Control of Cell Adhesion

- **Critical** to many physiological processes such as *leukocyte adhesion* to endothelium, tissue growth and differentiation (embryogenesis), and cell migration and tissue organization
- **Cell-Cell interaction**; cell surface receptors bind to receptors on the surface of another cell => *tight junction formation*
- **Cell-Extracellular Matrix (ECM) interaction**; cell surface binding to insoluble ECM proteins to mediate, for example, *cell migration and ECM organization*
- *Cell adhesion* and migration determine the characteristics of “engineered tissues”, including electromechanical properties
- **Aim** is to develop and characterize *relationship between ligand/receptor binding and cell adhesion*, eventually leading to cell motility and/or differentiation
Growth factors

Growth factors are gene-signaling molecules (e.g., proteins) that are released by one cell type but exert dramatic effects on neighboring and/or distant cells.

- Often growth factors are ligands for receptors: e.g.
- insulin, growth hormone, nerve growth factor (NGF), epidermal growth factor (EGF), transforming growth factor-β (TGF-β), interferon
- Small molecules such as steroids and thyroid hormones diffuse across the cell membrane
- Larger molecules have specific receptors expressed on the cell surface, e.g., insulin receptors and EGF receptors (likely protein kinase)
- Receptor-ligand binding activates a number of signal transduction cascades and/or complexes may be endocytosed
Signaling Pathway of TGF-β
Endogenous Growth Factors

- During natural tissue repair/regeneration, a complex and orchestrated delivery of various growth factors is observed, e.g., wound healing or liver regeneration after toxic injury.
- The orchestrated and timed release of growth factors trigger cell proliferation, immune system activation, and initiate angiogenesis.
- Further complicating fact is that there is no one set of rules for growth factor release => tissue specific.
- More attention paid to growth factor delivery mechanisms to mimic the endogenous processes of growth factor production and release during natural tissue morphogenesis or regeneration.
- In addition to cell-cell and cell-matrix adhesions, tissue formation is also likely to depend growth factors and ECM, suggesting the important role of “microenvironment”
VEGF triggered signaling
Growth Factors in TE Applications

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Receptor-Mediated Signal Transduction Pathways

By what mechanisms do cells/tissues respond to chemical messengers (e.g., growth factors, hormones, etc)?

- **Receptor activation** can be defined as conformational changes in the receptor upon binding a chemical messenger.

- **Receptor activation** is always the initial step that leads to cellular responses, including changes in cell permeability and membrane potentials, metabolism, secretory activities, and rate of proliferation and differentiation.

- **Receptor activation** by a chemical messenger is only the initial event. In order to produce cellular responses, many other molecular steps are necessary. The sequences of these events is generally referred to as “signal transduction pathways”
Signaling by Lipid-soluble Messengers

Most lipid-soluble messengers are small hormones, such as steroid hormones

- readily **diffuses** across the plasma membrane
- **bind** with cytosolic receptors or directly **insert into nucleus** and then
  bind intranuclear receptors
- these **activated** receptors now **act like transcription factors**, leading to
  production of mRNA
- protein synthesis or **increased rate of secretion** follows
- these are typically called, **“first messengers”** because they do not rely on
  other molecular interactions that may be required to achieve the end
  results
- now the **cellular response** by the particular first messenger is completed
- how this cellular response **affect the neighboring cells and**
  microenvironment is the **next set of questions** to be addressed later
Lipid signaling
Plasma Membrane Receptors

- Generally speaking, chemical messengers (e.g., growth factors, antibodies, and drugs) do not readily diffuse across the plasma membrane. Rather, these rely on the specific receptors expressed in the plasma membrane.
  - **Specificity** is very high but, in no way, absolute.
  - Upon ligand binding, some receptors form clusters that are pinched off from the cytosol side => endocytosis.
  - Other receptors, upon ligand binding, initiate a sequence of biochemical reactions that require “other messengers” to be produced. These are called second messengers and include cAMP, cGMP, DAG, IP₃, and Ca²⁺ ions.
- Once activated, receptors could function as:
  - Ion channels
  - Enzymes (i.e., protein kinases: phosphorylate other proteins)
  - G-protein activators (i.e., guanine triphosphate binding proteins)
Voltage-Gated Calcium Channels
Second Messenger Pathways

