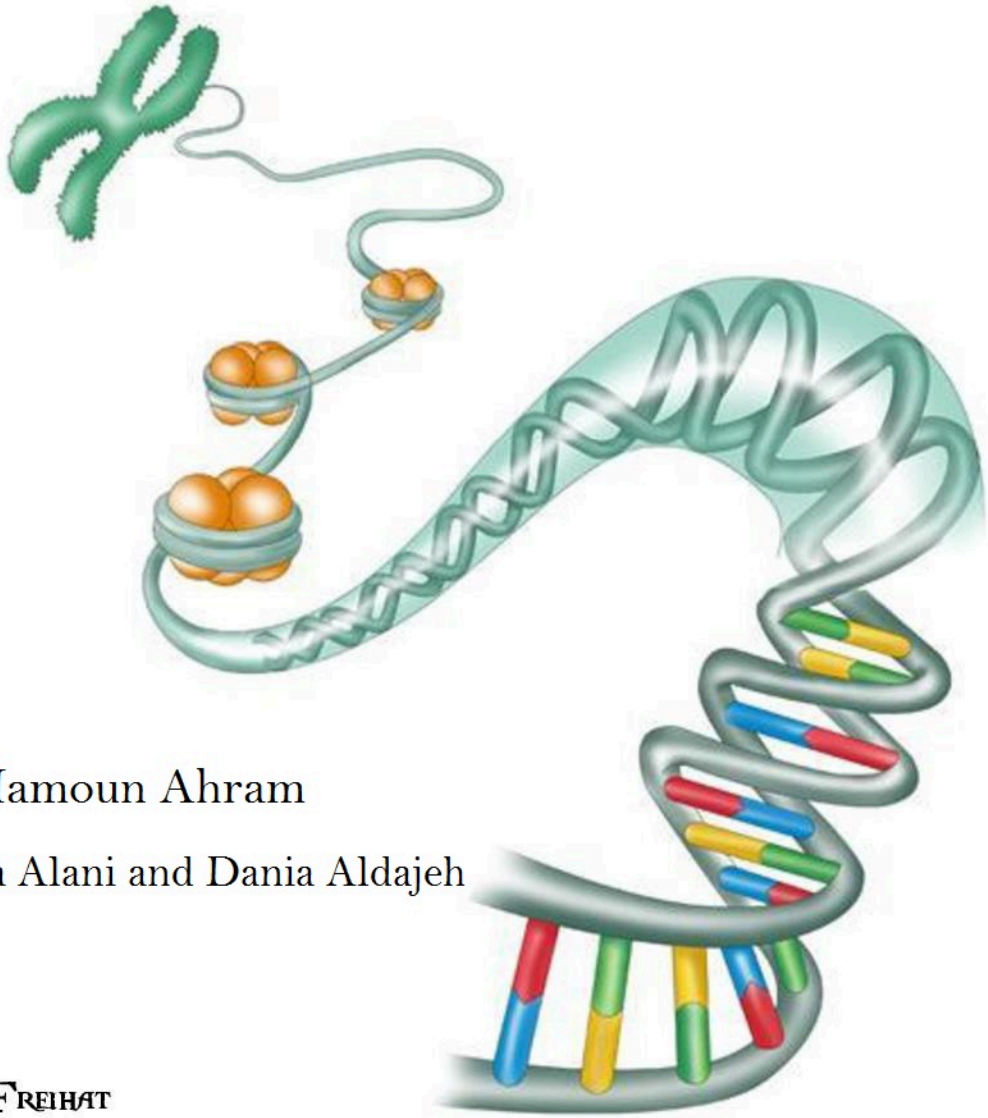
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ○ Sheet ○ Handout ○ other.....



Sheet#: 10

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Extracellular matrix

Hello everyone ……today we will finish with Intermediate Filaments and start with Extracellular Matrix ….

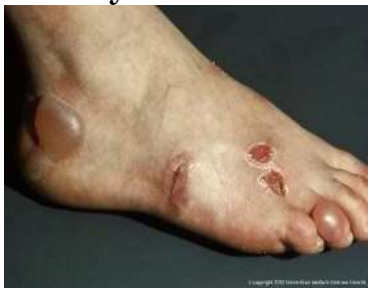
The keratin filaments of epithelial cells are tightly anchored to the plasma membrane at two areas of specialized cell contacts, desmosomes and **hemidesmosomes**. First we will start with desmosomes ; desmosomes are connections between two receptors on the cell surface exactly cadherin so we have cadherin- cadherin interaction and each one of these receptors is binding to IFs, so the desmosomes is one of the mechanisms by which cells can connect to each other.

