Tissue Repair: Regeneration and Fibrosis

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Lecture Outline

• Control of Cell Proliferation – cell cycle
• Growth Factors
• Extracellular matrix
• Cell and Tissue Regeneration
• Repair (scar)
• Cutaneous wound healing
• Pathologic repair

Tissue Types

• Continuously Dividing (labile)
  – Hematopoietic and surface epithelia
• Stable
  – Liver, kidney, pancreas, smooth muscle, endothelial cells, fibroblasts
• Permanent
  – Neurons and cardiac muscle

Proliferation

Baseline cell population

Differentiation

Stem cells

Cell death
Apoptosis

Tissue Steady State
Signaling of Growth Factor Receptors

- Autocrine – lymphocytes, liver
- Paracrine – macrophages in wound healing
- Endocrine - hormones

Growth Factors in Tissue Repair

- Vascular Endothelial growth factor (VEGF) – increased vascular permeability
- Transforming Growth Factor-Beta (TGF-B)
- Platelet Derived Growth Factor (PDGF)
- Epidermal Growth Factor (EGF)
- Fibroblast Growth Factor (FGF)

TGF- beta

- Produced by:
  - Platelets and macrophages
  MOST IMPORTANT FACTOR IN WOUND HEALING
- Actions:
  - Monocyte chemotaxis
  - Fibroblast migration and proliferation
  - Angiogenesis and fibronectin synthesis
  - Collagen and ECM:
    • Increased synthesis
    • Decreased degradation by MMP's, increased TIMP's

PDGF

- Produced by platelets, macrophages, endothelial cells
- Chemotactic for neutrophils, macrophages, fibroblasts, smooth muscle cells
- Stimulates production of MMP's, fibronectin and hyaluronic acid
- Stimulates angiogenesis

VEGF

- Produced by mesenchymal cells
- Increases vascular permeability
- Mitogenic for endothelial cells

EGF

- Produced by activated macrophages
- Mitogenic for keratinocytes and fibroblasts
- Stimulates granulation tissue formation
FGF

- Produced by macrophages, T cells
- Chemotactic for fibroblasts
- Mitogenic for fibroblasts and keratinocytes
- Stimulates keratinocyte migration, angiogenesis, wound contraction and matrix production

Role of ECM

- Mechanical Support – anchorage, cell migration, cell polarity
- Cell growth control
- Maintenance of cell differentiation -
FGF stimulates keratinocyte migration, wound contracture and matrix deposition

Model of Leukocyte Transmigration

Macrophages in inflammation and repair

Flowing and Inflammation

Model of Leukocyte Transmigration

Macrophages in inflammation and repair

Flowing and Inflammation
Key Points

• How does each tissue restore itself to prevent scar?
• Humans lose the ability to prevent scar after fetal life
• Scar prevents tissue regeneration
• What is the purpose of the scar?

Wound Repair and Regeneration

Patch of fibroblasts with disorganized ECM → Injury to tissue → Functional tissue

Scar
Non-functional tissue

Lung
Kidney
Heart
Skin
Liver
Spleen

Angiogenesis

• Proteolysis of vessel basement membrane
• Endothelial cell migration and proliferation
• Pericyte recruitment

Repair By Connective Tissue

• Formation of new blood vessels (angiogenesis)
• Migration and proliferation of fibroblasts
• Deposition of ECM (scar)
• Maturation and reorganization of fibrous tissue (remodeling)

Growth factor receptors in angiogenesis

Two types of angiogenesis
Scar Formation

- Fibroblast proliferation and migration
  - PDGF, FGF, TGF-beta mainly from macrophages
- ECM deposition
  - TGF-beta – potent agent of fibrosis

ECM and Tissue Remodeling

- Outcome of repair: balance between synthesis and degradation of matrix
- MMP’s are synthesized by fibroblasts, macrophages, neutrophils, epithelial cells, etc destroy matrix (inactive form) activated by proteases and plasmin and inhibited by TIMP’s-synthesized by mesenchymal cells
Matrix Metalloproteinase Regulation

1. Stimulation
   - PDGF
   - EGF
   - IL-1/TNF

2. Inhibition
   - TGF-beta
   - Steroids

3. Activation
   - Procollagenases
   - Prostromelysins
   - Collagenase
   - Stromelysin

4. ECM Degraded ECM
   - TIMPs
   - Plasmin
   - Plasminogen Activators

Possible Outcomes after Injury

- **Response**
  - Acute Inflammation
    - Stimulus Promptly Destroyed
    - Minimal necrosis
  - Stimulus Not Destroyed
    - Necrosis

