Lecture 5: Cell-Matrix and Cell-Cell Adhesions

What keeps cells and tissues together?
How can cells move?
How do they shape the matrix components?

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Content

• Overview on cell adhesion receptors
• Difference between heterophilic and homophilic adhesions
• Integrins – general structure and function
• Selectins and Ig-G superfamily – important for immune cells
• Cadherins – cell adhesion in epithelial cells
• Tight junctions, desmosomes, gap junctions
• Proteoglycan receptors - syndecans
Epidermis Bullosa (EB)

Blistering of epidermis due to defective anchoring of keratinocytes to basal lamina and dermis
Junctional EB
→ defective **hemidesmosomal** adhesion

dermaamin.com
Role of Adhesion Receptors in Tumor Angiogenesis & Metastasis

Role of Adhesion Receptors in Endocytosis of Particles
Cellular Adhesion Molecules (CAMS)

**Integrins**
- Generally heterophilic
- Cell–ECM interactions
  - Integrins generally mediate adhesion to ECM and partly to other cells
  - Require divalent cations for proper function (Mg$^{2+}$, Mn$^{2+}$) Ca$^{2+}$ decreases

- Ca$^{2+}$ independent

**IgG superfamily**
- Homophilic/heterophilic (same/different cells stick together)
- Involved in adhesion of neurons, muscle, glial cells, endothelia cells and leukocytes
### Cellular Adhesion Molecules (CAMS)

**Ca\(^{2+}\) dependent**

#### Cadherins
- Homophilic
- Found at cellular junctions in epithelial and other cells

Tissue distribution, such as epithelial (E-cadherins), placental (P-cadherins), neural (N-cadherins), retinal (R-cadherins), brain (B-cadherins and T-cadherins), and muscle (M-cadherins)

#### Selectins
- Bind to carbohydrate receptors (e.g., glycoproteins, glycolipids)
- Involved mainly in endothelia and blood cell interactions (heterophilic)

- E-selectin (endothelial), L-selectin (leukocyte), and P-selectin (platelet)
Cell Adhesion Molecules (CAM) for Cell-Cell and Cell-Matrix Adhesion
Integrins as Major Cellular Receptors for ECM Components

- Integrins \(\rightarrow\) heterodimeric transmembrane proteins composed of \(\alpha\) and \(\beta\) subunits (not covalently bound)

- Longer cyCombinations of \(\alpha\) and \(\beta\) subunits \(\rightarrow\) Receptors for different ligands

- Cytosolic tail of \(\beta\) subunit \(\rightarrow\) Interaction with cytoskeletal and signaling molecules

- Complex of \(\alpha\) and \(\beta\) subunit \(\rightarrow\) binding site for ECM proteins

- Ligand binding \(\rightarrow\) dependence on divalent cations, such as \(\text{Mg}^{2+}\), \(\text{Mn}^{2+}\)

[Diagram showing integrin structure with binding sites for ECM and cytoskeletal molecules]
Integrin Functions

Motility

See next lecture on signal transduction

growth
differentiation

apoptosis

Motility

growth
differentiation

Matrix adhesion

ECM Synthesis

Nucleus

Cytosol
Integrin Family of Cell Receptors

- Integrins require minimal sequence to bind ligands – a versatile binding sequence recognized by many integrins is RGD (Arg-Gly-Asp) that is present in many adhesive proteins such as fibrinogen (FBG), fibronectin (FN), von Willebrandt factor (vWV) and vitronectin (VN)

- Integrins may be specific for a single adhesive protein (e.g., $\alpha_7\beta_1$ for laminin) or a multitude (e.g., $\alpha_{IIb}\beta_3$ in blood platelets for FBG, FN, vWF, and VN)

Hynes 2002
Integrins as Cell Adhesion Molecules

• Integrins are mobile in the cell membrane

• Contact of cells with ECM \(\rightarrow\) formation of integrin clusters \(\rightarrow\) Connection with intracellular signaling molecules and cell cytoskeleton – „focal adhesion plaques“
Integrins „Integrate“ ECM Components with Cytoskeletal Structures

Transmission electron micrograph → connection of extracellular fibronectin cables with intracellular actin cytoskeleton via integrins
Simplified Model of Focal Adhesion PLAques

- Binding of integrins to actin cytoskeleton via linker proteins – α-actinin, filamentin, talin, tensin
- Binding to signaling molecules (tyrosine kinases)
- Binding of integrins to ECM ligands and connection to cytoskeleton builds up mechanical tension necessary for cell attachment, spreading, and migration