The other cellular structure is the hemidesmosomes and here the connection is between the cell and the matrix protein so we have the IFs followed by numbers of proteins like plectin which connect to cell surface receptors and these receptors are known as integrins then these integrins will bind to the extra cellular matrix.

*** **Desmosomes** connect cells with each other mediated by cadherin but **hemidesmosomes** connect cells with extracellular matrix mediated by integrins.

People tried to study the Ifs; they eliminated the gene of IFs and they wanted to see the effect on cell structure, they found that there is basically no effect => cells can survive and grow and there is nothing wrong with them, so they came up with the hypothesis saying that these IFs are needed to strengthen the cytoskeleton or to maintain cell structure. They created transgenic mice, we talked about transgenic mice in the first lecture. Transgenic mice are mice with an altered gene so they have a gene that they either added or eliminated and they found that these mice are fine except that they had blisters (skin injury or damage) and they said this is due to epidermal cell lysis and this supports the hypothesis that IFs strengthen the structure of cells or the shape of cells.

So that was in mice, and there are number of human diseases related to defective keratins or Ifs one of them is known as **human epidermolysis bullosa**, where you can see blisters in feet as in the picture.



Another disease is the **Amyotrophic lateral sclerosis** also known as **lou Gehrig's disease** which is characterized by the accumulation and abnormal assembly of neurofilaments, so the nervous system would be defective.
There is a table in slides (table 7.2) that summarizes the cytoskeleton, please take a look at it.
*** We should know that the cell has all the three kinds of cytoskeleton components not one of them and they are connected to each other and work together.

Now, we will move to the ECM (slide8)

The Extracellular Matrix

The matrix is a network of proteins that exist outside the cell, and these proteins are synthesized by cells, so you have a community of cells in certain tissues and each cell can secret a certain type of proteins that assemble to make a network.

Now, the ECM is a space, filled between cells, and there are 2 types of these tissues:

1. BASAL LAMINA: This is a very thin layer that exists underneath the epithelial cells.

So there is array of epithelial cells, and below it there is a line where the epithelial cells sit on, this line is known as "basal lamina" and this basal lamina separates the epithelial cells from the cells in the matrix.

One of the mechanisms by which cancer cells become aggressive, metastasize and spread, is the production of protease that degrades the basal lamina allowing the cells to move forward and invade the tissue into the blood vessels and then spread all over the body.

The other mechanism is the changing in the shape of an epithelial cell ->when it becomes fibroblast like or mesenchymal cells like; they become elongated, motile and express Vimentin.

2. THE CONNECTIVE TISSUE: Which is the loose network that exists underneath the epithelial cells, it contains scattered cells, different types of cells, like: fibroblasts, inflammatory cells, endothelial cells, muscle cells, and so on...

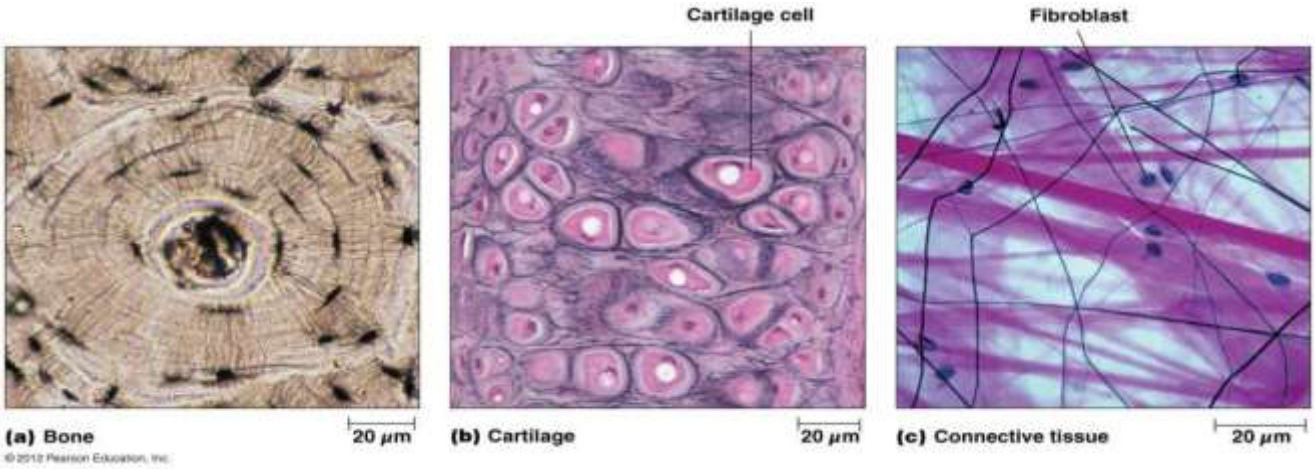
The matrix is also made of proteins; these proteins are tough, fibrous, embedded in a gel-like polysaccharides ground substance. It also contains adhesion proteins that link matrix proteins to each other and link the cells to matrix proteins (So we have 2 types of these adhesion proteins). So we have fibrous proteins, sugars that bind to the proteins, and adhesion proteins.

