Tissue Renewal and Repair
Regeneration
Healing & Fibrosis

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Regeneration vs. Healing

**Regeneration**
Growth of cell and tissue to *replace lost structures*
- Amputated tails in amphibians
- Liver/kidney regeneration after resection
- Hematopoietic system and epithelium

Intact **connective tissue scaffold** (ECM framework)

**Healing**

*Tissue response* to wound, inflammation, or cell necrosis
Regeneration (c.f, cutaneous wound, erosion, blister) and scar (c.f, dermal incision) formation
- Constrictive pericarditis, liver cirrhosis, peptic ulcer, organizing pneumonia
- Damaged **connective tissue scaffold**
Differential host response against CCl$_4$ injury

CCl$_4$ injection

- acute injury by high dosage
  : more than 50% hepatocyte necrosis
  : Regeneration

- chronic injury by small dosage
  : ECM framework damage
  : Fibrosis
  : healing scar formation
Tissue Response to Injury

NORMAL HOMEOSTASIS
(balance of proliferation and apoptosis)

INJURY

REGENERATION

Renewing tissues
- Epidermis, GI tract epithelium, hematopoietic system

Stable tissues
- Compensatory growth of liver and kidney

HEALING

Wound
- Wound healing, scar formation

Chronic inflammation
- Fibrosis
Extracellular matrix (ECM) and cell-matrix interactions: critical for tissue repair

Tissue homeostasis
- Cell cycle
- Stem cell
- Growth factor
- Cell signaling mechanism

Repair by healing, scar formation and fibrosis
- Angiogenesis
- Scar formation
Role of ECM in Regeneration and repair

Liver regeneration with restoration of normal tissue after injury requires an intact cellular matrix.

If the matrix is damaged the injury is repaired by fibrous tissue deposition and scar formation.
Mechanisms Regulating Cell Population

1. Proliferation
2. Differentiation
3. Cell death (apoptosis)

Ex) Uterine Cervix
Squamous Epithelium

Apop

Diff

Pro

Stem
1. Continuously dividing tissue (labile ts)
   - continue to proliferate through the life
   - surface epithelium, hematopoietic cells, most parenchymal tissues

2. Quiescent ts (stable ts)
   - low level of replication
   - capable of reconstituting the tissue of origin in case of injury
   - **G0 phase, but can be stimulated to G1**
   - parenchymal tissues (liver, kidney, pancreas)
   - mesenchymal cells (fibroblast, smooth muscle, vascular endothelial cell, resting lymphocyte, leukocyte, chondrocyte, osteocyte)

3. Non-dividing ts (permanent ts)
   - never undergo mitosis in postnatal life
   - nerve cell, skeletal, cardiac muscle cells,
   - gliosis, myocardial infarct (scar)
Cell Cycle Landmarks

- Continuously cycling labile cells (e.g., epidermis, GI tract epithelium)
- Chromosome duplication
- Check for DNA damage ($G_1$/S checkpoint)
- Restriction point
- Centrosome duplication
- Growth in mass
- $G_0$
- Quiescent, stable cells (e.g., hepatocytes)
- Permanent cells (e.g., neurons, cardiac myocytes)
- Mitosis
- Check for damaged or unduplicated DNA ($G_2$/M checkpoint)
- $G_1$
- $S$
- $G_2$
- $M$
- Cell division
Cyclin E activation is required for cell cycle entry

GROWTH INHIBITORS (TGF-β, p53, others)

GROWTH FACTORS (EGF, PDGF)

CDK Inhibitors e.g., p16 (INK4a)

Cyclins D/CDK 4,6
Cyclin E/CDK2

Hyperphosphorylated pRb

DP1

E2F

E2F site

S Phase genes

Transcriptional activation

Hypophosphorylated pRb

E2F

E2F site

S Phase genes

Transcriptional block

Cyclin E transcription

R point entering
Stem Cells: Regenerative Medicine

*Tir na n’Og (=Celtic land of the ever-young)*

Prolonged self-renewal capacity
Asymmetric replication
Some self-replicate, others differentiate
Embryonic stem cell (ES): pluripotency
Blastocyst at 32-cell stage
Nanog (Homeobox protein) as transcriptional factor
Wnt-β-catenin signaling
Adult stem cell
More restricted differentiation
Lineage-specific differentiation
Hematopoietic stem cells (HSC): transdifferentiation, developmental plasticity
Multipotent adult progenitor cells (MAPC): adult counterpart of ES
Tissue stem cells: niche
Cancer stem cell
Therapeutic Cloning, using ES

Nuclear Transplantation, Embryonic Stem Cells, and the Potential for Cell Therapy

Konrad Hochedlinger, Ph.D., and Rudolf Jaenisch, M.D.

