



Escuela de Verano 2006  
Curso Células Cancerosas  
Facultad de Medicina



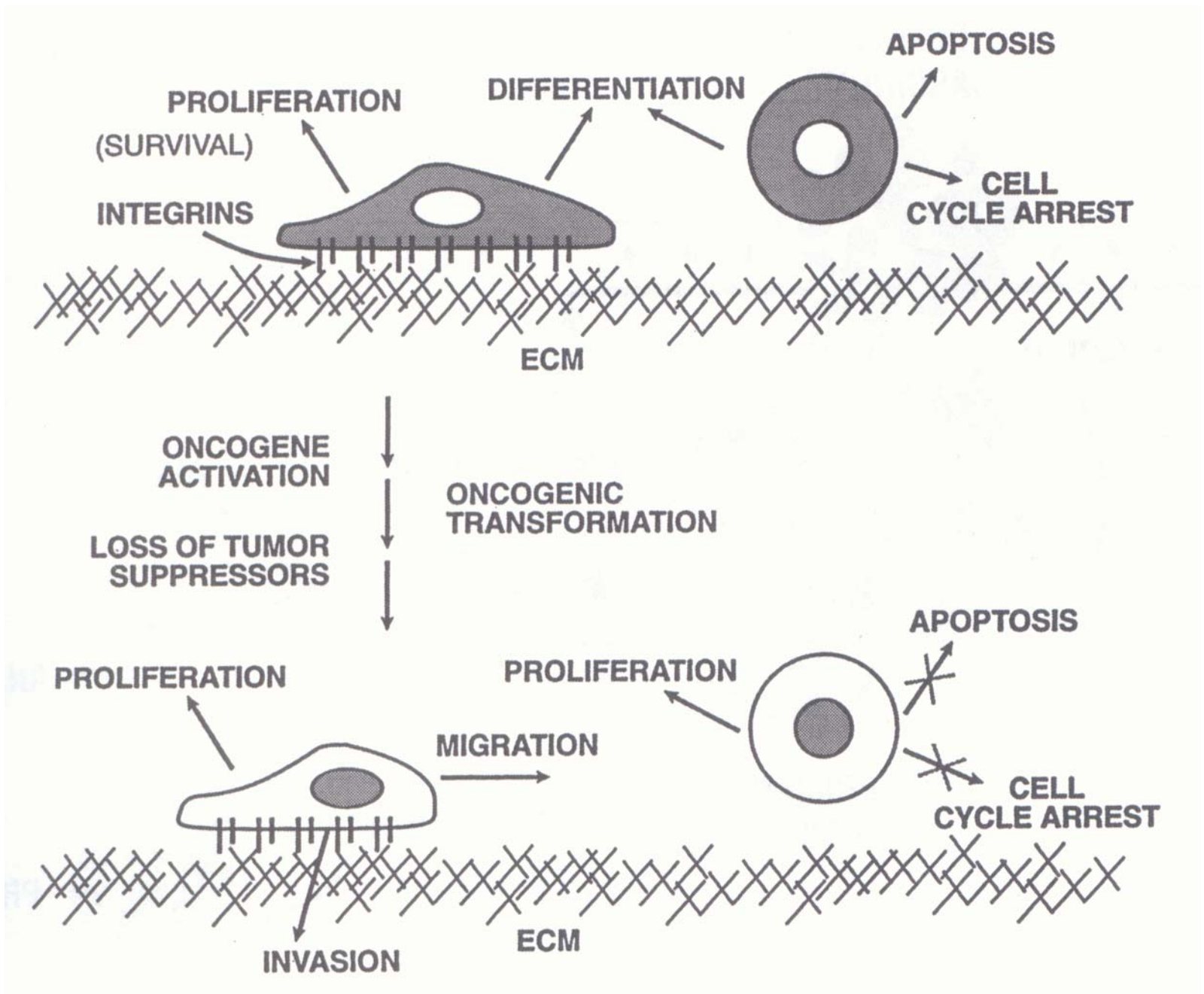
## ***Adhesión, invasión, metástasis y angiogénesis: mecanismos asociados***

**Héctor R. Contreras M.**

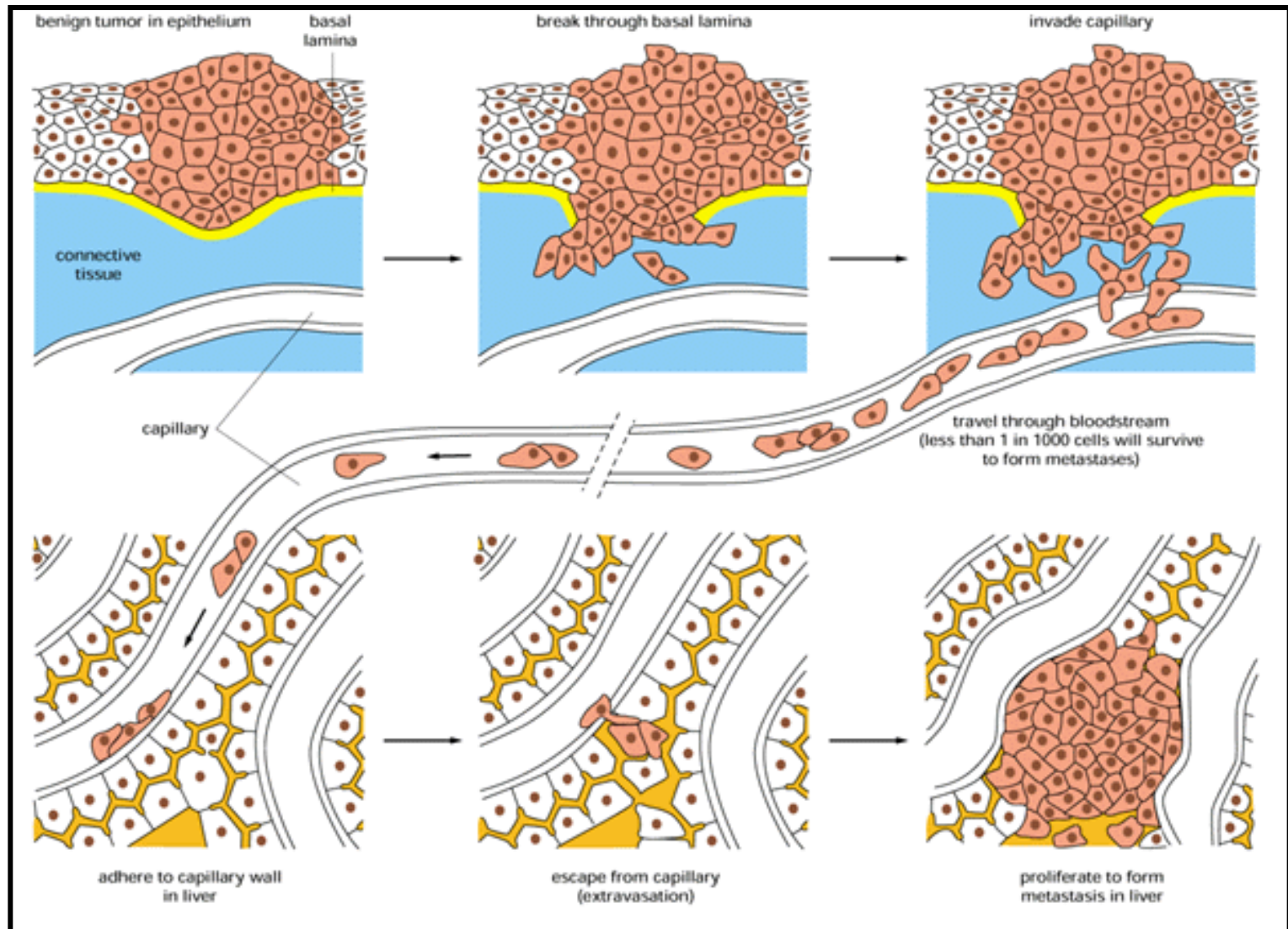
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# Cáncer simplificado

- Inicio
  - Tumor localizado
- Malignidad
  - Invasividad
    - Tumor invade tejidos vecinos
  - Metastasis
    - Celulas colonizan diferentes tejidos del cuerpo