The basal lamina is formed by matrix components which are different from those in the CT, for example: **Tendons** (composed of high proportion of fibrous proteins).

Cartilage (contains high concentration of polysaccharides- because these polysaccharides are negatively charged, they can be compressed but once the compression is gone then it can go back to its original structure because of the repulsion between the negative charges) .

In bone the matrix is hardened by deposition of calcium phosphate crystals.

So if you look at these different connective tissues, you can notice that they are completely different.



To sum up: There are 3 components of the matrix:

1) **The matrix proteins**

2) Polysaccharides

3) Adhesion proteins (link the matrix proteins to each other and link the cells to the matrix as well), different tissues have different types of these proteins.

Collagen: it is a filamentous, long, stiff, triple-stranded protein.

It is made of a lot of Glycine, Proline, Hydroxyproline, and Hydroxylysine.

(The purpose of hydroxylysine is to add polysaccharides to the protein(attachment of polysaccharides// the purpose of hydroxyproline is to stabilize the structure // the lysine and aldolysine – oxidized lysine- link the helical chains together).

**there are different types of collagen molecules:

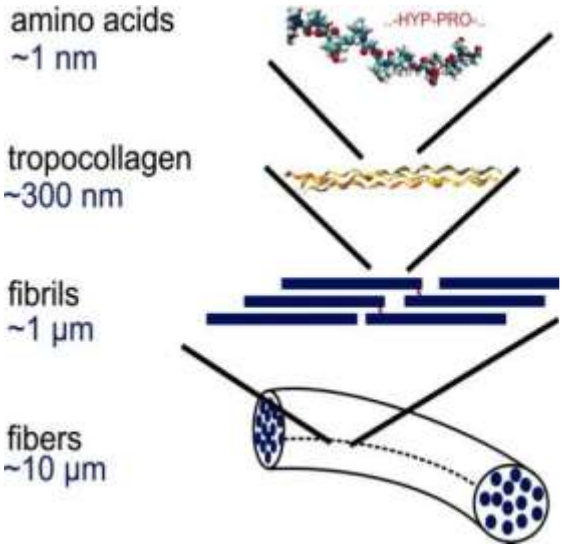
1. **Fibrillar collagen**
2. **Fibril associated collagens**
3. **Network forming collagens**
4. **Anchoring fibrils (proteins attached to other proteins like fibronectin)**

1*The fibrillar collagens: they have different types:
- **Type one** (also called type 1 collagen): mainly found in the **connective tissue.**
-**type II** (also called type II collagen): mainly in **cartilage**, usually it is smaller than type I, oriented randomly, rigid but compressible.
-**type III** (also called type III collagen): found in **extensible tissues**, like the lung and skin.
- Type XI: found in the cartilage.

Each one of them is found in different tissues but the most abundant is type 1 collagen found in CT.

Collagen filaments are assembled after secretions; first they are synthesized in the cell and then secreted, once they are secreted they can assemble into an ordered structure.

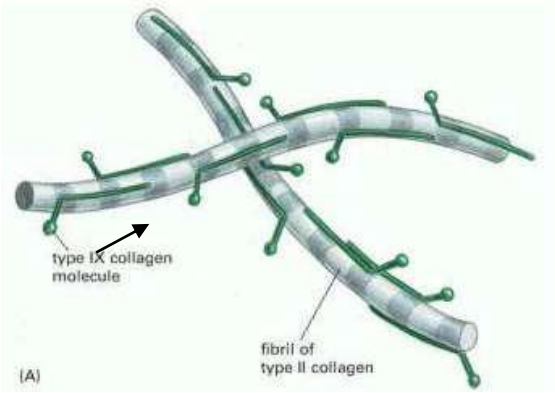
The tropocollagen is the basic unit of the collagen molecule, which is a triple helical molecule .....> after secretion, these tropocollagens come together to form collagen fibril ...>and these fibrils come together to form collagen fiber. We can observe the collagen fibers under the light microscope, but the fibrils can be seen under the EM.



