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Course C001: Structure and Function of the Skin: Development, Cell Biology and Skin Structure

EXTRACELLULAR MATRIX AND ASSOCIATED DISEASES

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The extracellular matrix of connective tissue of the skin consists of a large number of distinct macromolecules which play a critical role in providing physiological properties to normal skin. Within the dermis, collagen and elastic fibers are the two major fibrillar components of connective tissue, and they exist in a fibrous interwoven meshwork structure associated with other extracellular macromolecules of the dermis, such as proteoglycan/glycosaminoglycan complexes, and a number of non-collagenous glycoproteins, including fibronectin, fibrillins and fibulins (Tables 1 and 2). Encased in the fibrillar meshwork are cells, such as fibroblasts that interact with the extracellular matrix components through cell-matrix receptors, such as integrins.

THE COLLAGENS:

Biology: The collagens comprise a family of genetically distinct, yet closely related proteins. Currently, as many as 28 distinct collagen types, designated as type I – type XXVIII, have been identified in vertebrate tissues and numbered in the order of their discovery. All collagens consist of a triple-helical molecule composed of three subunit polypeptides, so-called α-chains. Some of the collagens are homotrimers, in which all three α-chains are identical, while others are heterotrimers so that two or three different kinds of subunit polypeptides assemble into the collagen triple helix. Thus, there are as many as 40 different collagenous polypeptides which have been identified in mammalian tissues, each of them being a unique gene product.

Table 1. Genetically distinct collagens in the skin

Collagen Type	Size of Native Molecule (daltons)	Tissue Distribution	Functional Role
Type I	285,000	Most connective tissues, including skin, bones, tendons, ligaments, etc.	Major structural component providing tensile strength
Type III	285,000	Skin, lung, blood vessels; predominant in fetal tissues	Contributes to tensile and elastic properties
Type IV	540,000	Basement membranes	Major structural component of basement membranes
Type V	300,000	Ubiquitous	Pericellular location; interaction of cell surface and the surrounding matrix
Type VI	530,000	Extracellular microfibrils	Matrix assembly
Type VII	510,000	Skin, cornea, mucous membranes	Structural component of anchoring fibrils
Type XII	666,000	Similar to type I	Regulation of fibril diameter by association with type I
Type XIV	666,000	Similar to type I	Regulation of fibril diameter by association with type I
Type XVII	540,000	Cutaneous basement membrane zone	Stable association of basal keratinocytes to lamina lucida as part of hemidesmosomes

Table 2. Noncollagenous components of the extracellular matrix in the skin*

Component	Size (daltons)	Structure	Distribution	Functional Role
Elastic Fibers				
Elastin	70,000	Cross-linked polymer of fibers	Blood vessels, skin and lungs	Resilience and elasticity
Fibrillin-1	350,000	Microfibrillar network	Similar to elastin	Structural component of lens ligaments, periosteum, and elastic network
Fibrillin-2	350,000	Elastin-associated	Same as elastin	Regulation of elastogenesis
Basement Membrane-Associated Macromolecules				
Proteoglycans and glycosaminoglycans	$>10^6$	Complex aggregates	Cartilage, skin	Maintenance of water balance; regulation of growth, migration and attachment of cells
Fibronectin	450,000	Disulfide-linked dimers	Cell surface, plasma	Attachment of cells to the extracellular matrix
Laminins	900,000	α, β, γ chains	Basement membranes	Cell attachment and differentiation
Nidogens	150,000	Stoichiometric binding to laminin	Co-localizes with laminin	Cell binding

*These are the major, relatively well characterized matrix components in the skin. Several additional components, including fibulins, SPARC/BM-40/osteonectin, vitronectin, tenascin, and epinectin, are currently under further investigation.

Currently, at least 12 different collagen types have been detected in the human skin, and each of these genetically distinct collagens has an important functional role within its compartmentalized distribution in the skin (Table 1).

