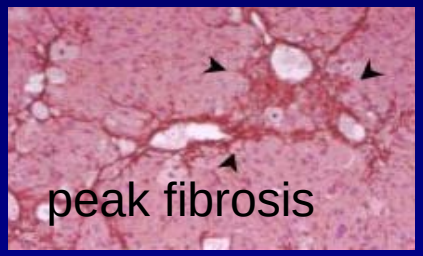


Macrophages can be fibrogenic or fibrolytic

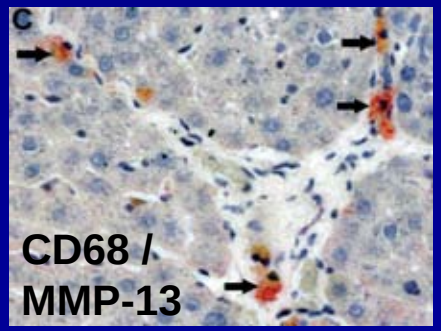


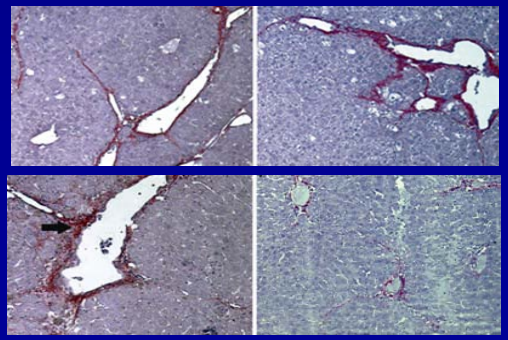


- **Conditional depletion of SAMs during injury decreases fibrosis**



- **Depletion of SAMs during recovery attenuates regression of fibrosis**

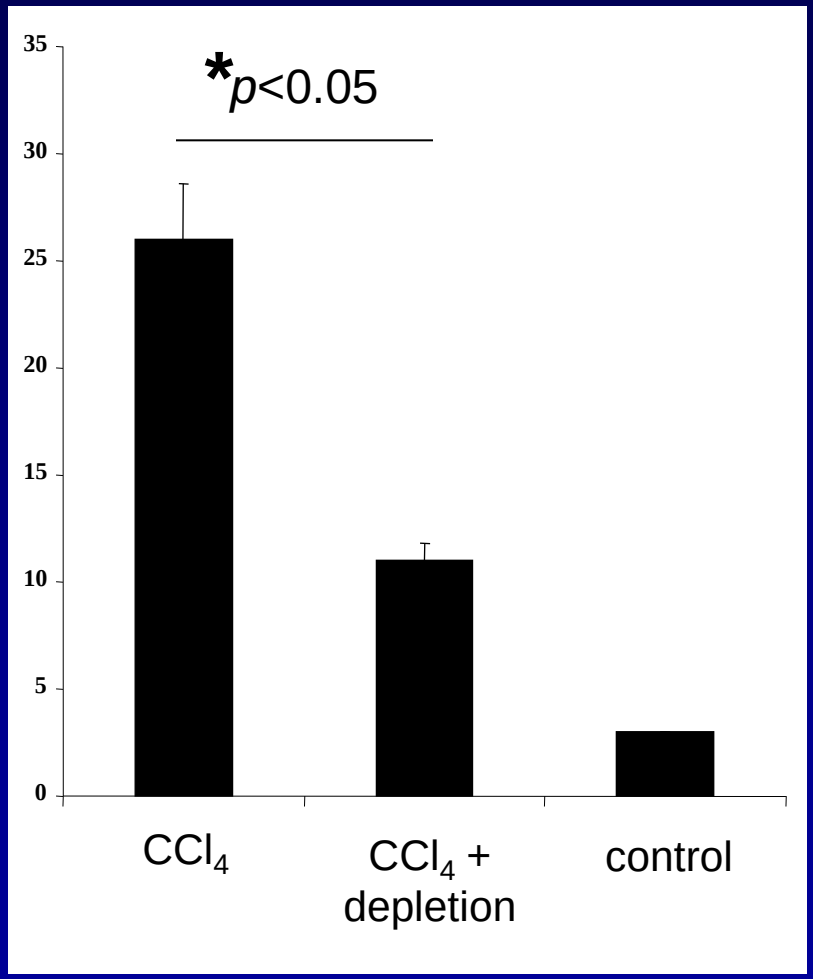


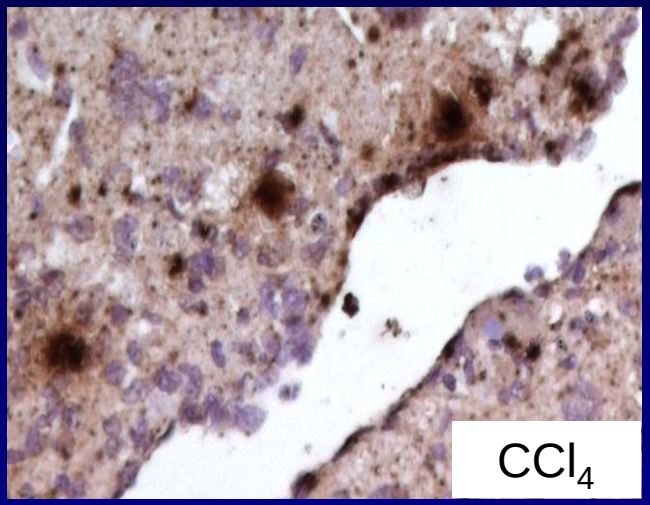


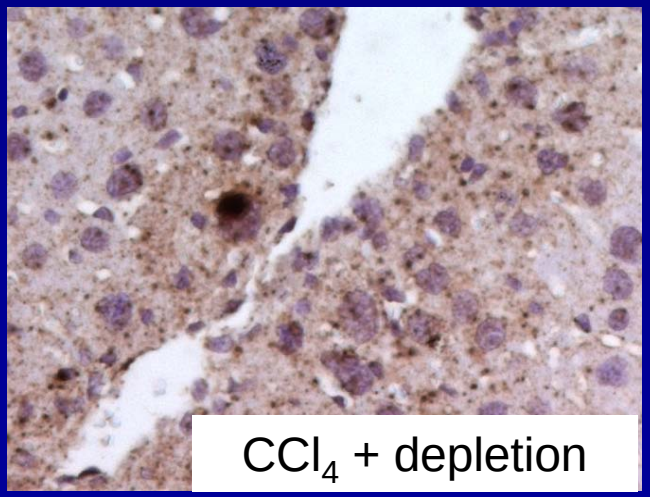
SAM induce and use collagenase (MMP-13) during regression of fibrosis

Duffield et al JCI 2005, Fallowfield J et al, J Immunol 2007

Effect of conditional macrophage depletion on MMP-13 mRNA: *in situ* hybridisation

