Connective Tissue Response in IBD

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Intestinal response to Chronic Inflammation

Control

UC

CD

Trichrome Staining of Collagen
Balancing ECM deposition and degradation

Collagens
- Elastin
- Fibronectin
- Laminin
- TIMPs
- Plasmin/PA
- MMPs
- Collagenases
- Gelatinases
- Stromelysins

Proteoglycans
- PAI
- Deposition
- Degradation
Balancing ECM deposition and degradation

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Deposition

 Degradation
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Deposition vs. Degradation
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**Deposition**

**Degradation**
Balancing ECM deposition and degradation

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Degradation
Balancing ECM deposition and degradation

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Stromelysins
Immunohistochemistry Collagen III and I

Control

Inflamed UC

Fibrosed CD

Collagen III

Collagen I

Ratio Collagen type III:I

LP
Lamina Propria

SM
Submucosa

ME
Muscularis Externa

A
Adventitia

Lawrance et.al. IBD 2001
Isolated IBD Intestinal Fibroblasts-like cells

Phase contrast light microscopy

Electron microscopy

Rough endoplasmic reticulum (thick arrows)
Collagen fibrils (thin arrows).

Lawrance et al. IBD 2001
Intestinal Fibroblasts

Lawrance et al. IBD 2001
Fibroblast $\rightarrow$ Myofibroblast

TGF-β1

IGF-1
bFGF

Myofibroblast $\rightarrow$ Smooth Muscle Cell

Smooth Muscle Cell
Regulators of ECM - TGF-β1

- TGF-β1
- Plasmin
- PAI-1
- PDGF

Recruitment
- Macrophages
- Leukocytes
- Fibroblasts
- Myofibroblasts

- IGF-1

- MMP-1
- MMP-3
- MMP-9

- COL I
- TIMP-1
TGF-β1 and IGF-1 expression in IBD

**TGF-β1 In situ hybridisation**

No. Positive cells per HPF

LP - Lamina Propria
SM - Submucosa
ME - Muscularis Externa
A - Adventitia

**IGF-1 In situ hybridisation**

**TGF-β1 Immunohistochemistry**

**IGF-1 Immunohistochemistry**

Lawrance et al. IBD 2001
Regulators of ECM - SPARC

SPARC (Secreted Protein Acidic and Rich in Cysteine)
Transimently secreted in actively inflamed tissue
Microarray analysis of ECM-related genes

UC & CD compared to Control

- UC: 24 genes
  - 5 genes upregulated
  - 19 genes downregulated

- CD: 15 genes
  - 7 genes upregulated
  - 8 genes downregulated

7 genes in common

- Trefoil factor 1
- Fibronectin
- Proteoglycan
- Mucin 4
- MMP-12
- MMP-9
- MMP-1

Inflamed UC:
- Fibrosed CD:

Lawrence et al. Human Mol Genetics 2001
SPARC Immunohistochemistry
Control Intestine

Lawrance et.al. Gastro 126 (4): Suppl. 2 2004
SPARC Immunohistochemistry

Inflamed UC

Inflamed Ischaemic Colitis

Neo-endothelial cells

Inflammatory cells
SPARC Immunohistochemistry

Fibroshed CD

Neo-endothelial cells

Fibroblasts-like cells

Inflammatory cells

Fibroshed Diverticular Disease

Inflammatory cells
Methods

TNBS murine model of Chronic Inflammation-induced intestinal fibrosis

12 week old female CD-1 and BALB/c mice sensitised to TNBS

Weekly enemas for 6-8 weeks

Colons removed cleaned & weighed

Lawrance, Wu et.al. Gastroenterology 2003
Methods

TNBS murine model ± Indomethacin or Retinoic Acid

12 week old female CD-1 mice sensitised to TNBS

Retinoic Acid
50ug/day
or
Indomethacin
0.2mg/kg/day

Weekly enemas for 6 weeks

Colons removed
cleaned & weighed

Lawrance et al. Gastroenterology 126 (4): Suppl. 2 2004
Histology at 2 weeks

TNBS alone or with 0.2mg/kg/day indomethacin or 50ug RA/day
SPARC mRNA at 2 weeks

Ratio to control mice SPARC/βActin

- Control: p=0.047
- TNBS Low Fibrosis: p=0.016
- TNBS High Fibrosis: p=0.0090
- RA/TNBS: p=0.005
- Indo/TNBS: p=0.047
SPARC Immunohistochemistry

Da: TNBS High Fibrosis
Db: Trichrome

Ea: TNBS and Indomethacin
Eb: SPARC
SPARC +ve Cells per High Power Field

- Water Control: n=10
- RA Control: n=5
- Indo Control: n=5
- TNBS High: n=6
- TNBS Low: n=6
- RA: n=6
- Indo: n=10

Cell count

- p=0.005
- p=0.007
- p=0.01
Regulators of ECM - SPARC

Indomethacin

SPARC → COX-2 → PGE2

Fibroblast

Macrophage

MMP-1
MMP-3
MMP-9

MMP-1
MMP-3

PGE2

?
SPARC-null and WT mice

TNBS murine model
Weekly enemas for 2 weeks and 6 weeks

SPARC-null
11 week female
C57bl6 x 129SV mice

SPARC WT ± Indomethacin
11 week female
C57bl6 x 129SV mice

Lawrance et al. Gastroenterology 128 (4): Suppl. 2 Apr 2005
SPARC-null and WT mice at 2 weeks

SPARC +/- mice TNBS ± with 0.2mg/kg/day indomethacin
SPARC -/- mice TNBS alone
Extracellular Matrix (ECM)

- Epithelial cells
- Smooth Muscle Cells
- Macrophage
- Fibroblast
- Myofibroblast

Molecules:
- TGF-β1
- SPARC
- IGF-1
- Collagen III
- Collagen I

TGF-β1 and SPARC are shown to be key factors in the ECM.