- Types I and III collagens are the major interstitial fiber-forming collagens in normal human skin, and they constitute the bulk of collagen fibers in dermis.
- Type IV collagen is the major constituent of the basement membrane at the dermal-epidermal junction.
- Type V collagen is ubiquitously present in a variety of tissues, including skin, where it regulates the diameter of major fibers consisting of type I and III collagens.
- Type VI collagen forms specific microfibrils that play a role in providing physiologic properties to the skin.
- Type VII collagen, is the major, if not the exclusive, constituent of the anchoring fibrils, structures that extend from the dermal-epidermal basement membrane to the upper papillary dermis and facilitate demo-epidermal adhesion.
- Type VIII collagen is present as a minor component in the dermis, being primarily a product of endothelial cells.
- Type XII and XIV collagens, members of the group called **FACIT** collagens (**F**ibril-**A**ssociated-**C**ollagens with **I**nterrupted **T**riple-helices), have been found to be ubiquitously distributed in small amounts in many, but not all, tissues that contain the major fibrillar collagens, type I and III.
- Type XIII collagen has been detected by mRNA hybridization in the epidermis. Its role is currently unknown.
- Type XVII collagen, also known as the 180-kDa bullous pemphigoid antigen, is a transmembrane collagen present in hemidesmosomes.

Pathology: Several unique features in the biology of collagens predispose these proteins to be candidate gene/protein systems in heritable diseases. Specifically, based on current information about normal biology and biochemistry of collagen fibers, we can recognize several distinct levels at which errors could be introduced into the collagen structure or metabolism in a manner that they could be manifested phenotypically as a disease. In fact, several heritable connective tissue diseases with cutaneous involvement are known to result from specific molecular defects in collagen genes (Table 3). Many of these diseases involve insertions, deletions or single base substitutions in the collagen genes which then alter the primary sequence of the protein. In addition, defects leading to altered activities of enzymes, which post-translationally modify collagen polypeptides, can result in a clinical disease.

Table 3. Heritable connective tissue diseases with cutaneous involvement due to defects in collagens

Disease Entity	Inheritance*
Ehlers-Danlos syndrome	AD, AR
Osteogenesis imperfecta	AD, AR
Homocystinuria	AR
Menkes syndrome	XR
Focal dermal hypoplasia	XD
Tuberous sclerosis	AD
Familial cutaneous collagenoma	AD
Epidermolysis bullosa	AD, AR

*AD = autosomal dominant; AR = autosomal recessive; XD = X-linked dominant; XR = X-linked recessive

The diversity of collagen pathology is exemplified by a group of clinical diseases, collectively known as the **Ehlers-Danlos syndrome**, characterized by hyperextensible skin, loose jointedness, and fragility of the dermis and other connective tissues. Previously, at least eleven different types of the Ehlers-Danlos syndrome were recognized in humans on the basis of clinical, genetic and molecular considerations. Currently, the classification recognizes six subtypes, and new candidate genes have been identified (Table 4).

Table 4. Genetic and biochemical heterogeneity and current subclassification of the Ehlers-Danlos syndrome

EDS type*	Traditional Classification	Clinical Features	Inheritance	Mutated gene/protein system
Classical	I, II	Hyperextensible skin and joint hypermobility, atrophic scars, easy bruising	AD, AR†	Type V collagen Tenascin X
Hypermobility	III	Joint hypermobility, pain, dislocations	AD	Not known
Vascular	IV	Thin skin, arterial, gastrointestinal or uterine rupture, bruising, small joint hypermobility, fragility of different tissues	AD	Type III collagen
Kyphoscoliosis	VI	Hypotonia, joint laxity, congenital scoliosis, ocular fragility	AR	Lysyl hydroxylase

Table 4 (continued)

Arthrochalasia	VIIa, VIIb	Severe joint hypermobility with congenital hip dislocations, skin involvement, scoliosis, bruising	AD	Type I collagen (defective procollagen to collagen conversion)
Dermatosparaxis	VIIc	Severe skin fragility and hyperextensibility, bruising	AR	Procollagen N-peptidase
Other‡	V, VIII, X, XI			

*According to The 1997 Villefranche Consensus Meeting.
‡The previous EDS type IX has been reclassified as occipital horn syndrome, a disorder allelic with Menkes syndrome. Previous types V, VIII and XI have been eliminated because of their poorly defined clinical features.
†The classical forms due to type V collagen mutations are inherited in an autosomal dominant (AD) fashion, while those caused by tenascin-X deficiency are autosomal recessive (AR).

