Genetics
& Cell biology

◉ Sheet ○ Slides

Number

10

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In this sheet we will move on to a new topic in cell biology which is called **extra cellular matrix (ECM).**

So the first question to ask, **what is ECM?**
-It's a collection of components that surround the cell from the outside, they differ in their specific composition from one cell type to another, but there are some components and materials in common between cells in general (that may differ in their specific composition and distribution) .For e.g: if we talk about a certain protein type that is present in ECM, it may exist in a certain isoform for ECM of a specific cell type but another isoform for another cell in a different tissue.

-Sometimes we will notice that some tissues are mostly composed of ECM rather than cells, for e.g.: connective tissues in general ,let's take the bone as an example, osteocytes are widely distributed and the space between them is filled with special type of ECM that's composed of minerals in general and calcium more specifically. So the point in cell biology is that the structure of a certain tissue must always be suitable for its function.

-There are also some tissues, for e.x epithelial cells, that need support and separation from other tissues, which are provided by the basement membrane (Basal lamina) they are "sitting" on which is one type of ECM that surrounds the cells.

So –as mentioned above- ,we said that there are certain **general components that we can find in different ECMs , what are they?**

1)**Fibrous proteins:** as the name indicates ,they are arranged in **fibers**. This fiber structure gives **support** to the components of the ECM. There are different types (different composition) of fibrous protein in different ECMs and we will talk about them later.

2)**Adhesion Proteins:** their function is to link and attach the cells to other ECM components.

These 2 components are suspended within:
3) Ground substance ,it's a gel-like structure that's composed of polysaccharides, those polysaccharides may attach to proteins to form what we call proteoglycans.

- We mentioned examples about different tissues with different ECMs that suit their functions (epithelial and bone tissues), the third e.x is: cartilage, to provide some sort of flexibility and shock absorption, making its compression better than the bone, its ECM is composed of an amount of polysaccharides that are arranged as GAGs as a part of proteoglycans, and these GAGs act as cushions giving cartilage the required flexibility.

-Tendons for example , for every muscle contraction and relaxation they will be exposed to strong pull forces , in order to bear these forces they must contain in their ECMs high amounts of fibrous proteins.

SO DON'T FORGET: these general components are present in all ECMs in different cell types, but their levels, isoforms and distributions differ from one tissue type to another in order to suit their different functions.

-During the development of stem cells in embryos, we call ECMs in these cells **(Stem cells' niche)** which means the microenvironment of ECM that surround these cells and drive them to differentiate into different types of cells and also to give stem cells the maintenance of their function and structures as well . And many researches have been done on this topic (Stem cells' niche) in stem cells field in general and particularly in cancer stem cells.

Now let's talk in details about the general components we've mentioned above:
1)Fibrous proteins : there are many types of them including :
a)Collagen : the most common type , it's arranged in **triple helices** , it's composed of high levels of (**Proline ,Lysine and Glycine**).Both **proline and lysine get into hydroxylation process** ,in which we add OH group , facilitating the formation of hydrogen bonds that contribute in binding between these collagens with other ECM components providing the required mechanical support eventually.

- -In addition to that , **HydroxyLysine** specifically serves as attachment site for sugar ,making it a glycoprotein. Also there are crosslinking processes that happen in HydroxyLysine and Lysine –remember that Lysine is already a basic amino acid containing amino group making it able on its own to make hydrogen bonds even without hydroxylation unlike proline- ,to provide better strength of collagen fibers.

-Another thing about collagen it has the highest expression level in our body compared to other proteins, however, it's the most difficult one to isolate thus it's hard to study .

-What are the types of collagen ?

1) Fibril forming collagen

2) Fiber associated collagen (more flexible than the first one)

3) Network forming collagen (also more flexible than the first one), this high flexibility comes from structural changes compared to fibril forming collagen, for e.x: some structures are composed of short sequences forming non-helical structures that are separated from the rigid basic triple helix structure.

4) Anchoring fibers, they attach between network and fibril forming collagens.

5)Some types of collagen act as transmembrane proteins, so they facilitates interaction between the cell with its ECM.

