Biochemistry of Connective Tissue

Lecture # 34

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Introduction

• The family of connective-tissue cells includes *fibroblasts*, *chondrocytes* (cartilage cells), and *osteoblasts* (bone-forming cells).

• They are specialized to secrete extracellular proteins, particularly collagens, and mineral substances, which they use to build up the *extracellular matrix*.

• By contrast, *osteoclasts* dissolve bone matter again by secreting $\text{H}^+$ and collagenases.
Bones

Functions:
- Mechanical support
- Storage for Ca\(^{2+}\) and phosphate
- Synthesis of blood cells
- Maturation of B cells

Composition:
- Inorganic: Apatite, Carbonate, Water
- Organic: Type I collagen, Proteoglycans, Phosphatases

Hormones of the calcium metabolism:
- Parathyroid hormone
- Calcitonin
- Calcitriol

Absence leads to rickets
Teeth

Hardest substance in the body:
- Dental enamel: 97%
- Dentin: 70%
- Cement: 65%
- Jawbone: 45%

Crown - Dental plaque

Inorganic components:

Plaque

Bacterium

Dental enamel

Protect bacteria
- Saccharose
- Glucose
- Fructose

Sugar

Dextran metabolism

Anaerobic metabolism
- Bacterium E.g., Streptococcus mutans

Attack apatite
- Lactic acid
- Propionic acid
- Acetic acid
- Butyric acid
- Calcium salts

De-mineralization
The extracellular matrix (ECM) is a complex structural entity surrounding and supporting cells that are found within mammalian tissues.

The ECM is often referred to as the connective tissue.
Extracellular Matrix (cont’d)

• The ECM is composed of 3 major classes of biomolecules:

  2. Specialized proteins: e.g. fibrillin, fibronectin, and laminin.
  3. Proteoglycans: these are composed of a protein core to which is attached long chains of repeating disaccharide units termed of glycosaminoglycans (GAGs) forming extremely complex high molecular weight components of the ECM.
Structure of Collagens

- The fundamental higher order structure of collagens is a long and thin diameter rod-like protein.
  - Type I collagen for instance is 300nm long, 1.5nm in diameter and consists of 3 coiled subunits composed of two $\alpha_1(I)$ chains and one $\alpha_2(I)$ chain.
  - Each chain consists of 1050 amino acids wound around each other in a characteristic right-handed triple helix.
    - There are 3 amino acids per turn of the helix and every third amino acid is a G.
  - Collagens are also rich in proline and hydroxyproline. The bulky pyrrolidone rings of proline reside on the outside of the triple helix.
Formation of Collagen Fibrils

- Lateral interactions of triple helices of collagens result in the formation of fibrils roughly 50nm diameter.
- The packing of collagen is such that adjacent molecules are displaced approximately 1/4 of their length (67nm).
  - This staggered array produces a striated effect that can be seen in the electron microscope.
Procollagens

• Collagens are synthesized as longer precursor proteins called **procollagens**.
• Type I procollagen contains an additional 150 amino acids at the N-terminus and 250 at the C-terminus.
  – These pro-domains are globular and form multiple intrachain disulfide bonds.
  – The disulfides stabilize the proprotein allowing the triple helical section to form.
Collagen processing (1)

- Collagen fibers begin to assemble in the ER and Golgi complexes.
- The signal sequence is removed and numerous modifications take place in the collagen chains.
  - Specific proline residues are hydroxylated by prolyl 4-hydroxylase and prolyl 3-hydroxylase.
  - Specific lysine residues also are hydroxylated by lysyl hydroxylase.
  - Both prolyl hydroxylases are absolutely dependent upon vitamin C as co-factor.
  - Glycosylations of the O-linked type also occurs during Golgi transit.
Collagen processing (2)