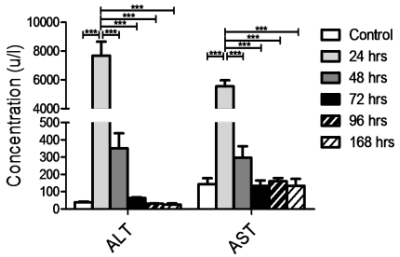


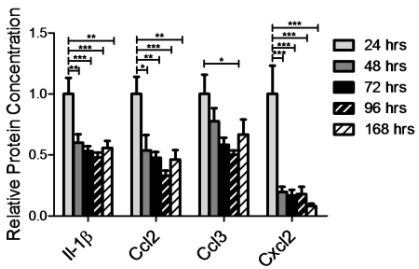


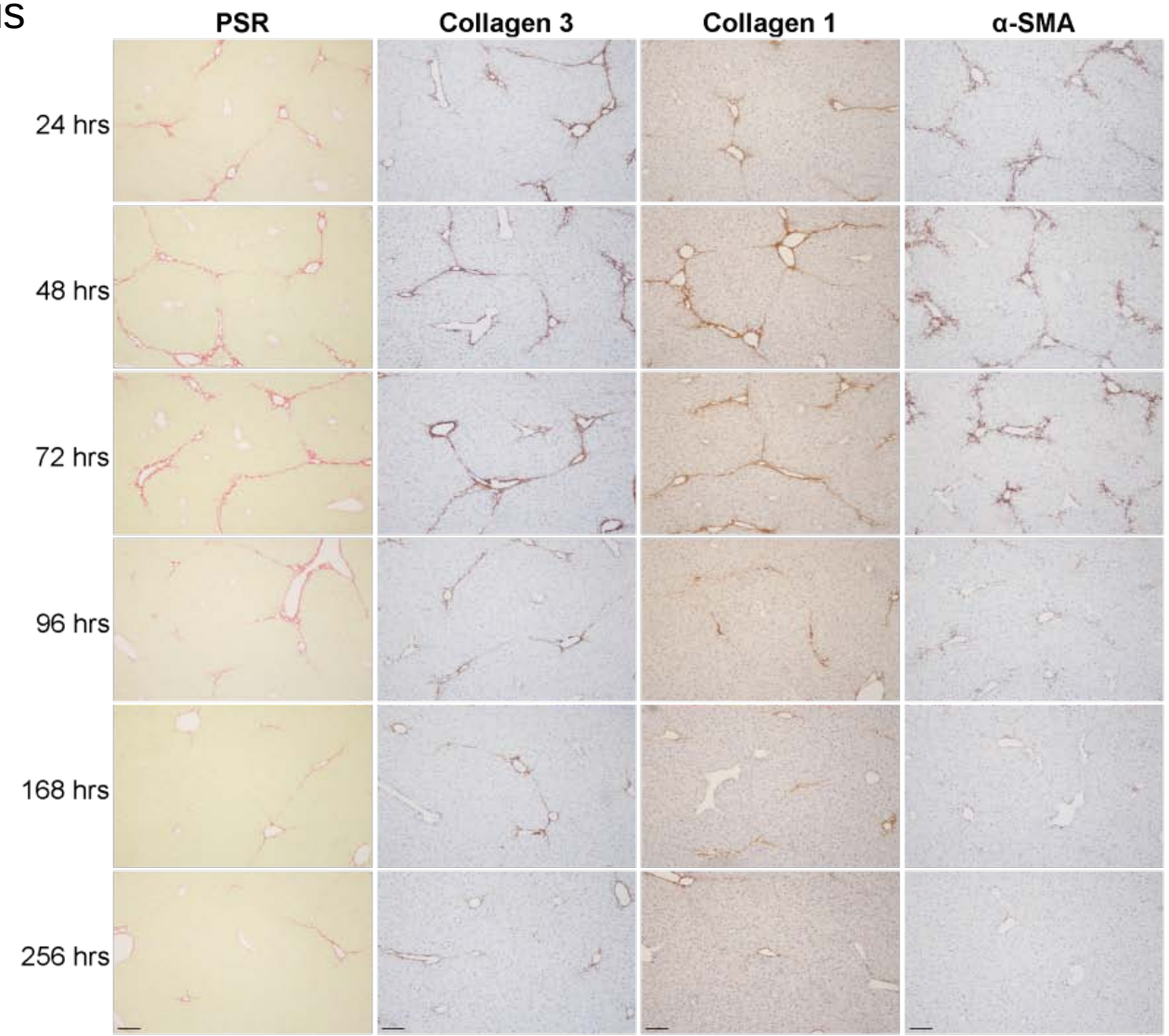


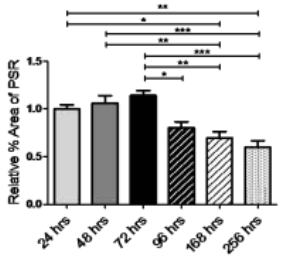
Fallowfield et al JI 2007

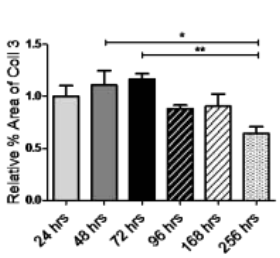
Detailed Model of fibrosis Resolution

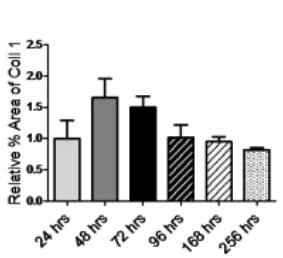


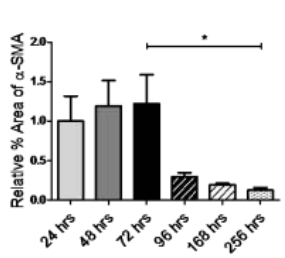




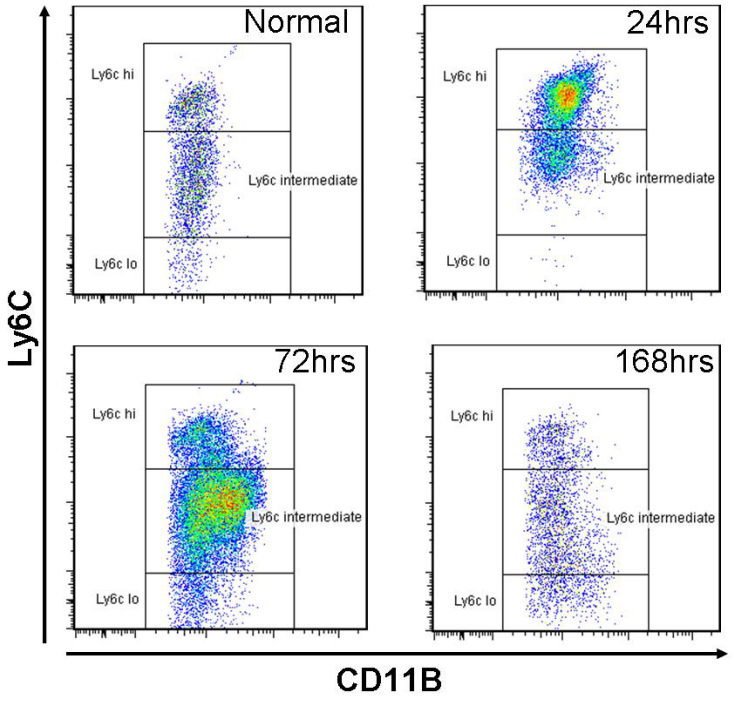


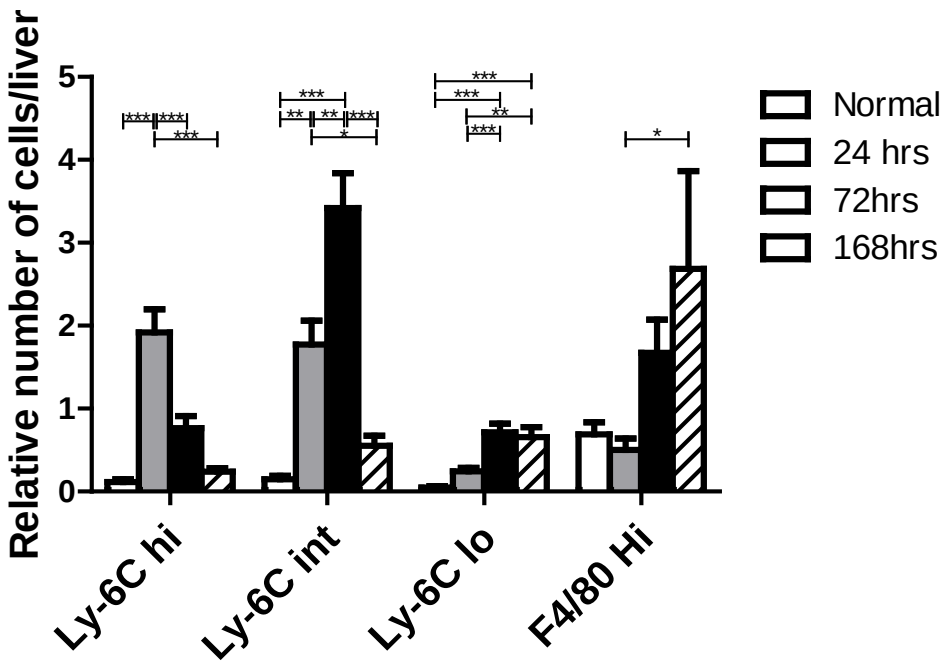






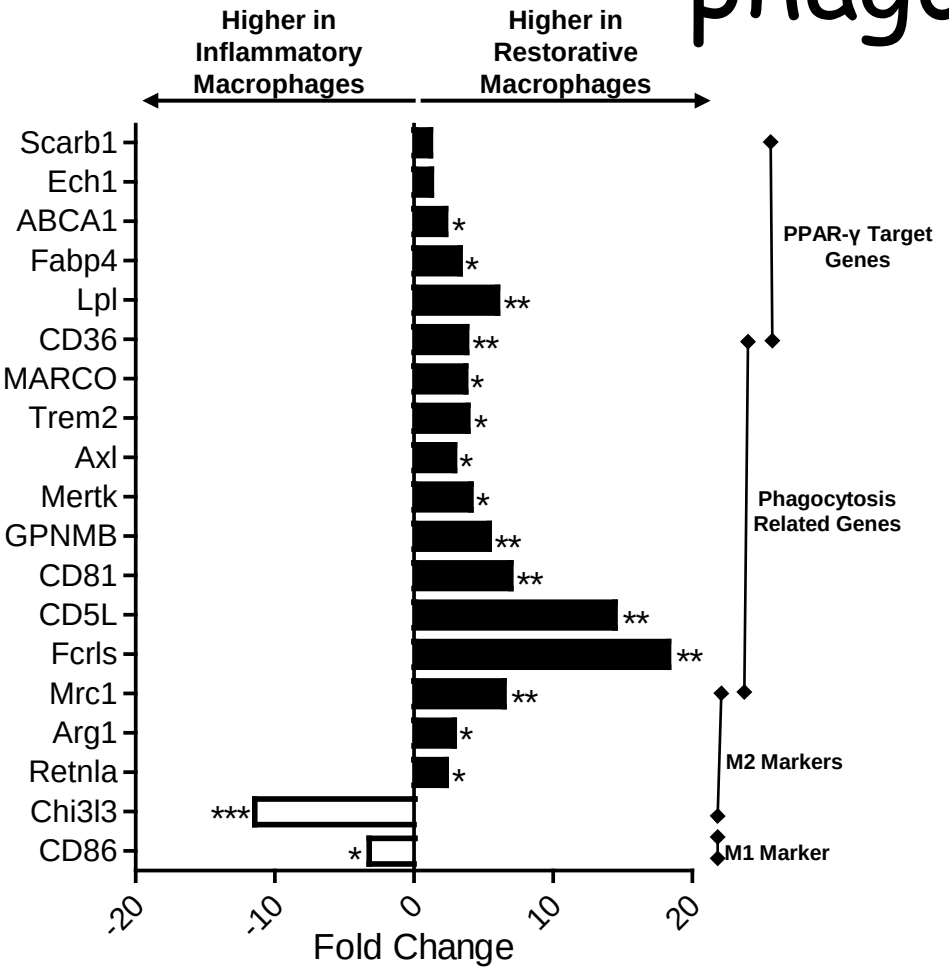
Ly-6C^{int} macrophages accumulate at time of scar resolution