Sigaling: FAK – Focal Adhesion Kinase, ILK – Integrin linked Kinase,
Linker to cytoskeleton: α-Act- actinin, Fil – Filamentin
Tal – Talin, Ten – Tensin, Pax – Paxillin, Vin - Vinculin
Integrins “Integrate” Extra- and Intracellular Proteins in Focal Adhesions
Function of Integrins – Ligand Binding and Outside-in-Signaling

• Integrin transmit signals from outside into the cell

→ Change of conformation upon ligand binding

→ exposure of cryptic binding sites for cytoskeletal and signaling proteins

→ necessary for survival, motility, growth and function

• (outside-in-signalling – next lecture)

www.chuv.ch/cpo_research/integrins.html
Integrin Function – Inside-out-Signaling, Ligand Binding, Outside-in-Signaling

• Intracellular processes regulate affinity of integrins for ligands (inside-out-signaling)

• Ligand binding to integrins changes conformation of integrin with exposure of cryptic binding sites inside the cells.

• Example: activation of $\alpha_{Ib}\beta_3$ integrin in blood platelets upon activation by thrombin

Figure 7
Integrin Function - Cell Movement

Integrins and cytoskeletal structures cooperate in cell movement → integrins provide anchoring points to substratum, while cytoskeletal structures (actin/myosin) generate traction for movement of cells body.

www.utm.utoronto.ca/~w3bio315
Integrin Function- ECM Synthesis

• Integrins play a crucial role for structuring of extracellular matrix component fibronectin

• Core process is fibrillization of fibronectin, which may guide assembly of further matrix components, such as collagens and GAGs

• Cooperation of specific fibronectin modules and $\alpha_5\beta_1$ integrins necessary to polymerize fibronectin

• $\alpha_5\beta_1$ integrins move centripetally from focal adhesions by actin-myosin contractile activity $\rightarrow$ fibrillar adhesions
Integrin and Fibronectin (FN) Fibrillization

1. Receptor-Ligand (FN) binding (Outside-in-Signaling)
2. Signaltransduction outside-in-signalling
3. Gen expression, protein synthesis
4. Fibronectin secretion
5. Clustering of integrins in focal adhesions
6. Move of fibronectin receptor (a5b1 integrin)
7. Fibronectin fibrillization
Model for the Formation and Segregation of Focal Contact and Fibrillar Adhesions

Movement of Fibronectin Receptor (a5b1 Integrin) and Formation of FN Fibrills

Altankov, Zlatanov, Groth, Biophysical Journal
Segregation of Fibronectin and Vitronectin Receptor

α₅-Integrin green, αᵥ-Integrin red
Cell Adhesion Molecules (CAM) for Heterophilic Cell-Cell Adhesion

- **IgG-superfamily** – homology with immune globulins – e.g. neural-cell adhesion molecules (N-CAM) for adhesion of nerve cells or ICAM - Intercellular Adhesion Molecules for adhesion of leukocytes on endothelial cells

- **Selectins** – bind specific carbohydrate residues on cells (glycokalyx) – P-Selectin, E-Selectin (P – Platelets, E - Endothelial)
Survey on Selectins & Ligands

Prototype of selectin ligands
(PSGL- P-Selectin Glycoprotein Ligand)

Varki 1994
Cell-Cell Adhesion is Necessary for the Defence Against Invading Microorganisms

- Inflammatory (soluble) signals from infected tissues upregulate expression of **selectins** and **ICAMs** on endothelial cells.
Cell-Cell Adhesion is Necessary for the Defence Against Invading Microorganisms

- Leukocytes in the blood stream may interact first via their carbohydrate chains on the surface (sialic acids) with E- and P- selectin on the endothelial cell surface
Cell-Cell Adhesion is Necessary for the Defence Against Invading Microorganisms

- Leukocytes attach loosely and start to roll over the endothelium.

- Leukocytes make stable adhesion via $\alpha_L\beta_2$ integrin (CD11/CD18) with ICAM-1 on endothelial cells.
Cell-Cell Adhesion is Necessary for the Defence Against Invading Microorganisms

- Leukocytes transmigrate through the epithelium into the tissue to attack bacteria, etc.
# Distribution & Function of HA (Hyaladherin) Receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>CD44</th>
<th>RHAMM</th>
<th>ICAM-1</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>ECs, SMCs, fibroblasts, leukocytes, tumor cells, adhesion, proliferation, migration, HA internalization, cytokine release, metastasis</td>
<td>many cell types</td>
<td>ECs, SMCs, macrophages, lymphocytes</td>
<td>TLR-4</td>
</tr>
<tr>
<td>Functions</td>
<td>adhesion, migration, metastasis</td>
<td>adhesion, migration, metastasis</td>
<td>adhesion, HA internalization</td>
<td>hepatic HA clearance receptor (liver)</td>
</tr>
<tr>
<td>Cell Signaling</td>
<td>FAK, GTPase, gene expression</td>
<td>FAK, Ras, gene expression</td>
<td>FAK, gene expression</td>
<td>LYVE-1 (lymph vessels)</td>
</tr>
</tbody>
</table>