(A) Ion channel receptor

(B) Enzyme receptor

(C) G-protein activator
G-protein-Mediated Signal Pathways
Ca\textsuperscript{2+}-Mediated Signal Pathways

Calmodulin is one of the best characterized calcium binding proteins

- can either activate or inhibit calmodulin-dependent protein kinases
- thus, critically involved in phosphorylation
Extracellular Matrix (ECM)

- Extracellular matrix once thought to provide only structural support for tissue
- Now “dynamic reciprocity model” proven correct
  - ECM molecules interact with receptors, transmitting signals across the cell membrane to cytoplasmic molecules that, in turn, initiate cascades of events involving cytoskeleton and nucleus
  - these “nuclear events” affect specific gene expression that reciprocally regulates ECM structures and contents
- ECM now shown directly to participate cell adhesion, migration, growth, differentiation, apoptosis
- ECM modulates the activity of growth factors and cytokines, indicating delicate and mutual interplay ⇒ integrated picture is required
ECM Composition

- **Basic components** include collagen, hyaluronic acid, proteoglycan (e.g., glycosaminoglycan), elastin
- **Harbors** growth factors, cytokines, matrix degrading enzymes (e.g., MMPs)
- **Content and organization not static but dynamics**, and depend on tissue type
  - mesenchymal cells: embedded in interstitial matrix
  - epithelial and endothelial cells: base membrane contact
  - divalent ions such as Ca$^{2+}$ affect matrix organization
  - for TE applications, tissue constructs be selectively engineered
- **ECM integrity**, i.e., proper functionality, not only regulated by biochemical interactions but also by mechanical homeostasis
- **Mechanical factors** including pore size, mesh network distribution, polymer strength, and the overall stiffness now emerged just as important
Receptors for ECM molecules

How do ECM molecules affect cell homeostasis?

- **transmembrane proteins identified to bind ECM molecules**
- recent data confirmed that ECM molecules containing **specific amino acid motifs** are capable of binding directly to **cell surface receptors**
  - tripeptide **RGD motif**: Arginine (positively charged)-Glycine (uncharged)-Aspartic Acid (negatively charged)
  - **fibronectin**: first found to demonstrate the tripeptide motif
  - peptides containing this sequence shown to promote cell adhesion
  - RGD motif now identified in laminin, entracin, thrombin, tenacin, fibrinogen, vitronectin, collagen, osteopondin
  - other motifs shown to bind to integrins
- **first ECM receptors** identified in 1985 ➔ integrins
- **other ECM receptors** include transmembrane proteoglycan, laminin-binding protein, and CD36
Adhesion Receptors

At least four superfamilies of adhesion receptors identified

1) **Integrins**
   - heterodimeric glycoproteins composed of non-covalently linked a and b subunits
   - binds to proteins containing tripeptide sequence arginine-glycine-aspartate (RGD) (e.g., ECM proteins such as fibronectin, laminin, and collagen)
   - RGD motif NOT universal, however

2) **Selectins**
   - at least 3 subtypes (L, E, P)
   - typically found in leukocytes for “cell rolling”

3) **Immunoglobulins** (e.g., ICAMs)

4) **Cadherins** mainly found in the cell-cell junction
Integrins

- Heterodimer glycoproteins consisting of \( \alpha \) and \( \beta \) subunits
- At least 21 subtypes defined by pairing of distinct \( \alpha \) and \( \beta \) subunits
- Constitutively expressed in almost all cell types but cell-specific, e.g., \( \beta_2 \)-integrins found on leukocytes only
- Mediate cell adhesion to substrate (divalent ions required)
- Participate in signal transduction cascades and known to transduce mechanical signals => “mechanotransducers”
- Bidirectional signaling mechanism
  1) outside-in signals regulate cellular homeostasis (e.g., focal adhesions and cytoskeletal reorganization)
  2) inside-out signals regulate the integrin affinity state (activation state)
Integrin Structural Features

