Extracellular Matrix (ECM)
Connective tissue

Structural and metabolic support for other tissues

- ECM
- Cells

Mesenchymal cell
- Collagen fibers
- Elastic fibers
- Reticular fibers

Protein fibers
- Extracellular matrix

Ground substance
- Mesoderm origin
- Blood vessel
- Macrophage
- Adipocyte (fat cell)
- Fibroblast
Extracellular matrix (ECM)

- Proteoglycan molecule
- Proteoglycan complex
- Polysaccharide molecule
- Collagen fiber
- Extracellular matrix
- Intracellular matrix
- Plasma membrane
- Integrin
- Microfilaments of cytoskeleton
- Cytoplasm

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ECM

• Ground substance
• Fibers
• Structural glycoproteins
Ground substance

• **Physical property:**
  – an amorphous transparent material, a semi-fluid gel

• **Function:**
  – medium for passage of molecules and exchange of metabolites

• **Chemical property:**
  – a mixture of long, unbranched glycosaminoglycans (GAGs) with negative charge
Ground substance: Glycosaminoglycan (GAG)  
(unbranched polysaccharide chains composed of repeating disaccharide units)

- **Hyaluronic acid**  
  - The predominant GAG in the loose supporting tissues  
  - without sulfate side group

**GAGs that form proteoglycans:**
- Chondroitin sulfate  
- Dermantan sulfate  
- Heparan sulfate  
- Heparin  
- Keratan sulfate
Highly charged side groups render them extremely hydrophilic --- attracting large volume of water and positive ions (Na\(^+\)): extracellular fluid
The linkage between a GAG chain and its core protein

(Heparan and chondroitin sulfate chains in proteoglycans)
Pentasaccharide GAG sequence that regulates the activity of antithrombin III

Heparin
Keratan sulfate

- KSI: Asn
- KSII: Ser/Thr
- KSIII: KS-Man-O-Ser/Thr
An aggregcan aggregates from fetal bovine cartilage
What is proteoglycan?
- Fibrinectin
- Hyaluronan
- Fibrillar collagen
- Laminin
- Tenascin
- Type IV collagen
- Decorin
- Perlecan
- Aggrecan

Scale: 100 nm
The relative dimensions and volumes occupied by various macromolecules

- Not flexible
- A huge volume for relatively small mass
Electron micrograph of proteoglycans in the extracellular matrix of rat cartilage

(reinforce ground substance)
The fibers of supporting tissue

- **Collagen** (including reticulin)
  - Tensile strength

- **Elastin**
  - Stretching and recoil
Collagen

28 types of collagen

• Type I collagen:
  – fibril, fibrous supporting tissue (bone, skin…)

• Type II collagen:
  – fibril, hyaline cartilage

• Type III collagen: reticulin
  – fibril, branched reticular supporting tissue (lymphoid organs)

• Type IV collagen:
  – mesh-like network, basement membrane

• Type VII collagen:
  – anchoring fibril, beneath stratified squamous epithelia
## Some Types of Collagen and Their Properties

<table>
<thead>
<tr>
<th>Type</th>
<th>Molecular Formula</th>
<th>Polymerized Form</th>
<th>Tissue Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FIBRIL-FORMING (FIBRILLAR)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>[a 1(I)]$_2$a2(I)</td>
<td>fibril</td>
<td>bone, skin, tendon, ligaments, cornea, internal organs (accounts for 90% of body collagen)</td>
</tr>
<tr>
<td>II</td>
<td>[a 1(II)]$_3$</td>
<td>fibril</td>
<td>cartilage, intervertebral disc, notochord, vitreous humor of the eye</td>
</tr>
<tr>
<td>III</td>
<td>[a 1(III)]$_3$</td>
<td>fibril</td>
<td>skin, blood vessels, internal organs</td>
</tr>
<tr>
<td>V</td>
<td>[a 1(V)]$_2$a2(V)</td>
<td>fibril (with type I)</td>
<td>as for type I</td>
</tr>
<tr>
<td>XI</td>
<td>a1(XI) a2(XI) a3(XI)</td>
<td>fibril (with type II)</td>
<td>as for type II</td>
</tr>
<tr>
<td><strong>FIBRIL-ASSOCIATED</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>a1(IX) a2(IX) a3(IX) with type II fibrils</td>
<td>lateral association</td>
<td>cartilage</td>
</tr>
<tr>
<td>XII</td>
<td>[a 1(XII)]$_3$ with some type I fibrils</td>
<td>lateral association</td>
<td>tendon, ligaments, some other tissues</td>
</tr>
<tr>
<td><strong>NETWORK-FORMING</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>[a 1(IV)]$_2$a2(IV)</td>
<td>sheetlike network</td>
<td>basal laminae</td>
</tr>
<tr>
<td>VII</td>
<td>[a 1(VII)]$_3$</td>
<td>anchoring fibrils</td>
<td>beneath stratified squamous epithelia</td>
</tr>
</tbody>
</table>
Electron micrograph of fibroblasts surrounded by collagen fibrils in the connective tissue of embryonic chick skin.
The structure of a typical collagen molecule
Hydroxylysine and hydroxyproline residues
The intracellular and extracellular events involved in the formation of a collagen fibril:
The covalent intramolecular and intermolecular cross-links formed between modified lysine side chains within a collagen fibril.
How type IV collagen molecules are thought to assemble into a multilayered network
Type IX collagen
(a) Type-I collagen fibrils

