Fibrogenesis and fibrolysis in opisthorchiasis: an implication for chemoprevention

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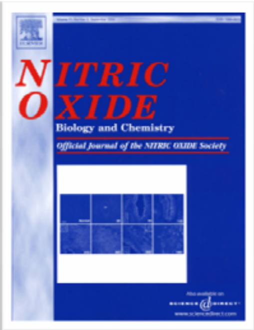
CONTENTS

Opisthorchis viverrini (OV) induces DNA damage contribution to the disease and CCA



OV infection

↓

Inflammation

↓

Free radical: ROS & RNS

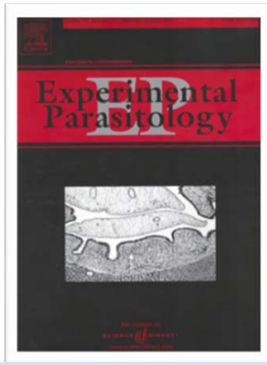
↓

Oxidative & Nitrative DNA damage

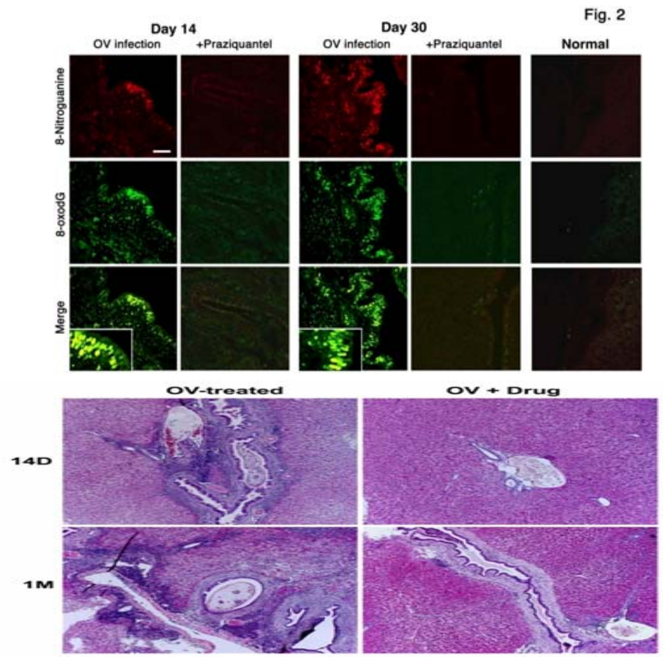
↓

CCA development

Pinlaor, S et al.
BBRC, 2003;
Nitric Oxide,2004;
Carcinogenesis,2004



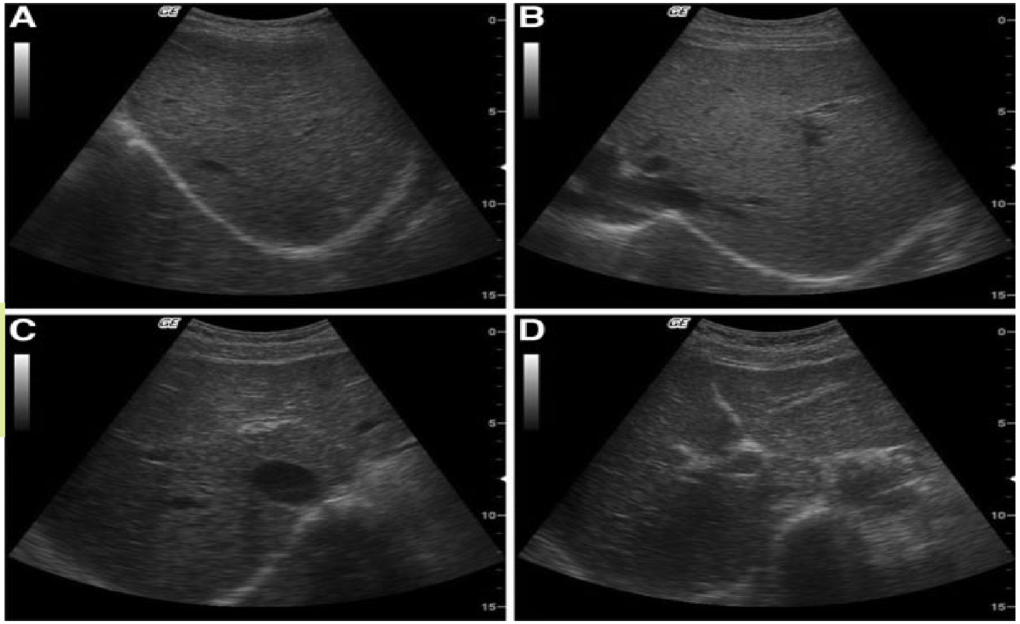
Pinlaor, et al. Exp. Parasitol, 2004

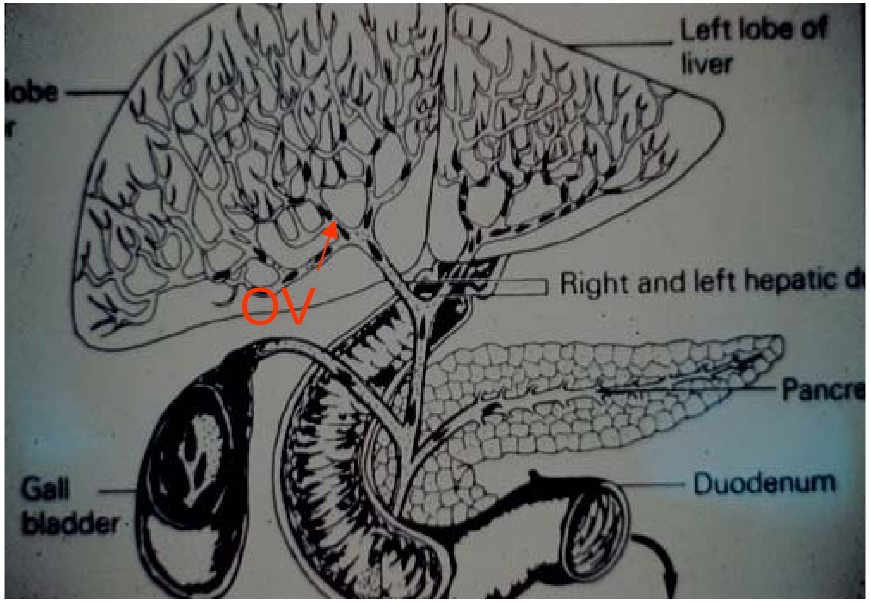


Pinlaor, Int J Cancer, 2006

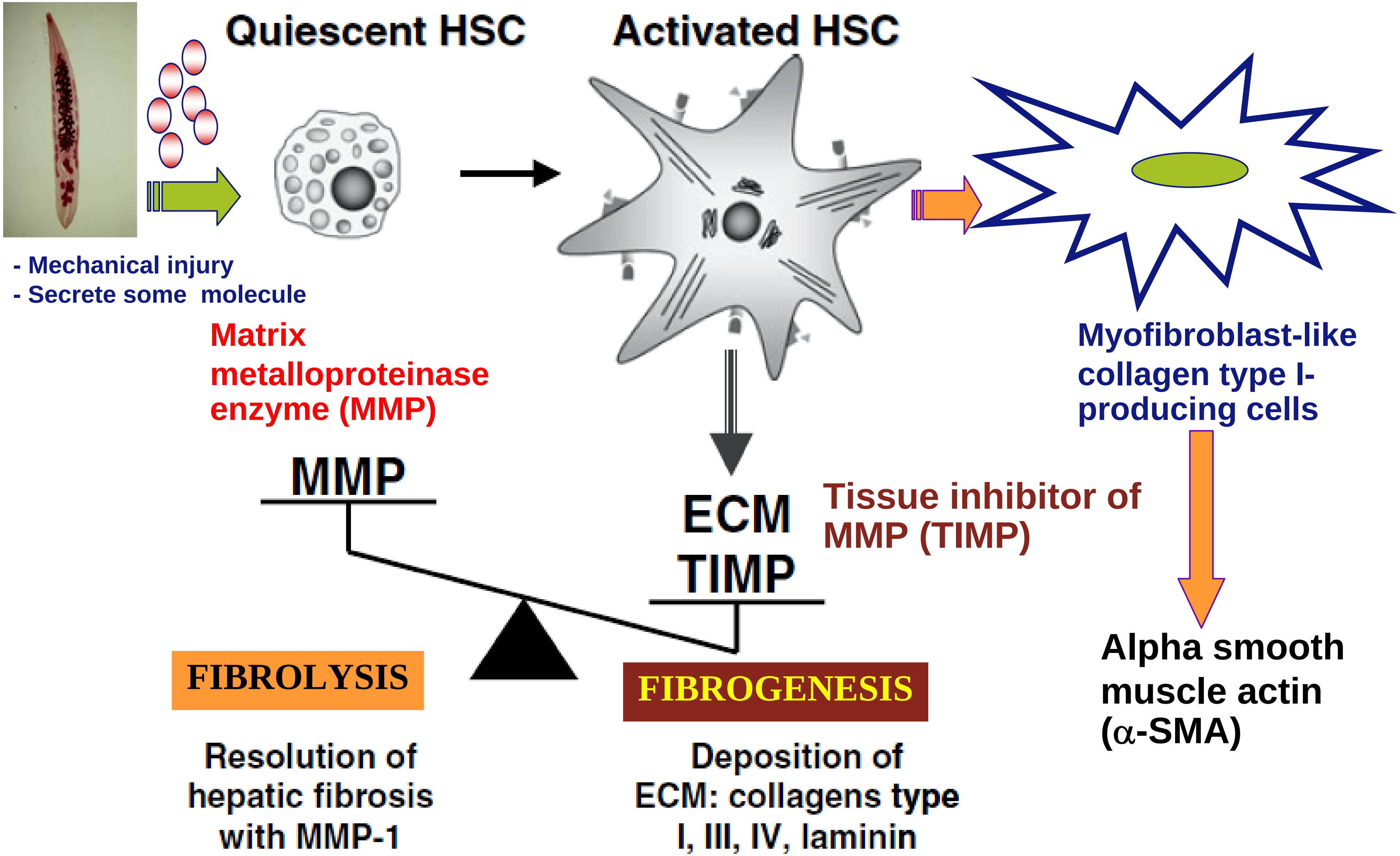
Advanced periductal fibrosis of OV-infected patient associated with the elevated level of IL-6

Sripa et al., Hepatology 2009



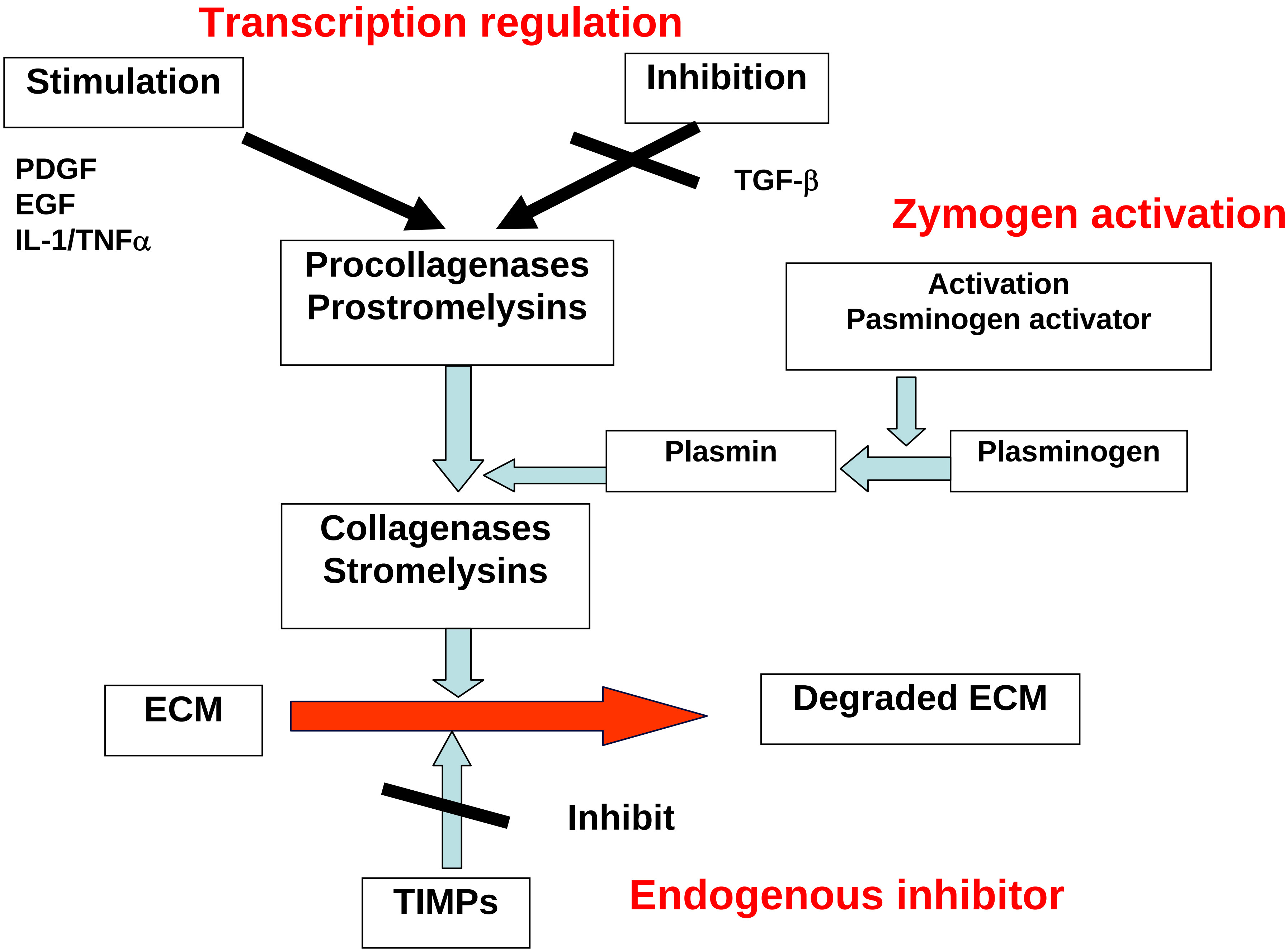


Fibrogenesis & Fibrolysis



Modified from Tatiana Kisseleva and David A Brenner,2006

MMP regulation and ECM metabolism



International Journal for Parasitology 39 (2009) 825–835

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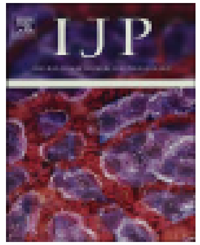