We won't talk about the details of every type , particularly we will discuss the major type which is **fibril forming collagen** , like any other protein it's encoded by a gene and going to be transcribed and synthesized by ribosomes. After synthesis with some modifications, it will start the folding process (process of association of more than one chain with each others), which in other words is the quaternary structure.**How is that ?**

-The **basic units** of mature collagen is called **(TropoCollagens) ,** which is going to start arranging in a mechanically rigid structure containing overlaps between them giving the better mechanical properties.

So imagine if we put these tropocollagens parallel to each other and there are spaces between each one with the other, we will have these spaces also parallel to each other which will eventually weaken the structure of collagen , **but when these tropocollagens are overlapped with each other , this will help in obtaining the needed function they are for.**

In terms of more details about the synthetic process , it **begins inside the cell** – specifically **in ER**- where **1)transcription and synthesis of the proteins** and then **2)most of modifications are going to happen (For e.x: hydroxylation of Proline and Lysine and also glycosylation).**

After that , the **3)formation of quaternary structure** begins ,**HOW?** the 3 helices get "braided" with each other forming **ProCollagen** , **4)this ProCollagen with all modifications and extra structures get packaged by vesicles** , **5)exocytosis** happens and **6)more modification will happen in ECM**. bearing in mind that in some places they're going to be loose and these loose parts are going to be removed later so there's no need to make them rigid.

At this stage , there won't be formation of fibrils and fibers inside the cell,**Why?** as the size will increase so these processes occur in ECM (outside the cell).

-What exactly happens in ECM? As we said there will be **cleavage** for certain additional loose areas (called propeptides) by an enzyme called **ProCollagen Peptidase ,** transforming the molecule into the basic unit which is **TropoCollagen** that's going to be arranged –as we said- in an overlapped fashion making what's called **Fibrils ,** these fibrils will aggregate forming the final **Fiber**.

-Why did we remove the loose propeptides ? as they will make the process of fibrils formation difficult.
NOTE: check the picture in slide 8 to make it easier imagining the whole synthetic process.

Now we will move to talk about **certain diseases that are related to collagen**.

1)Scurvy , what happens is that hydroxylation of proline is missed making the crosslinking between the fibers weaker and less (here crosslinking will happen only with Lysine) , this will eventually make the connective tissue weaker which is mostly obvious in case of oral and skin lesions ,also blood vessels (easily bleeding when exposed to traumas) because there's no rigid collagen that gives the needed support.

2)*Osteogenesis imperfecta(OI)* , also called **(Brittle-bone disease) :**
in which there's a deficiency in bone formation (fracture of bone is much easier compared to normal) , the patient's bone will have bends (تقوسات) (they are obvious via Xray). The patient may be referred to you by a dentist as they suffer from oral lesions and deficiencies in formation of teeth and the bone supporting them.

-It's a **genetic disorder (**it has **autosomal dominant** pattern of inheritance means that **one copy** of the altered gene is sufficient to cause the condition) , happens due to **mutations in COL1A1 and COL1A2 genes** that interfere with the assembly of type I collagen which is the most important type in the formation of collagen fibrils.

- **Four types of OI designated** as type I through type IV :
Type I : the mildest form of the condition
Type II: the most severe form that results in death in utero or shortly after birth
NOTE: Milder forms generate a **severe crippling disease.**

3) Chondrodysplasia (Achondroplasia) , it's due to mutations affecting **type II collagen** causes –in general- bone and joint deformities. The cases of this disease (particularly in this disease they have wide face and small body) have dwarfism as the cartilage is present in the epiphyseal plate which affects the height of the bone. Other special features in fingers and toes also help us in the diagnosis of this disease.

4) Ehlers-Danlos syndrome , patients with this disease suffer from excessive flexibility in joints due to mutation in different types of collagens and collagen processing enzymes (The most clinically important mutations are found in the gene of type III collagen). For e.x : **lysyl hydroxylase** ,also if the enzyme **ProCollagen Peptidase** is mutated then there will be no cleavage for the loose peptides leading to formation of very loose fibers that will eventually make the tendons and joints more loose and extremely flexible in movement. In addition to that , Since type III collagen is a major component of arteries, mutations affecting type III collagen result in fragile blood vessels.
NOTE: you can check the pictures in slides 13 and 14 to see how patients with this disease look like.