- At least 12 distinct $\alpha$-subunits and 9 $\beta$-subunits identified; single subunit can associate with more than one partner
- Difficult to crystallize and therefore 3D structures now readily available
- Cloning experiments indicate that integrins have short cytoplasmic tail (~50 amino acids) and 4 Ca$^{2+}$ binding sites identified in the $\alpha$ subunit
  - $10^{-6}$ M range Ca$^{2+}$ required to maintain $\alpha-\beta$ association
  - $10^{-4}$ M range Ca$^{2+}$ required for ligand binding
- Numerous disulfide bonds, difficult to identify all of them
- From EM data, globular head is about 80-120 A$^\circ$ and typical extension about 280 A$^\circ$
Schematic for Integrin

- All β subunits contain transmembrane domains and cytoplasmic tails
- Most α subunits have the heavy and light chain. Light chain contains the transmembrane domain and short cytoplasmic tail
- ~50 amino acids

Prototypical model of integrin receptors with major disulfide bonds.
Integrin Subtypes
Focal Adhesion Complexes

- Cell adhesion mediated by a localized aggregation of receptors and linking proteins (referred to as focal adhesion complexes or FACs)
- FAC formation is dynamic, not static
- Integrins critical in formation of FACs; interact with extra- and intra-cellular proteins
- $K_D$ for integrin-ECM protein binding $\sim 10^{-6}$ to $10^{-7}$ M
  - low affinity binding $\rightarrow$ easily reversible
  - facilitate cell migration by FAC formation/de-formation
  - affinity modulated to control adhesiveness
  (varies with the logarithm of $K_D$)
  - localization and aggregation of integrins thought to be mediated by diffusion and/or (?) by motor proteins along the “cytoskeletal tracks”
Proteins found in FACs

- **Structural proteins:**
  - talin, filamin, actin, α-actinin, vinculin
  - function of each protein not clearly defined; talin links F-actin to phospholipid bilayer and α-actinin crosslinks actin filaments

- **Signaling proteins:**
  - focal adhesion kinase (FAK), Src, Paxillin, rho-GTPase, p130<sup>cas</sup>
  - feedback mechanism in which elevated and/or transient increase detected

- **Integrin-mediated calcium signaling**
  - found in platelets, neutrophils, monocytes, lymphocytes, fibroblasts, endothelial cells, osteoclasts, and epithelial cells
  - PLC-induced release of IP<sub>3</sub> to activate internal Ca<sup>2+</sup> pools
  - [Ca<sup>2+</sup>]<sub>i</sub> increases regulate integrin-ECM binding affinity
    => dynamic adhesion/de-adhesion
Selectin-Mediated Leukocyte Rolling

- More physiological studies of leukocyte binding to endothelium
- Two distinctive processes
  - initial arrest mediated by selectin binding to carbohydrate ligands expressed on endothelial venules
  - subsequent adhesion and transmigration mediated by integrins
- Transient adhesion mediated by selectin likely to depend on kinetics and force of selectin-ligand bond => this condition represents the physiological environment
- Reverse arrangement, i.e., selectins on substrate and ligands on cell surface, may help to elucidate and understand, for example, leukocytes rolling on other leukocytes and interleukocyte adhesion responsible for leukocyte aggregates
Selectin Subtypes

- **Ca\(^{2+}\)-dependent** carbohydrate-binding selectins are classified into three categories
  1) **L-selectins**
     - found on all leukocytes
     - bind to activated endothelial cells (ECs)
  2) **E-selectins**
     - found on activated ECs
     - bind to neutrophils, monocytes, and T cells
  3) **P-selectins**
     - found on platelets
     - bind to neutrophils, monocytes, and T cells
Transcription Factor Regulation
(CREB, Elk-1 and c-Fos)
The Mitogen-activated Protein Kinase (MAPK) Cascades
Integrin Signaling
Schematic for Leukocyte Adhesion

See: http://www.youtube.com/watch?v=UB6G9GD2KFk