Time profiles of the expression of metalloproteinases, tissue inhibitors of metalloproteases, cytokines and collagens in hamsters infected with *Opisthorchis viverrini* with special reference to peribiliary fibrosis and liver injury

Suksanti Prakobwong[a,d], Somchai Pinlaor[a,d,*], Puangrat Yongvanit[b,d], Paiboon Sithithaworn[a,d], Chawalit Pairojkul[c,d], Yusuke Hiraku[e]

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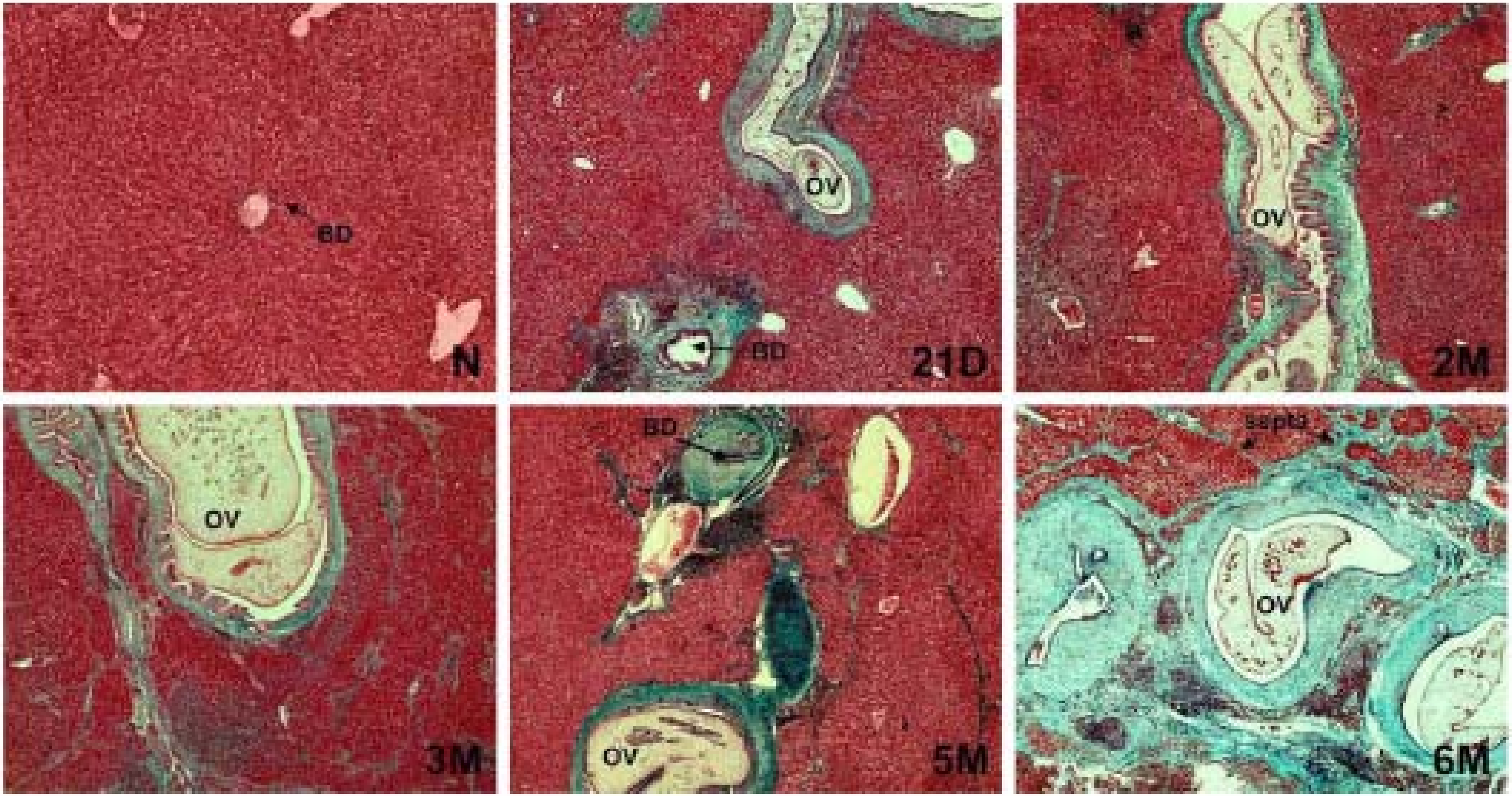
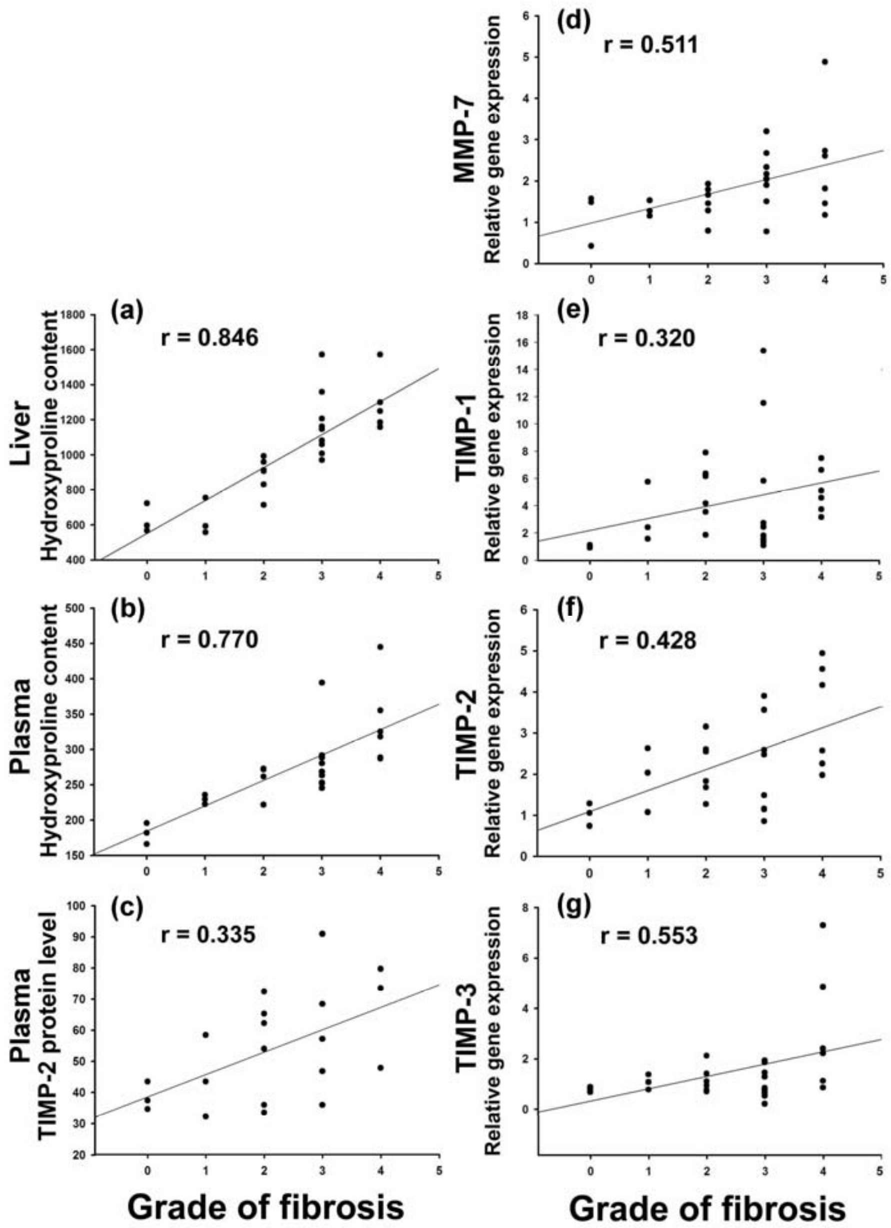
[c] *Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*
[d] *The Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*
[e] *Department of Environmental and Molecular Medicine, Mie University Graduate School of Medicine, Tsu, Mie 514-8507, Japan*

OV infection increased fibrosis with time-dependent



The correlation of grading score of fibrosis and the fibrotic markers



CONCLUSION I

- **OV infection increased fibrosis with time-dependent**
- **Grading score of fibrosis positively correlated with:**
  - **The level of hydroxyproline in the liver and in the plasma**
  - **Plasma TIMP-2 level**
  - **mRNA expression level of *MMP-7, TIMPs-1, -2*, and *-3***

CONCLUSION II

- Curcumin had no effect on periductal fibrosis at the short-term in OV-infected hamsters
- Curcumin decreased the thickness of periductal fibrosis at the long-term in OV-infected hamsters by:
  - Suppresion TIMPs and TNF-α genes
  - Increasing IL-1, TGF-β and MMPs-7, -13
  - Enhancing MMPs activities
- Curcumin may prove a valuable anti-fibrogenic agent including in the OV-induced fibrosis and prevent opisthorchiasis associated the risk of CCA development.

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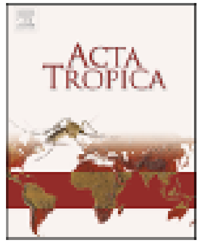

Effect of praziquantel treatment on the expression of matrix metalloproteinases in relation to tissue resorption during fibrosis in hamsters with acute and chronic *Opisthorchis viverrini* infection

Somchai Pinlaor[a,d,*], Suksanti Prakobwong[a,d], Thidarut Boonmars[a,d], Chaisiri Wongkham[b,d], Porntip Pinlaor[c,d], Yusuke Hiraku[e]

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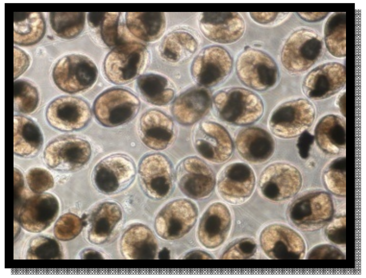
[e] Department of Environmental and Molecular Medicine, Mie University Graduate School of Medicine, Tsu, Mie 514-8507, Japan