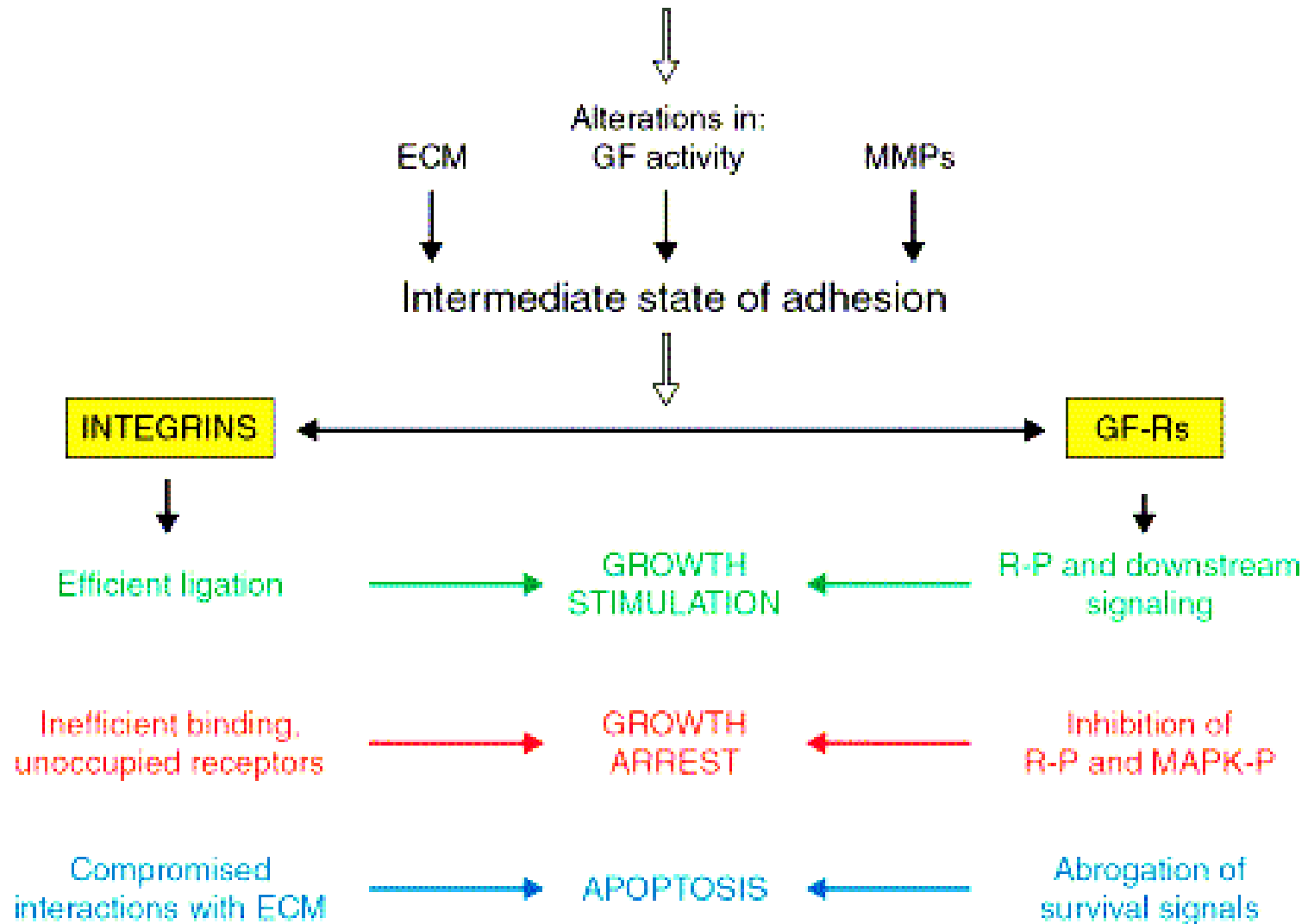
# DEGRADACIÓN DE LOS COMPONENTES DE LA MATRIZ EXTRACELULAR

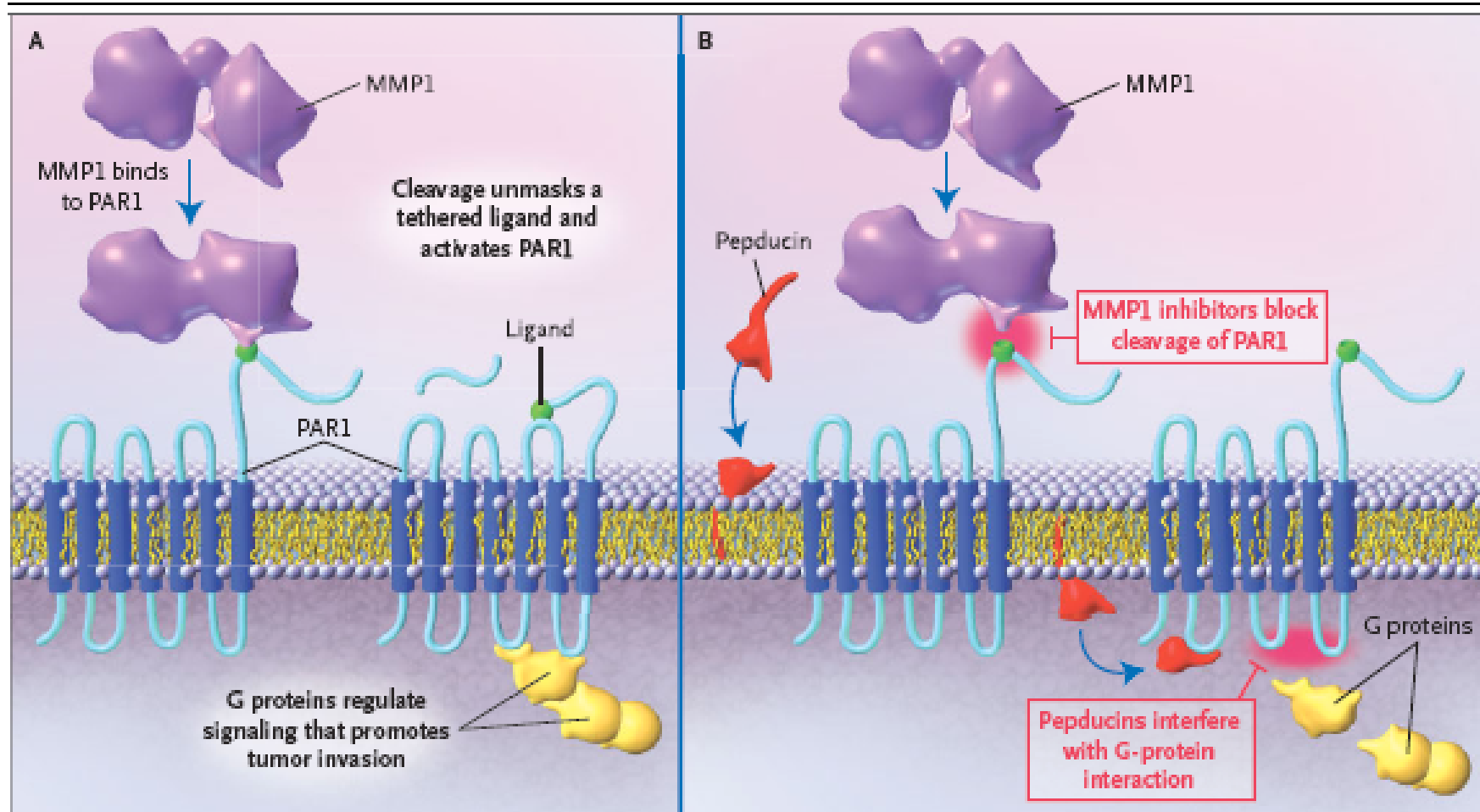


**Table 1** Proteases participating in degradation of ECM components

Protease family	Protease	Protease function	Protease inhibitors
Aspartyl protease	Cathepsin D	Degradation of ECM components Conversion of cysteine procathepsins into cathepsins	
Cysteine proteases	Cathepsins B, L, H, K	Degradation of ECM components Conversion of pro-MMPs into MMPs	Cystatins, stefins, kininogen
Serine proteases	Plasmin	Degradation of ECM components Activation of uPA Conversion of inactive elastase into elastase	$\alpha_2$ -antiplasmin, $\alpha_2$ -macroglobulin
	Urokinase-type plasminogen activator (uPA)	Conversion of plasminogen into plasmin	PAI-1, 2, 3
	Tissue-type plasminogen activator (tPA)	Conversion of plasminogen into plasmin	
Neutrophil serine proteases	Elastase	Degradation of ECM components	$\alpha_2$ -antiplasmin $\alpha_2$ -macroglobulin secretory leukoprotease inhibitor
	Cathepsin G		
Matrix metalloproteinases		Degradation of collagens and other ECM proteins Activation another pro-MMPs into MMPs	TIMP-1, 2, 3, 4 $\alpha_2$ -macroglobulin
	Collagenases [MMP-1, 8, 13]	Degradation of collagens: I, II, III, VII, X and gelatins	
	Stromelysins [MMP-3, 10]	Degradation of proteoglycans, laminin, gelatins, collagens III, IV, V, IX, fibronectin, entactin, SPARC, collagenases-1	
	Gelatinases [MMP-2, 9]	Degradation of gelatins, collagens: I, IV, V, VII, X, fibronectin, elastin, procollagenase-3	
	Membrane-type [MMP-14, 15, 16, 17, 24, 25]	Degradation of collagen I, II, III, gelatins, aggrecan, fibronectin, laminin, vitronectin, MMP-2,13, tenascin, nidogen	
	Others [MMP-7, 11, 12, 19, 20, 23]	Degradation of proteoglycans, laminin, fibronectin, gelatins, collagens IV, elastin, entactin, tenascin, $\alpha_1$ -antiproteinase, amelogenin	