Comparison of Motility of Cancer Cell Lines on Glycosaminoglycan Coatings

- Thiolated GAGs were covalently bound to „vinylated“ glass
- MDA_MB_231 overexpressing CD 44 (HA receptor) and are highly metastatic compared to MDA_MB_468 with normal expression levels and low metastatic potential seeded and studied 24 h with time lapse microscopy
- Hyaluronan (t-HA) promotes migration of highly metastatic cancer cell line MDA_MB_231

Hyaladherin CD 44 in the Immune System

- T lymphocyte rolling on endothelium can be mediated by CD44 → interaction between CD44 on T cells and hyaluronan on the surface of the endothelium → initial “rolling” interaction, which is required for the extravasation to tissues

Rolling interaction with chemokines (red) facilitates firm adhesion, mediated by integrins (grey) and their counter receptors (green) on endothelial cells → diapedesis and migration to the site of inflammation and infection
Hyaladherin CD 44 in the Immune System

The "sandwich" model of CD44:HA:CD44 mediated cell-cell interaction

• CD44 expressed on activated T cells binds to hyaluronan on the surface of endothelial cells by CD44

• The level of hyaluronan expressed by endothelial cells is increased under inflammatory conditions (Nguyet M Nguyen, et al. 2006)
Cell Adhesion Molecules in Epithelia

- Cadherin (E-cadherin)
- Ig-superfamily CAMs (N-CAM)
- Mucin-like CAMs
- Integrin ($\alpha_3 \beta_1$)

- Homophilic interactions
- Heterophilic interactions

- Ca$^{2+}$ binding sites
- Carbohydrate
- Lectin domain
- Selectins (P-selectin)
- Type III fibronectin repeats
- Fibronectin

- BEAM - Master Joint Mobility Project
- Grant Agreement, 2014-1543/001-001-CPT EU/CH/SCP

Martin Luther University
Halle-Wittenberg
Cell Adhesion Molecules (CAM) for Homophilic Cell-Cell Adhesion

- Epithelial junctions → separation of different compartments (basal-apical) controlling exchange of substances and protection against microorganisms

- Tight junctions – Occludin; Adherens junctions – Cadherins, Desmosoms – Placoglobin

Structure of gut epithelial cell
Cadherin – based Adherens Junctions

- The term **Cadherin** denotes calcium-dependent intercellular adhesions molecules

- Structure of the extracellular part → homophilic adhesion to cadherin molecules on neighbouring cells → Strong cell-cell adhesions

- Loss of E-cadherin expression or function → Tumor metastasis of epithelial cancers (carcinomas)

Protein interactions that mediate the association of $\alpha$-catenin with the actin cytoskeleton

- $\beta$-catenin bind to $\alpha$-catenin and links cadherins to actin cytoskeleton

- $\alpha$-catenin is related to vinculin (an actin binding protein found at integrin-based focal contacts)
Loss of Cadherins in Tumor Development
Loss of Cadherins in Pancreatic \( \beta \) Cell Cancer

A tissue section of a \( \beta \)-cell tumour from a Rip1Tag2 transgenic mouse (a model of pancreatic \( \beta \)-cell carcinogenesis) has been stained with antibodies against E-cadherin (a) and \( \beta \)-catenin (b), and with DAPI to visualize the nuclei (c). Tumour cells on the left-hand side of the tumour have lost E-cadherin expression, whereas the tumour cells on the right-hand side still express E-cadherin. Note the marked change of nuclear shape and downregulation of \( \beta \)-catenin concomitant with the loss of E-cadherin expression. d | Merged image of the stainings shown in a–c.
Occluding Junctions – Tight Junction

- The tight junction → belt-like region connecting two membranes together

- In epithelial and endothelial cells → tight junctions → selective (semipermeable) diffusion barriers between apical and basal part of the cell layer (example: gut)

- → Maintainance of different composition of proteins and lipids between the apical and basolateral plasma membrane domains

Freeze-fracture replica of a tight junction in the intestinal epithelium
Junction Complexes in Epithelial Cells

Histochemistry and Cell Biology © Springer-Verlag 200810.1007/s00418-008-0424-9

Review

Tight junctions and the modulation of barrier function in disease

Carola Förster
Junction Complexes in Epithelial Cells
Effect of Pathogens on Barrier Function of Epithelia by Disruption of Tight Junctions
*Yersinia enterocolitica* induces epithelial barrier dysfunction through regional tight junction changes in colonic HT-29/B6 cell monolayers.