MATERIALS AND METHODS

Fish digestion



Metacercaria collection

50 MC infected hamsters
4-6 week-old male golden hamsters

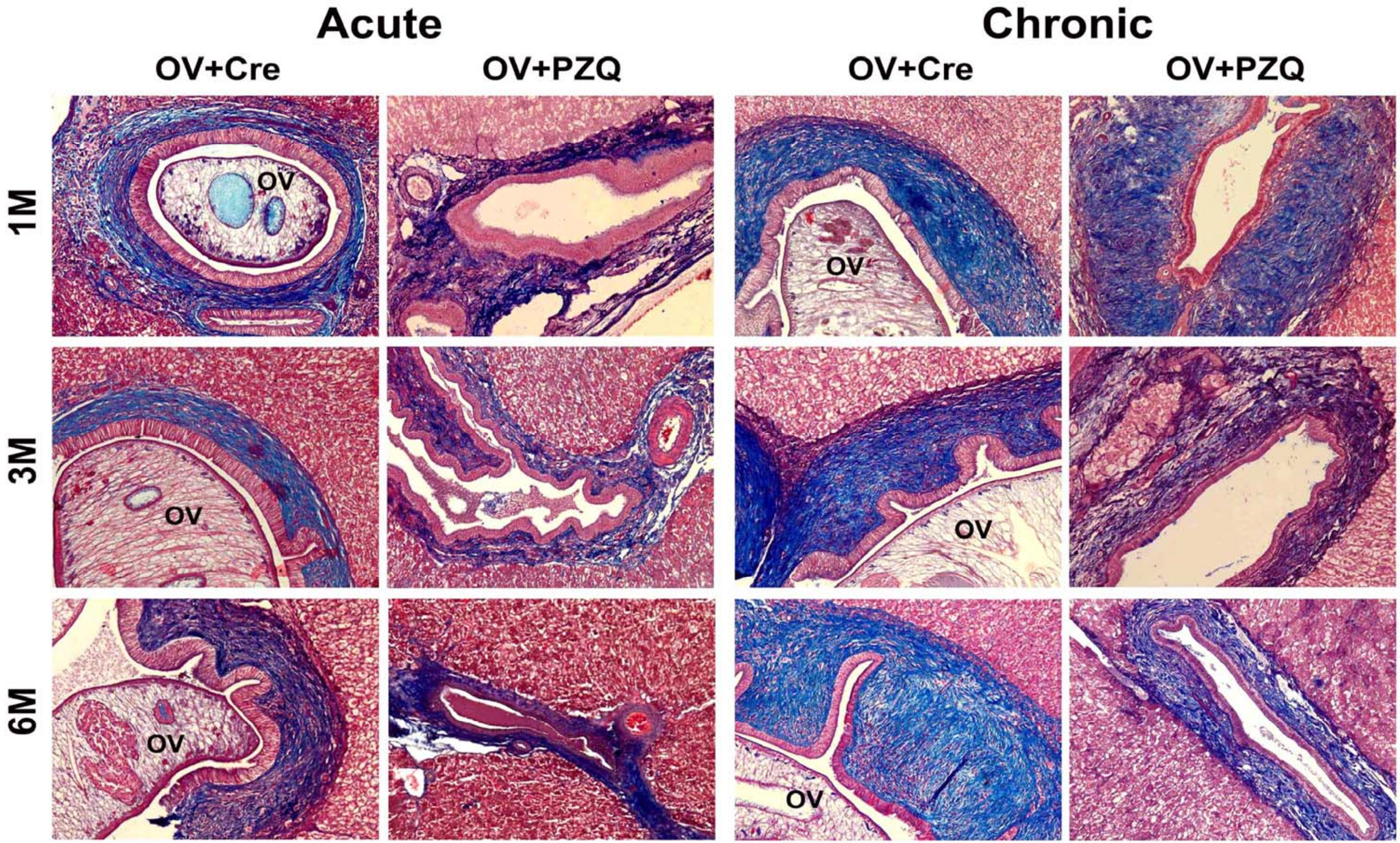
OV-infected for 21 days (acute infection,AI)

OV-infected for 4 months (chronic infection, CI)

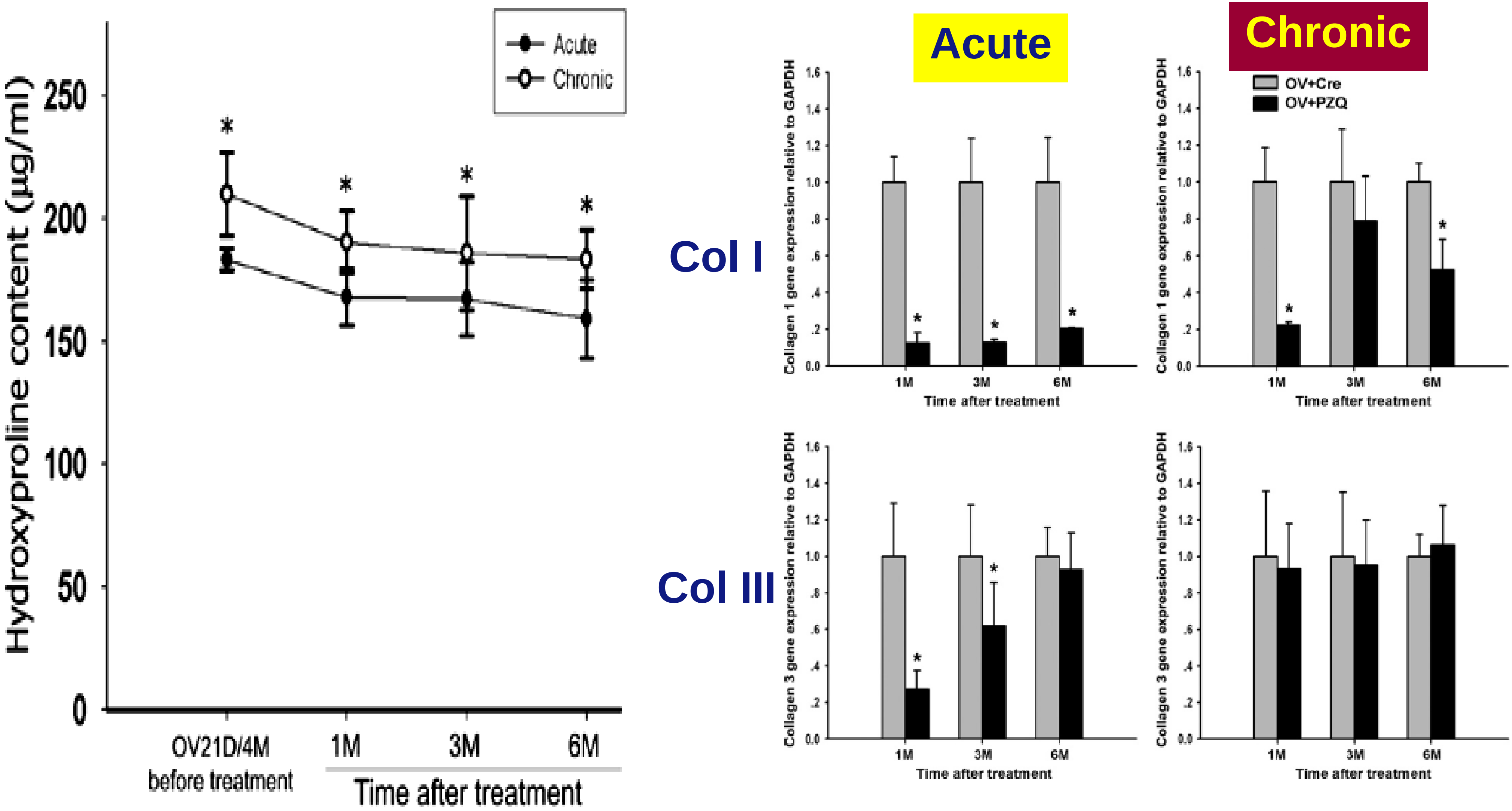
Treated with praziquantel 2 days

Sacrificed at 1, 3 and 6 months post-treatment

Slower tissues resolution in praziquantel (PZ) treatment of chronic *O. viverrini* -infected hamsters compared with acute infection



Reduction of hydroxyproline content and collagens gene expression



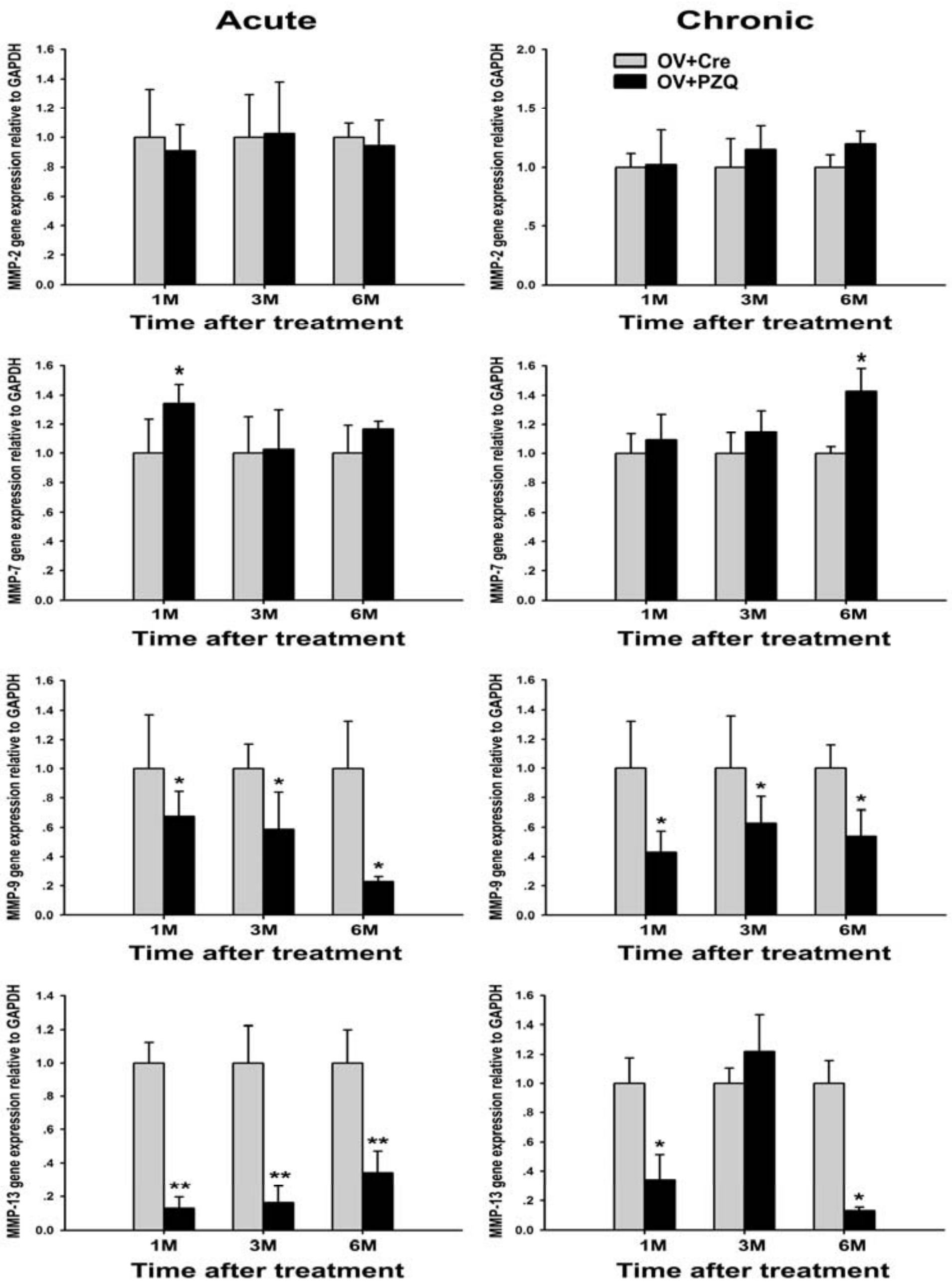
Profiles of mRNA expression of MMPs in OV - infected hamsters and the effect of PZ treatment

MMP2

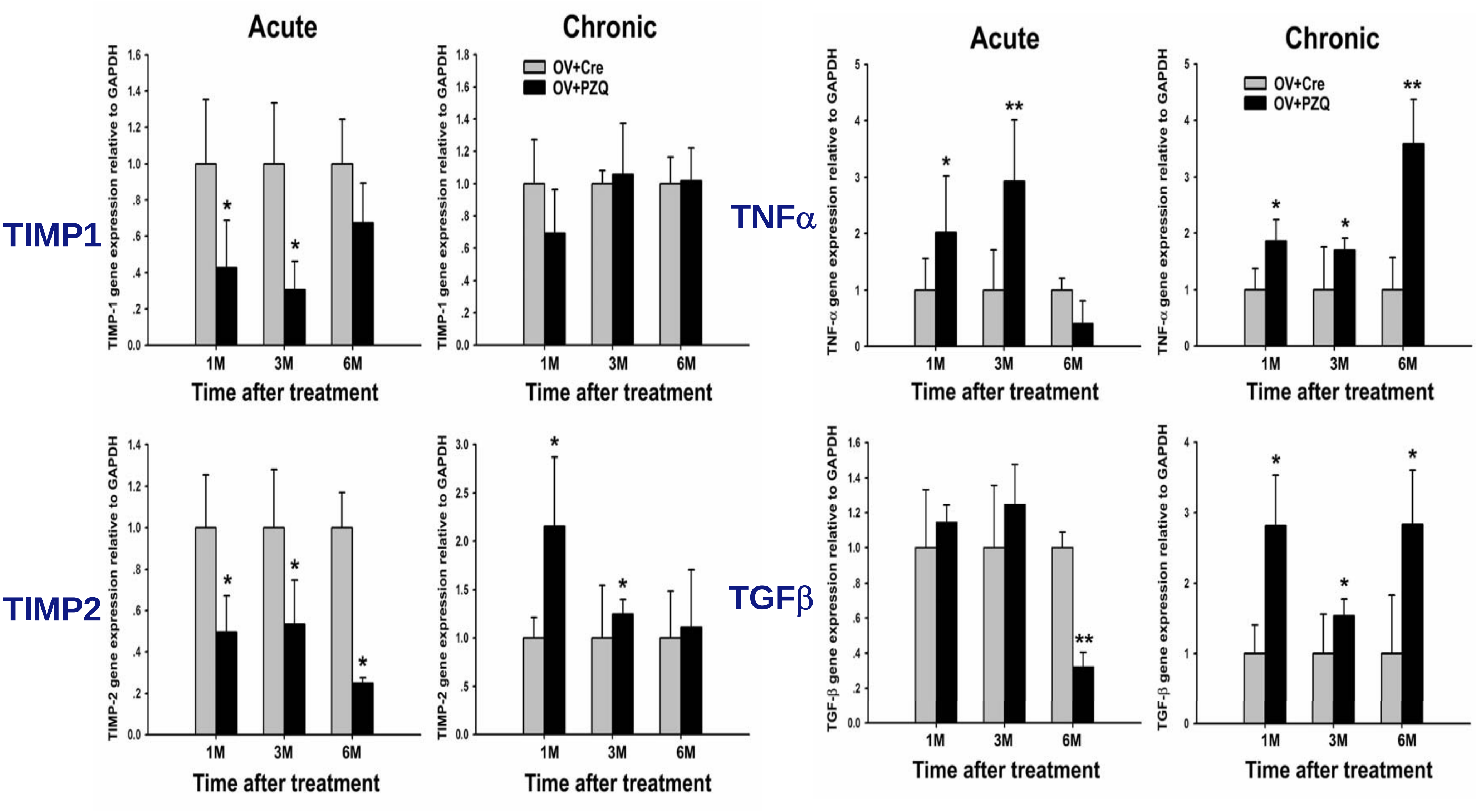
MMP7

MMP9

MMP13



Profiles of mRNA expression of TIMPs and cytokines



CONCLUSION III

- **Tissue resolution with AI and CI treatment is different and its is correlation with the expression levels of TIMPs, MMP7, TNF-α and TGF-β gene after praziquantel treatment**