# MATRICELLULAR PROTEINS

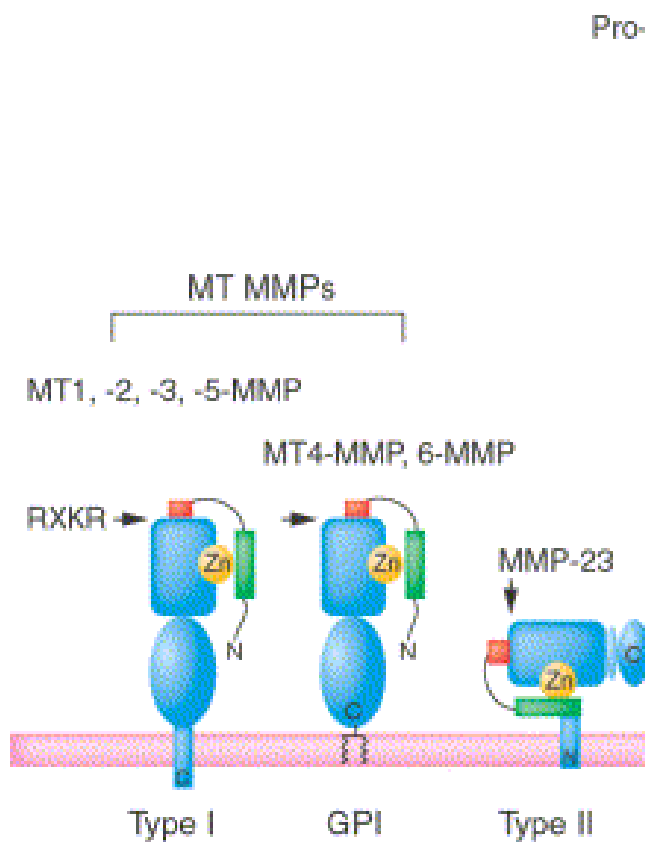




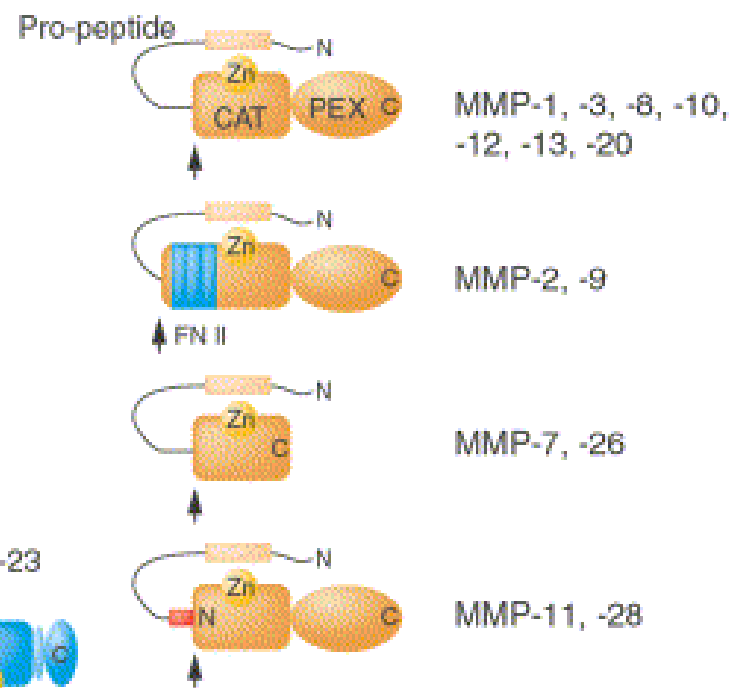
**Figure 1. Picking Apart PAR1.**

Nontransformed host cells secrete matrix metalloproteases (MMPs) into the extracellular milieu (Panel A). A member of this family, MMP1, cleaves protease-activated receptor 1 (PAR1). The cleavage unmasks a tethered ligand that activates PAR1. Activated PAR1 couples to G proteins, which regulate cell responses leading to tumor invasion. In a recent study, Boire et al.<sup>1</sup> showed that inhibitors of MMP1 abrogate PAR1 signaling by blocking its cleavage (Panel B). Alternatively, short peptides known as pepducins can block signaling by interfering with the interaction between PAR1 and G proteins. Boire et al. showed that both types of compounds block metastasis from breast-cancer xenografts in nude mice.

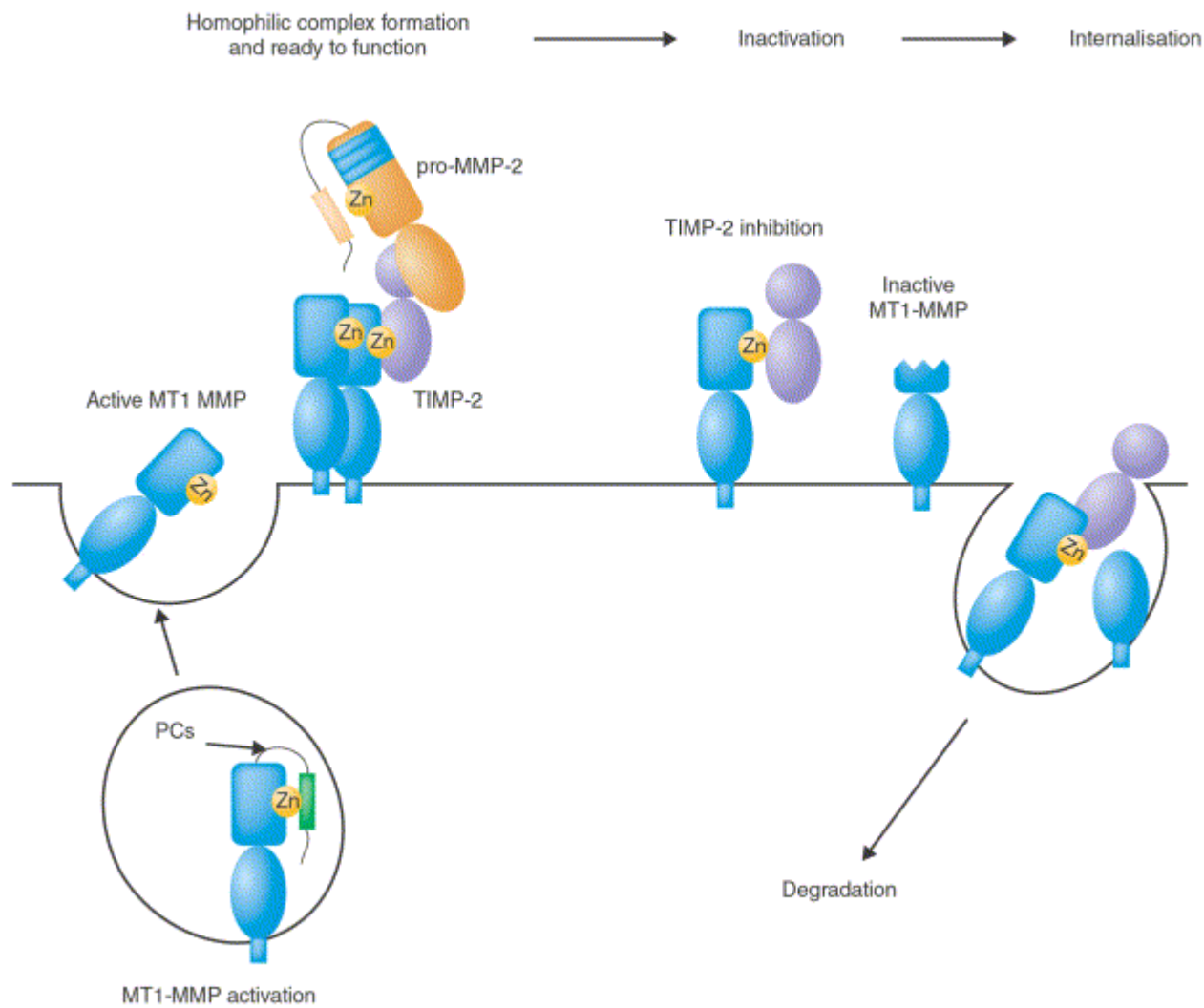
## Membrane-anchored MMP



## Secreted-type MMP

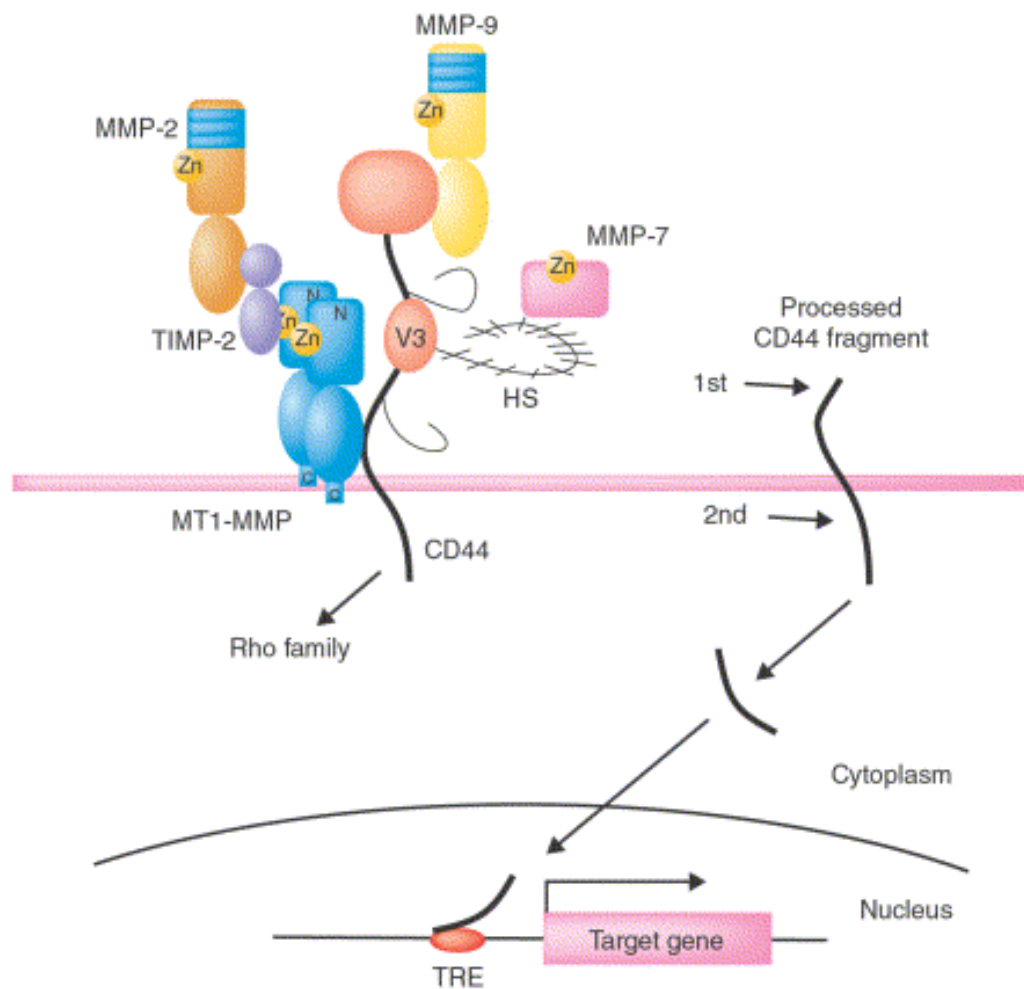






### Substrates near CD44

MT1-MMP	CD44, proMMP-2, $\alpha\beta 3$ Integrin, collagen I
MMP-2	TGF- $\beta$
MMP-7	pro-HB-EGF, Osteopontin
MMP-9	TGF- $\beta$