2 *** Fibril associated collagens (collagen types IX, XII)**

They are the structure in green

They are found along the fibrils, they connect collagen fibrils to other molecules, proteins or to other collagen fibrils.



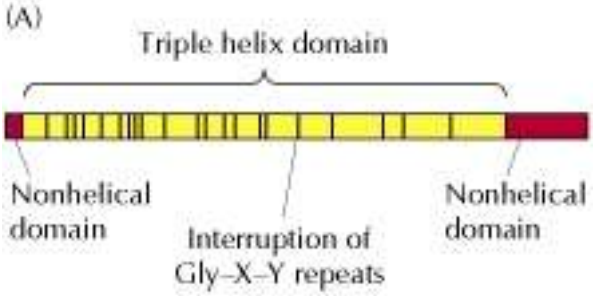
3* network forming collagens

The most important type is **type IV**, which is found in the basal lamina (again, the basal lamina is the layer that exists underneath the epithelial cells).

4* anchoring fibrils

Which associate network forming collagen with fibrillar collagen. They anchor to proteins, and these proteins can interact with other protein, such as fibronectin.

The difference in anchoring proteins is that their helical structure is actually interrupted, (if you look at the structure of fibril collagen it is mainly helical) and the anchoring fiber is helical as well ,but this helical structure is interrupted , so this interruption gives flexibility to the molecule, because if it is helical without that interruption ,it will be rigid.



Now, **HOW ARE COLLAGEN MOLECULES SYNTHESIZED??**

Firstly, there will be a translocation of pro α-chain into the ER and Golgi. Once it is in the ER, then:

- Signal sequence is cleaved off
- It gets glycosylated
- Formation of tropocollagen
- Modification (by hydroxylation and glycosylation)

**So once the protein gets into the ER >> the signal sequence is cleaved off
..>>>then modification by sugars occurs (it gets glycosylated and hydroxylated, by which give hydroxylysin)
...>> and finally, the formation of tropocollagen.
**then it goes to secretary vesicles.

In pink, we have a helical structure.

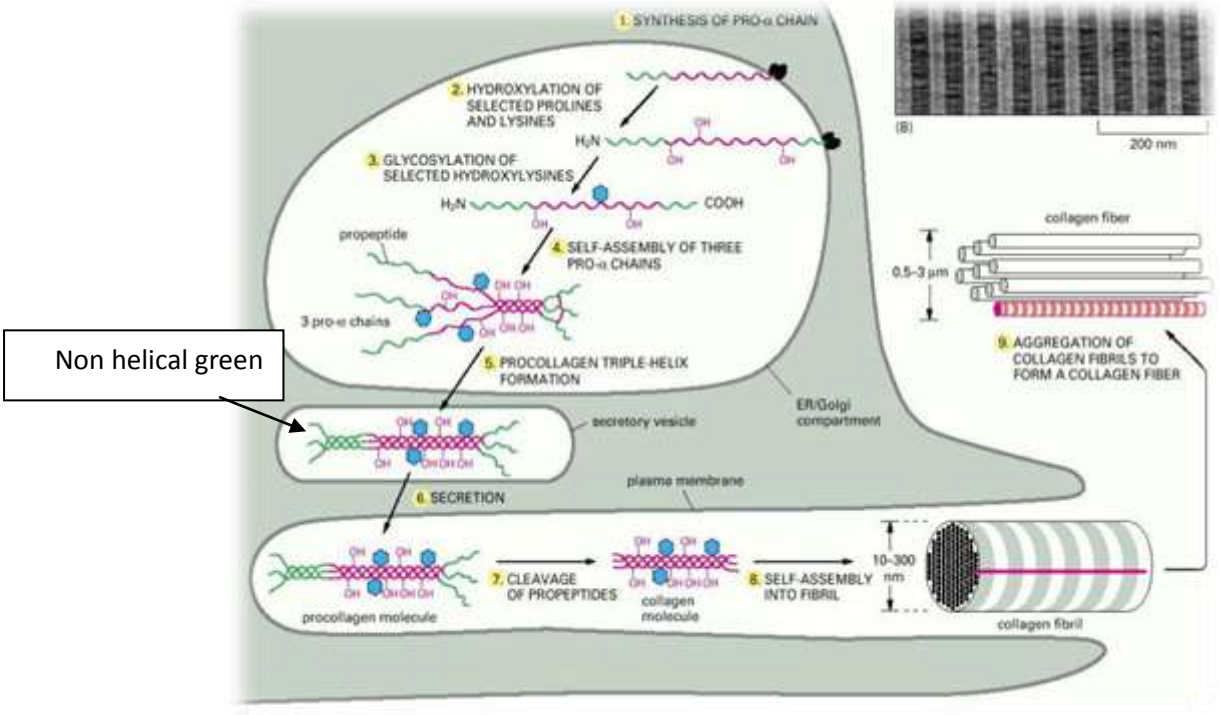
Notice the green fragments, which are at the end of the molecule and they are not helical.