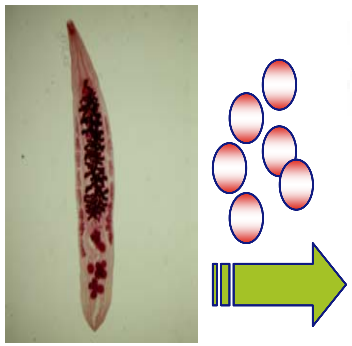
- **In animals with chronic OV infection, praziquantel treatment induces the inhibition of MMP activity via the expression of TGF-β, and TIMPs, resulting in slow resorption of hepatic fibrosis**

SUMMARY

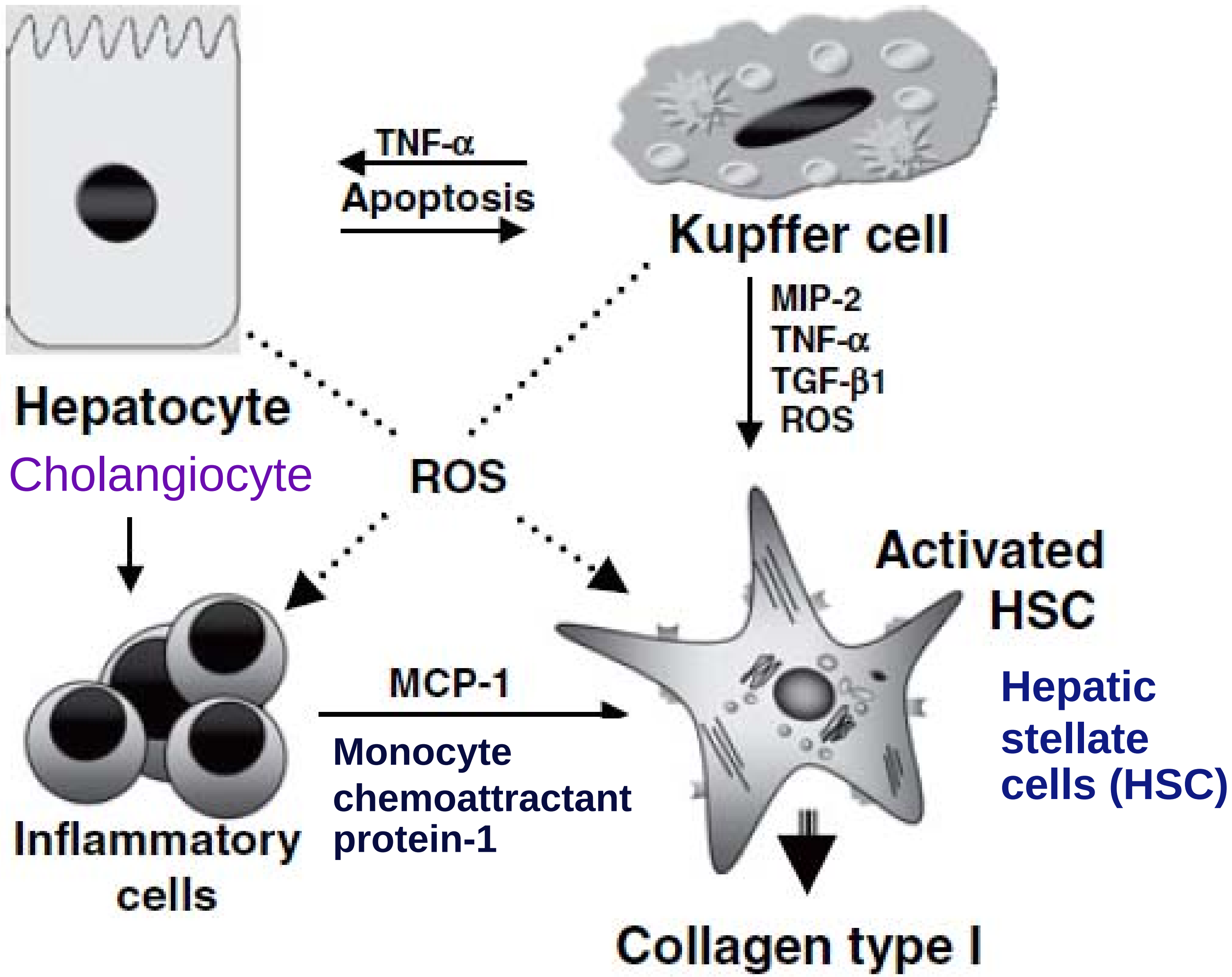
- OV infection increases periductal fibrosis time-dependent in the correlation with fibrotic markers
- Curcumin decreases fibrosis at the long-term treatment which it may use as chemopreventive agent in opisthorchiasis patient
- Reduction rate of tissue resolution is different between PZ-treated in acute and chronic OV infection
- PZ-treated at chronic OV infection has slow tissue resolution