Some of the heritable diseases of the extracellular matrix affect exclusively skin and mucous membranes and are considered to be non-syndromic, while some of them also affect the number of other tissues and organs, thus being syndromic. One of such conditions is **epidermolysis bullosa**, a highly heterogeneous group of disorders due to mutations in as many as 21 distinct genes. EB is divided into four broad categories based on the level of blistering within the skin, *viz.*, epidermolysis bullosa simplex (EBS), junctional EB (JEB), the dystrophic form of EB (DEB), and Kindler syndrome. Some patients with JEB harbor mutations in *COL17A1* gene encoding type VII collagen, an integral component of hemidesmosomes. The dystrophic forms of EB are associated with mutations in *COL7A1*, encoding type VII collagen which is the major, if not the exclusive, component of anchoring fibrils, critical attachment structures required for stable association of epidermis to the underlying dermis. A large number of mutations in these two genes have already been identified, and the application of next generation sequencing techniques, including gene targeted sequencing arrays, whole exome sequencing, whole genome sequencing, and RNA transcriptome analysis by RNA-Seq, has recently facilitated identification of mutations in these candidate genes. Delineation of candidate genes and specific mutations in different families with EB allows identification of carriers in extended families, forming the basis for genetic counseling regarding the risk of recurrence of affected individuals to the same biological parents or to other members of the family. Identification of mutations also allows implementation of DNA-based prenatal testing and preimplantation genetic diagnosis, as well as development of gene therapy approaches in the realm of allele-specific therapy within the evolving concept of personalized medicine.

THE ELASTIC FIBERS:

Biology: The elastic fibers of human skin can be visualized by special histopathologic staining, such as Verhoeff-van Gieson stain. The elastic fibers are relatively thick in the reticular dermis,

where they are oriented parallel to the surface of the skin, intertwined among the collagen bundles. In the papillary dermis, thinner elastic fibers, known as elaunin, are organized in the arcade-like arrangement. In the most superficial papillary dermis, even thinner fibrils, known as oxytalan fibers, ascend vertically to terminate at the dermal-epidermal junction.

Examination of elastic fibers by transmission electron microscopy has revealed that these fibers consist of two distinct components. The major component is elastin, a well characterized connective tissue protein. Surrounding the elastin core are less well characterized electron-dense structures, known as the elastin associated microfibrils. Although the precise composition of the microfibrils is currently unknown, recent studies have indicated that fibrillins, another family of extracellular matrix proteins, are an integral part of the microfibrillar structure. Thus far, two distinct fibrillins have been characterized. The genes encoding fibrillin 1 and fibrillin 2 reside in the chromosomes 15 and 5 respectively, indicating that they are distinct gene products. Furthermore, a number of additional microfibrillar proteins have been recently characterized by molecular cloning (Table 5).

Table 5. Elastin associated microfibrillar proteins

Microfibrillar Protein	**Characteristic Features**	**Human Chromosomal Locus**
Fibrillins	350 kDA	
FBN1	Contain EGF and TGFβ binding protein motifs	**15q15-q21**
FBN2		**5q23-q31**
Latent TGF-β Binding-Proteins (LTBP)	150-205 kDA	
LTBP1	Contain EGF and TGFβ binding protein motifs.	**2p12-q22**
LTBP2	Secreted as a complex with latent TGF-β, but	**14q24**
LTBP3	also found as a free protein	**11q12**
LTBP4		**19q13**
Fibulins	350 kDA	
FBLN1	Contain EGF and anaphylatoxin motifs; bind	**22q13.3**
FBLN2	elastin and other microfibrillar components	**3p24-25**
FBLN3		**2p16**
FBLN4		**11q13.1**
FBLN5		**14q31**
Microfibril Associated Glycoproteins (MAGP)		
MAGP1 (MFAP2)	31 kDa; widely distributed in microfibrils.	**1p36.1-p35**
MAGP2 (MP25)	25 kDa	**12p12.3-p13.1**
Microfibril Associated Protein		
MFAP1	Very acidic	**15q15-q21**
MFAP3		**5q23-q31**
MFAP4	Frequently deleted in Smith-Magenis Syndrome	**17p11.2**
MP70/78 (Big-h3)		**5q31**
Lysyl Oxidase		**5q23-q31**
Emilin (gp115)	Probably not a structural component	**Not known**

Elastin fibrillogenesis involves a large number of intricate steps, many of which are enzymatically mediated. One of them, the oxidative deamination of certain lysyl residues, mediated by a copper-dependent enzyme, lysyl oxidase, takes place in the extracellular space. The resultant aldehyde derivates of lysine, known as allysines, then participate in the formation of complex cross-links known as desmosines, which stabilize the elastin molecules into an insoluble fiber network.