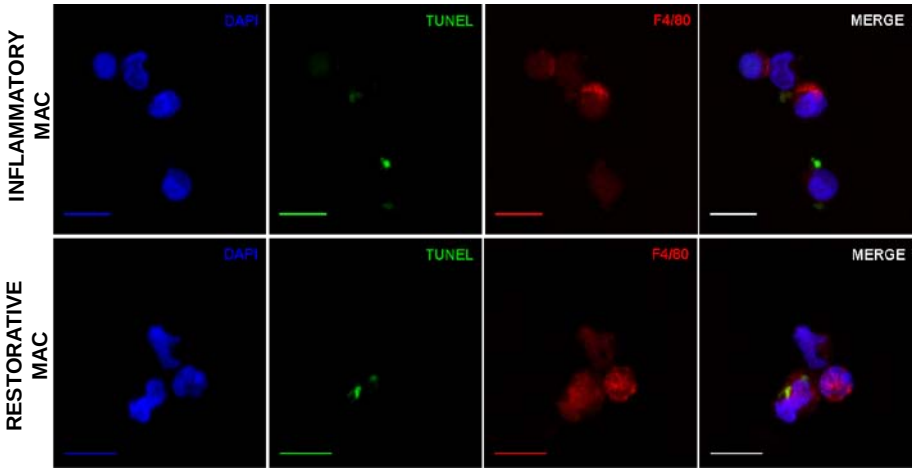


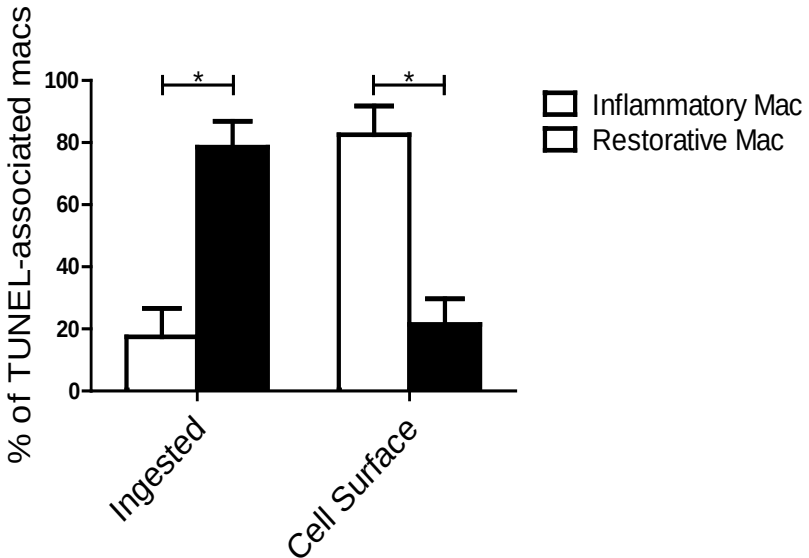


Ramachandran et al PNAS 2012

Ly-6C^{int} macrophages are post-phagocytic

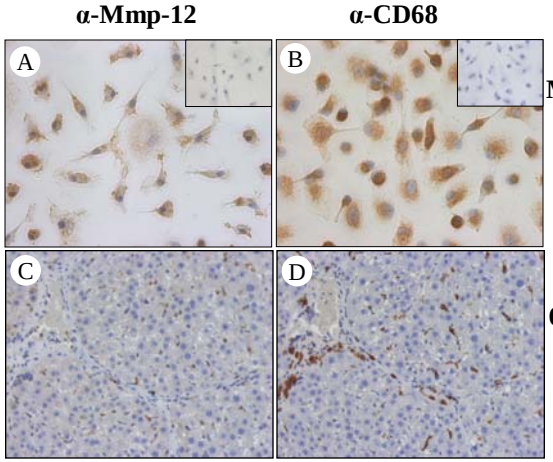






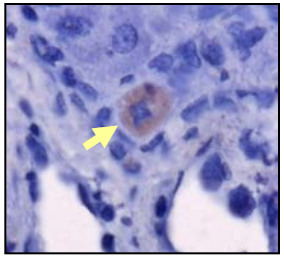
Ramachandran et al PNAS 2012

Macrophages are Major Source MMP-12

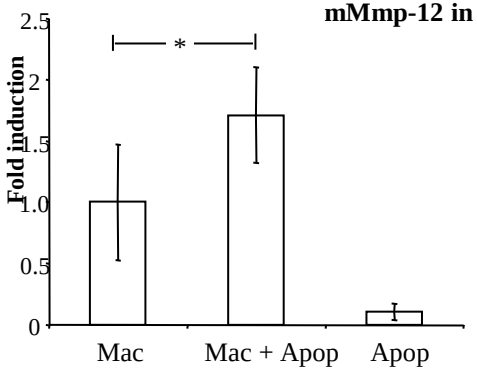


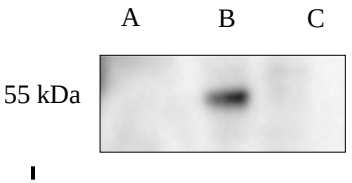
Human Macrophages

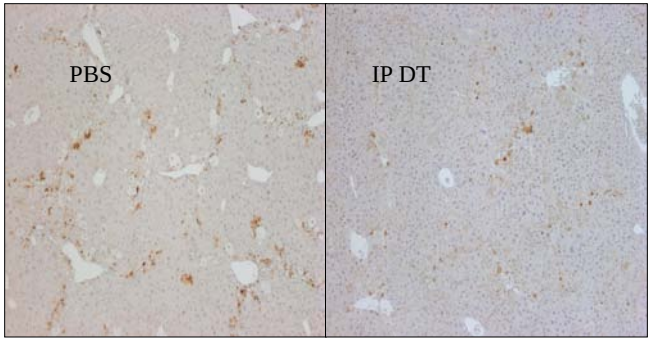
Rat liver 6 weeks CCl_4

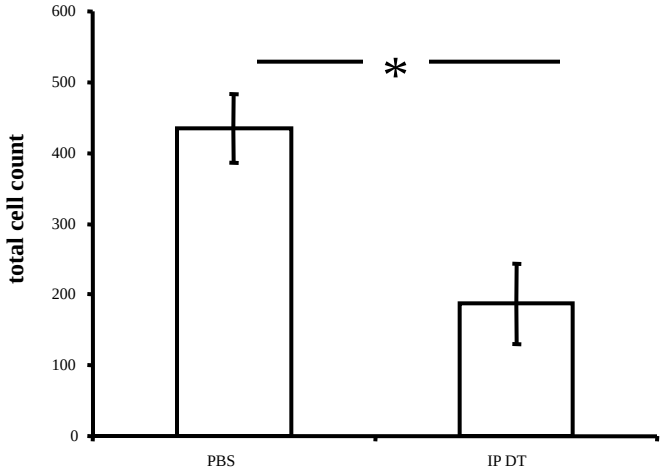


Human liver MMP-12 ISH



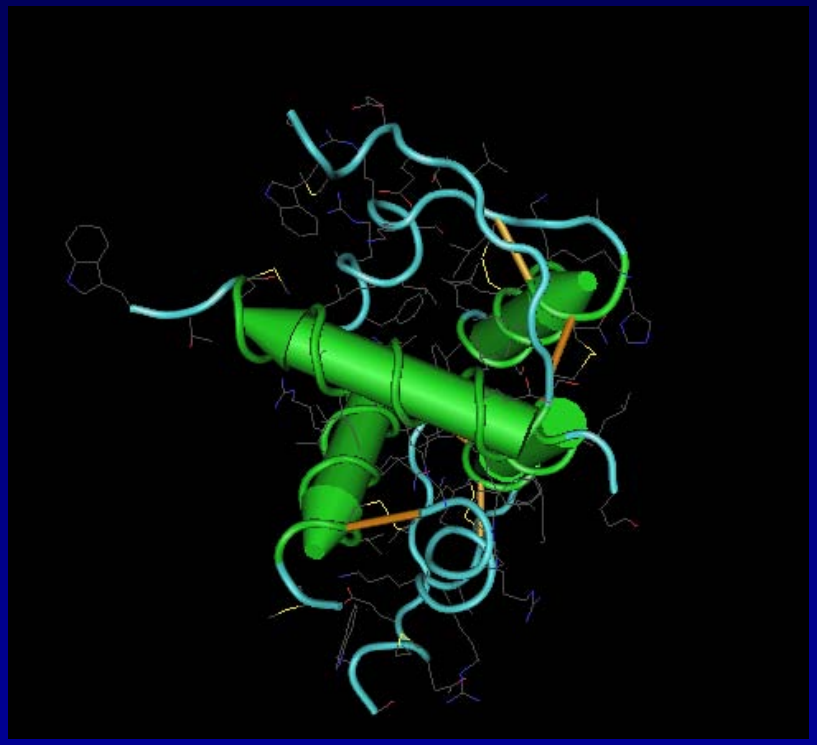






Pellicoro et al Hepatology 2012 and Popov Am J Phys 2010

Relaxin



- Relaxin is a hormone and was first described 75 years ago it has a specific function in pregnancy and birth
- Assist pregnancy maintenance, facilitate delivery and prepare the mammary gland for lactation
- Rlx=peptide, structurally similar to insulin
- Potentially other roles in non-pregnant females and males
- Ticks the boxes:
- Reduces TIMP, Scar Tissue and
- Accelerates scar breakdown.

Active, dynamic component of PHT

- Encircling the sinusoid, myofibroblasts (HSC-MFs) in scars contract
- HSC-MF density/ coverage of sinusoids enhanced in cirrhosis
- Cell contraction represents a dynamic contribution to PHT due to inbalance of vasoactive mediators
- **Potentially reversible/ modifiable by drugs**

Agent	Effect	Proposed mechanism
Endothelin-1	Contraction	$[Ca^{2+}]_i$
Thrombin	Contraction	$[Ca^{2+}]_i$
Angiotensin II	Contraction	$[Ca^{2+}]_i$