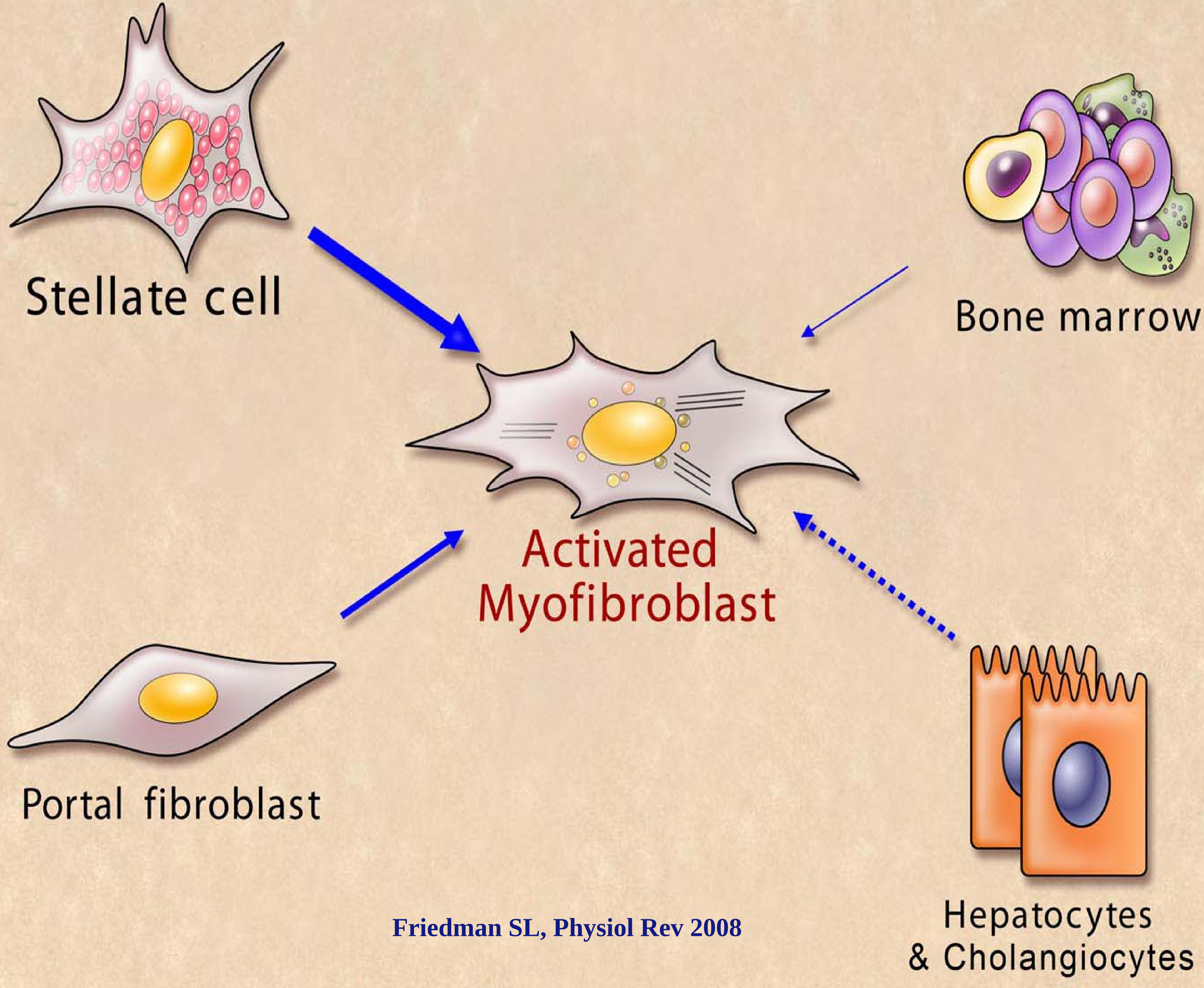
Thanks you for your attention

Fibrogenesis induced by OV infection

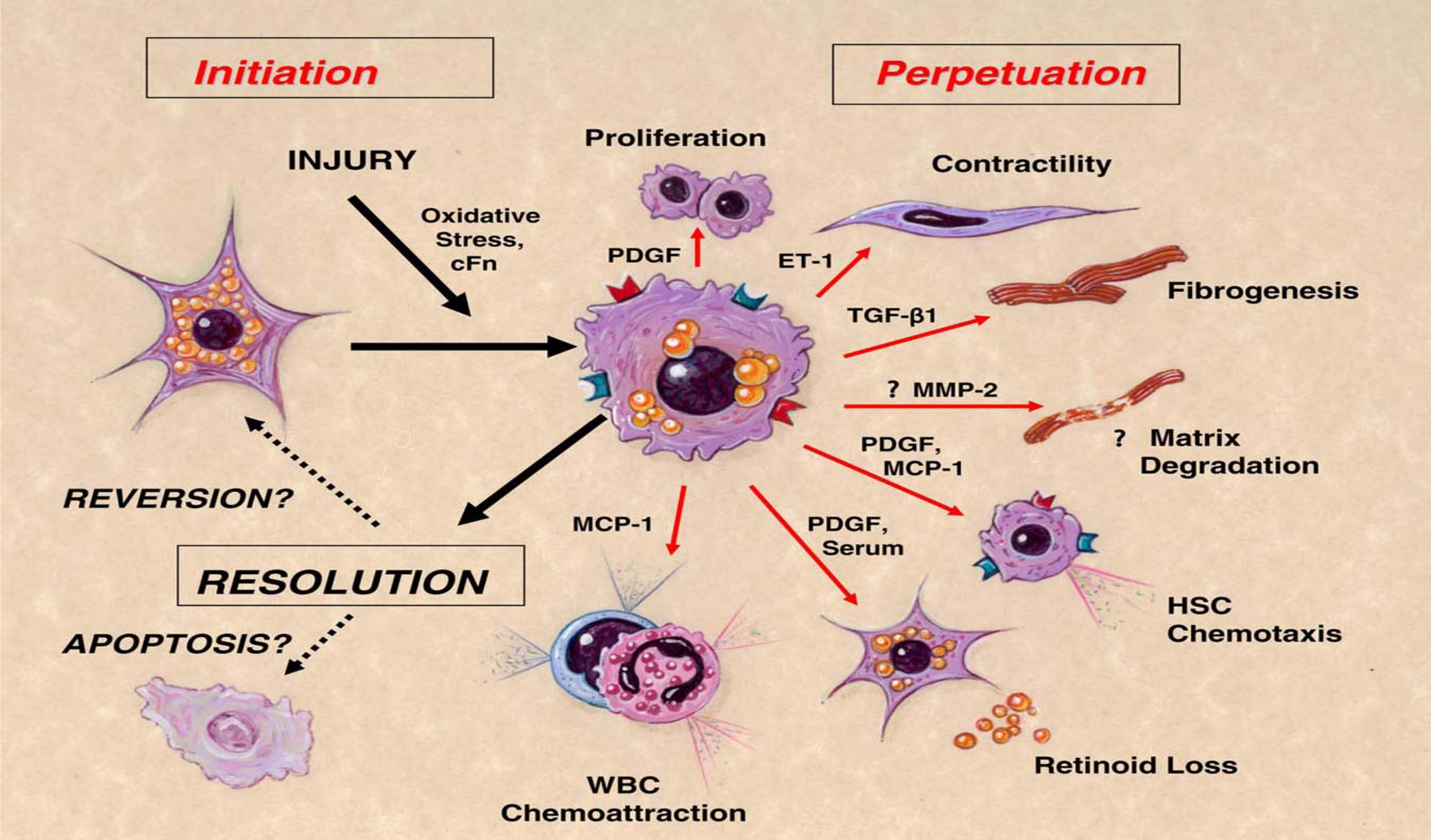


- Mechanical injury
- Secrete some molecule

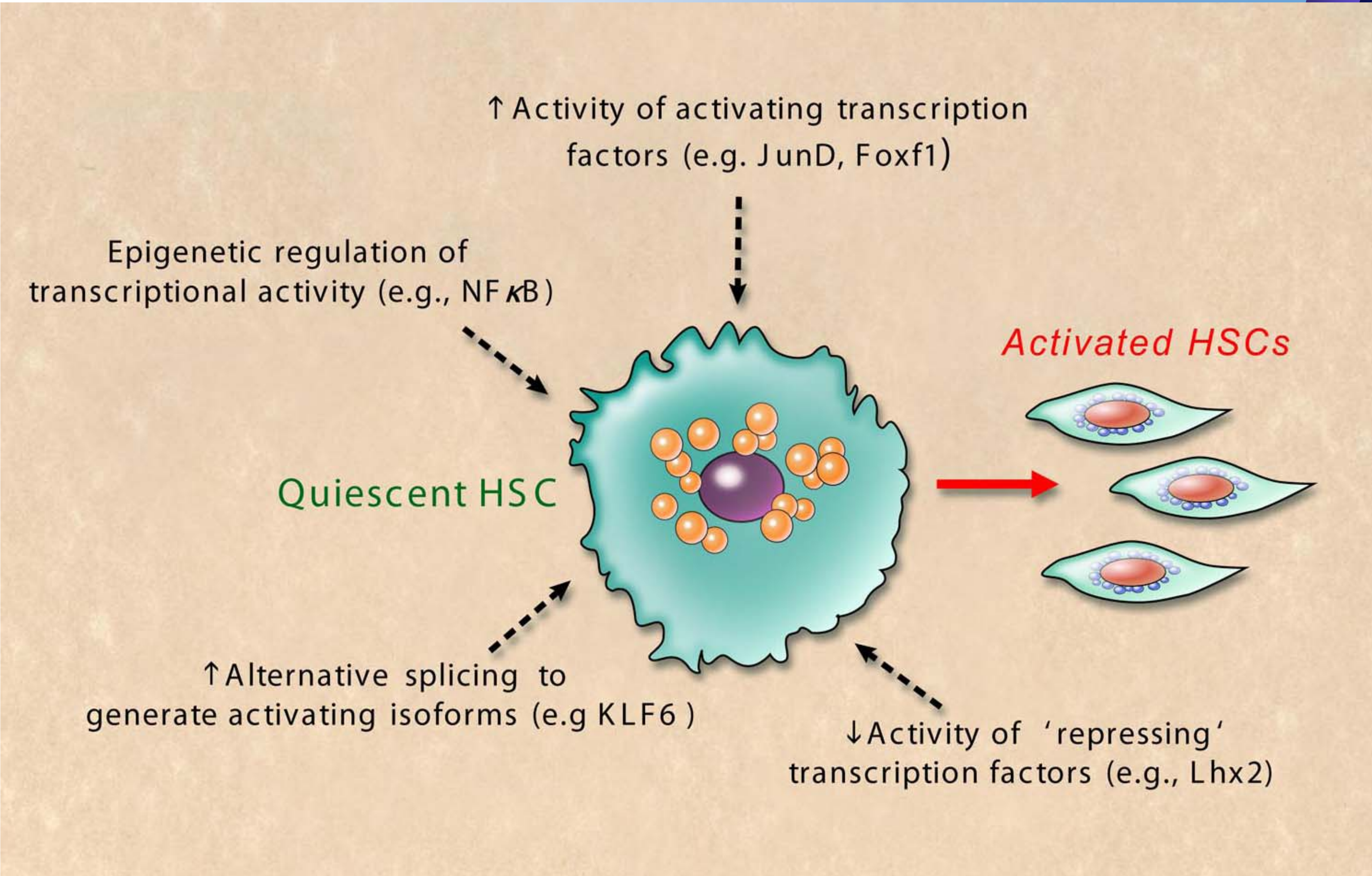




Friedman SL, Physiol Rev 2008

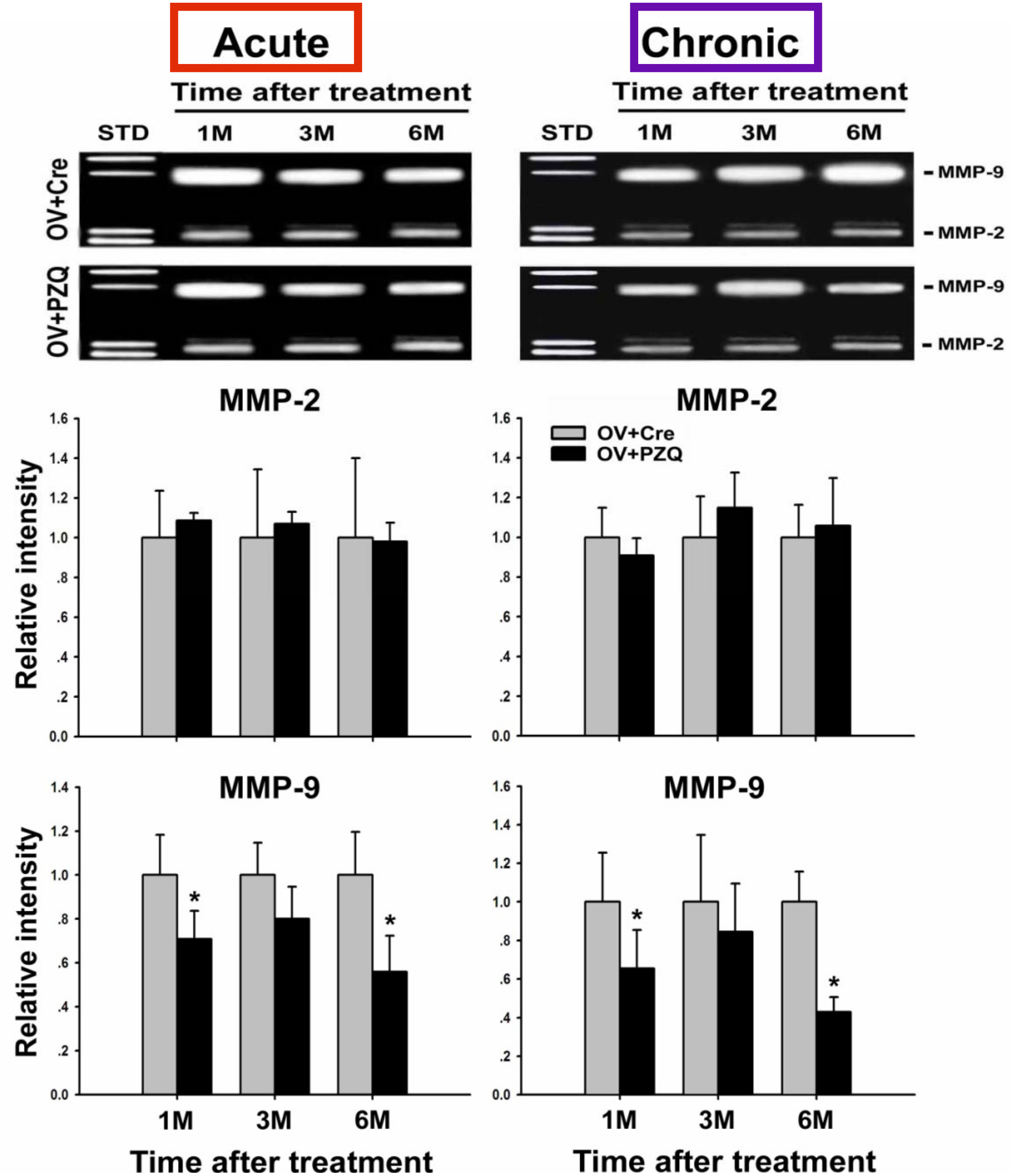


Friedman SL, Physiol Rev 2008



Friedman SL, Physiol Rev 2008

Gelatinase activity in OV-infected hamsters and the effect of PZ treatment



Family of metalloproteinases

Enzyme	MMP	Main substrate (s)
Collagenases		
Collagenase I	MMP 1	collagen
Collagenase II	MMP 8	collagen, gelatin, proteoglycan
Collagenase III	MMP 13	collagen, gelatin, proteoglycan
Collagenase IV	MMP 18	Not defined
Gelatinases		
Gelatinase A	MMP 2	gelatin, collagen, elastin, fribonectin
Gelatinase B	MMP 9	gelatin, collagen, elastin, fribonectin
Stromelysins		
Stromelysins	MMP 3	proteoglycan, fribonectin, laminin, collagen, elastin
Stromelysins	MMP 10	proteoglycan, fribonectin, laminin, collagen, elastin
Stromelysins	MMP 11	alph-1 proteinase
Membrane-type MMPs		
MT-MMP	MMP 14	progelatinase A

MMP expression regulation

- Transcription regulation
- Zymogen activation
- Tissue inhibitor of metalloproteinases (TIMPs1-4)