- Type-VI collagen
- Proteoglycan

(b) Type-II collagen fibril

- Chondroitin sulfate
- Kink
- Type-IX collagen
The shaping of the extracellular matrix by cells

This micrograph shows a region between two pieces of embryonic chick heart (rich in fibroblasts as well as heart muscle cells) that has grown in culture on a collagen gel for four days. A dense tract of aligned collagen fibers has formed between the explants, presumably as a result of the fibroblasts in the explants tugging on the collagen. (From D. Stopak and A.K. Harris, *Dev. Biol.* 90:383-28 398, 1982.)
Elastin

- Streach and elastic recoil: skin, lung, blood vessels
- Produced by fibroblasts
- Polymerize in the ECM
- Require microfibrils of structural glycoprotein fibrillin
Marfan's syndrome: mutations in the fibrillin gene

1 在骨骼系统方面：体型特别高瘦，手脚及手指特别长（俗称蜘蛛指），胸廓异常（俗称鸡胸或漏斗胸）。

2 眼睛系统方面：早期有深度近视、眼内水晶体脱垂、甚至造成视网膜剥离而双眼失明。

3 心脏系统方面：早期易有心脏的二尖瓣膜脱垂、主动脉根部纤维坏死造成瘤样扩大，有发生剥离的危险。
Structural glycoproteins

• **Fibril-forming molecules**
  – Fibrillin
  – Fibronectin

• **Non-filamentous proteins**
  – Laminin
  – Entactin
  – Tenascin
Scanning electron micrograph of a basal lamina in the cornea of a chick embryo
Basement membrane consists of three layers

- Lamina lucida
- Lamina densa
- Lamina fibroreticularis
  - Type III collagen (reticulin)
Three ways in which basal laminae *(yellow lines)* are organized
Basement membrane
— mainly synthesized by the cells resting on it

• **GAG**: perlecan (heparan sulfate)

• **Fibrous protein**: type IV collagen

• **Structural proteins**: laminin, entactin
# Basement membrane proteins

## Table 1. Mammalian basement membrane proteins

<table>
<thead>
<tr>
<th>Protein (family)</th>
<th>Oligomeric structure, isoforms, etc.</th>
<th>Cellular receptors$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminin</td>
<td>At least 15 heterotrimers formed from 5α, 3β, and 3γ chains; further diversity created by proteolytic processing and alternative splicing</td>
<td>Integrins (α1β1, α2β1, α3β1, α6β1, α6β4, α7β1); dystroglycan; heparan sulfate proteoglycans; sulfatides; HNK-1 (α1 chain); Lutheran (α5 chain)</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>At least 3 heterotrimers formed from 6 homologous α chains</td>
<td>Integrins (α1β1, α2β1)</td>
</tr>
<tr>
<td>Nidogen/entactin</td>
<td>Single chain; 2 isoforms</td>
<td>Integrins (α3β1, αVβ3)</td>
</tr>
<tr>
<td>Perlecanc</td>
<td>Single chain; proteoglycan</td>
<td>Dystroglycan</td>
</tr>
<tr>
<td>Agrin</td>
<td>Single chain; proteoglycan; biological activities regulated by alternative splicing</td>
<td>Dystroglycan; MuSK/agrin receptor</td>
</tr>
<tr>
<td>Collagen XV</td>
<td>Homotrimer; proteoglycan</td>
<td></td>
</tr>
<tr>
<td>Collagen XVIII</td>
<td>Homotrimer; proteoglycan; alternative splicing</td>
<td>Heparan sulfate proteoglycans</td>
</tr>
<tr>
<td>Fibulin</td>
<td>5 isoforms; alternative splicing (fibulin-1); monomers and disulfide-linked dimer (fibulin-2)</td>
<td>Integrins (fibulin-2: αIββ3; fibulin-5: αVβ3, αVβ5, α9β1)</td>
</tr>
<tr>
<td>Osteonectin/SPARC/BM-40</td>
<td>Single chain; several poorly characterized homologues</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Additional receptors have been described for BM protein fragments (Kalluri, 2003).
Monomer (single α-chain)