The metabolic turnover of mature elastic fibers in the extracellular space is relatively slow, but it is clear that a portion of elastin is continuously degraded and replaced during early stages of development. In addition, degradation of elastic fibers is markedly increased in a variety of pathological conditions. Degradation of elastic fibers is initiated by elastases, a family of proteolytic enzymes capable of degrading elastic fibers. The most powerful elastolytic enzymes are present in polymorphonuclear leukocytes and monocyte/macrophages, which initiate elastin degradation in inflammatory processes.

Pathology: The complexity of elastin gene expression and the biosynthetic pathway can introduce errors into the assembly of elastic fibers in a manner that may be manifested as a clinical disease. The mechanisms leading to elastic fiber pathology can occur at one of several different levels in elastin fibrillogenesis. In fact, abnormalities in the elastic fiber network have been detected in various diseases affecting the skin (Table 6).

The prototype of skin diseases affecting the cutaneous elastic fibers is **cutis laxa**, characterized by redundant, loose and pendulous skin that frequently forms sagging folds on the face, and gives the patient a prematurely aged appearance. The skin is inelastic and it lacks recoil. Both the clinical presentation and the mode of inheritance of cutis laxa reveal considerable heterogeneity. The newborn patients frequently show evidence of more generalized connective tissue involvement, such as pulmonary emphysema and dislocation of the hips. Most of the cases with the inherited forms of the disease suggest autosomal recessive inheritance, but cases with autosomal dominant or X-linked recessive patterns have also been reported. In addition to the heritable forms of the disease, typical cutaneous changes can develop as a result of an extensive inflammatory reaction, such as a severe drug reaction, in the acquired forms of cutis laxa.

The major histopathologic feature of cutis laxa is the diminuition and/or fragmentation of elastic fibers. In some cases, excessive degradation of elastic fibers by the elastases in the skin could explain the clinical manifestations of cutis laxa. Also, acquired cutis laxa manifests as a post-inflammatory condition and may result from increased degradation of elastic fibers by leukocyte- or monocyte/macrophage-derived elastases. Alternatively, the paucity of elastic fibers in some patients with cutis laxa can be explained by a reduction in elastin gene expression or by defects in the elastin gene. Consequently, cutis laxa appears to have multiple underlying etiologic factors that contribute to the heterogeneous phenotype seen in this condition. Recently, genetic mutations either in the elastin, fibulin-5 and fibulin-4 genes have been identified in a limited number of cases with features of cutis laxa, attesting to the critical importance of the elastic fibers in skin physiology. Isolated cases of cutis laxa with mutations in the ATP6V02/V-ATP-ase $\alpha 2$ subunit and PYCR1/pyrroline-5-carboxylate reductase 1 genes, with unknown pathomechanisms, have been reported.

Table 6: Clinical features, histopathology, inheritance, associated biochemical findings, gene defects, and predisposing clinical conditions in diseases with elastic fiber abnormalities*

DISEASE	INHERITANCE†	CLINICAL MANIFESTATIONS	HISTOPATHOLOGY OF ELASTIC FIBERS	GENE DEFECTS, BIOCHEMICAL FINDINGS‡, AND PREDISPOSING CLINICAL CONDITIONS
Pseudoxanthoma elasticum	AR	Yellowish papules coalescing into plaques of inelastic skin Cardiovascular and ocular abnormalities	Accumulation of pleomorphic and calcified elastic fibers in the mid-dermis	Deposition of calcium hydroxyapatite crystals; D-penicillamine treatment; *ABCC6* or *GGCX* gene mutations
The Buschke-Ollendorff syndrome	AD	Dermatofibrosis lenticularis disseminata and osteopoikilosis	Accumulation of interlacing elastic fibers in the dermis	Increased desmosine content in the skin; loss-of-function mutations in *LEMD3*
Cutis laxa	AR, AD, or NH	Loose, sagging, inelastic skin Pulmonary emphysema Urinary and gastrointestinal tract diverticuli	Fragmentation and loss of elastic fibers	Decreased desmosine content and reduced elastin mRNA levels; *ELN*, *FBLN4* and *FBLN5* gene mutations or increased elastase activity in some cases; D- penicillamine treatment; Inflammatory and urticarial skin lesions (e.g., drug reaction)
Copper deficiency syndromes	XR	A spectrum of clinical manifestations – Menkes syndrome; Occipital horn syndrome (previously X-linked cutis laxa)	Paucity of the central amorphous component of elastic fibers while the microfibrillar material is normal. Frayed and split arterial intima reflecting defects in elastin and collagen cross-linking.	Reduced activity of lysyl oxidase and other copper dependent enzymes due to mutations in the *ATP7A* gene
DeBarsy syndrome	AR	Cutis laxa-like skin changes Mental retardation Dwarfism	Rudimentary, fragmented elastic fibers	Reduced elastin mRNA levels
The wrinkly skin	AR	Decreased elastic recoil of the	Decreased number and length of	Variant of cutis laxa?