Vasopresssin	Contraction	$[Ca^{2+}]_i$
Adenosine	Contraction	$[Ca^{2+}]_i$?
Substance P	Contraction	$[Ca^{2+}]_i$
Leukotriene D_4	Contraction	$[Ca^{2+}]_i$
PGF_2/thromboxane	Contraction	$[Ca^{2+}]_i$
Lysophosphatidic acid	Contraction	Rho kinase
NO	Relaxation	cGMP
ANP	Relaxation	cGMP/$[Ca^{2+}]_i$
Adrenomedullin	Relaxation	cAMP
Somatostatin	Relaxation	$[Ca^{2+}]_i$/rho kinase?
Agents increasing cAMP/cGMP	Relaxation	cAMP/cGMP
PGI_2/PGE_2	Relaxation	cAMP
Y-27632 (rho kinase inhibitor)	Relaxation	Rho kinase

Reynaert H *et al*., Gut 2002

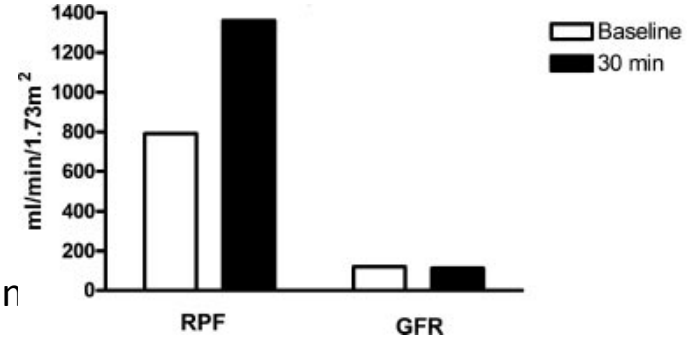
Scientific rationale for use of relaxin in PHT

- Antifibrotic effect of RLN well established
  - Liver, lung, heart, kidney, skin fibrosis models
  - Serum relaxin levels ↑ in patients with cirrhosis
  - RLN receptor expressed in fibrotic rat/human liver

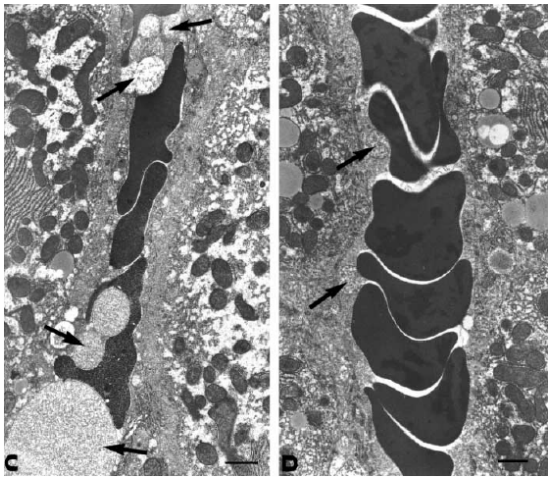
- Vasoactive effects of RLN ('anti-vasoconstrictor')
  - Vasodilatory responses in tissues, generally with preservation of MAP
  - Reduced myogenic activity in isolated human arteries
  - Attenuated vasoconstrictor response to Ang II
  - 47% ↑ RBF in male and female healthy volunteers

- RLN induced morphologic changes in hepatic (sinusoidal) microcirculation in normal rats

- RLN safe and generally well-tolerated in diverse human trials- Is this dynamic HSC function an effective way to establish POC of RLX targeting in Hu Model?
  - Scleroderma (up to 6 months), cervical ripening
  - Acute heart failure (Ph2 PRE-RELAX, Ph3 RELAX)