Triple-helical domain (Gly-X-Y motif)

7S ~1,400 aa ~230 aa

NC1 domain

NC1 interactions initiate type IV protomer formation

Protomer (a trimer of α-chains)

Protomer

Dimer

NC1 hexamer

Type IV collagen tetramer

7S domain

Type IV collagen suprastructure

NC1 hexamer

7S domain
Cancer invasion through BM

Scanning electron micrograph of two human fibrosarcoma cells, having digested the BD Matrigel Matrix occluding the membrane and migrating through the 8 μm of the PET membrane.
**a Induction**

VBM degradation (MMPs)

- Proliferation
- Migration

MMPs, VEGF, bFGF, PDGF

- Fibroblast
- Immune cells

Pericyte detachment

Tumour cells

**b Resolution**

Downregulation of proliferation and migration
Reformation of VBM

- VBM assembly
- Pericyte attachment

- Provisional matrix
- Intermediate matrix
- Mature VBM
- Degraded VBM
The structure of laminin

- > 12 isoforms: 5α, 3β, 3γ
- > 20 receptors
- laminin-5: α3β1, α6β4 integrins
YIGSR from β1 chain
Decreased lung colonies, tumor growth, invasion angiogenesis
Receptor: 32/67kDa protein

α1 Chain

VAYI from α1 and YVRL from γ1 chains
Increased lung colonies, tumor growth, angiogenesis
Receptors: α5β1 and αvβ3

β1 Chain

IKVAV from α1 chain
Increased lung colonies, tumor growth, proteases, angiogenesis
Receptor: APP?

γ1 Chain

LQVQLSIR from α1 chain
Increased lung colonies, liver and bone metastases, migration, invasion/proteases (no effect on angiogenesis)
Receptor: syndecan-1 or heparan/chondroitin sulfate proteoglycan
20 isoforms from alternative splicing of a single gene
dimer
α5β1 integrin
Integrins: ECM receptors

Inside-out and outside-in signals

A
Low-affinity

B1
Intermediate affinity

B2
High affinity

C

Inside-out signaling

Outside-in signaling

Retrograde flow

Fibronectin
Actin
Talin
Kindlin

https://www.mechanobio.info/
www.accessmedicine.com
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## Common integrins and ECM ligands bound