• Following completion of processing the procollagens are secreted into the extracellular space where extracellular enzymes remove the pro-domains.
  – The collagen molecules then polymerize to form collagen fibrils.
  – Accompanying fibril formation is the oxidation of certain lysine residues by the extracellular enzyme lysyl oxidase forming reactive aldehydes.
  – These reactive aldehydes form specific cross-links between two chains thereby, stabilizing the staggered array of the collagens in the fibril.
Biosynthesis of Collagen: Overview

1. Removal of the prepeptide
2. Hydroxylation of Pro and Lys residues
3. Glycosylation of 5Hyl and Asn
4. Oxidation of Cys in propeptides
5. Assemblage to form triple helix
6. Removal of the propeptide
7. Staggered deposition to form fibrils
8. Oxidation of Lys and 5Hyl to aldehydes
9. Cross-linking to form supramolecules

Procollagen-proline 4-dioxygenase 1.14.11.2 [ascorbate, Fe]
Procollagen-lysine 5-dioxygenase 1.14.11.4 [ascorbate, Fe]
Protein-lysine 6-oxidase 1.4.3.13 [Cu]
### Types of Collagen (1)

<table>
<thead>
<tr>
<th>Types</th>
<th>Chain Composition</th>
<th>Structural Details</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>([\alpha_1(I)]_2[\alpha_2(I)])</td>
<td>300nm, 67nm banded fibrils</td>
<td>skin, tendon, bone, etc.</td>
</tr>
<tr>
<td>II</td>
<td>([\alpha_1(II)]_3)</td>
<td>300nm, small 67nm fibrils</td>
<td>cartilage, vitreous humor</td>
</tr>
<tr>
<td>III</td>
<td>([\alpha_1(III)]_3)</td>
<td>300nm, small 67nm fibrils</td>
<td>skin, muscle, frequently with type I</td>
</tr>
<tr>
<td>IV</td>
<td>([\alpha_1(IV)]_2[\alpha_2(IV)])</td>
<td>390nm C-term globular domain, nonfibrillar</td>
<td>all basal lamina</td>
</tr>
<tr>
<td>V</td>
<td>([\alpha_1(V)][\alpha_2(V)][\alpha_3(V)])</td>
<td>390nm N-term globular domain, small fibers</td>
<td>most interstitial tissue, assoc. with type I</td>
</tr>
<tr>
<td>VI</td>
<td>([\alpha_1(VI)]_2[\alpha_2(VI)][\alpha_3(VI)])</td>
<td>150nm, N+C term. globular domains, microfibrils, 100nm banded fibrils</td>
<td>most interstitial tissue, assoc. with type I</td>
</tr>
<tr>
<td>VII</td>
<td>([\alpha_1(VII)]_3)</td>
<td>450nm, dimer</td>
<td>epithelia</td>
</tr>
</tbody>
</table>
## Types of Collagen (2)

<table>
<thead>
<tr>
<th>Types</th>
<th>Chain Composition</th>
<th>Structural Details</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIII</td>
<td>$[\alpha_1\text{(VIII)}]_3$</td>
<td>? , ?</td>
<td>some endothelial cells</td>
</tr>
<tr>
<td>IX</td>
<td>$[\alpha_1\text{(IX)}][\alpha_2\text{(IX)}][\alpha_3\text{(IX)}]$</td>
<td>200nm, N-term. globular domain, bound proteoglycan</td>
<td>cartilage, assoc. with type II</td>
</tr>
<tr>
<td>X</td>
<td>$[\alpha_1\text{(X)}]_3$</td>
<td>150nm, C-term. globular domain</td>
<td>hypertrophic and mineralizing cartilage</td>
</tr>
<tr>
<td>XI</td>
<td>$[\alpha_1\text{(XI)}][\alpha_2\text{(XI)}][\alpha_3\text{(XI)}]$</td>
<td>300nm, small fibers</td>
<td>cartilage</td>
</tr>
<tr>
<td>XII</td>
<td>$\alpha_1\text{(XII)}$</td>
<td>? , ?</td>
<td>interacts with types I and III</td>
</tr>
</tbody>
</table>
## Classification of Collagens, Based Primarily on the Structures That They Form