**TABLE 1. Matrix Metalloproteinases**

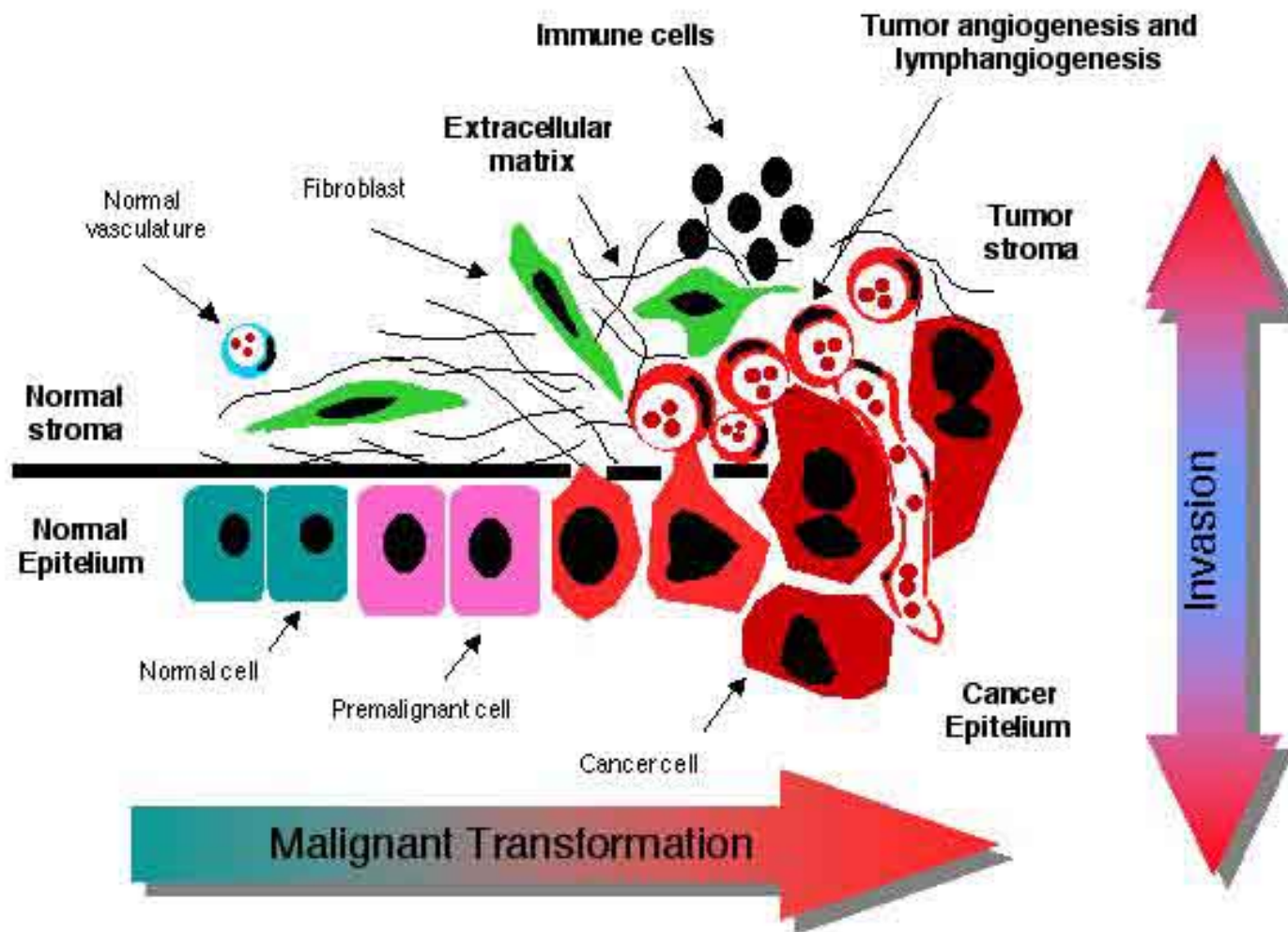
Enzyme	MMP	Human Chromosome	3D Structure (PDB Code)
<b>Collagenases</b>			
Interstitial collagenase; collagenase 1	MMP-1	11q22-q23	Mature protein; 1FBL cat domain; 1CGF, 2TCL, 1AYK, 2AYK, 1HFC, 1CGL, 1CGE, 966C, 3AYK, 4AYK
Neutrophil collagenase; collagenase 2	MMP-8	11q21-q22	Cat domain; 1MNC, 1I76, 1JAO, 1MMB, 1JAN, 1JAP, 1JAO, 1I73, 1KBC, 1A85, 1A86, 1BZS, 1JJ9, 1JH1
Collagenase 3	MMP-13	11q22.3	Cat domain; 1CXV, 1FM1, 1FLS, 456c, 830c, 1EUB Hpx domain; 1PEX
Collagenase 4 ( <i>Xenopus</i> )	MMP-18	NA	
<b>Gelatinases</b>			
Gelatinase A	MMP-2	16q13	proMMP-2; 1CK7; proMMP-2-TIMP-2 complex; 1GXD; cat domain; 1QIB, 1HOV, 1EAK; Hpx domain; 1GEN, 1RTG; Fn; 1CXW, 1KS0
Gelatinase B	MMP-9	20q11.2-q13.1	Pro-cat domain; 1LGJ; cat domain; 1GKC, 1GKD; Hpx domain; 1ITV
<b>Stromelysins</b>			
Stromelysin 1	MMP-3	11q23	Pro-cat domain; 1SLM; cat domain; D8M, 1CI2, 1CAQ, 1B8Y, 2SRT, 1HFS, 1SLN, 2USN, 1USN, 1D5J, 1BQO, 1D7X, 1D8F, 1BIW, 1UMS, 3USN, 1UMT, 1BM6, 1B3D, 1CQR, 1G4K, 1G49, 1HY7, 1G05; complex with N-TIMP-1; 1UEA
Stromelysin 2	MMP-10	11q22.3-q23	
Stromelysin 3	MMP-11	22q11.2	1HV5
<b>Matrilysins</b>			
Matrilysin 1; Pump-1	MMP-7	11q21-q22	Cat domain; 1MMP, 1MMQ, 1MMR
Matrilysin 2	MMP-26	11p15	
<b>Membrane-type MMPs</b>			
<b>Transmembrane</b>			
MT1-MMP	MMP-14	14q11-q12	Cat domain in complex with TIMP-2; 1BQO, 1BUV
MT2-MMP	MMP-15	15q13-q21	
MT3-MMP	MMP-16	8q21	
MT5-MMP	MMP-24	20q11.2	
<b>GPI anchor</b>			
MT4-MMP	MMP-17	12q24.3	
MT6-MMP	MMP-25	16p13.3	
<b>Others</b>			
Macrophage elastase	MMP-12	11q22.2-q22.3	Cat domain 1JK3, 1JIZ
No trivial name	MMP-19	12q14	
Enamelysin	MMP-20	11q22.3	
XMMP ( <i>Xenopus</i> )	MMP-21	ND	
CA-MMP	MMP-23	1p36.3	
CMMP ( <i>Gallus</i> )	MMP-27	11q24	
Epilysin	MMP-28	17q21.1	

Groups of MMPs are listed with their trivial names and chromosomal location. The names of the PDB files of structures determined by x-ray crystallography and NMR are listed. These files with their references can be downloaded from the protein databank ([www.rcsb.org](http://www.rcsb.org)). An extended version of this table including the substrates cleaved by the individual MMPs is available in online Table 1 (see online data supplement available at <http://www.circresaha.org>).

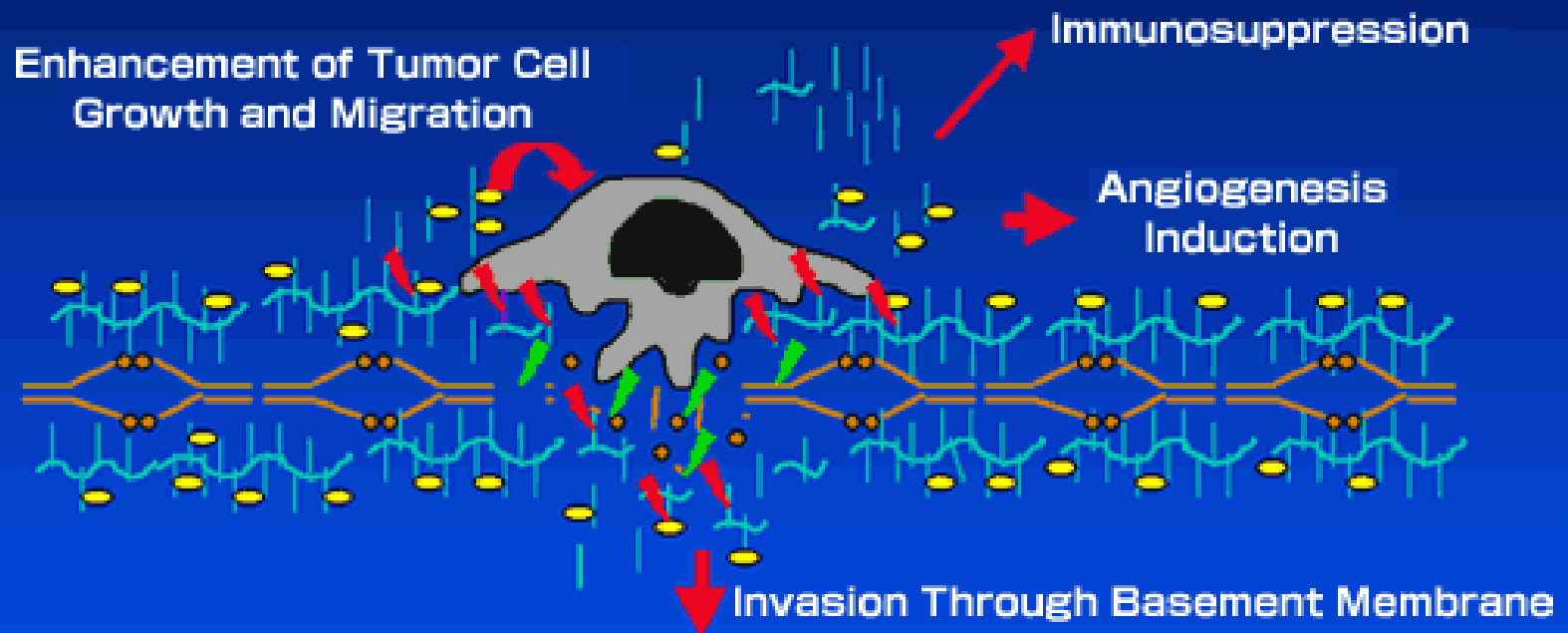
**TABLE 2. Biological Activities Generated by MMP-Mediated Cleavage**