Smith M *et al*., JASN 2006



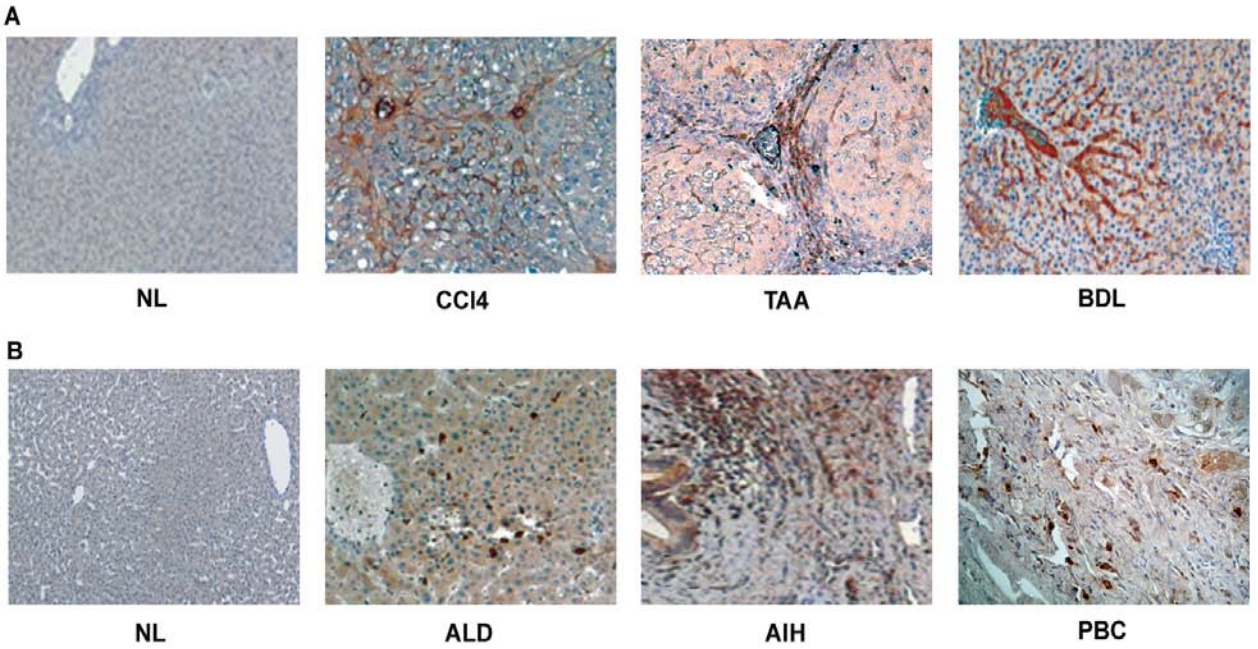
Control Relaxin

Bani D *et al*., J Endocrinol 2000

Hypothesis

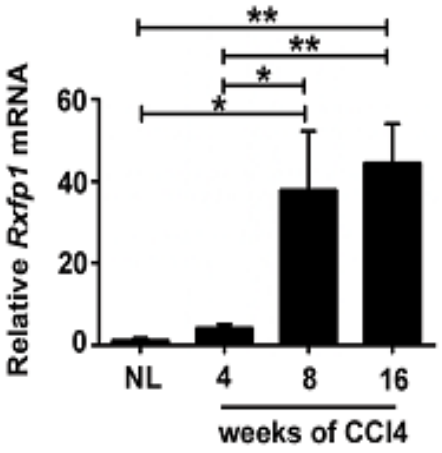
- Relaxin can modulate the dynamic component of cirrhosis-related (sinusoidal) portal hypertension

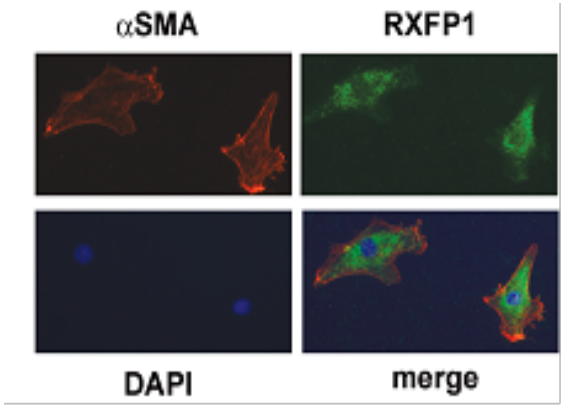
RXFP1 is expressed in rat and human cirrhosis and myofibroblasts are the major cellular source

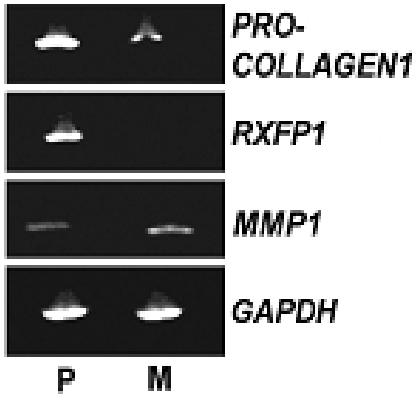


RAT

HUMAN







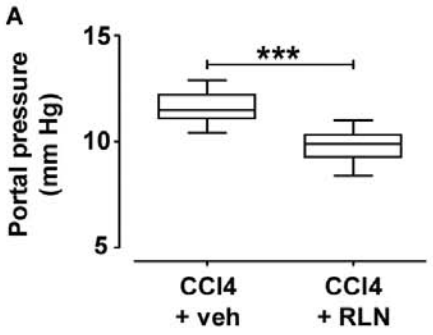
Relaxin reduces portal pressure in experimental cirrhosis

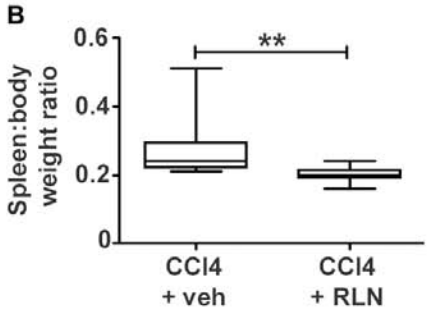
8 weeks CCl_4 rat model of early cirrhosis

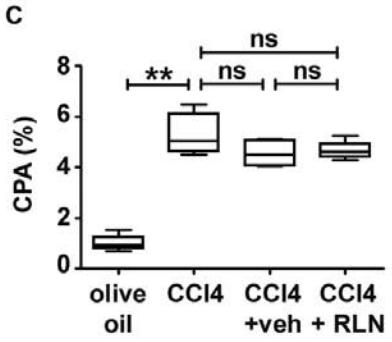
Relaxin or vehicle for 72 hours s.c. via osmotic minipump

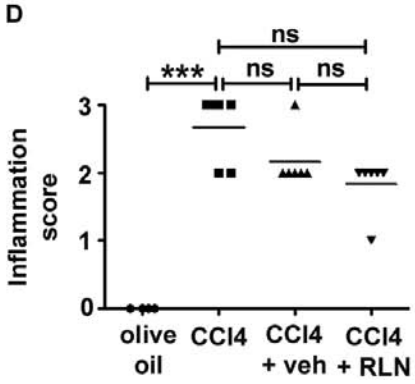
Reduction in PHT independent of fibrosis or inflammation

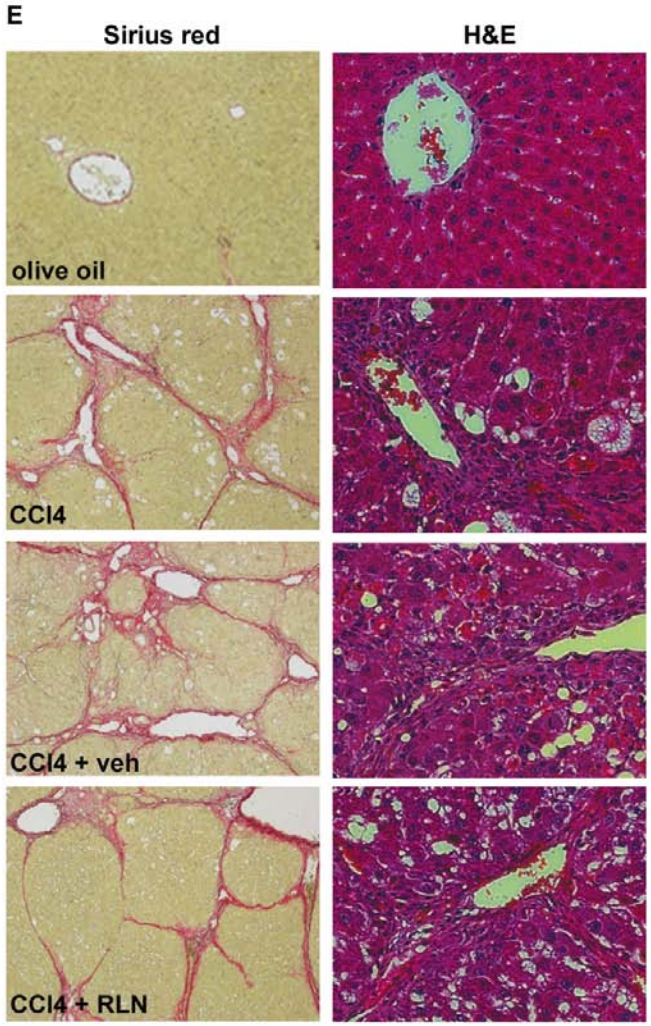
Key profibrotic marker genes down at gene level