<table>
<thead>
<tr>
<th>Class</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibril-forming</td>
<td>I, II, III, V, and XI</td>
</tr>
<tr>
<td>Network-like</td>
<td>IV, VIII, X</td>
</tr>
<tr>
<td>FACITs</td>
<td>IX, XII, XIV, XVI, XIX</td>
</tr>
<tr>
<td>Beaded filaments</td>
<td>VI</td>
</tr>
<tr>
<td>Anchoring fibrils</td>
<td>VII</td>
</tr>
<tr>
<td>Transmembrane domain</td>
<td>XIII, XVII</td>
</tr>
<tr>
<td>Others</td>
<td>XV, XVIII</td>
</tr>
</tbody>
</table>

*Murray, 2006*
Fibronectins

- Fibronectins contain 6-8 tightly folded domains each with a high affinity for a different substrate such as **heparan sulfate**, **collagen** (separate domains for types I, II and III), **fibrin** and **cell-surface receptors**.
Fibronectins

- The cell-surface receptor-binding domain contains a consensus amino acid sequence, **RGDS**.
Cell surface

Proteoglycan

Hyaluronate

Collagen

Link protein

-S-S-
The Role of Fibronectins

- The role of fibronectins is to attach cells to a variety of extracellular matrices.
  - Fibronectin attaches cells to all matrices except type IV that involves laminin as the adhesive molecule.
  - Fibronectins are dimers of 2 similar peptides. Each chain is 60-70nm long and 2-3nm thick.
  - At least 20 different fibronectin chains have been identified that arise by alternative RNA splicing of the primary transcript from a single fibronectin gene.
Integrins are heterodimers, containing various types of \( \alpha \) and \( \beta \) polypeptide chains.
Major Components of the Basal Lamina
Basal Lamina Components: Laminin

- All basal laminae contain a common set of proteins and GAGs.
- These are type IV collagen, heparan sulfate proteoglycans, perlecan, entactin and laminin.
  - The basal lamina is often referred to as the type IV matrix.
  - Each of the components of the basal lamina is synthesized by the cells that rest upon it.
  - Laminin anchors cell surfaces to the basal lamina.
### Representative matrix types produced by vertebrate cells

<table>
<thead>
<tr>
<th>Collagen</th>
<th>Anchor</th>
<th>Proteoglycan</th>
<th>Cell-Surface Receptor</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>fibronectin</td>
<td>chondroitin and dermatan sulfates</td>
<td>integrin</td>
<td>fibroblasts</td>
</tr>
<tr>
<td>II</td>
<td>fibronectin</td>
<td>chondroitin sulfate</td>
<td>integrin</td>
<td>chondrocytes</td>
</tr>
<tr>
<td>III</td>
<td>fibronectin</td>
<td>heparan sulfate and heparin</td>
<td>integrin</td>
<td>quiescent hepatocytes, epithelial; assoc. fibroblasts</td>
</tr>
<tr>
<td>IV</td>
<td>laminin</td>
<td>heparan sulfate and heparin</td>
<td>laminin receptors</td>
<td>all epithelial cells, endothelial cells, regenerating hepatocytes</td>
</tr>
<tr>
<td>V</td>
<td>fibronectin</td>
<td>heparan sulfate and heparin</td>
<td>integrin</td>
<td>quiescent fibroblasts</td>
</tr>
<tr>
<td>VI</td>
<td>fibronectin</td>
<td>heparan sulfate</td>
<td>litegrin</td>
<td>quiescent fibroblasts</td>
</tr>
</tbody>
</table>
Proteoglycans

Disaccharide units
- Uronic acid - Amino sugar -

Hyaluronate

Dermatan sulfate
IduUA = Iduronate
GlcUA = Glucuronate
GalNAc = N-Acetyl-galactosamine
GlcNAc = N-Acetyl-glucosamine