syndrome		skin Increased palmar and plantar creases	elastic fibers	
Mid-dermal elastolysis	NH	Fine wrinkling of the skin, primarily in exposed areas	Fragmentation and loss of elastic fibers in mid-dermis	Sun exposure
Anetoderma	NH	Localized areas of atrophic, sac-like esions	Loss and fragmentation of elastic fibers in the dermis	Reduced desmosine content of the lesions; often secondary to inflammatory reactions
Elastosis perforans serpiginosa	NH	Hyperkeratotic papules, commonly on the face and neck	Accumulation and transepidermal elimination of elastic fibers	D-penicillamine-induced abnormalities in elastin cross-linking; frequent in Down's syndrome
Elastoderma	Unknown	Loose and sagging skin with loss of recoil	Accumulation of pleiomorphic elastotic material without calcification in the mid and lower dermis and the subcutaneous tissue	Variant of pseudoxanthoma elasticum?
Isolated elastomas	NH	Dermal papules or nodules	Accumulation of thick elastic fibers in dermis	
Elastofibroma dorsi	NH	Deep subcutaneous tumors, usually on subscapular area	Accumulation of globular elastic structures encased in collagenous meshwork	Trauma on the lesional area; strong genetic component
Actinic (solar) elastosis	NH	Thickening and furrowing of the skin	Accumulation of irregularly thickened elastic fibers in dermis	Chronic sun exposure
Marfan syndrome	AD	Skeletal, ocular, and cardiovascular abnormalities, hyperextensible skin; striae distensae	Fragmentation of the elastic structures in aorta	Mutations in the *FBN1* gene
Congenital contractural arachnodactyly	AD	Joint contractures		Mutations in the *FBN2* gene

Williams syndrome	AD	Supravalvular aortic stenosis; velvety skin; dysmorphic facies	Disruption of smooth muscle and matrix relationship affecting blood vessels	Allelic deletion of the elastin gene; contiguous gene deletion syndrome
Variants of corneal dystrophy	AD, AR	Progressive opacification of the cornea leading to severe visual handicap	Progressive accumulation of corneal deposits (amyloid)	Mutations in the gene encoding βig-h3

* Most of these conditions represent a group of diseases with clinical, genetic, and biochemical heterogeneity.
† AD, autosomal dominant; AR, autosomal recessive; XR, X-linked recessive; NH, not a heritable disease.
‡ The genetic defects and biochemical abnormalities have been demonstrated in only a limited number of patients in each group, and it is not known whether they are the same in each patient with any given disease. The biochemical findings listed are those related to elastic fibers.

Quite recently, major progress has been made in understanding the molecular basis of **pseudoxanthoma elasticum** (PXE), a heritable connective tissue disorder characterized by progressive mineralization of elastic fibers in the skin, eyes, and the cardiovascular system, with considerable morbidity and mortality. Specifically, PXE has been shown to result from mutations in a gene, *ABCC6*, which encodes a transmembrane protein ABCC6. Curiously, this gene is expressed primarily in the liver and the kidneys, tissues which are not known to be affected in PXE. It has been recently shown that ABCC6 facilitates ATP release in the liver, and ATP is rapidly converted to AMP and inorganic pyrophosphate, the latter being a powerful anti-mineralization factor. Thus, in the absence of functional ABCC6, as in PXE due to genetic mutations, the plasma levels of inorganic pyrophosphate is reduced, allowing ectopic mineralization to ensue. Irrespective of the mechanism, however, identification of mutations in the *ABCC6* gene now provides a means for molecular confirmation of the diagnosis of patients suspected of having PXE, identification of carriers in families with history of PXE, and presymptomatic testing in individuals at risk of developing PXE.

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