<table>
<thead>
<tr>
<th>Integrin</th>
<th>ECM ligands</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1β1</td>
<td>Collagens</td>
<td>Binds to fibrillar collagen domains</td>
</tr>
<tr>
<td></td>
<td>Laminins</td>
<td></td>
</tr>
<tr>
<td>α2β1</td>
<td>Collagens</td>
<td>Binds to fibrillar collagen domains</td>
</tr>
<tr>
<td></td>
<td>Laminins</td>
<td>Binding dependent on increased activation state</td>
</tr>
<tr>
<td>α3β1</td>
<td>Collagens</td>
<td>Binding to NC1 domains, possibly fibrillar forms</td>
</tr>
<tr>
<td></td>
<td>Laminins</td>
<td>Binds to the Laminin ‘Toe’, GD6 peptide</td>
</tr>
<tr>
<td></td>
<td>Thrombospondin</td>
<td>Binds to TSP-678 peptide</td>
</tr>
<tr>
<td>α5β1</td>
<td>Fibronectin</td>
<td>Binds to RGD site in the cell-binding domain</td>
</tr>
<tr>
<td></td>
<td>Fibrin</td>
<td>Binds to cryptic sites in polymerized fibrin</td>
</tr>
<tr>
<td>α6β1</td>
<td>Laminins</td>
<td>May bind several sites in laminin</td>
</tr>
<tr>
<td>α6β4</td>
<td>Laminins</td>
<td>May bind several sites in laminin</td>
</tr>
<tr>
<td>αvβ3</td>
<td>Fibronectin</td>
<td>Binds to RGD sequence near PEX</td>
</tr>
<tr>
<td></td>
<td>Laminin</td>
<td>Binds to the RGD site in tenth FN-III (CBD)</td>
</tr>
<tr>
<td></td>
<td>Thrombospondin</td>
<td>Binds in an activation-dependent manner</td>
</tr>
<tr>
<td></td>
<td>Tenascin</td>
<td>Cryptic RGD site</td>
</tr>
<tr>
<td></td>
<td>Del-1</td>
<td>Binds RGD in a FN-III domain</td>
</tr>
<tr>
<td></td>
<td>Osteopontin</td>
<td>Binds RGD in an EGF-type domain</td>
</tr>
<tr>
<td></td>
<td>Bone Sialoprotein</td>
<td>RGD near a thrombin cleavage site</td>
</tr>
<tr>
<td></td>
<td>Nonfibrillar collagen</td>
<td>RGD-dependent and independent binding</td>
</tr>
<tr>
<td></td>
<td>Denatured collagen</td>
<td>RGD-dependent binding to NC1 domains</td>
</tr>
<tr>
<td></td>
<td>MMP2</td>
<td>RGD-independent binding to PEX domain</td>
</tr>
<tr>
<td></td>
<td>bFGF</td>
<td>Binds to a DGR motif</td>
</tr>
<tr>
<td></td>
<td>von Willebrand’s Factor</td>
<td>RGD-dependent binding</td>
</tr>
<tr>
<td></td>
<td>thrombin</td>
<td>RGD-dependent binding</td>
</tr>
<tr>
<td>αvβ5</td>
<td>Vitronec tin</td>
<td>Binds to RGD and KKQFRHRNRKG</td>
</tr>
<tr>
<td></td>
<td>Del-1</td>
<td>Binds RGD in an EGF-type domain</td>
</tr>
</tbody>
</table>
Integrin binding sites on various BM molecules
Effects of MMPs on angiogenesis

- Endothelial cells
- Pericytes
- Vascular basement membrane
- Blood vessel/capillary

**Extraction of basement-membrane collagen**

- Type IV collagen
- Type XVIII collagen

**MMPs, elastase or cathepsins (basement-membrane-degrading enzymes)***

**Anti-angiogenic activity***

**Basement-membrane collagen-derived endogenous inhibitors**

<table>
<thead>
<tr>
<th>Basement-membrane collagen-derived endogenous inhibitors</th>
<th>Integrin binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrestin (26 kDa)</td>
<td>α1β1</td>
</tr>
<tr>
<td>Canstatin (24 kDa)</td>
<td>αvβ3, α3β1</td>
</tr>
<tr>
<td>Tumstatin (28 kDa)</td>
<td>αvβ3, α6β1</td>
</tr>
<tr>
<td>Endostatin (20 kDa)</td>
<td>α5β1, αvβ3</td>
</tr>
</tbody>
</table>