Biological Effect	Responsible MMPs	Substrate Cleaved	Reference
Keratinocyte migration and reepithelialization	MMP-1	Type I collagen	79
Osteoclast activation	MMP-13	Type I collagen	78
Neurite outgrowth	MMP-2	Chondroitin sulphate proteoglycan	84
Adipocyte differentiation	MMP-7	Fibronectin	130
Cell migration	MMP-1, -2, -3	Fibronectin	
Cell migration	MT1-MMP	CD44	83
Mammary epithelial cell apoptosis	MMP-3	Basement membrane	131
Mammary epithelial alveolar formation	MMP-3	Basement membrane	132, 133
Epithelial-mesenchymal conversion (mammary epithelial cells)	MMP-3	E-cadherin	134, 135
Mesenchymal cell differentiation with inflammatory phenotype	MMP-2	Not identified	136
Platelet aggregation	MMP-1	Not identified	137
Generation of angiostatin-like fragment	MMP-3	Plasminogen	138
	MMP-7	Plasminogen	139
	MMP-9	Plasminogen	139
	MMP-12	Plasminogen	140
	MMPs	Type XVIII collagen	141
Enhanced collagen affinity	MMP-2, -3, -7, -9, -13 (but not MMP-1)	BM-40 (SPARC/osteonectin)	142
Kidney tubulogenesis	MT1-MMP	Type I collagen	143
Release of bFGF	MMP-3, -13	Perlecan	144
Increased bioavailability of IGF1 and cell proliferation	MMP-1, -2, -3	IGFBP-3	145
	MMPs	IGFBP-5	146
	MMP-11	IGFBP-1	147
	MMPs	CTGF	148
Activation of VEGF	MMPs	CTGF	148
Epithelial cell migration	MMP-2, MT1-MMP	Laminin 5 $\gamma$ 2 chain	81, 82
Apoptosis (amion epithelial cells)	Collagenase	Type I collagen	80
Proinflammatory	MMP-1, -3, -9	Processing IL-1 $\beta$ from the precursor	149
Tumor cell resistance	MMP-9	ICAM-1	150
Antiinflammatory	MMP-1, -2, -9	IL-1 $\beta$ degradation	151
Antiinflammatory	MMP-1, -2, -3, -13, -14	Monocyte chemoattractant protein-3	152, 153
Increased bioavailability of TGF- $\beta$	MMP-2, -3, -7	Decorin	154
Disrupted cell aggregation and increased cell invasion	MMP-3, MMP-7	E-cadherin	155
Reduced cell adhesion and spreading	MT1-MMP, MT2-MMP, MT3-MMP	Cell surface tissue transglutaminase	156
Fas receptor-mediated apoptosis	MMP-7	Fas ligand	157
Reduced IL-2 response	MMP-9	IL-2R $\alpha$	158