Differentiation Pathway for Pluripotent BM stromal cells

The Missing Bone Minireview
Gideon A. Rodan and Shunichi Harada
Department of Bone Biology and Merck Research Laboratories
West Point, Pennsylvania 19486
Cell, (89), 677–680, 1997,

PPAR: peroxisome proliferator–activated receptor
Stem cells—Tissue Homeostasis

- Liver
  - Oval cells in canals of Hering
- Brain
  - Neural stem cells in olfactory bulb, and the dentate gyrus of hippocampus
  - Stem cell marker: Nestin
- Muscle
  - Satellite cell beneath basal lamina
  - Myogenic or osteogenic or adipogenic
  - Not found in cardiac muscle, yet
- Epithelial tissue

**Renewal system strategy:** non-mutually exclusive principle
1. Increasing the number of actively dividing stem cells
2. Increasing the number of replication of cells in amplifying compartment
3. Decreasing the cell–cycle time for cell replication

(-and)
Stem cell niches

A. Skin

B. Intestine

C. Liver

D. Cornea

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# Growth Factors

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF (TGF-α)</td>
<td>EGFR 1, 2 (Her2/neu)</td>
</tr>
<tr>
<td>HGF/scatter factor</td>
<td>c–MET</td>
</tr>
<tr>
<td>VEGF A</td>
<td>2 (main angiogenesis)</td>
</tr>
<tr>
<td>VEGF B</td>
<td>1 (myocardium)</td>
</tr>
<tr>
<td>VEGF C/D</td>
<td>3 (lymphangiogenesis)</td>
</tr>
<tr>
<td>TGF–β 1(main)</td>
<td>TGFβR</td>
</tr>
</tbody>
</table>

- Cell cycle block by increasing CIP & INK4/ARF
- Proliferation of fibroblast and smooth muscle cells
- Potent fibrogenic agent enhancing collagen, fibronectin, proteoglycan
- Anti-inflammatory activity
EGFR Signaling Pathway: EGF Activation
Her Receptor Family and Their Ligands

- HB-EGF
- Betacellulin
- Amphiregulin
- TGFα
- EGF

**Extracellular Ligand Binding Domain**

- EGFR (HER1, ErbB-1)
- HER2 (ErbB-2)
- HER3 (ErbB-3)
- HER4 (ErbB-4)

**Tyrosine Kinase Domain**

- NRG3
- NRG2
- Betacellulin
- Heregulin
- Hersegulin
General Pattern of Intracellular Signaling

**Autocrine**
- Cell responds to the signaling substances that they themselves secrete.
- Hepatic regeneration, tumor cells, T-lymphocytes, proliferation

**Paracrine**
- Affect only a target cell in close proximity.
- Tissue repair and wound healing (granulation tissue)

**Endocrine**
- Act on target cells distant from their sites of synthesis.
Signal Transduction System

- Posttranslational modification -fos/jun heterodimerization
- STAT activation
- NFkB–nuclear migration

C-myc, fos, jun, p53, E2F
Liver regeneration after partial hepatectomy

Partial Hepatectomy

Growth factors

Cytokines

TNF
IL-6
Others

G1
Quiescence

G2
Progression

S

DNA replication
DNA replication and mitosis

Transcript levels

c-myc
p53
mcm2
bclx
c-fos
jun
cyclins

Normal

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Adjuvants
Norepinephrine
Insulin
Thyroid hormone
Growth hormone

Cell cycle inhibitors
Growth factors
Metabolic demands

Growth inhibition

Proliferation

Priming

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Major Components of ECM

- Basement Membrane
  - Type IV collagen
  - Laminin
  - Proteoglycan

- Interstitial Matrix
  - Fibrillar collagens
  - Elastin
  - Proteoglycan and hyaluronan

- Epithelium
- Integrons
- Fibroblast
- Integrons
- Endothelial cells
- Capillary
ECM 기능