Analyzes of tight junction proteins in leaky and intact regions by confocal laser scanning microscopy. The z-stack projections of infected monolayers displaying parallel biotinylation (red) and immunostaining for (a) ZO-1, (b) claudin-4 and (c) claudin-8 (green). ‘Leaky regions’ are characterized by an intense biotin permeation (red), which was absent in ‘intact regions’ that are characterized by lacking biotinylated cell boundaries. *Laboratory Investigation* (2011) 91, 310–324.
Cell-Cell Adhesions - Desmosomes

- Desmosomes are specialized junctional plaque-like structures → tight connection between all epithelial cells (and cardiac myocytes) - related to adherens junctions
- Anchorage of intermediate filaments at membrane-associated plaques in adjoining cells
- Components divided into three superfamilyes: desmosomal cadherins, the armadillo family of nuclear and junctional proteins, and plakins

Personalpages.umist.ac.uk
Downregulation of Desmosomal Adhesion During Early Stage of Cancer

Rachel L. Dusek & Laura D. Attardi
Nature Reviews Cancer 11, 317-323 (May 2011)
Cell-Matrix Adhesions in Epithelia - Hemidesmosomes

- Hemidesmosomes are multimeric protein complexes that attach epithelial cells to the underlying matrix via integrins and interkeratin cytoskeleton.

- Hemidesmosomes bind via integrins α6β4, facilitating attachment of epithelium to the underlying basal lamina.

- Morphological similarity to desmosomes but contain α6β4 integrins.

- Genetic defects of α6β4 lead to epidermis bullosa.

Left: Ultrastructure of tracheal hemidesmosomes. Control tracheas (a) had well-defined, organized hemidesmosomes with darkened areas in the lamina densa abutting the hemidesmosome (arrows). In contrast, hemidesmosomes in Lamc2-/- tracheas (b) were less organized, with a more diffuse intracellular component, and the lamina densa directly below the hemidesmosomal areas lacked the electron density seen in the littermate control (arrows).
Cell-Cell Adhesions - Gap Junctions

- **Gap junctions** as intercellular structures → passive diffusion of ions and small (signaling) molecules (2 kDa) through intercellular channels (connexons made of connexin)

- Most cells of the normal tissues communicate via these junctions, except skeletal muscle cells, erythrocytes and circulating lymphocytes

- Special: caridomyocytes - contraction

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Cell Surface Proteoglycan (Syndecans, Glycipans) Functions

A. Coreceptor for Insoluble Ligands e.g. ECM components
   - Integrin
   - Fibronectin
   - Cell Adhesion

C. Internalization Receptor e.g. enzymes
   - Lipoprotein lipase
   - Ligand Internalized

3. Coreceptor for Soluble Ligands e.g. growth factors
   - FGF
   - FGFR
   - Receptor Activated

D. Soluble Paracrine Effector
   - Ectodomain shed

Example of Function of Syndecan 4

Binding to ECM of syndecan-4 → immobilization in the membrane → V region can bind both the catalytic domain of protein kinase C-α (PKC-α), activating this Ser/Thr kinase, and phosphatidylinositol-4,5 bis phosphate (PtdIns-4,5-P2) → promotes oligomerization of the cytoplasmic domain and results in calcium independence of PKC-α.

PKC-α activation → focal adhesions formation and syndecan-4 accumulation

PtdIns-4,5-P2 levels increase after integrin ligation → control cytoskeletal rearrangements via the Rho family of GTP-binding proteins
Contribution of Syndecans in Tumor Progression

A) Involvement in signal transduction by co-signaling with integrins and after shedding with growth factor receptors

B) Shedded syndecans with HS „improves“ tumor microenvironment by recruitment of tumor associated macrophages & fibroblasts plus stimulation of angiogenesis

Front. Oncol., 03 February 2014 | http://dx.doi.org/10.3389/fonc.2014.00004
Summary

• Multitude of adhesion receptors for different kinds of fixed ligands like proteins and glycans

• Cell-Cell adhesions either homophilic or heterophilic

• Specific tissue distribution of adhesion receptors that may represent ubiquitous ligands like integrin $\alpha 5\beta 1$ or specific like P-Selectin

• Adhesion ligands often not only for attachment of cells but also linked to transduce signals from extracellular space to cell interior or vc.vs.
Literature