There is a reason for having these non-helical molecules.

So what is secreted outside the cell is **the pro-collagen**, which is the tropocollagen molecule that is glycosylated and contains the hydroxyproline as well as pro-regions, which exist at the end of both sides.

The purpose of these pro-sigments ››› is to prevent the assembly of a larger structure (of tropocollagen together) - because if we have a formation of the tropocollagen inside the cell, the cell will explode (because it is larger than the cell).

So, what prevents the formation of fibrils or fibers inside the cell is the **pro-region.**



Again, once the pro-collagen is secreted, the pro-regions will be removed, and then the assembly of fibrils and fibers happens.

The difference between the fibril collagens and the network-forming collagens is that:

1*The pro-regions of the network-forming collagens are not cleaved, they are retained and that is the reason why the network forming collagens don't form larger fibrils and fibers, so they don't aggregate with each other in the matrix outside the cell.

2* the other difference is that the network-forming collagen has non helical domains, and these non-helical domains provide flexibility to the molecule, while these are not found in fibrillar collagen.

So, there are 2 reasons for not removing the pro-region inside the cell or for not forming a large molecule inside the cell:

1) Because of the presence of pro-peptide that is removed outside the cell.

2) The enzyme "lysyl oxidase" which forms the reactive aldehyde and helps in cross linking between the different tropocollagen, exists outside the cell. So collagen molecules (tropocollagen) are cross linked via lysine or hydroxylysine and the enzyme **that is responsible for the modification of lysine,** is present outside the cell (these cross links are important to stabilize the collagen fibrils)

There are number of collagen related disease (Those are not mentioned the book):

1) If the cross linking of collagen is inhibited then the strength of collagen molecules is greatly reduced.

In case there is no cross linking (i.e. there is a problem in the enzyme" lysyl oxidase " which forms the oxidized lysine) then what will happen is that the skin (for example) will tend to tear easily.

2) Scurvy results from the deficiency in vitamin C (vitamin C is important in the reaction that converts Proline to Hydroxyproline) so if there is no Hydroxyproline then the collagen molecule will be weak and a number of symptoms will appear on this person (skin and gum lesions, weak blood vessels).

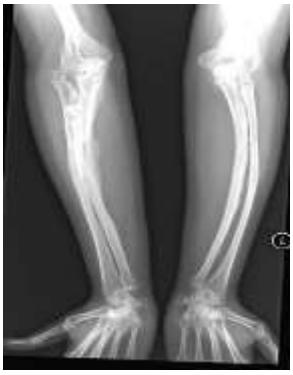
There are a group of diseases related to defected collagens, like:

Osteogenesis Imperfecta (Brittle bone disease) "imperfect bone formation"

-It is a Genetic disease
-The person will have weak bones that are easily broken
-There are 4 types of this disease: typeI,II,III,IV .
Type I is the mildest
Type II is the most severe

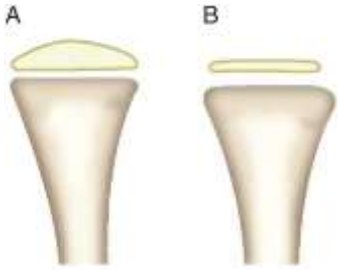
According to the severity, the milder forms generate a sever crippling disease (which most likely appear in type I)

So there are number of mutations related to Osteogenesis imperfecta:
-one of them is the mutation in genes called COL1A1/COL1A2 (these genes are important in the formation of type I fibrillar collagen). So you can see that the person has imperfect, fractured, weak bone.
-It is an autosomal dominant disease (meaning that you only need one defected gene to have the disease).



The second condition is known as (<u>Chondrodysplasia</u>):

In this case, the cartilage will be affected, as we said that type II collagen is the one that is present in the cartilage. So in this case, there might be crippling, and a person may have malformation, abnormal hands, legs and body structure, and also the cartilage between bones will be defected.