1. sequester water that provides turgor to soft tissue
2. sequester mineral that give rigidity to skeletal muscle
3. reservoir for growth factor controlling cell proliferation
4. cell-cell interaction
5. substratum for cells to migrate, adhere, and proliferate, directly modulating cell form and function
6. morphogenesis
7. wound healing and scar
8. tumor invasion and metastasis
CAM (Cell adhesion molecules)

- 4 family
  1. Immunoglobulin family CAMs
  2. Cadherin
  3. Integrins
  4. Selectins

- Cell membrane location
- Receptor function–ECM proteins ligand
- Stored in cytoplasm
- Homotypic (cadherin) and heterotypic (Ig) interaction
Integrins

- Heterodimeric (α and 5β) cell surface proteins, about consisting of 30 homologous proteins
- Cell–ECM interaction and adhesion (fibronectin and laminin…)
  - Extracytoplasmic RGD (Arg-Gly-Asp) binding sequence domain
  - Cytoplasmic domain interacting to cytoskeletal protein (Talin, vinculin, paxillin)
    - **FAC: focal adhesion complex in cell membrane**
  - Fibronectin
    - Tissue form: wound healing
    - Plasma form: binding to fibrin and forming provisional blood clot, served as substratum for ECM
  - Laminin
    - Collagen IV network
- Cell–cell interaction–complement components or surface proteins
Cadherin

- Calcium dependent adherence protein
- 90 members
- Homotypic interaction
- Cell junction forming
  - Zonula adherence-apical surface of epithelial cells
  - Desmosome-lateral surface, stronger
- Binding to cytoskeleton (actin) through catenin
  - Cell motility, proliferation, and differentiation regulation
  - Contact inhibition—normal cell line in vitro
- Free-β-catenin
  - Independent to cadherin
  - Nucelar TF regulation in Wnt signalling pathway
Zonula adherence

Tight junctions prevent fluid from moving across a layer of cells.

Gap junctions

Desmosomes

Intermediate filaments

Space between cells

Plasma membranes of adjacent cells

Tight junction

0.5 μm

0.1 μm

Plasma membrane

Cadherin

α-actinin

p120ctn

Catenins

Vinculin

Actin

Actin filaments

Catenin

Vinculin

α-actinin

ZONULA ADHERENS
ECM & Growth factor cross-talk

- ECM fibers (Laminin, Collagen, Fibronectin)
- Growth factor receptors
- Integrin binding
- Focal adhesion complexes
- Actin cytoskeleton
- Cytoskeleton-mediated signals
- Cytoplasmic signal transduction pathways
- Nucleus
- Proliferation, differentiation, protein synthesis, attachment, migration, shape change
Other adhesive proteins

- SPARC (secreted protein acidic and rich in cysteine)/osteonectin
  - Tissue remodelling
  - *Angiogenesis inhibitor (??)–variable in tumors*
- Thermobospondins
  - *Angiogenesis inhibitor (??)–paradoxical effect*
- Osteopontin
  - Calcification regulation
  - Leukocyte migration through CD44 receptor
- Tenacin
  - Cell adhesion and morphogenesis
Proteoglycans and hyaluronic acids

• Proteoglycans
  • Notable diversity
  • Heparan sulfate, chondroitin sulfate, dermatan sulfate
  • Integral membrane protein binding to FGF

• Hyaluronic acids
  • Binding to large water and forming viscus hydrated gel
    • for resistance against compression forces
  • Resilience and lubrication in cartilage matrix
  • Cell-cell adhesion, cell motility inhibition
  • CD44 binding – T-cell retain in tissue
Proteoglycans, glycosaminoglycans, and hyaluronan.