## The microenvironment at the tumor-host interface



# Role of Heparanase in Tumor Cell Invasion and Angiogenesis



🔴 Heparanase

🔵 HSPG

🟢 MMPs

🟡 Type IV collagen

🟡 HS fragments

● bFGF, KGF, HG, VEGF, IL-8, TGF- $\beta$

**Carcinoma of the lung**

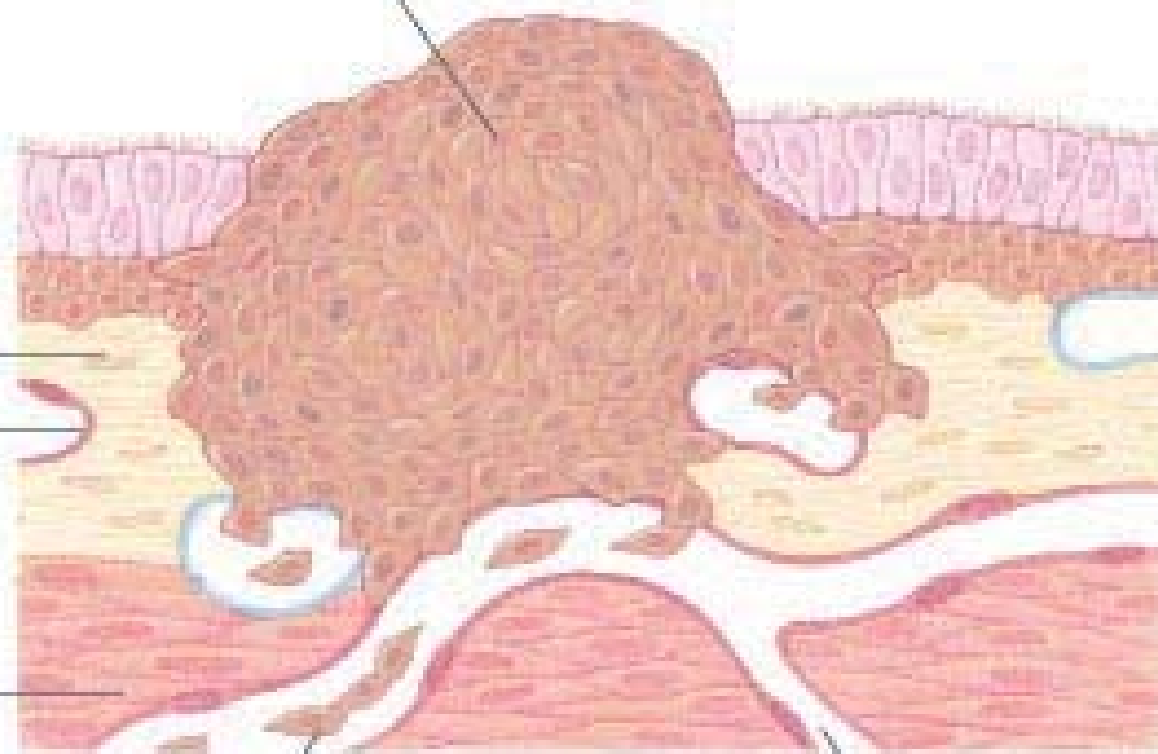
**Connective tissue**

**Blood vessel**

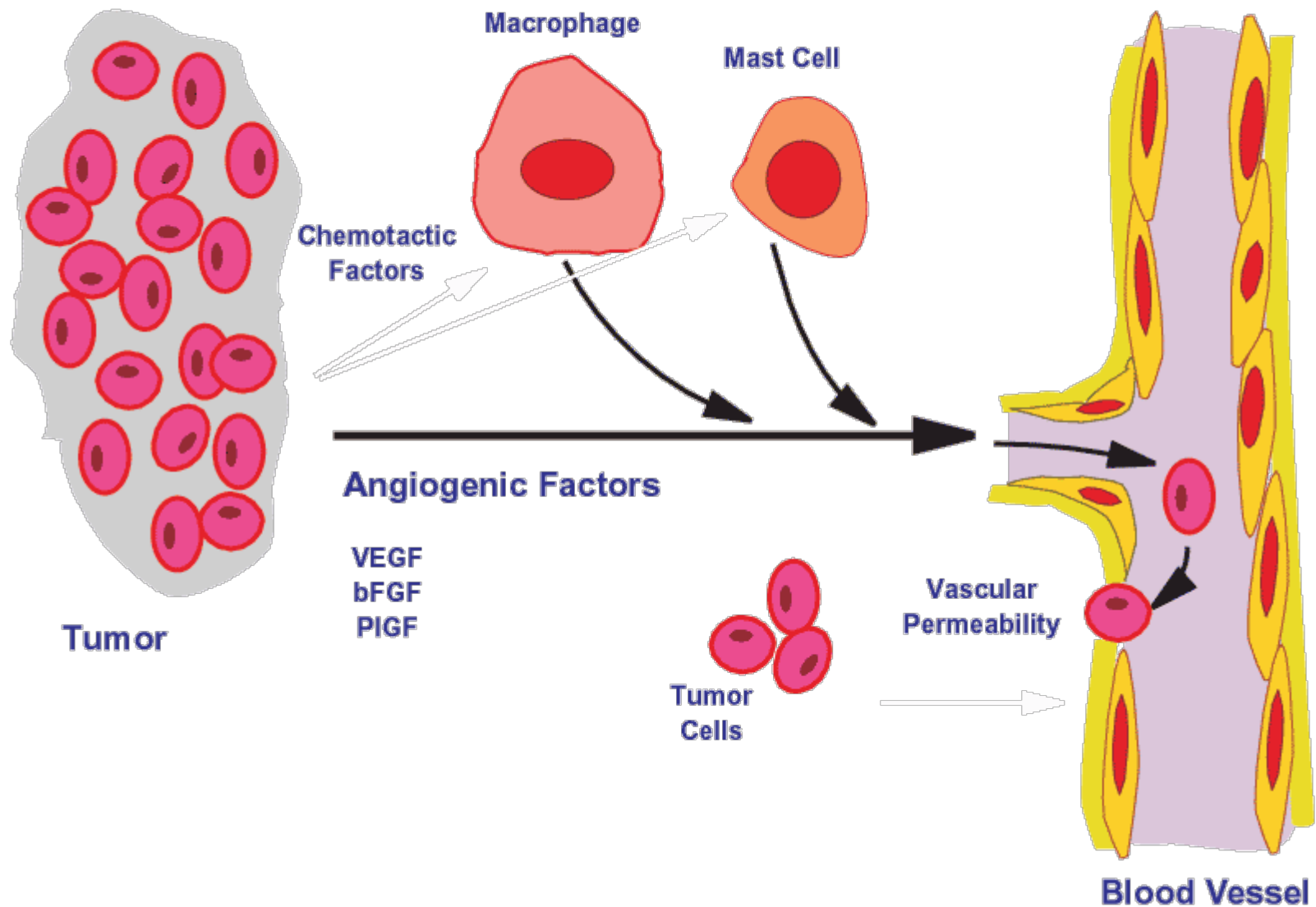
**Smooth muscle**

**Metastatic cells**

**Blood vessel**



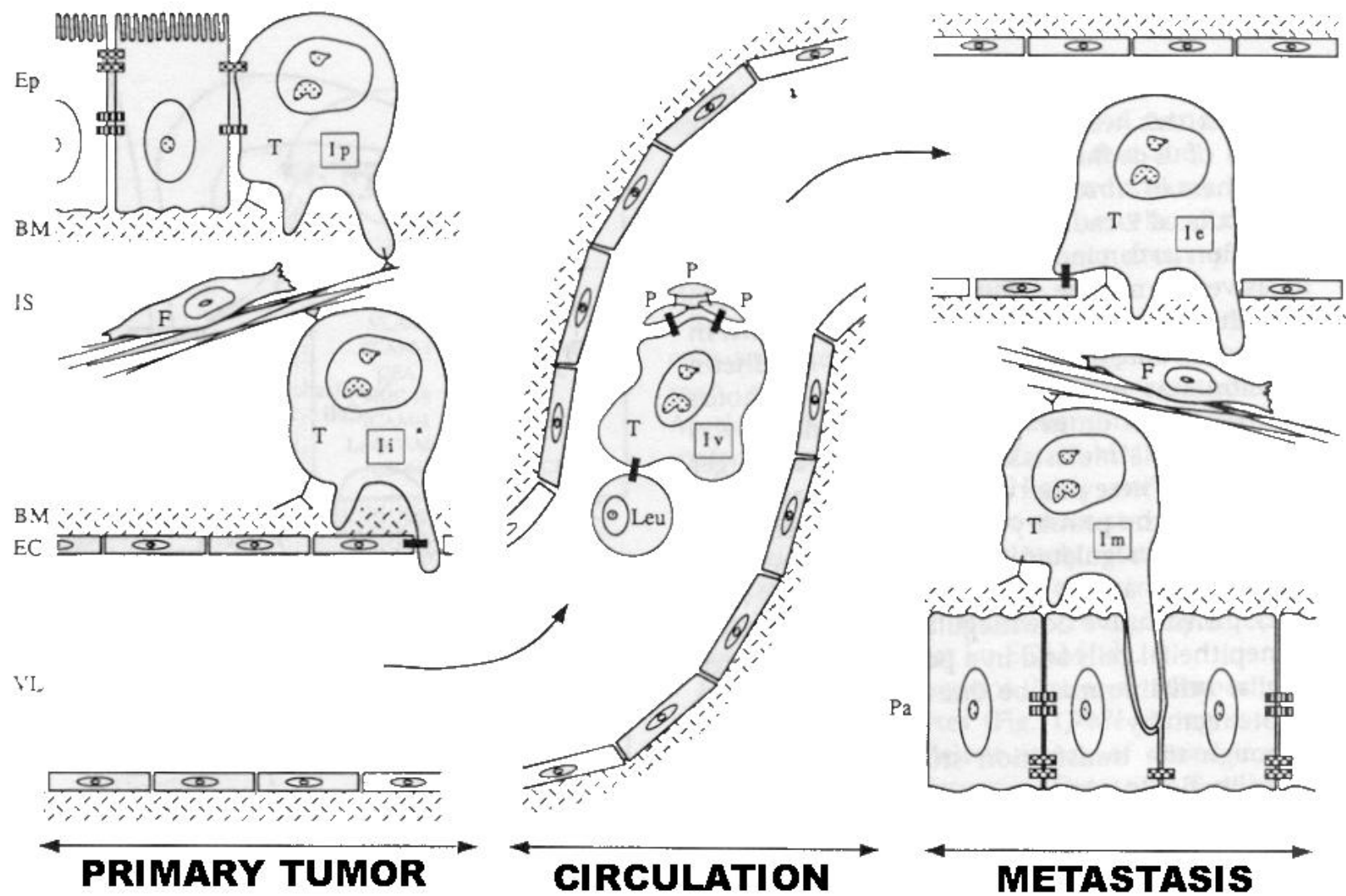