**No anti-angiogenic activity in this form**
<table>
<thead>
<tr>
<th>MMP designation</th>
<th>Structural class</th>
<th>Common name(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>Simple hemopexin domain</td>
<td>Collagenase-1, interstitial collagenase, fibroblast collagenase, tissue collagenase</td>
</tr>
<tr>
<td>MMP-2</td>
<td>Gelatin-binding</td>
<td>Gelatinase A, 72-kDa gelatinase, 72-kDa type IV collagenase, neutrophil gelatinase</td>
</tr>
<tr>
<td>MMP-3</td>
<td>Simple hemopexin domain</td>
<td>Stromelysin-1, transin-1, proteoglycanase, procollagenase-activating protein</td>
</tr>
<tr>
<td>MMP-7</td>
<td>Minimal domain</td>
<td>Matrilysin, matrin, PUMP1, small uterine metalloproteinase</td>
</tr>
<tr>
<td>MMP-8</td>
<td>Simple hemopexin domain</td>
<td>Collagenase-2, neutrophil collagenase, PMN collagenase, granulocyte collagenase</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Gelatin-binding</td>
<td>Gelatinase B, 92-kDa gelatinase, 92-kDa type IV collagenase</td>
</tr>
<tr>
<td>MMP-10</td>
<td>Simple hemopexin domain</td>
<td>Stromelysin-2, transin-2</td>
</tr>
<tr>
<td>MMP-11</td>
<td>Furin-activated and secreted</td>
<td>Stromelysin-3</td>
</tr>
<tr>
<td>MMP-12</td>
<td>Simple hemopexin domain</td>
<td>Metalloelastase, macrophage elastase, macrophage metalloelastase</td>
</tr>
<tr>
<td>MMP-13</td>
<td>Simple hemopexin domain</td>
<td>Collagenase-3</td>
</tr>
<tr>
<td>MMP-14</td>
<td>Transmembrane</td>
<td>MT1-MMP, MT-MMP1</td>
</tr>
<tr>
<td>MMP-15</td>
<td>Transmembrane</td>
<td>MT2-MMP, MT-MMP2</td>
</tr>
<tr>
<td>MMP-16</td>
<td>Transmembrane</td>
<td>MT3-MMP, MT-MMP3</td>
</tr>
<tr>
<td>MMP-17</td>
<td>GPI-linked</td>
<td>MT4-MMP, MT-MMP4</td>
</tr>
<tr>
<td>MMP-18</td>
<td>Simple hemopexin domain</td>
<td>Collagenase-4 (<em>Xenopus</em>; no human homologue known)</td>
</tr>
<tr>
<td>MMP-19</td>
<td>Simple hemopexin domain</td>
<td>RAS1-1, MMP-18†</td>
</tr>
<tr>
<td>MMP-20</td>
<td>Simple hemopexin domain</td>
<td>Enamelysin</td>
</tr>
<tr>
<td>MMP-21§</td>
<td>Vitronection-like insert</td>
<td>Homologue of <em>Xenopus</em> XMMMP</td>
</tr>
<tr>
<td>MMP-22</td>
<td>Simple hemopexin domain</td>
<td>CMMP (chicken; no human homologue known)</td>
</tr>
<tr>
<td>MMP-23</td>
<td>Type II transmembrane†</td>
<td>Cysteine array MMP (CA-MMP), femalyasin, MIFR, MMP-21/MMMP-22‡</td>
</tr>
<tr>
<td>MMP-24</td>
<td>Transmembrane</td>
<td>MT5-MMP, MT-MMP5</td>
</tr>
<tr>
<td>MMP-25</td>
<td>GPI-linked</td>
<td>MT6-MMP, MT-MMP6, leukolysin</td>
</tr>
<tr>
<td>MMP-26</td>
<td>Minimal domain</td>
<td>Endometase, matrilysin-2</td>
</tr>
<tr>
<td>MMP-27*</td>
<td>Simple hemopexin domain</td>
<td></td>
</tr>
<tr>
<td>MMP-28</td>
<td>Furin-activated and secreted</td>
<td>Epilysin</td>
</tr>
<tr>
<td>No designation</td>
<td>Simple hemopexin domain</td>
<td>Mcol-A (Mouse)</td>
</tr>
<tr>
<td>No designation</td>
<td>Simple hemopexin domain</td>
<td>Mcol-B (Mouse)</td>
</tr>
<tr>
<td>No designation</td>
<td>Gelatin-binding</td>
<td>75-kDa gelatinase (chicken)</td>
</tr>
</tbody>
</table>
Minimal-domain MMPs

Simple hemopexin-domain-containing MMPs

Gelatin-binding MMPs

Furin-activated secreted MMPs

Vitronectin-like insert MMPs

Transmembrane MMPs

GPI-anchored MMPs

Type II transmembrane MMPs
Expression of MMPs and TIMPs in breast tumours. In addition to cancer cells,
Promoting

a Growth

IGF-BP  MMP  IGF

Integrin

TGF-α

ECM

Inhibiting

Growth inhibition

TGF-β

Latent TGF-β
d Invasion

Invasive cell
Cleaved Lam-5
MMP
CD44
Cleaved CD44

CXCL12
ECM
Cleaved CXCL12

Cleaved E-cad
**Epithelial-to-mesenchymal transition**

- Latent TGF-β
- MMP
- TGF-β

**Differentiation**

- Cancer cell undergoing EMT
- Cleaved E-cad
- ECM
- Differentiated cells
f Inflammation and immune surveillance

- Macrophage
- Neutrophil
- Cleaved IL-2Rα
- IL-2Rα
- Chemokines
- Cleaved chemokines
- T lymphocytes
- TGF-β
- Cleaved α1-PI
- α1-PI
- MMP
- Cytotoxic T lymphocyte
- Natural killer cell