Moving to another fibrous component of ECM which is **Elastic fibers** .
-Compared to collagen , these fibers have some sort of elasticity , they are arranged in networks rather than fibers as in collagen .It's composed of a protein called **Elastin** which has two types of short segments that alternate along the polypeptide chain:
1. Hydrophobic segments, which are responsible for the elastic properties of the molecule
2. Alanine- and lysine-rich alpha-helical segments, which form cross-links between adjacent molecules

-Collagen and Elastin have some **similarities** and **differences** as follows:
1)They are both rich with Proline and Glycine (also hydroxyproline) ,but Elastic fibers don't have Lysine in high amounts and it doesn't contain HydroxyLysine(there's no hydroxylation for Lysine), less crosslinking and thus more flexible compared to collagen .That's why we can find elastic fibers abundant in organs to allow them to stretch then return to the original shape, e.g. lungs
2)Elastic is non-glycosylated unlike Collagen.

-**Synthesis of Elastin** as a protein ,like collagen, encoded by genes , synthesized by ribosomes , hydroxylation of proline residues happens , preparation and formation of basic structural unit that's called **TropoElastin,** and finally packaging and exocytosis to ECM to form elastic fibers that –as mentioned- will have crosslinkings via covalent bonds between lysines.

-**What is the role of Elastic fiber in the arterial walls**?
They sure give elasticity but also high levels of elastic fibers give the arterial walls some sorts of rigidity especially that we're talking about arteries not veins (they both differ widely in their wall thickness, flexibility and elasticity).

- The normal elasticity of an artery restrains the proliferation of smooth muscle cells. Sometimes because of **abnormal or deficiency of the elastin** results in **excessive proliferation of smooth muscle cells** in the arterial wall and narrowing of the arteries leading to hypertensions and other related problems .

-Elastic fibers (green in the picture in slide 18) also in some structures are going to be surrounded by certain proteins (blue in that picture) like **fibrillin** , forming a structure called **Microfibrils** .This is mostly found in structures that can move like hair and sperm tail.

-Clinical application on Microfibrils : mutations in Fibrillin (the protein) cause **Marfan's syndrome.**

Signs and symptoms: **1**)A tall, thin build, long arms, legs, fingers, and toes. **2**)Flexible joints **3**)Scoliosis, or curvature of the spine **4**)A chest that sinks in or sticks out **5**)Crowded teeth **6**) Flat feet. And also **may cause rapture of aorta**.

Another application is **emphysema (destructive lung disease),** Due to a **dysfunctional alpha-1 antitrypsin** leading to **increased activity of Elastase** in lungs , **HOW?** The lysine to-glutamate mutation causes protein misfolding that causes formation of an aggregate and block of ER export.
-This leads to losing the sac-like structure presenting in the lungs and disability to exchange the gases (the patient will appear bluish in color due to hypoxia). Smoking is a risk factor (by oxidizing essential methionine residues) in addition to genetic mutations.

We've finished talking about fibrous proteins as major ECM component and now we will discuss **polysaccharides** that form the gel-like structure inside ECM. We will discuss briefly few examples as there are a lot others.

-The basic structure of these polysaccharides is **GAGs (Glycosaminoglycans)** that form brush-like structures which are connected to **core protein** forming **ProteoGlycans ,** which some are **cell surface proteins** with either **transmembrane domains (syndecans)** or **GPI anchors (glycipans) interacting with integrins**.

- The **basic structure of GAGs are disaccharides units repeated many times** , one of these disaccharides is either **N-acetylglucosamine or N-acetylgalactosamine** , The other disaccharide is usually **acidic (either glucuronic acid or iduronic acid) → highly negatively charged and modified by sulfate groups**, (e.g. dermatan sulfate, chondroitin sulfate, keratan sulfate, heparin sulfate.), so they are **very polar** sugar molecules in order to attract water and makes gel-like structure ,making the structure more compressive and more suitable for shock absorption.