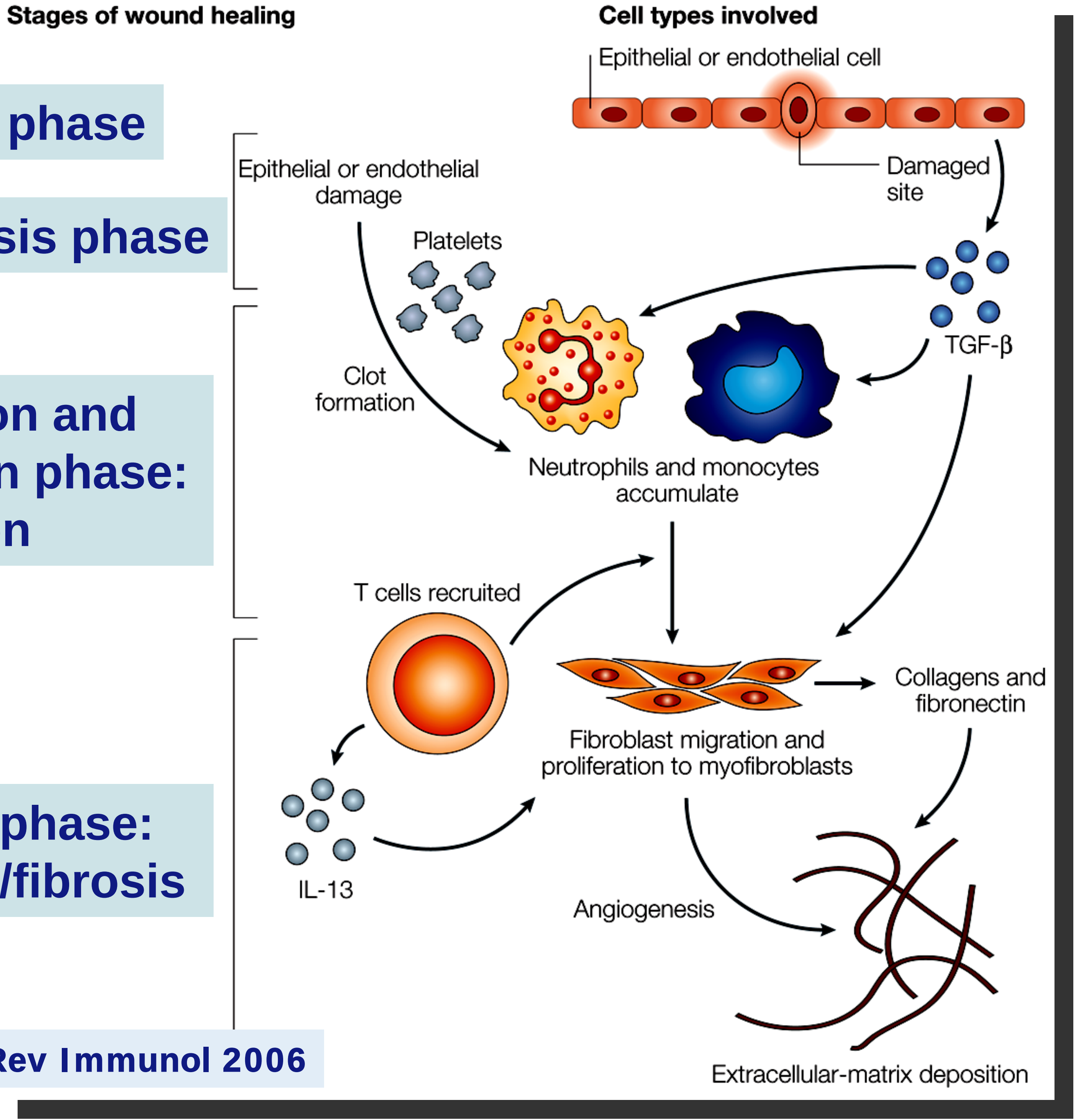
Stages of wound healing

Injury phase

Haematostasis phase

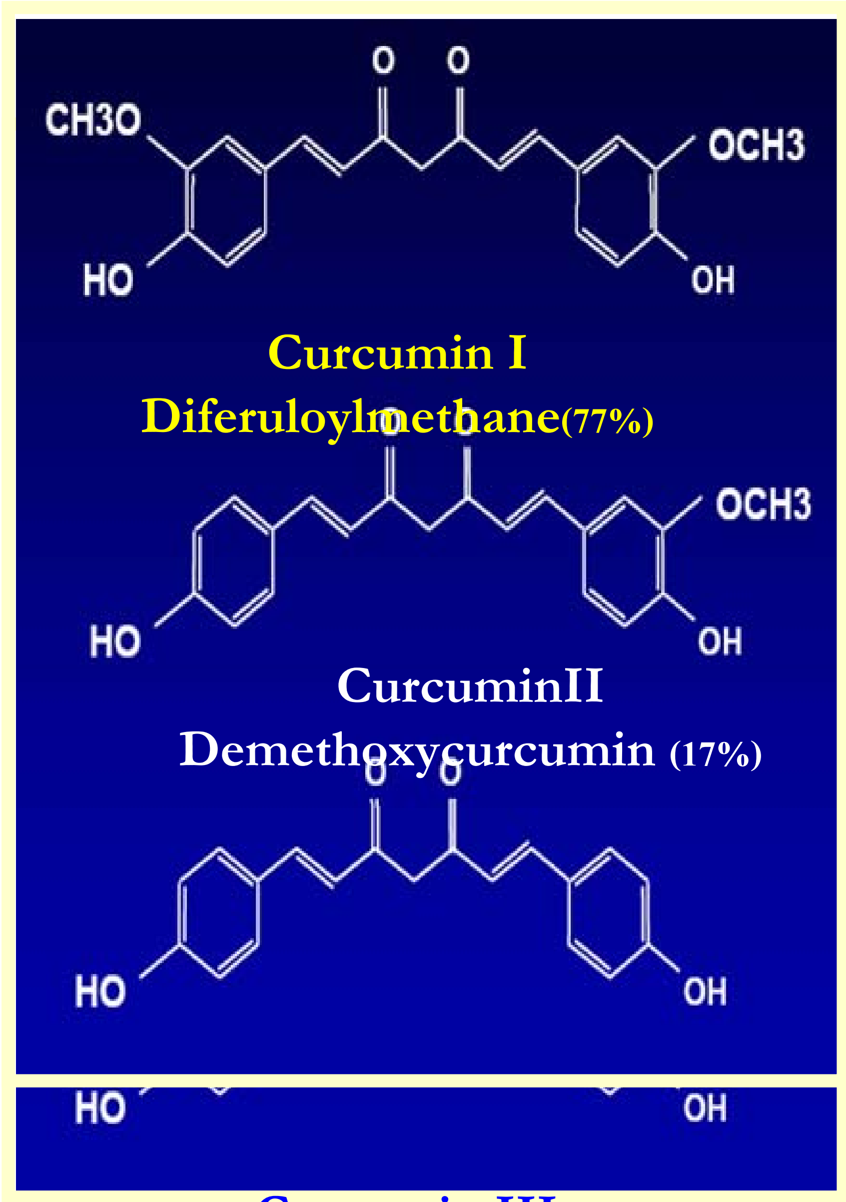
Inflammation and proliferation phase: regeneration

Maturation phase: remodeling/fibrosis



Wynn T., Nat Rev Immunol 2006

Properties of curcumin

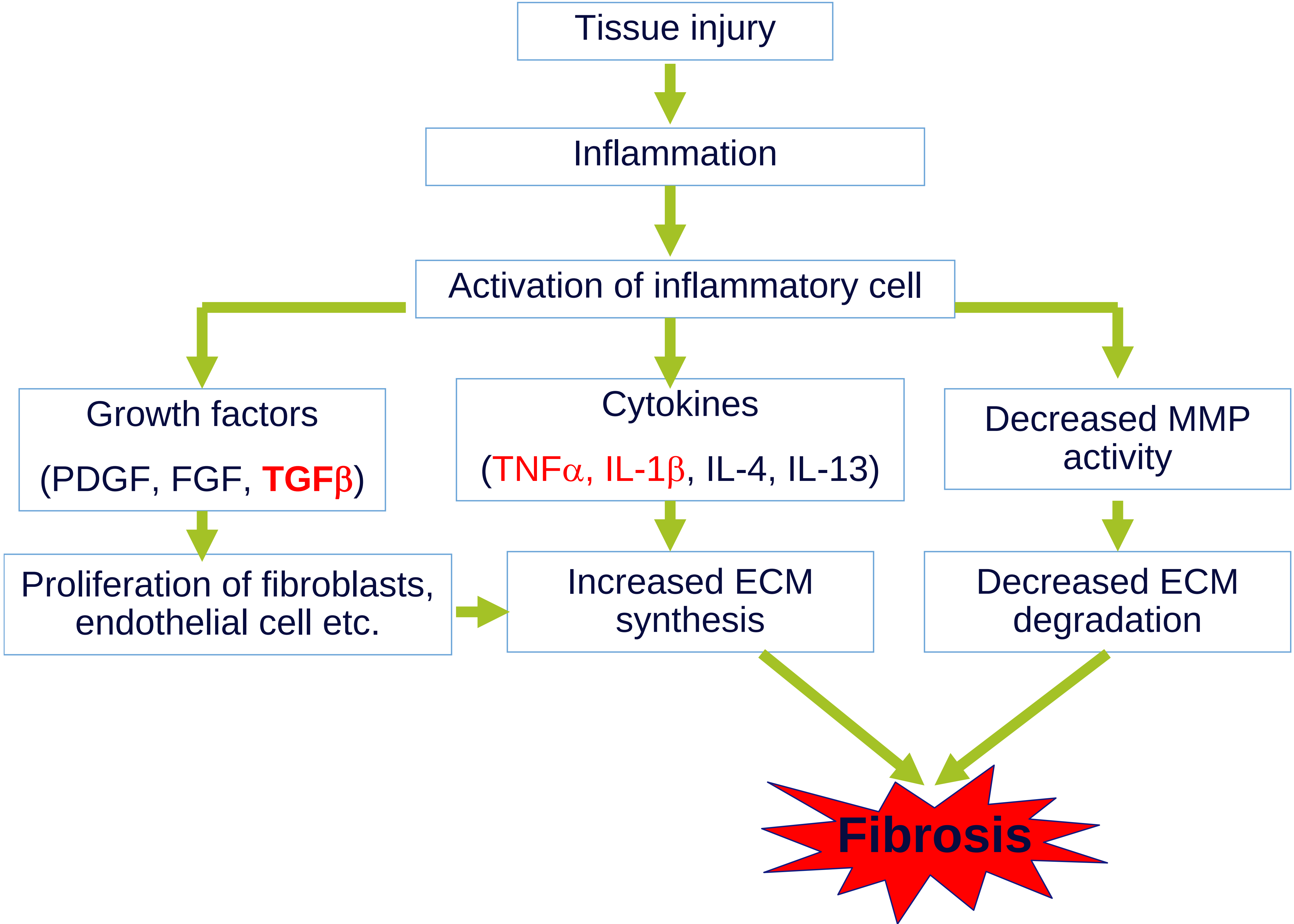


Curcumin I
Diferuloylmethane(77%)

CurcuminII
Demethoxycurcumin (17%)

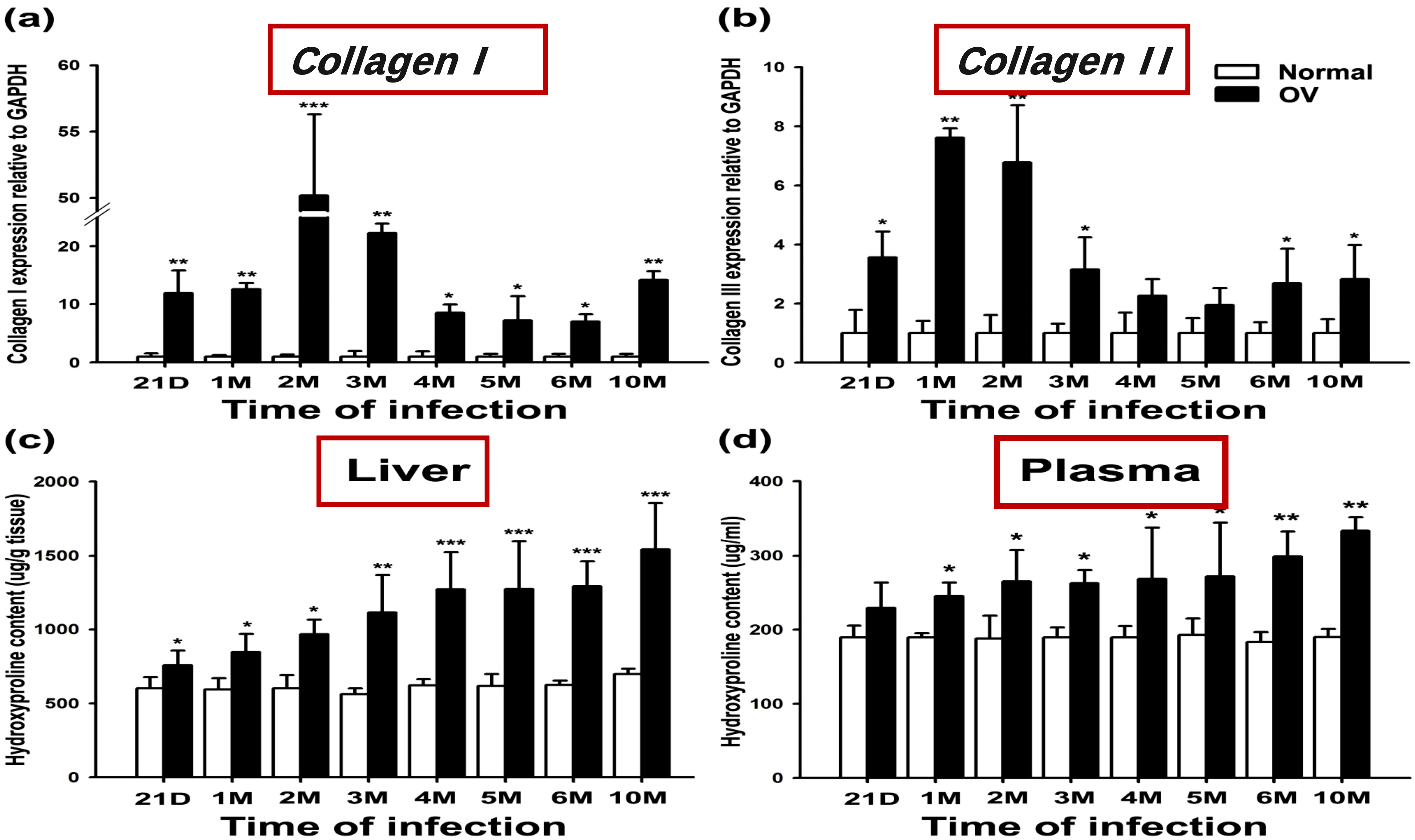
Curcumin III
Bis-Demethoxycurcumin (3%; less active)

- ➢ Free radical scavenging
- ➢ Anti-inflammation
- ➢ Antioxidant
- ➢ Increased bile secretion



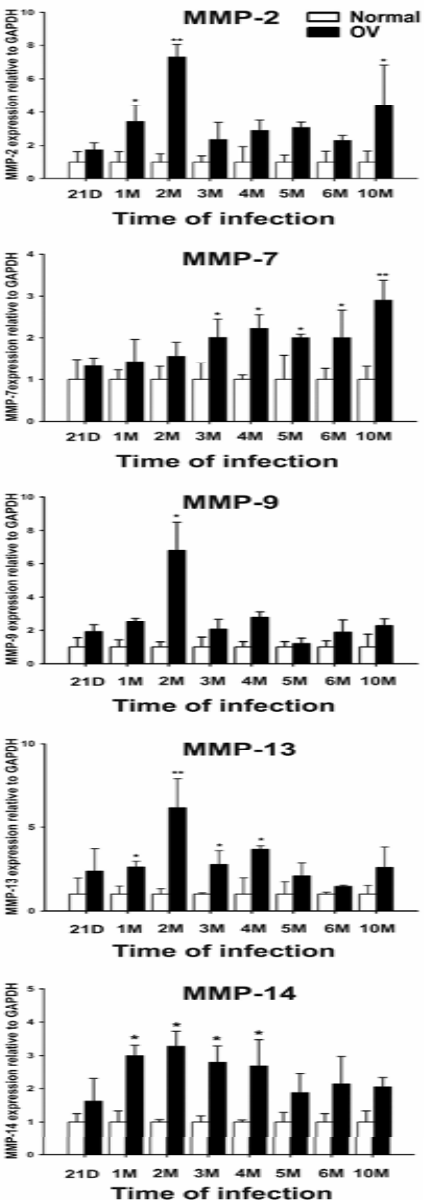
Tissue injury
Inflammation
Activation of inflammatory cell
Growth factors
(PDGF, FGF, TGFβ)
Cytokines
(TNFα, IL-1β, IL-4, IL-13)
Decreased MMP activity
Proliferation of fibroblasts, endothelial cell etc.
Increased ECM synthesis
Decreased ECM degradation
Fibrosis