# Tumor Angiogenesis and Metastasis

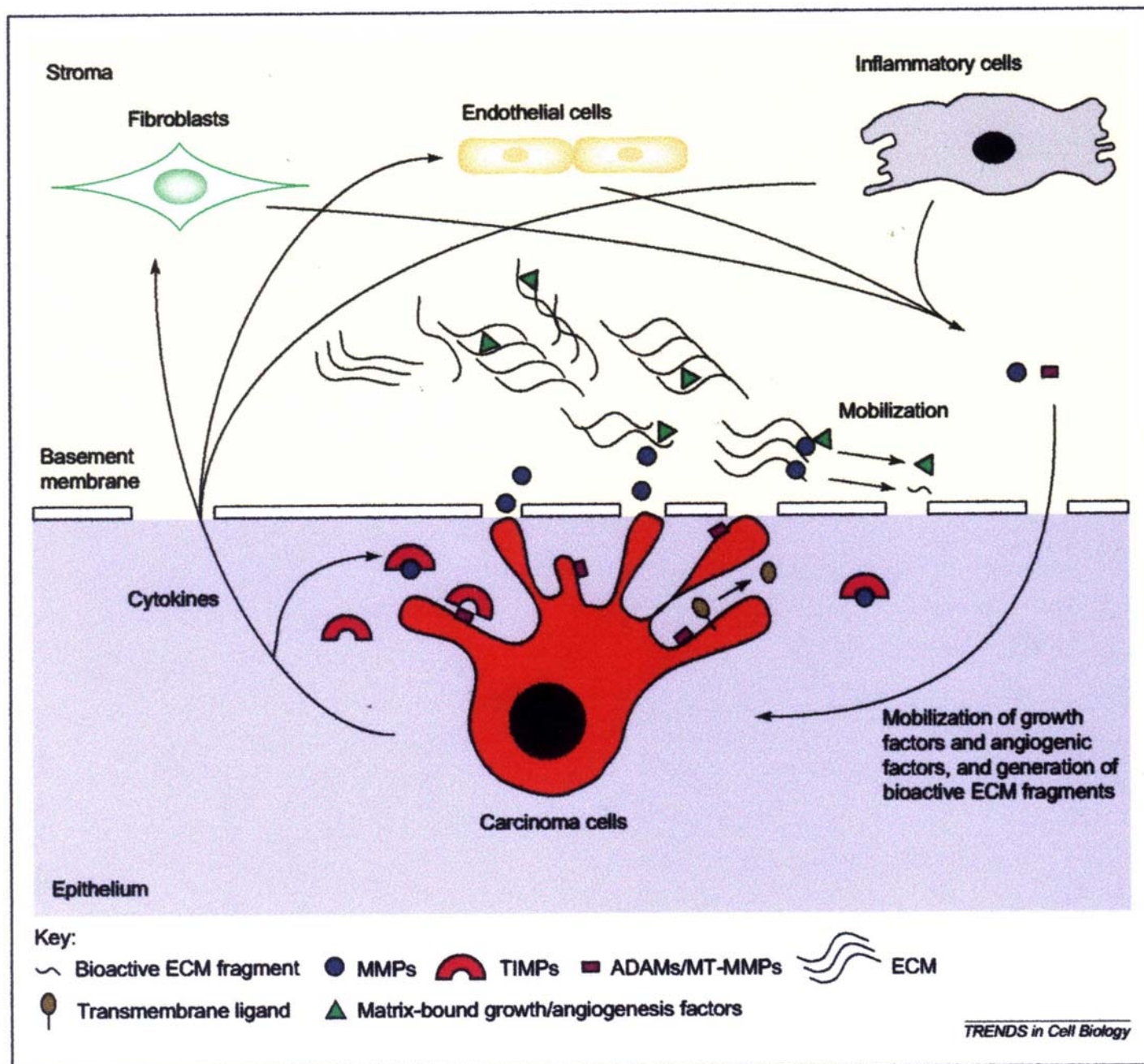


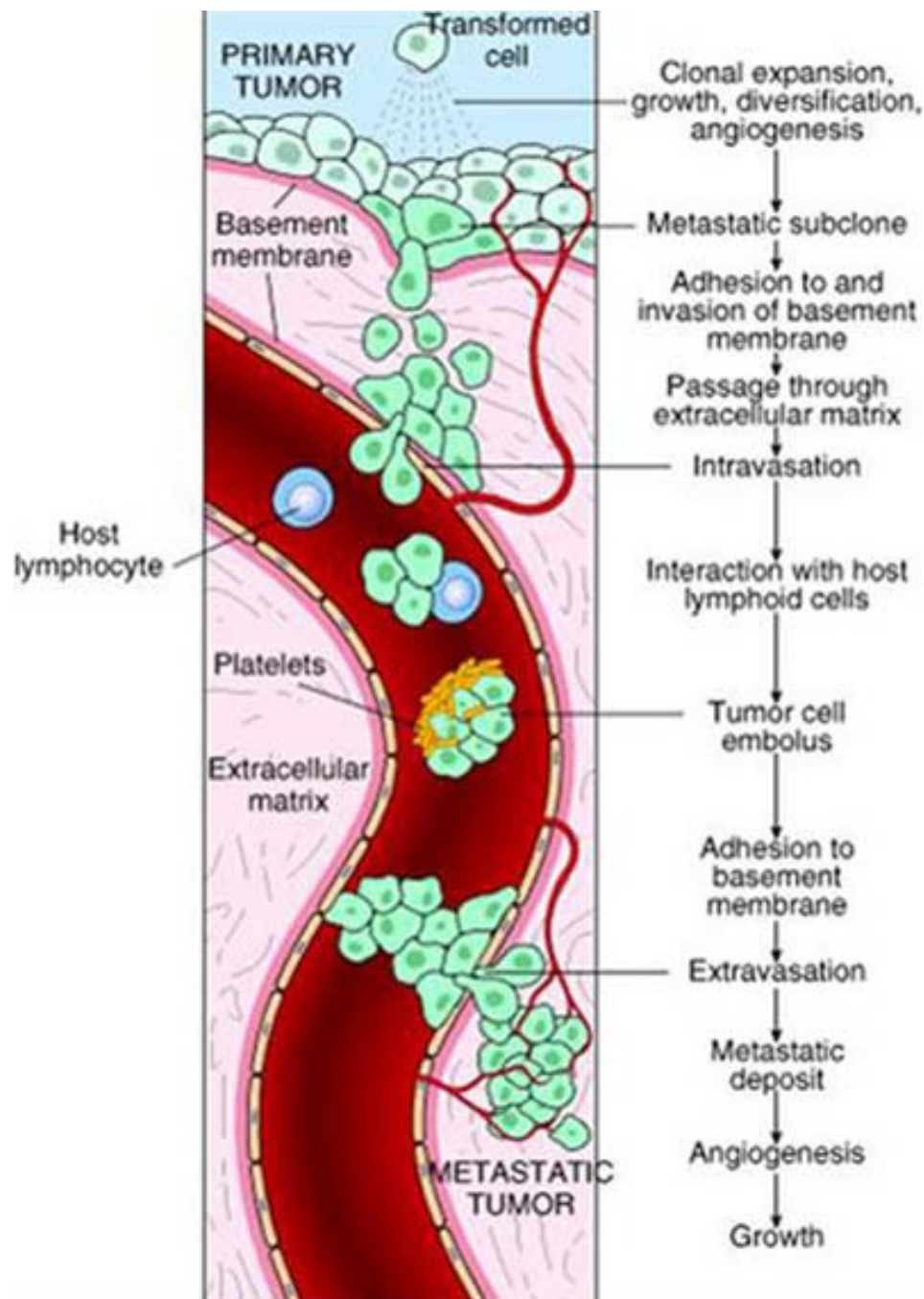


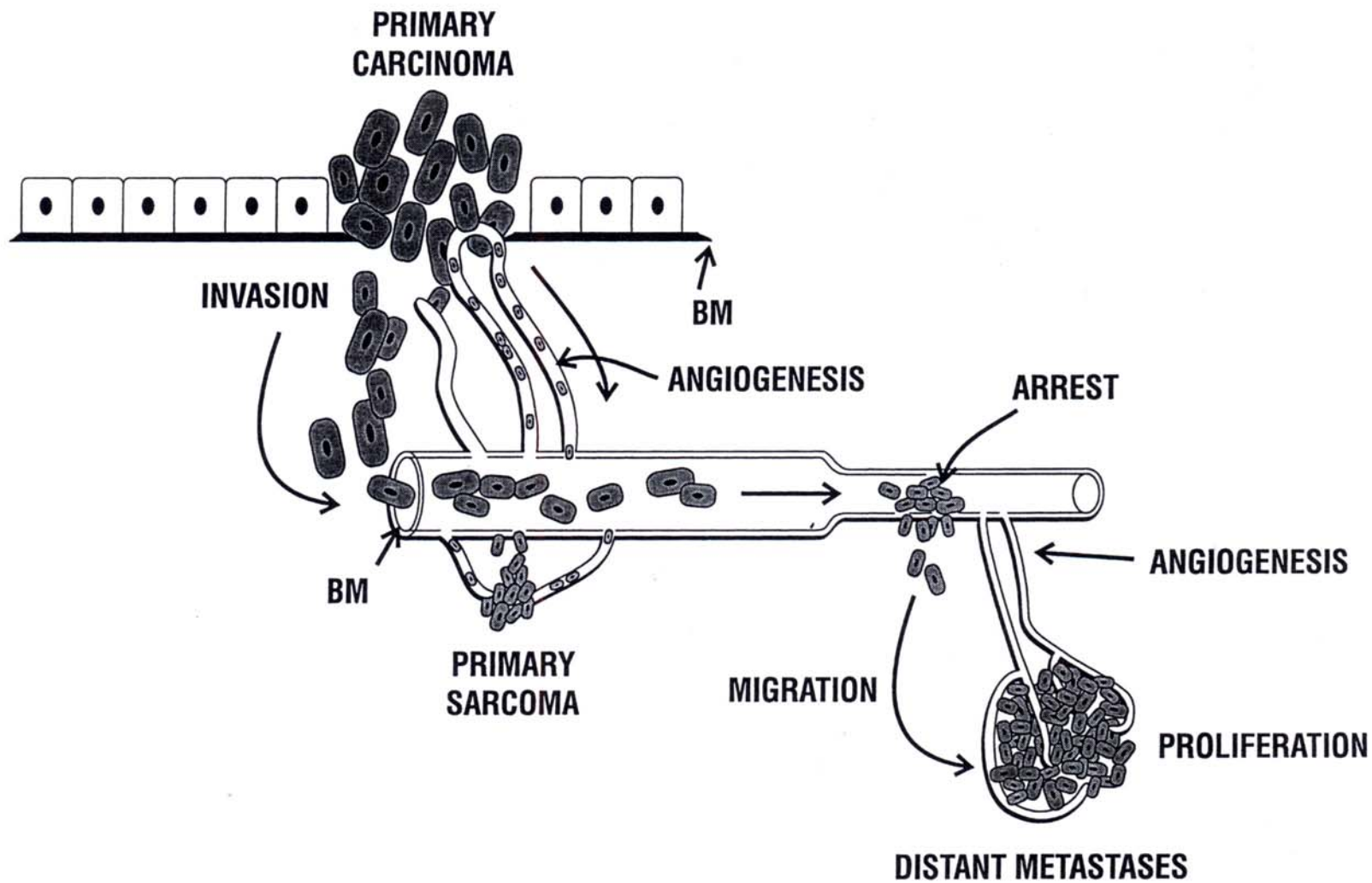
# Metástasis

- Metástasis se define como la transferencia de foco de tumor primario a lugares distantes del organismo.
- En este contexto del cáncer la migración de células de tumor a otro sitio estableciéndose un tumor secundario