The third condition is <u>Ehlers -Danlos syndrome</u>:

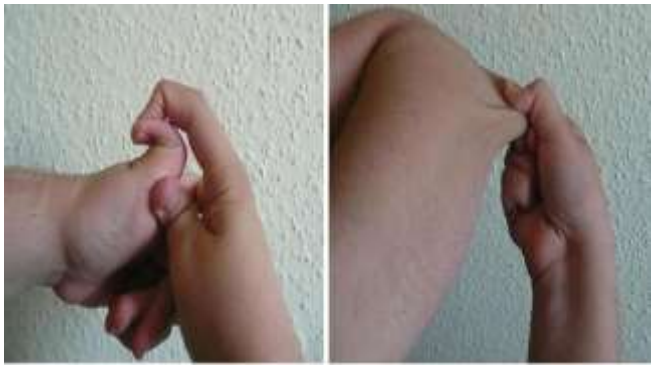
-It is a heterogenous (meaning that there are different forms of disease depending on the type of mutation, and severity; so it can be life threatening or mild)

-You have defective collagen synthesis

- You can have mutations in type I, III , V collagen related to any of the enzymes that modify the collagen molecule , such as if you have mutations in pro-collagen peptidase which is the enzyme that removes the pro-region => so you will have formation of pro-collagen but not a normal formation of collagen fibers. Or if you have a mutation in lysyl hydroxylase (this enzyme adds a hydroxyl group to lysine) then the glycosylated process will be affected so there will not be sugar molecules on collagen.

In this case, the main manifestations are: skin fragility ,hyper extensibility and joint hyper mobility , there will not be toughness in the molecule.

Type III EDS: Since type III collagen is a major component of arteries, mutations affecting type III collagen result in fragile blood vessels. Other symptoms include stretchy skin and hyper mobile joints.



Elastin

-intermixed with collagen
-it provides elastic features to the tissues , like: the blood vessels .
-it is rich in proline , glycine , and also has hydroxyprplin, but it doesn't have hydroxylysine ››so it can't be glycosylated.

****The formation of elastic fibers:**

-they form from tropoelastin.
-the elastic fibers are formed outside the cell, because we need the enzyme "lysyl oxidase" to cross link the elastin molecules to each other ... and as we said before, the enzyme lysyl oxidase is present outside the cell.

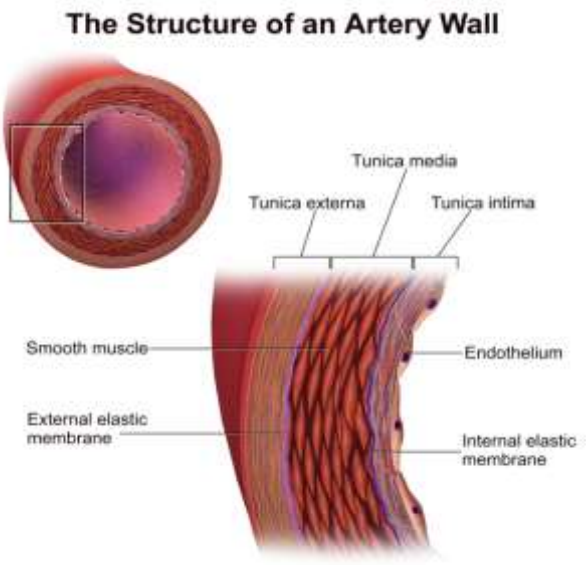
**** The structure of elastin:**

The elastin protein is composed largely of two types of short segments that alternate along the polypeptide chain:
hydrophobic segments, which are responsible for the elastic properties of the molecule;
and alanine- and lysine-rich helical segments, which form cross-links between adjacent molecules.

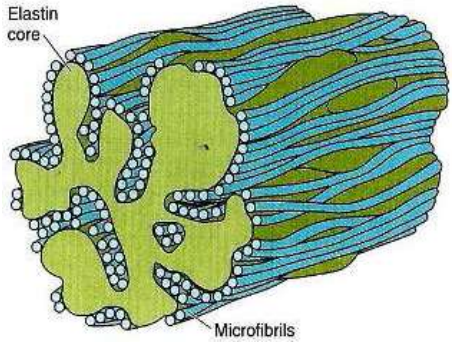
**** The function of Elastin :**

-it is present in arteries, it allows the tissues to be stretched
-if there is abnormal elastin that will result in excessive proliferation.
-- The main reason is the the artery is surrounded by elastin fibers , and they almost work as a belt , so they prevent (limit) the proliferation of muscle cells.