OV -induced collagen gene expression and increased hydroxyproline (HP) level

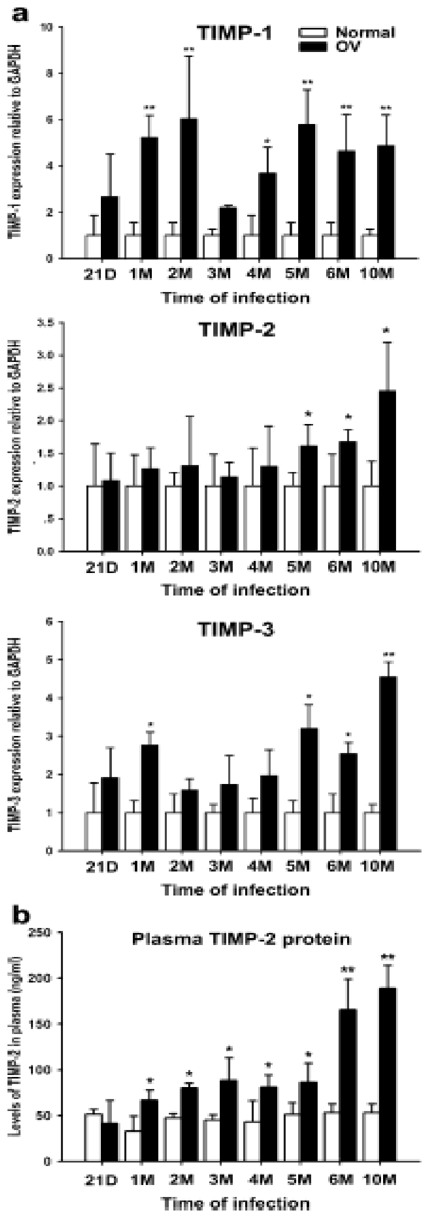


mRNA expression profiles of MMPs, TIMPs, cytokines

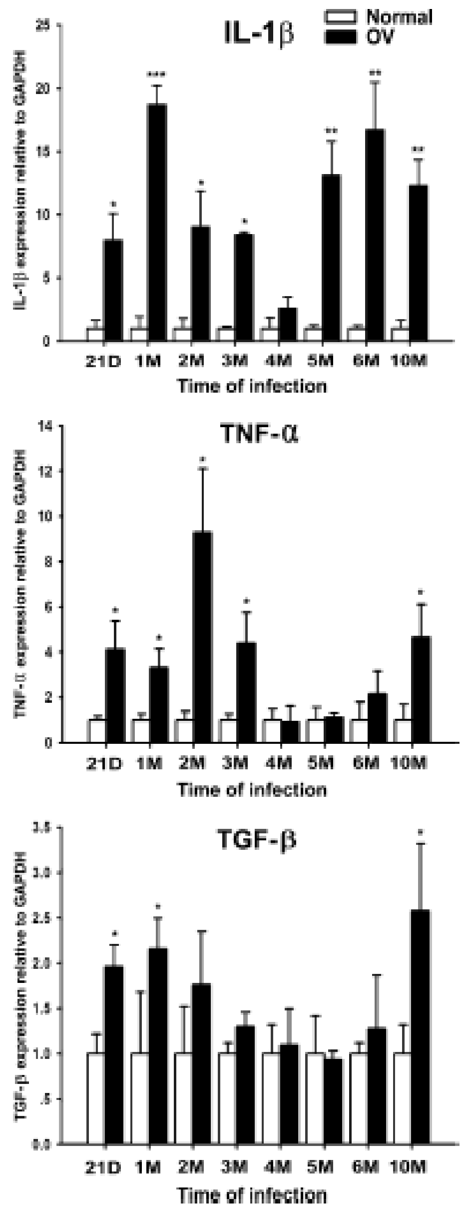
MMPs



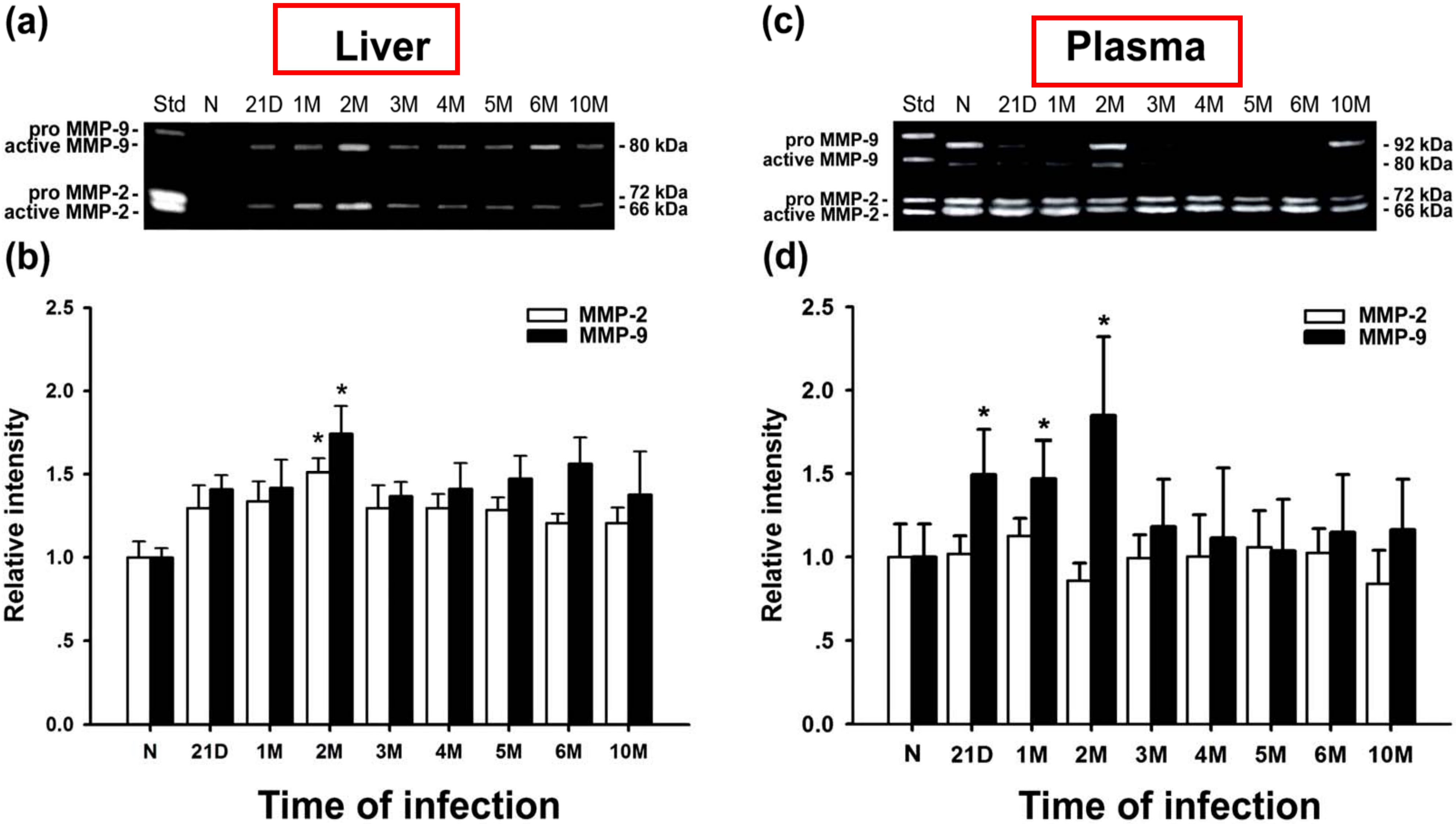
TIMPs



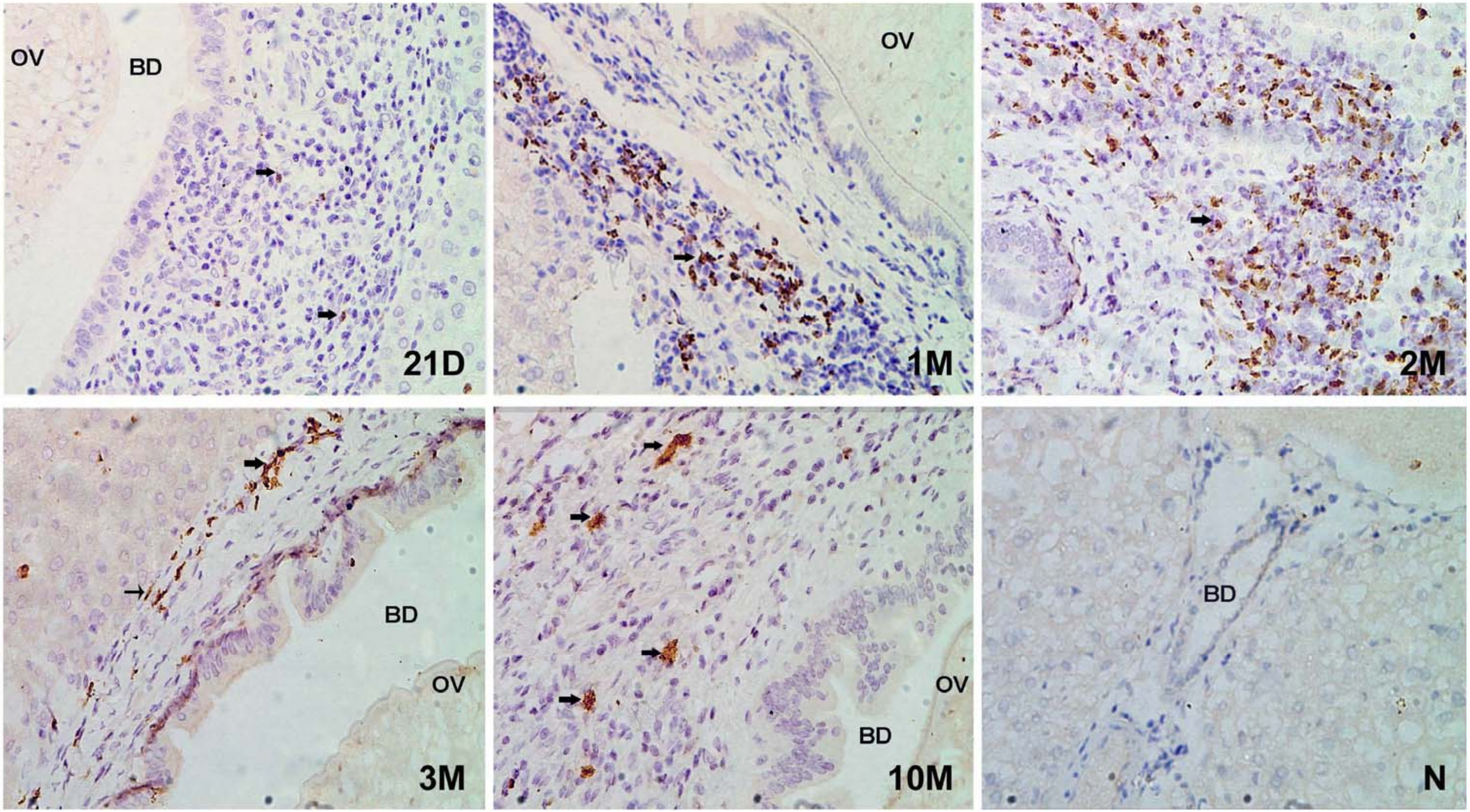
Cytokines



Gelatin zymography revealed MMPs-2 and -9 to be increased in the liver at all time points