Regulation of FGF-2 activity by ECM and cellular proteoglycans. Heparan sulfate binds FGF-2 (basic FGF) secreted into the ECM. Syndecan is a cell surface proteoglycan with a transmembrane core protein, extracellular GAG side chains that can bind FGF-2, and a cytoplasmic tail that binds to the actin cytoskeleton. Syndecan side chains bind FGF-2 released by damage to the ECM and facilitate the interaction with cell surface receptors. Synthesis of hyaluronan at the inner surface of the plasma membrane. The molecule extends to the extracellular space, while still attached to hyaluronan synthase. Hyaluronan chains in the extracellular space are bound to the plasma membrane through the CD44 receptor. Multiple proteoglycans may attach to hyaluronan chains in the ECM.
Angiogenesis by Mobilization of EPC

A. Angiogenesis by mobilization of EPCs from the bone marrow

- EPCs
- Bone marrow
- Homing
- Capillary plexus
- Mature network

B. Angiogenesis from pre-existing vessels

- Capillary sprouting
- Mature network
The Notch receptor binds a ligand (a delta-like ligand,Dll, is shown in the figure) located in an adjacent cell, and undergoes two proteolytic cleavages (the first cleavage by ADAM protease, the second by δ-secretase), releasing a C-terminal fragment known as Notch intracellular domain (Notch–ICD). Notch signaling in endothelial cells during angiogenesis, triggered by the binding of the Dll4 ligand in a tip cell to a Notch receptor in a stalk cell. Notch–ICD migrates into the nucleus and activates the transcription of target genes. Sprouting angiogenesis, showing a migrating tip cell and stalk cells connected to the endothelial cells of the main vessel.
Interactions between Notch and VEGF during angiogenesis

VEGF stimulates delta-like ligand 4 (Dll4)/Notch, which inhibits VEGFR signaling. Compared with unperturbed angiogenesis, Dll4 blockade causes an increase in capillary sprouting and endothelial cell (EC) proliferation, creating vessels that are disorganized and have a small lumen size.

VEGF blockade decreases capillary sprouting, and the proliferation and survival of ECs.
Angiogenesis

**Key component: motility and direct migration of EC**

Main effector: **VEGFR-2** (endothelial-specificity)
- Mobilization from BM EC precursors and cooperated with **FGF-2**

Stabilization factor
- **Ang-1** (periendothelial cell recruit), **Ang-2** (inhibitor), **PDGF** (smooth muscle cell), **TGF-β** (ECM production)

Regulator
- **Integrin** ($\alpha_v\beta_3$): critical for formation and maintenance of new vessels, MMP2 direct interaction
  - VEGFR-2 binding and activity regulation
  - adhesion to ECM (fibronectin, osteopontin, thrombospondin...)

**Matricellular protein**: **thrombospondin-1, SPARC, tenascin C**
- Destabilize cell-matrix interaction, and promote angiogenesis

**Proteinase**: plasminogen activator, MMP
- Tissue remodelling for endothelial invasion
Degradation of collagen/ECM by MMP, which activity depends on zinc −180 residue zinc–protease domain (metalloenzyme) in more than 20 members.

c.f) Neutrophil elastase, cathepsin G, kinin, plasmin: serine protease

1,2,3: interstitial collagenase
2,9: gelatinase
3,10,11: ECM lysis
Membrane-bound MMP(surface associated proteinase): MMP−14/15 (MT−MMP)
TIMP
ADAM (Disintegrin And MMP−domain family)
: TNF/TGF−α cleavage and release−activation
Phases of cutaneous wound healing: inflammation, proliferation, and maturation
Steps in Wound healing

Suture wound
Wounds with opposed edges
Primary union

5–7 d: stitch–out
1 wk: 10% of tensile strength
~ 2 wks: increased collagen synthesis
~ 4 wks: scar and permanent loss of dermal appendages
~ 2 m: collagen synthesis> degradation
~ 3 m: collagen synthesis cease collagen modification
70–80% of tensile strength
Second Intention

- Wounds with separated edges
- DDx 1st intention
  - Large defect
  - Debris removal essentially required
  - More intense inflammation
  - Larger granulation tissue
  - Wound contraction
    - Myofibroblast from edge
    - 6 wks 후 정상의 5-10% volume
    - Substantial scar formation
    - Epidermis thinning
    - MMP-3 (stromelysin-1)
• Angiogenesis
  • leaky and edematous
  • Fibroblast migration and proliferation

- **TGF-β**: most important factor
  - Angiogenesis
  - ECM production
  - Inhibition of ECM degradation
  - Macrophage influx

- **Macrophage**: scavenger role
  - Clearance of extracellular debris, fibrin, foreign materials
병리 총론 구성

Immunity    Infection    Neoplasia

Cell Injury - Cell adaptation - Cell death

Inflammation - Tissue repair, healing, fibrosis

Hemodynamic disorder - shock