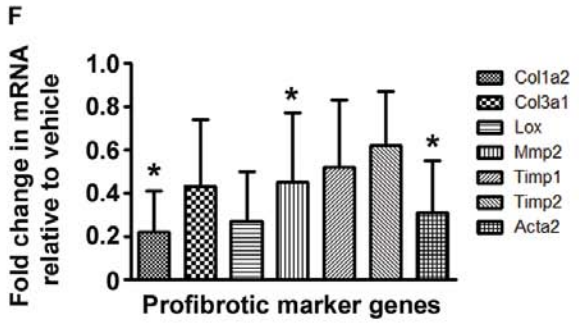






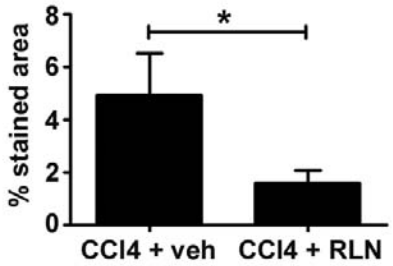


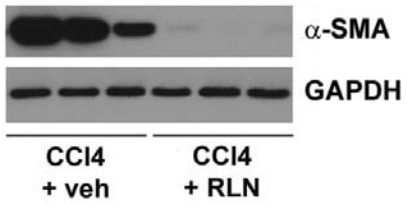




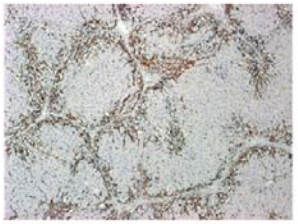
Relaxin reduces cytoskeletal turnover and reduces cytoskeletal tension in liver fibrosis

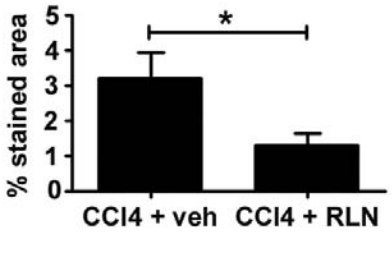
α-SMA

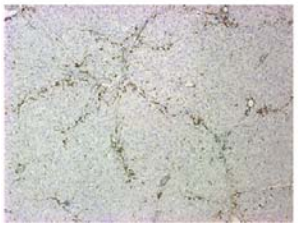


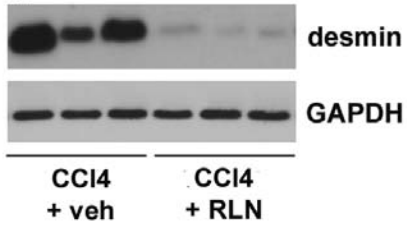


desmin

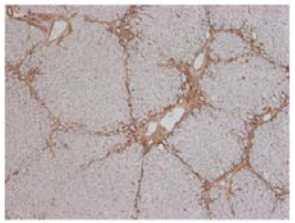


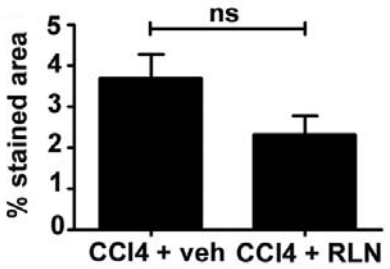


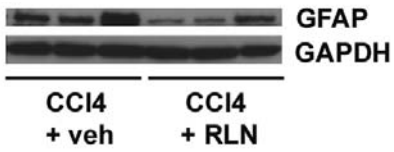


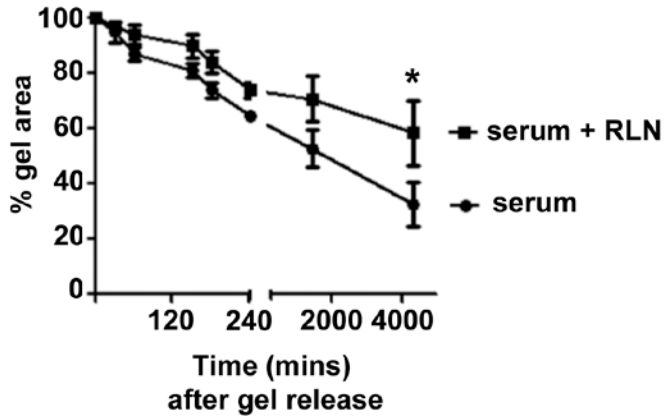


GFAP



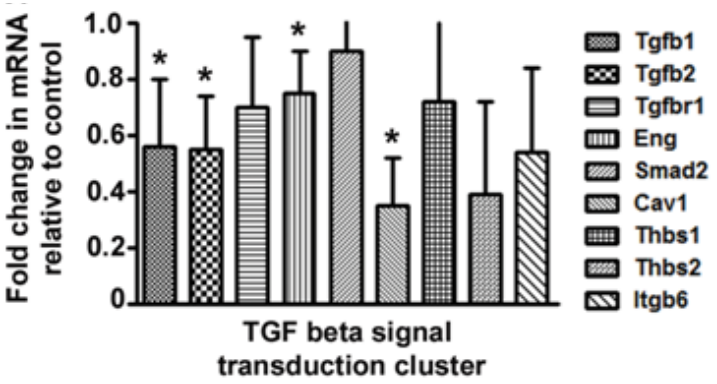


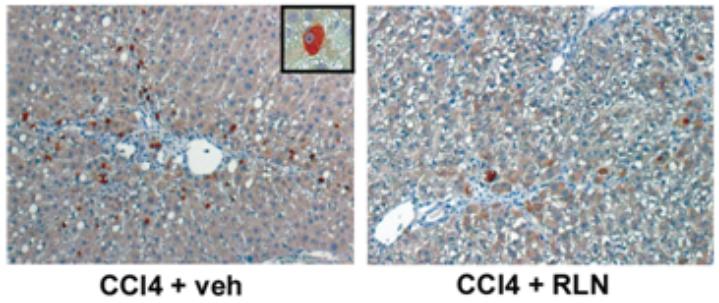


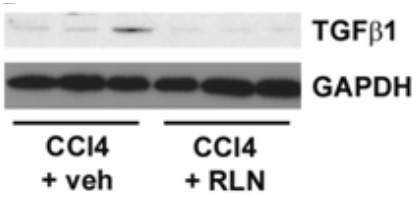


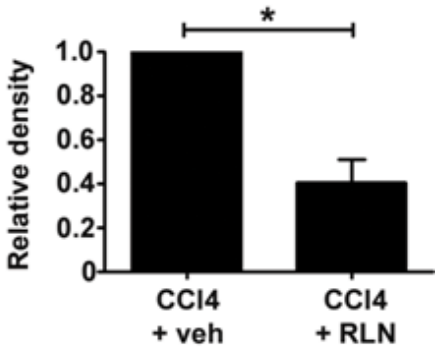
40% ↓ gel area after RLN – effect abrogated by pretreatment with RXFP1 siRNA