So now if we have defective elastin ...› that will give more space for the cells to grow and proliferate , so what will happen is that the artery becomes very thick (caused by the excessive growth of these smooth muscle cells and also there will be narrowing of the artery as well).



The elastin fiber is covered with certain proteins, one of them is known as microfibrils ,which is a long structure that covers the Elastin molecule and these microfibrils are formed from glycosylated proteins known as fibrilin .**Their function** is to provide or maintain the integrity of elastin ,so the structure of elastin itself is maintained and supported by the fibrillin which forms the microfibrils.



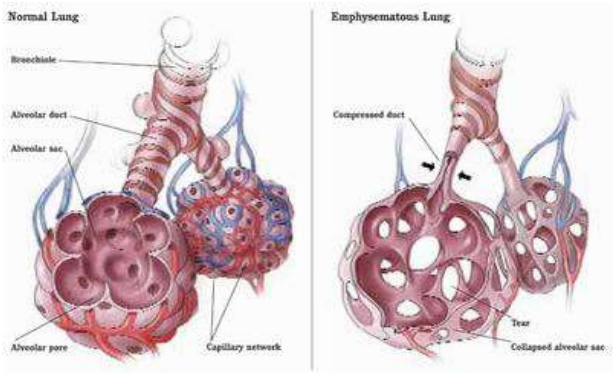
If there is a mutation in the fibrillin it will cause a condition known as **Marfan's syndrome** in which the structure of the artery is not maintained and when it expands it can be easily broken and ruptured.

*** **The symptoms of Marfan's syndrome:**

The person would be tall and have long legs, arms and fingers. There are other symptoms (like: flexible joints , scoliosis or curvature of the spine , a chest that sinks in or sticks out , crowded teeth and flat feet) but the doctor didn't mention them since they are less important.

Another disease that is related to elastin is **Emphysema** which is a destructive lung disease .we normally need a lot of elastin in our lung , so lung can expand and have their regular shape back. There is an enzyme known as elastase that degrades elastin, in our lungs there is always a balance between protease and protease inhibitor (elastase inhibitor) which is the alpha-1 antitrypsin, if the person **has a genetic defect or a genetic mutation** in alpha-1 antitrypsin molecule he will have an elevated activity of elastase, higher degradation of elastin in lungs and that causes inability to breath well.

Smoking also inactivates alpha-1 antitrypsin by oxidizing essential methionine residues , so increasing the activity of elastase and decreasing elastin fibers in lungs and cause emphysema, this why smokers have difficulty in breathing.



Now we finished talking about the two matrix proteins (collagen and elastin)
As we said, the extracellular matrix has three components :

1) The matrix proteins (collagen , elastin)
2) The sugars
3) Also we have adhesion proteins .

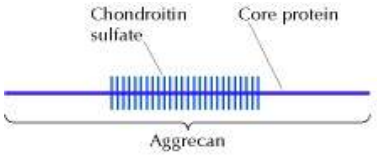
Matrix polysaccharides (sugars):

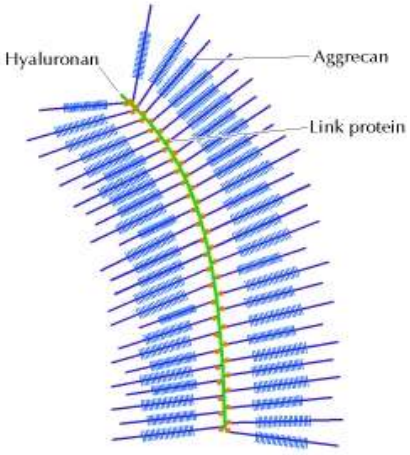
There are **glycosaminoglycans proteins** (GAGs) which are polysaccharides of repeated disaccharides and they either have N-acetylglucosamine or N-acetylgalactoamine and they are usually, at least one of them, are acidic so the whole molecule is negatively charged and it can be modified by a sulfate groups ,and some of them exist as proteoglycans that is when we have a structure of a little protein and a lot of sugar so these proteoglycans not only exist in the matrix it also can be part of cell surface proteins with either transmembrane domains (**syndecans**) or GPI anchors (**glycipans**) interacting with integrins.
*glycipans and syndecans are cell surface proteins that are modified with glycosaminoglycans.
***Skip (Perlican) slide.**

Now let's talk about **aggregan**

It is a large proteoglycan that consists of more than 100 **chondroitin sulfate** chains joined to a **core protein**.

* Multiple aggrecan molecules bind to long chain of **hyaluronan** (which is another glygosaminoglycan) and become trapped in the collagen network, forming large complex in the ECM of **cartilage** mainly. So again,there is a large structure with these components:
hyaluronan and **aggregan** (which consists of protein and chondroitin sulfate) present mainly in cartilage .