One example of these GAGs is **Hyaluronan** which is the only GAG with a single long polysaccharide chain.
-**Aggregan** which is the major proteoglycan of cartilage, it is a large proteoglycan consisting of more than 100 **chondroitin sulfate chains joined to a core protein.**
-Multiple **Aggregan** units bind to long chains of **hyaluronan (**acts like backbone) that become trapped in the collagen network, forming large complexes in the ECM of cartilage.

-Proteoglycans and GAGs also have a very important role in maintaining the homeostasis of tissues, for e.x there's a specialized structure in the eye that controls its pressure, in some cases of Glaucoma (high pressure of the eye), this tissue which is composed of certain cells and ECM , particularly in the brush-like structures of proteoglycans presenting in ECM ,the number of branches increase resulting in difficulties facing the fluid that must go outside the eye (preventing these fluids from going to the outside of the eye) leading to increase in the pressure .
-However , in normal people, when the pressure of the eye increases ,these branches start to get removed and modified and this will facilitate the fluids to go out returning the eye pressure to normal levels .

-Another type of proteoglycans is **Perlecan** which is specifically composed of Heparan-Sulfate. It binds to matrix proteins forming gel-like networks .The function is to provide a sort of communication between different components , for e.x between proteins that are protruding from the cells(integrin for example) with collagen and other proteins that are parts of ECM itself.

The last topic to discuss in ECM is **Matrix adhesion proteins** which are molecules whose function is to link matrix proteins with one another and to the surfaces of cells.
- They interact with collagen and proteoglycans and specify matrix organization and are major binding sites for cell surface receptors such as integrins.

-One of the most common examples is **Fibronectin** . In terms of structure it's composed of two chains that are connected with disulfide bridges and these chains contain many binding sites (cell, collagen and proteoglycans binding sites).

To sum up : Fibronectin is a dimeric glycoprotein that is crosslinked into fibrils by s-s bonds. It binds to collagen ,GAGs and to cell surface proteins like integrins linking cells to the ECM.

Another adhesion protein is **Laminin** which is found in basal laminae, it forms a (+)shape (T-shaped as described in the book) heterotrimers with binding sites for cell surface receptors (e.g. integrins), and ECM components, e.g. type IV collagen, and perlecan.

- Laminins are also tightly associated with another adhesion protein, called **nidogen**, which also binds to type IV collagen, all of which forms crosslinked networks in the basal lamina.

So the important question now , **how do all these components perform in terms of cell-cell interactions ?**
-Remember that interactions may be cell-cell or cell-ECM.
Also remember (focal adhesions) that are connected to actin filaments inside, also hemidesmosomes that are connected to intermediate filaments specifically.
(CHECK slide 26 to get the point).

-So we've mentioned previously **integrins** which are are a family of transmembrane heterodimers (α and β) ,**They bind to short sequences present in ECM proteins including collagen, fibronectin, laminin and proteoglycans**. In addition, they are one of the strongest molecules that can attach cell-ECM or cell-cell.

-Functions of integrins:
1. The major cell surface receptors that **attach cells to ECM**
2. They **anchor the cytoskeleton** at focal adhesions and hemidesmosomes.

-There are matrix binding sites on exposed surface of integrins so they can interact and bind with different molecules includings ions and so these integrins can be activated or inactivated depending on the status of the cell.

-How do integrins induce the formation and assembly of focal adhesions particularly ? (REFER to slide 27 for the pictures please)
-in the first picture we have the inactive form of integrin which has closed and unavailable binding site. When a stimuli (like ions) comes , they get activated and so the binding site becomes available. Binding on one site will stimulate other sites on other integrins ,leading to aggregation of integrins and their binding to ECM on one site and with Actin filaments (in addition to recruitment of formin, talin and vinculin) on the other sites and so we will have what we call **focal adehsions**.
-**NOTE:** Interactions between different types of proteins on both surfaces is called Heterophilic while if we have same types of proteins binding on both surfaces then we call these interactions homophilic.