- **Injury**
  - Exudate resolved
  - Exudate organized
  - Fibrinopurulent
  - Fibrinous pleurisy

Regeneration

- If the connective tissue framework is intact
- If the cells are not post-mitotic
- THEN:
  - Complete restoration of the structure and function of the tissue is possible

Classic Stages of Wound Repair

- Inflammation – until 48 hrs. after injury
- New tissue formation – 2-10 days after injury
- Remodeling – 1-12 months after repair
Possible Outcomes after Injury

Injury → Response → Acute Inflammation

- Stimulus Promptly Destroyed
  - Minimal necrosis
  - Exudate resolved
  - Normal tissue
  - Mild burn
  - Scarring
  - Fibrinospurulent pericarditis, peritonitis

- Stimulus Not Destroyed
  - Necrosis
  - Labile or stable cells
  - Framework intact
  - Framework destroyed
  - Permanent cells

- Lobar Pneumonia
  - Labile or stable cells
  - Permanent cells

- Scar
  - Bacterial abscess

- Myocardial Infarction

Possible Outcomes after Injury
Repair by Fibrosis

- Angiogenesis
- Granulation tissue
- Migration and proliferation of fibroblasts
- Deposition of extracellular matrix
- Organization of collagen "remodeling"
- Fibrosis – scar formation

Macrophages in healing and fibrosis

1. PERSISTENT STIMULUS (chronic inflammation)
   - Activation of macrophages and lymphocytes

2. Growth factors (PDGF, TGF-β)
3. Cytokines (TNF-α, IL-1, IL-6)
4. Decreased metalloproteinase activity
5. Proliferation of fibroblasts, endothelial cells, and specialized angiogenic cells
6. Increased collagen synthesis
7. Decreased collagen degradation
8. FIBROSISS

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Chronic Peptic Ulcer

Fibrosis below the ulcer bed

Scarring in the Liver

- Healing by fibrosis after inflammation
- TGF beta implicated in excessive collagen formation

Fibrotic response to toxin-mediated injury

- Poorly understood:
  - Liver Hepatitis B,C
  - Pulmonary fibrosis

Cirrhosis
Classic Stages of Wound Repair

• Inflammation – until 48 hrs. after injury
• New tissue formation – 2-10 days after injury
• Remodeling – 1-12 months after repair

Overview of Cutaneous Wound Healing

• A defect in the skin occurs
• Fibrin fills in defect – scab forms
• Epithelial regeneration beneath scab
• Granulation tissue – angiogenesis
• Wound contraction
• Collagen remodeling

Cell Migrations in Wound Healing

• Platelets form a blood clot and secrete fibronectin (FN), PDGF and TGF-beta
• Neutrophils arrive within minutes
• Macrophages move in as part of granulation tissue and secrete fibronectin
• Keratinocytes or other epithelial cells detach from the basement membrane at wound edge and migrate on fibronectin rich matrix across wound to fill in defect (cells switch receptors from those for BM to FN receptors)
Healing by Primary Intention

- Surgical incision
- Edges easily joined together
- Small amount of granulation tissue
- Little fibrosis
- Wound strength 70-80% of normal by 3 months

Healing by Second Intention

- Large wound, may be infected
- Edges not brought close together
- Large amount of granulation tissue
- Scar formation and contracture
Inhibition of Repair

- Infection with inadequate nutrition (Vitamin C is essential for collagen)
- Glucocorticoids inhibit inflammation with decreased wound strength and less fibrosis.
- Poor perfusion due to diabetes or atherosclerosis.
- Foreign bodies left in the wound.
- Chronic inflammation leads to excess, disabling fibrosis as in rheumatoid arthritis, pulmonary fibrosis and cirrhosis.

Diabetic Foot Ulcer Case #1

- A 52 year old woman has had fairly well controlled type 2 diabetes mellitus for the past 20 years.
- In the last three months, she has noticed a non-healing ulcer on her heel.
- She asks you what can be done to make it heal better.

Possible New Therapy

- Application of VEGF alone to wounds in an animal model of diabetes (wound repair is dysregulated in DM) can normalize healing

Diabetic Foot Ulcer Case #2

- A 63 year old male has had Type 2 diabetes mellitus for the past 10 years.
- He requires insulin.
- He presents to you with the complaint of a painless sore on the sole of his foot directly beneath a metatarsal head.
- He asks why his foot has difficulty healing.
Inhibition of Repair

- Foreign body in wound

Abnormal Repair Processes

- Inadequate scar formation - dehiscence, ulceration
- Excessive scar formation – keloids
- Contracture – exaggeration of normal process (soles, palms, thorax) especially with serious burns