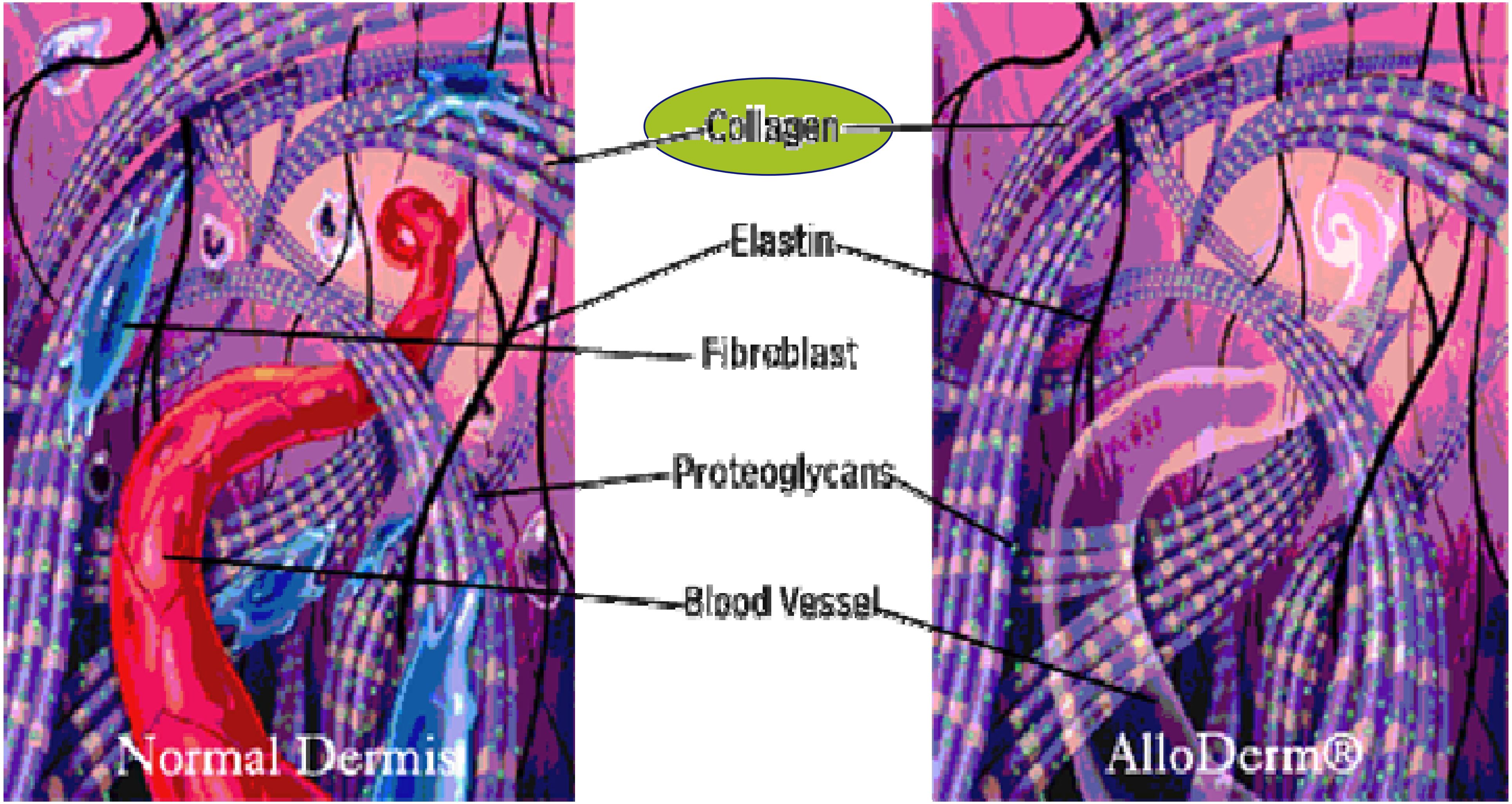


localized MMP-9 expression in liver sections

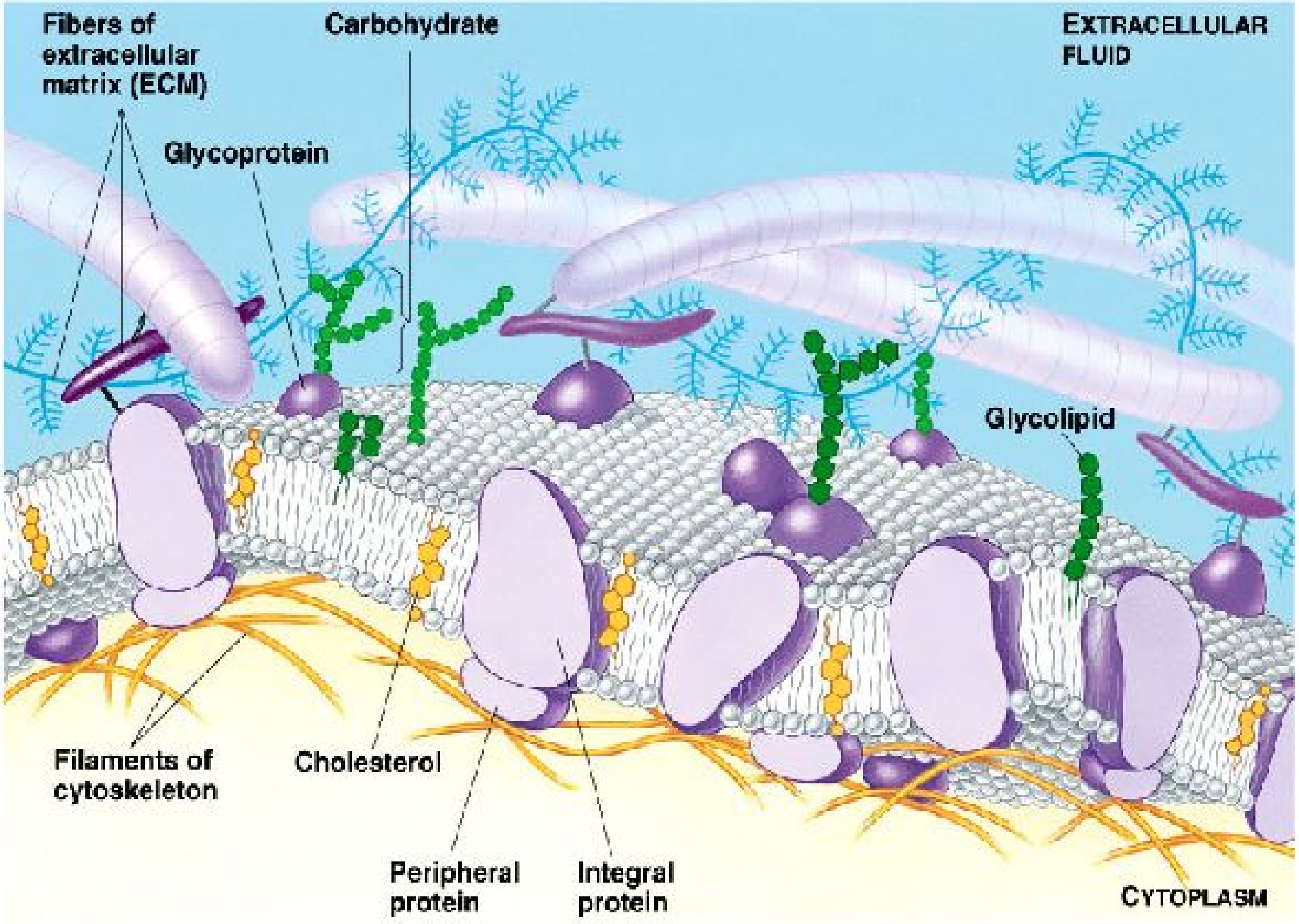


Fibrosis

Extra cellular matrix (ECM)

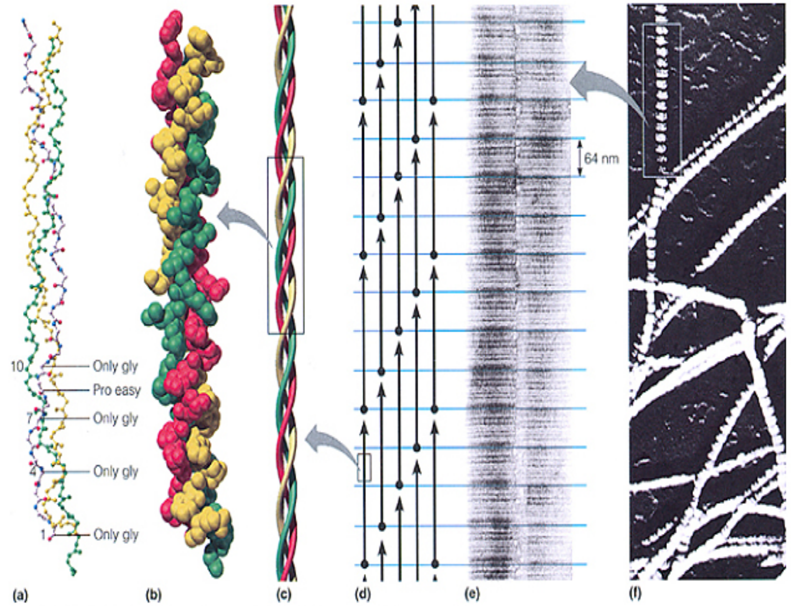


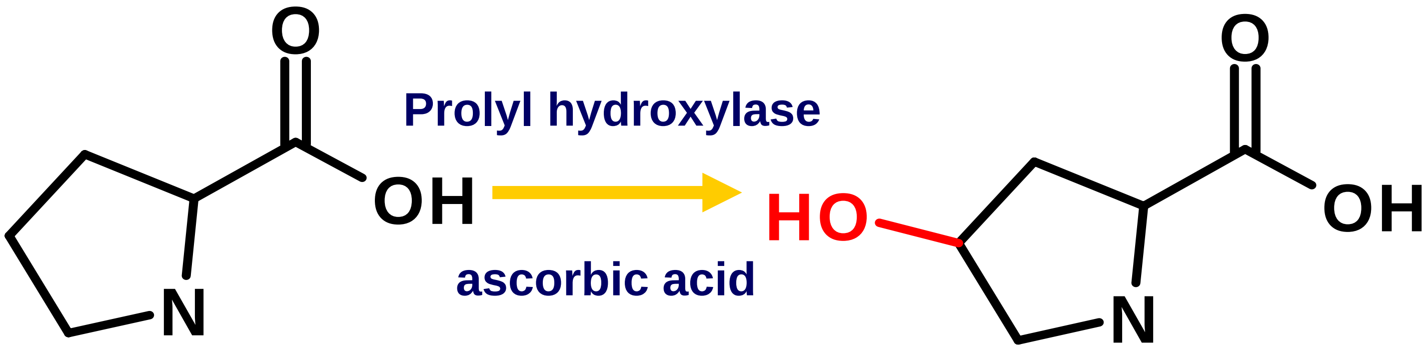
Fibrosis and ECM accumulation



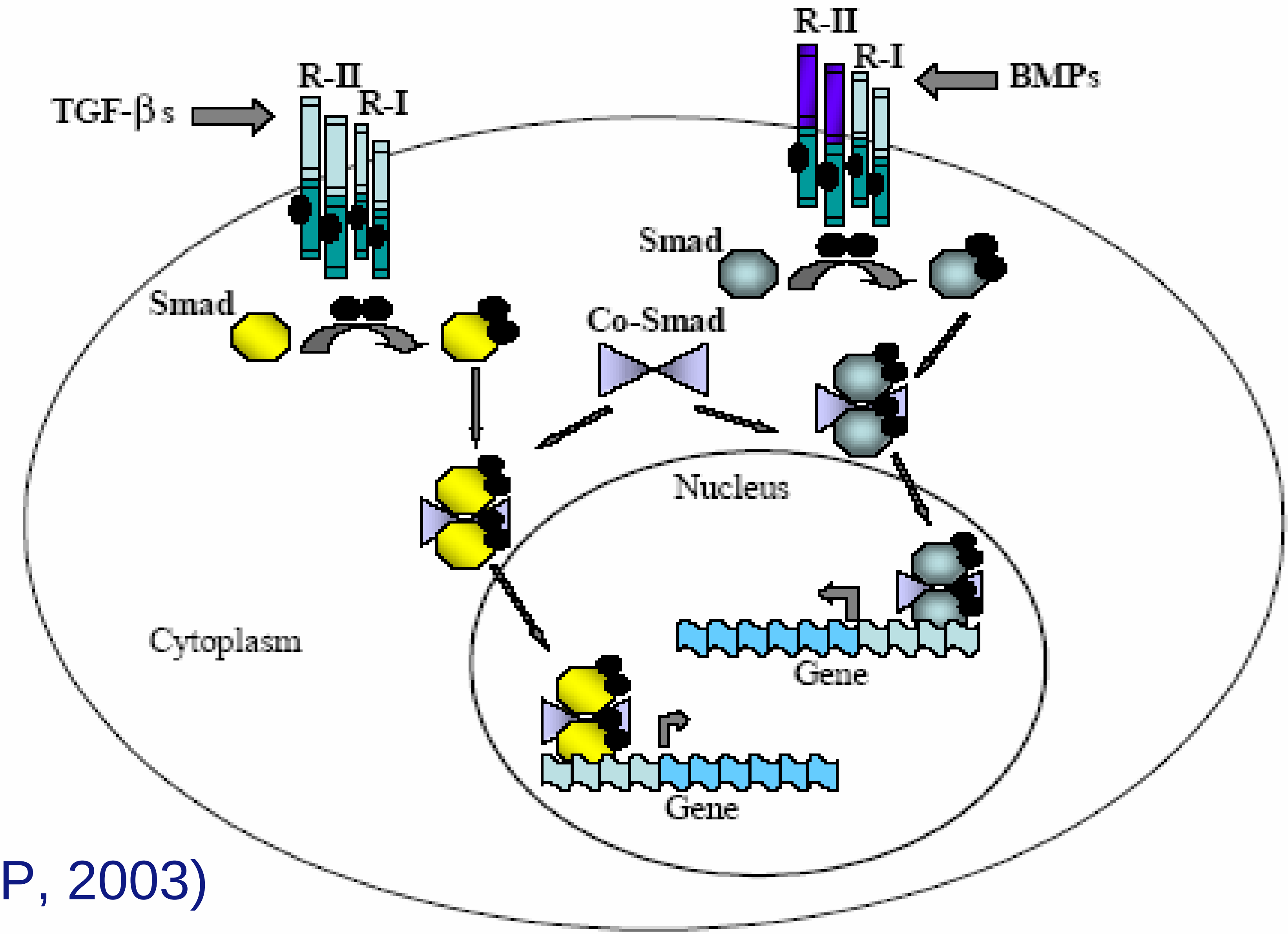
Collagen

- Hydroxyproline **is a major component of the protein collagen.**





Transcription regulation of MMPs



(Heidi P, 2003)