Relaxin regulates TGFβ pathway, but does not affect MFB numbers/viability

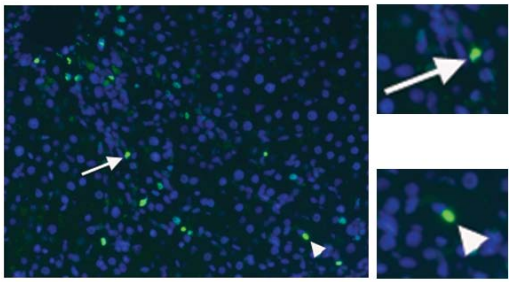


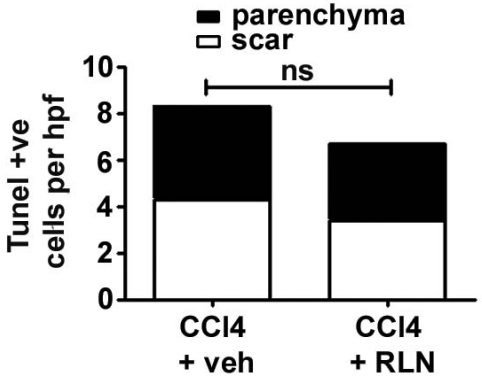




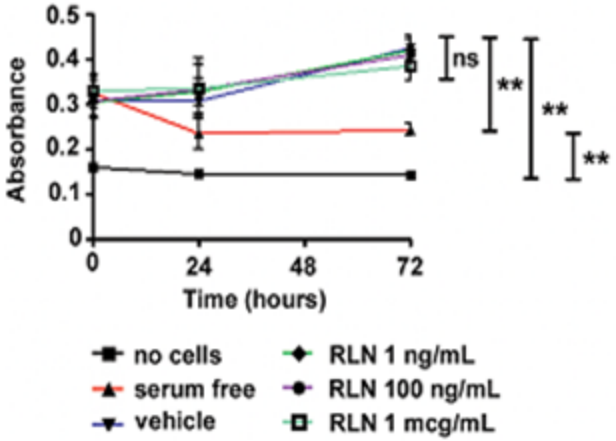


Tunel staining 8 week CCl_4 rat liver after 72 h RLN or vehicle





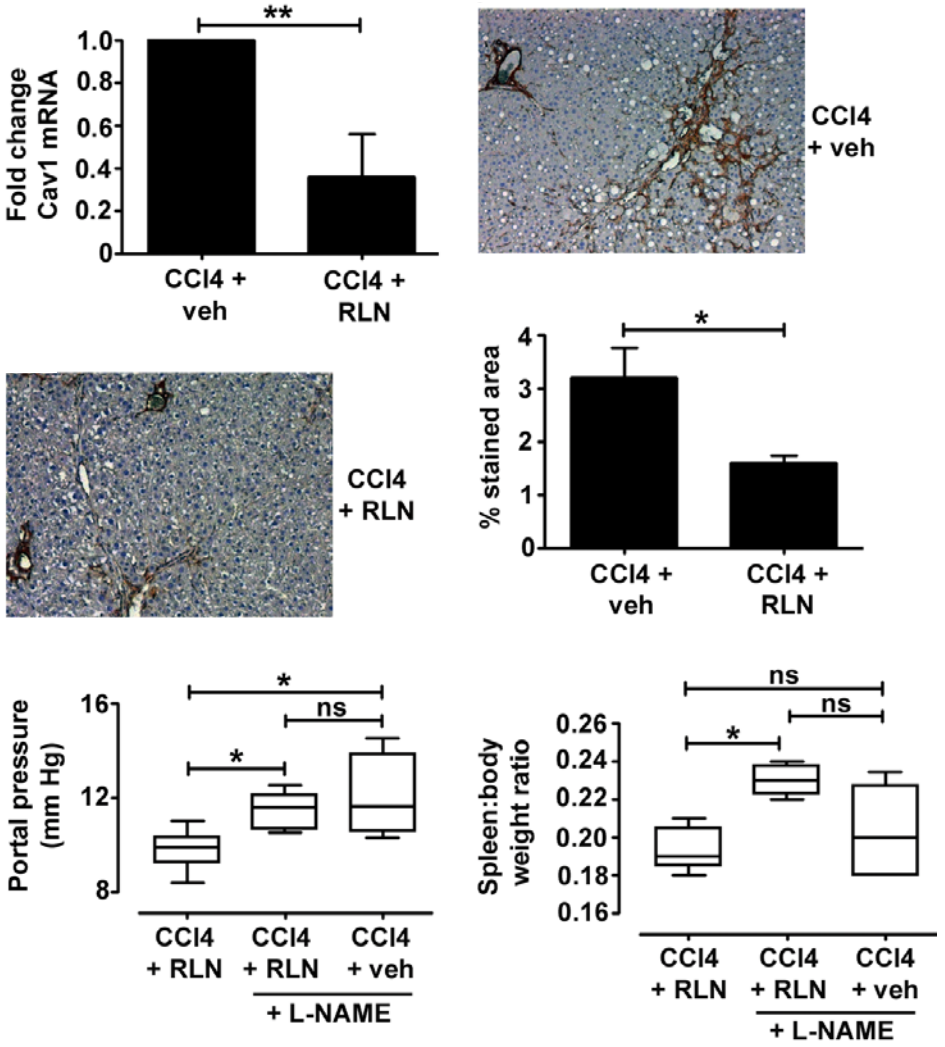
MTS assay: rat HSC-MFs



Relaxin augments intrahepatic (but not systemic) NO and NO signaling pathway

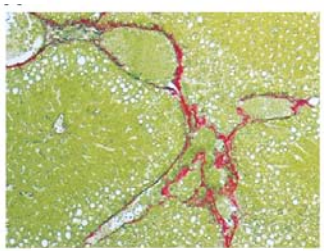
Relaxin downregulated Caveolin-1 gene and protein expression

Effect or relaxin on portal pressure abrogated by co-administration of NO synthase inhibitor L-NAME