Heparin
Keratan sulfate
Chondroitin 6-sulfate
Some Proteoglycans

- The known proteoglycans include a variety of structures.
- The carbohydrate groups of proteoglycans are predominantly glycosaminoglycans O-linked to serine residues.
- Proteoglycans include both soluble proteins and integral transmembrane proteins.
Role of Proteoglycans

- Proteoglycans serve a variety of functions on the cytoplasmic and extracellular surfaces of the plasma membrane.
- Many of these functions appear to involve the binding of specific proteins to the glycosaminoglycan groups.
Pathology of Connective Tissue
Collagen-Related Diseases

• Collagen provides an ideal case study of the molecular basis of physiology and disease.
  – The nature and extent of collagen cross-linking depends on the age and function of the tissue.
  – Collagen from young animals is predominantly *uncrosslinked* and can be extracted in soluble form, whereas collagen from older animals is highly cross-linked and thus insoluble. The loss of flexibility of joints with aging is probably due in part to increased cross-linking of collagen.
Lathyrisim

• Several serious and debilitating diseases involving collagen abnormalities are known.

• **Lathyrisim** occurs in animals due to the regular consumption of seeds of *Lathyrus odoratus*, the sweet pea, and involves weakening and abnormalities in blood vessels, joints, and bones.

  – These conditions are caused by \( \beta\)-aminopropionitrile (see figure), which covalently inactivates lysyl oxidase and leads to greatly reduced intramolecular cross-linking of collagen in affected animals (or humans).
Other Collagen Disorders

- Alterations in collagen structure resulting from abnormal genes or abnormal processing of collagen proteins results in numerous diseases, e.g. Larsen syndrome, scurvy, osteogenesis imperfecta and Ehlers-Danlos syndrome.
Osteogenesis Imperfecta

- Osteogenesis imperfecta also encompasses more than one disorder.
  - At least four biochemically and clinically distinguishable disorders have been identified all of which are characterized by multiple fractures and resultant bone deformities.
Ehlers-Danlos Syndrome

• Ehlers-Danlos syndrome is actually the name associated with at least ten distinct disorders that are biochemically and clinically distinct yet all manifest structural weakness in connective tissue as a result of defects in the structure of collagens.
Ehlers-Danlos Syndrome-2
Marfan's syndrome

- Marfan's syndrome manifests itself as a disorder of the connective tissue and was believed to be the result of abnormal collagens.
  - However, recent evidence has shown that Marfan's results from mutations in the extracellular protein, fibrillin, which is an integral constituent of the non-collagenous microfibrils of the extracellular matrix.
## Disorders of Collagen

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Collagen Defect</th>
<th>Symptomology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehlers-Danlos IV</td>
<td>decrease in type III</td>
<td>arterial, intestinal and uterine rupture, thin easily bruised skin</td>
</tr>
<tr>
<td>Ehlers-Danlos V</td>
<td>decreased cross-linking</td>
<td>skin and joint hyperextensibility</td>
</tr>
<tr>
<td>Ehlers-Danlos VI</td>
<td>decreased hydroxylysine</td>
<td>poor wound healing, musculo-skeletal deformities, skin and joint hyperextensibility</td>
</tr>
<tr>
<td>Ehlers-Danlos VII</td>
<td>N-terminal pro-peptide not removed</td>
<td>easily bruised skin, hip dislocations, hyperextensibility</td>
</tr>
<tr>
<td>Osteogenesis imperfecta</td>
<td>decrease in type I</td>
<td>blue sclerae, bone deformities</td>
</tr>
<tr>
<td>Scurvy</td>
<td>decreased hydroxyproline</td>
<td>poor wound healing, deficient growth, capillary weakness</td>
</tr>
</tbody>
</table>
Thank you for your attention