Matrix Adhesion Proteins

The matrix adhesion proteins link matrix proteins with one another and link the matrix proteins to cells. We will talk about two of them: Fibronectin and Laminin.

Fibronectin	**Laminin**
The principal adhesion protein of connective tissue	A main component of basal laminae
A dimeric glycoprotein that is cross-linked into fibrils by disulfide bonds	A heterotrimer protein with binding sites for cell surface receptors, type IV collagen and perlecan
Binds to collagen, GAGs and cell surface proteins like integrins (linking cells to the ECM)	Link cells to the basal lamina and also binds integrins.

* Role of integrins:

- The first structure is Focal adhesions:

Integrins are present in a structure that we talked about before, called **focal adhesions** (remember when we said that when the cell moves it forms these focal adhesions). These actin fibers are called stress fibers. What they do is that they eventually link to the cell surface via a complex protein known as "focal adhesion". In these focal adhesions we have integrins(the cell surface proteins). Linking integrins with actin filaments needs a number of proteins, one of them is Vinculin. So when cells move they form attachments to the substratum by integrins then detachment and attachment and so on..

- The second structure is hemidesmosomes:

Here the integrins are present in the **hemidesmosomes** that we talked about in the beginning of this lecture (it is a way by which cells link the Ifs not the actin to the outer side of the cell and this link is completed via integrins)

How this works is that integrins are inactive dimers and we activate them by
1) forming the initial binding with the matrix proteins,
2) recruitment (activation) of additional integrin molecules binding together forming **focal complex** or the hemidesmosomes
3) recruitment of different actin filaments forming the **focal adhesion**

Cell-Cell interaction:

There are many groups of cell adhesion molecules that play a role in cell-cell interaction, like selectins, integrins and cadherins.

*** Selectins:**

They are a class of proteins that exists in Leukocytes.

Types of selectins:

1) L-selectins (L- stands for Leukocytes)
2) E-selectins (E- stands for **endothelial** or epithelial)
3) P-selectins (P for platelets)

Interactions between these types of selectins, recruit integrins and send a signal.

By this mechanism, Leukocytes can transfer from the blood to the tissue.

Types of interactions:

1 - Homophilic interaction: which is between the same molecules, like selectin-selectin interaction or cadherin-cadherin interaction.

2- Heterophilic interaction: between different molecules.

* Cadherins:

They are cell surface receptors that exist as dimers, they interact with each other on two different cells.

And we have different types of **cadherin:**

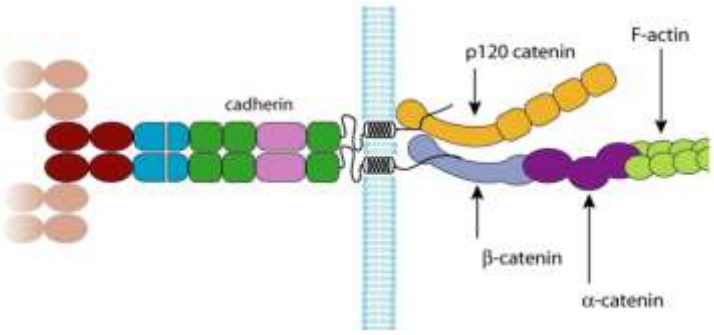
1) E- cadherin on epithelial cells (the most important cadherin)
2) N- cadherin on neural cells
3) P- cadherin on placental cells
4) desmosomal cadherin

Now when cells transfer into tumor they will be fibroblast- like cells, they start to express vimentin and lose the expression of E- cadherin, because E-cadherin connects different epithelial cells to each other. So if cadherin was defected, there is no cell-cell interaction and cells become free and motile.

There are three types of cell interactions **(Adherens Junctions, Desmosomes, and Tight Junctions**)

***Adherens junctions**

In which we have cadherin on cell surface that binds to β-catenin, and β -catenin binds to α- catenin, and α- catenin binds to actin. That's how the signal is moved, if one actin molecule moves, the signal will be sent to alpha- catenin, B-catenin and cadherins. (Memorize the names of these proteins)



*__Desmosomes__ is cadherin – cadherin interaction but here the cadherin binds to intermediate filaments not to actin and this cadherin is known as Desmosomal cadherin.

We will continue this subject in the next lecture, please take a look at the slides.

Special thanks to Yara AlKayed
Done by: Ruba Alani and Dania Aldajeh