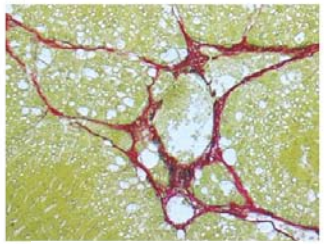


Relaxin selectively reduces portal pressure in advanced cirrhosis models

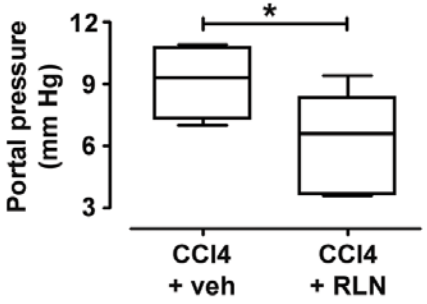
16 weeks CCl_4

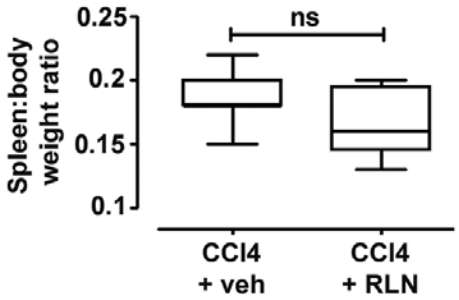


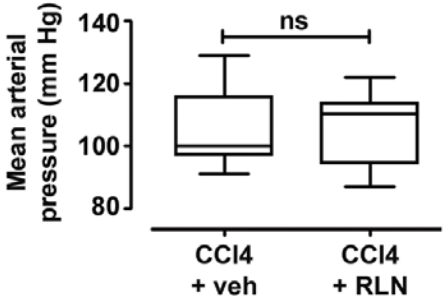
CCl_4 + veh



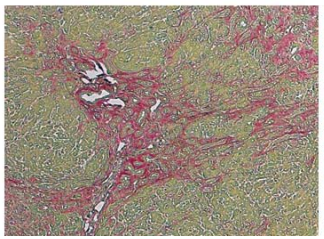
CCl_4 + RLN



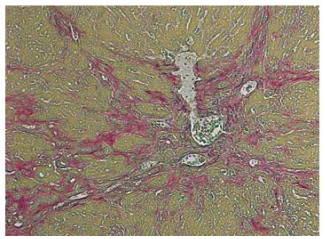




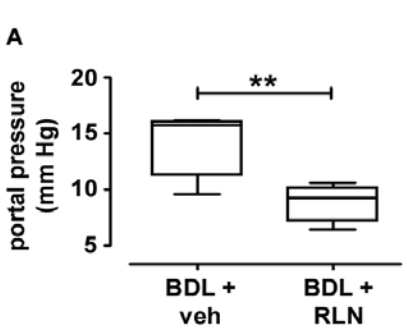
3 weeks bile duct ligation

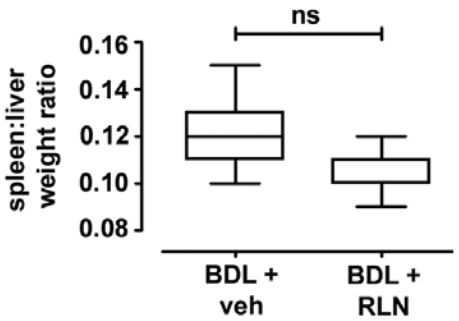


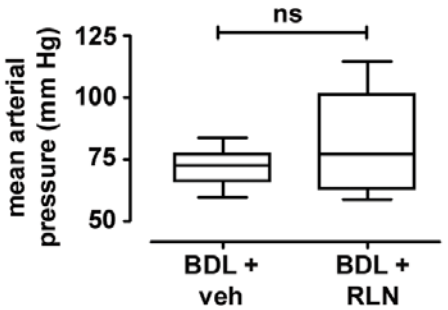
CCl_4 + veh



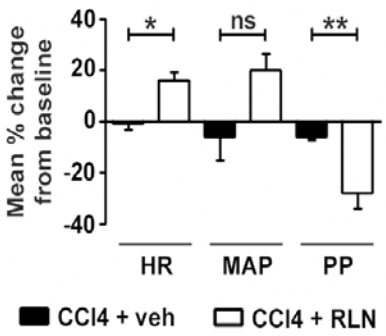
CCl_4 + RLN

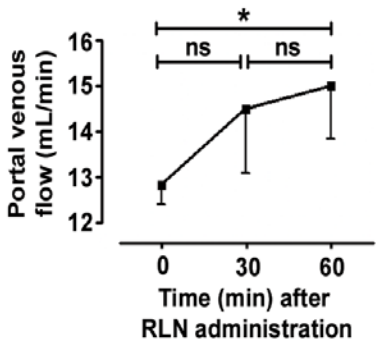




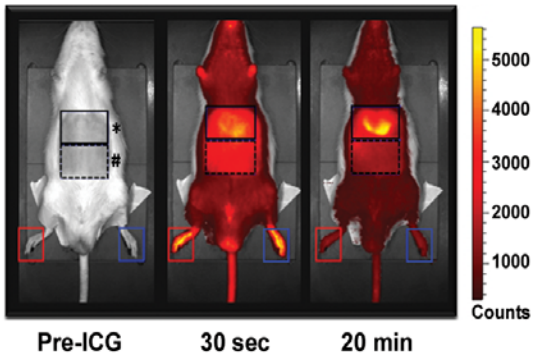


Acute i.v. relaxin administration reduces portal pressure, but sustains portal blood flow

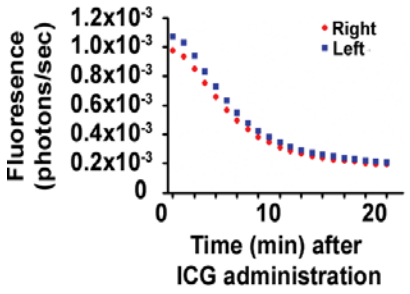


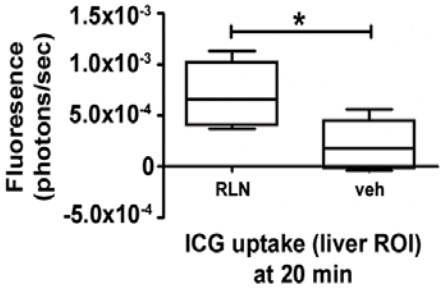


8 weeks CCl_4 rat: haemodynamic monitoring (HR, MAP, PP, PVBF) post i.v. RLN

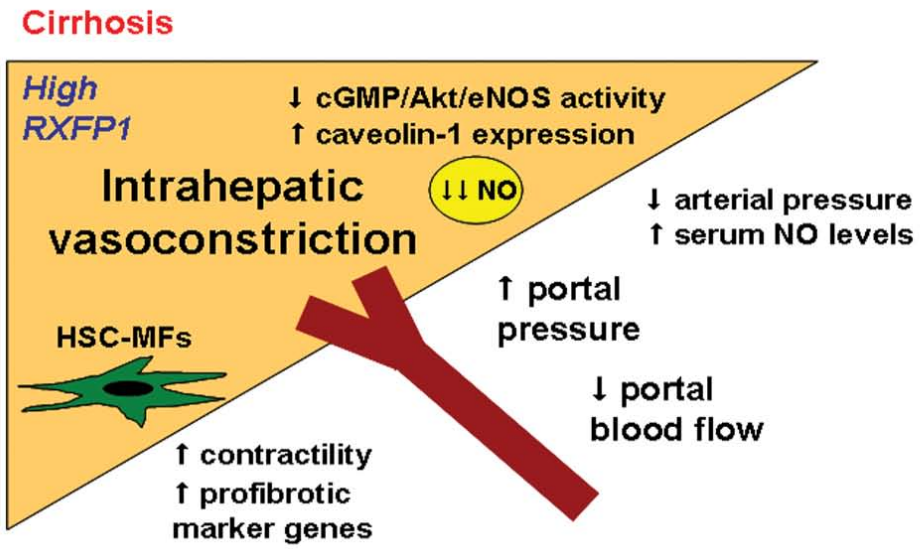


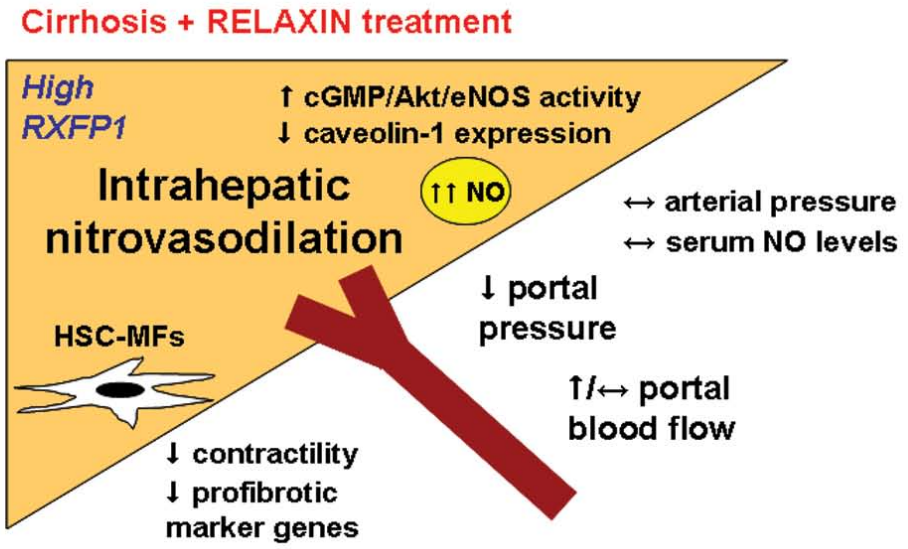
8 weeks CCl_4 rat: 30 min post i.v. RLN, ICG kinetics analysed over 20 min





Summary


Cirrhosis
High RXFP1
↓ cGMP/Akt/eNOS activity
↑ caveolin-1 expression
Intrahepatic vasoconstriction
↓↓ NO
↓ arterial pressure
↑ serum NO levels
↑ portal pressure
↓ portal blood flow
HSC-MFs
↑ contractility
↑ profibrotic marker genes


Cirrhosis + RELAXIN treatment
High RXFP1
↑ cGMP/Akt/eNOS activity
↓ caveolin-1 expression
Intrahepatic nitrovasodilation
↑↑ NO
↔ arterial pressure
↔ serum NO levels
↓ portal pressure
↑/↔ portal blood flow
HSC-MFs
↓ contractility
↓ profibrotic marker genes

ESHRE Workshop Edinburgh 2013

Take Home Messages:

•Wound healing and fibrosis are generic mechanisms which demonstrate common attributes across a range of organs. Identified targets may well be valuable across organs

•The hepatic scar is dynamic with respect to both its cellular and matrix components and makes an excellent model to examine scar plasticity.

•The wound healing MFBs of the liver and the hepatic macrophages are key players in progressive and resolving fibrosis.

•Therapies targeting the TIMP/MMP balance and the dynamic functions of the wound healing MFB show promise not only in reducing fibrosis, but through alterations in contractility, portal pressure and other plastic attributes of scars.

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