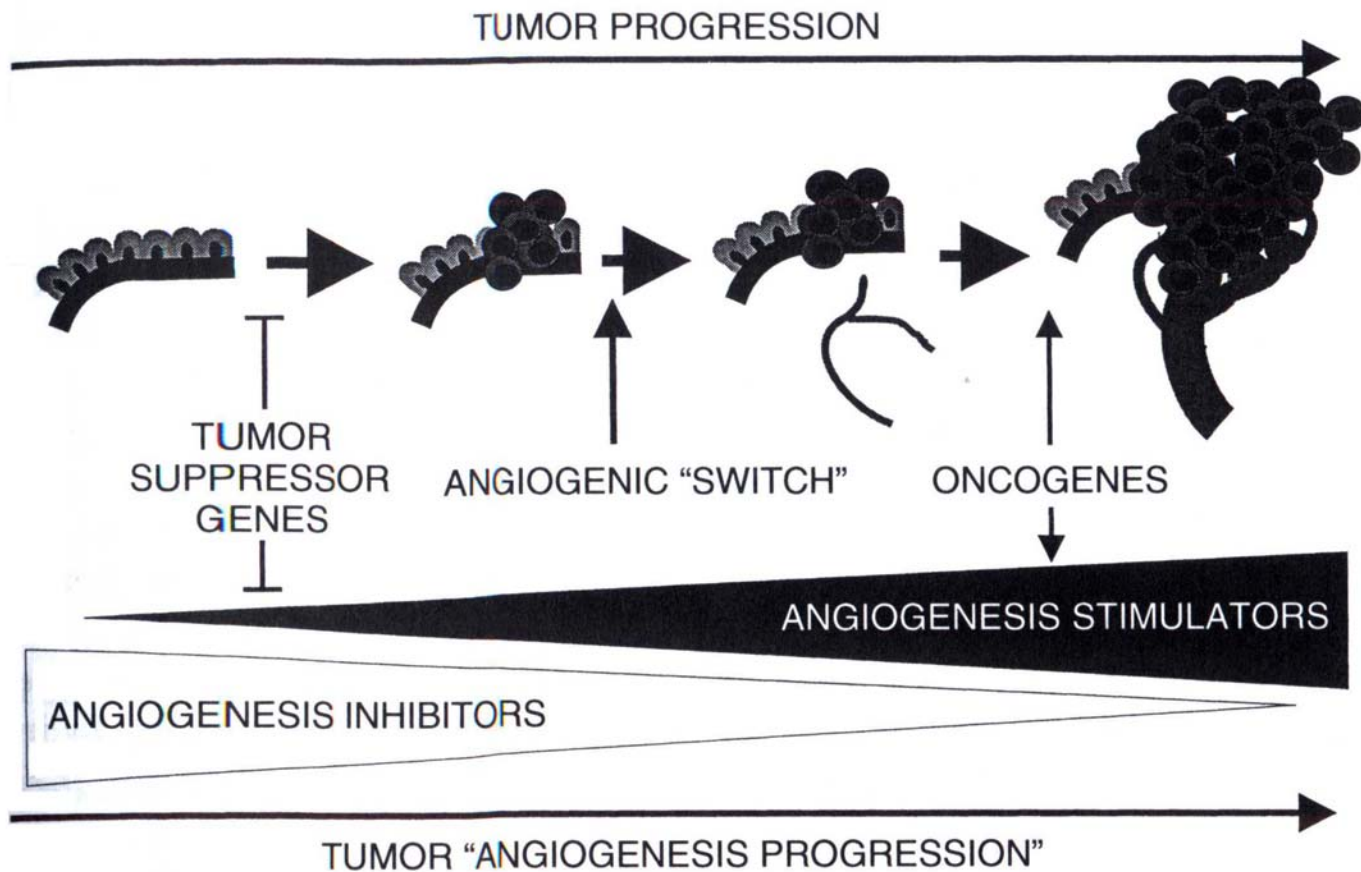


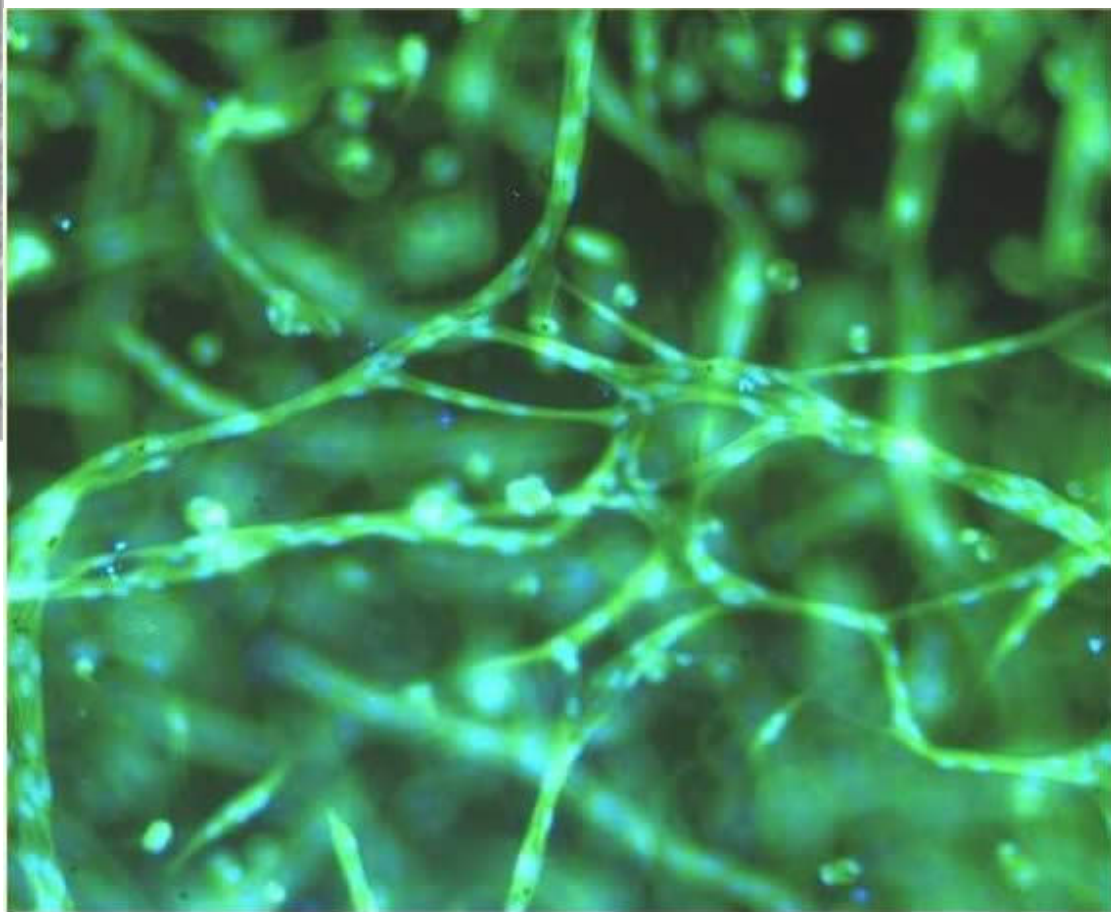
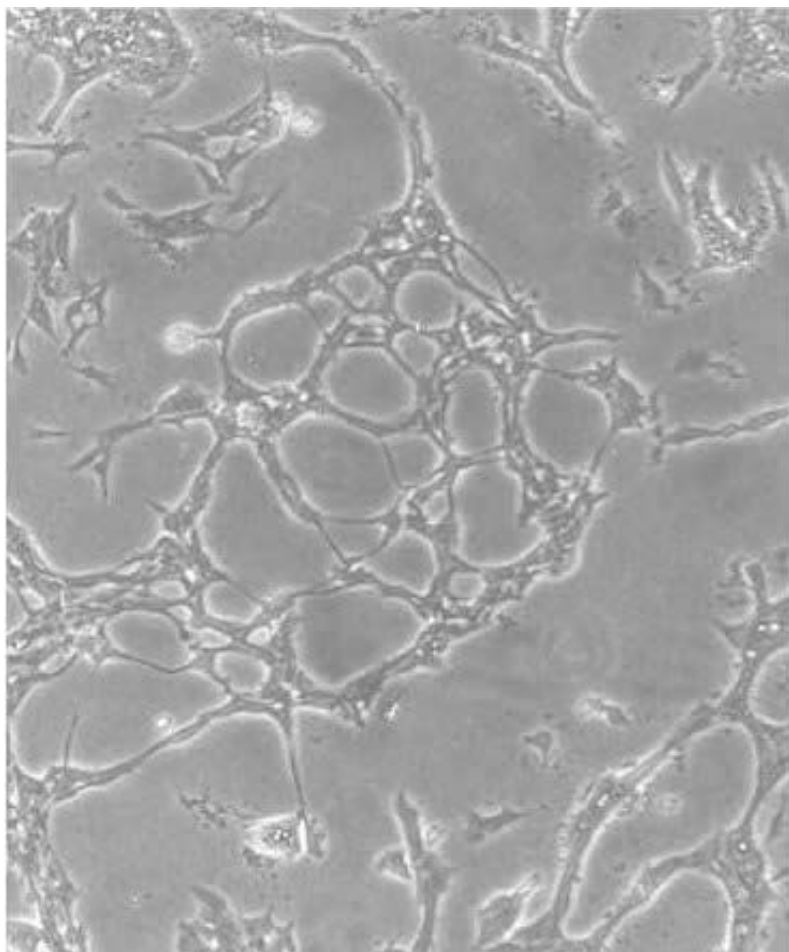


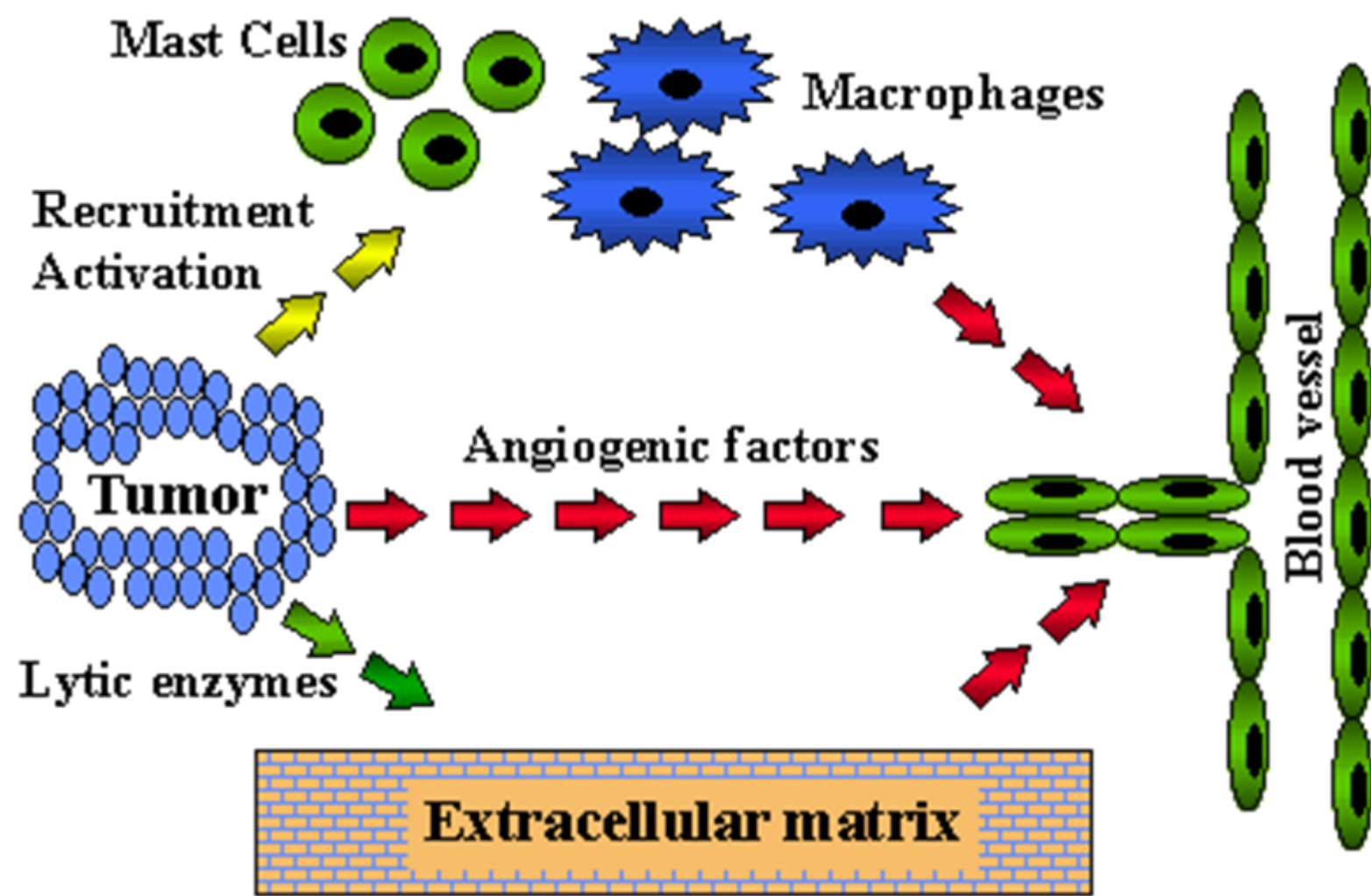




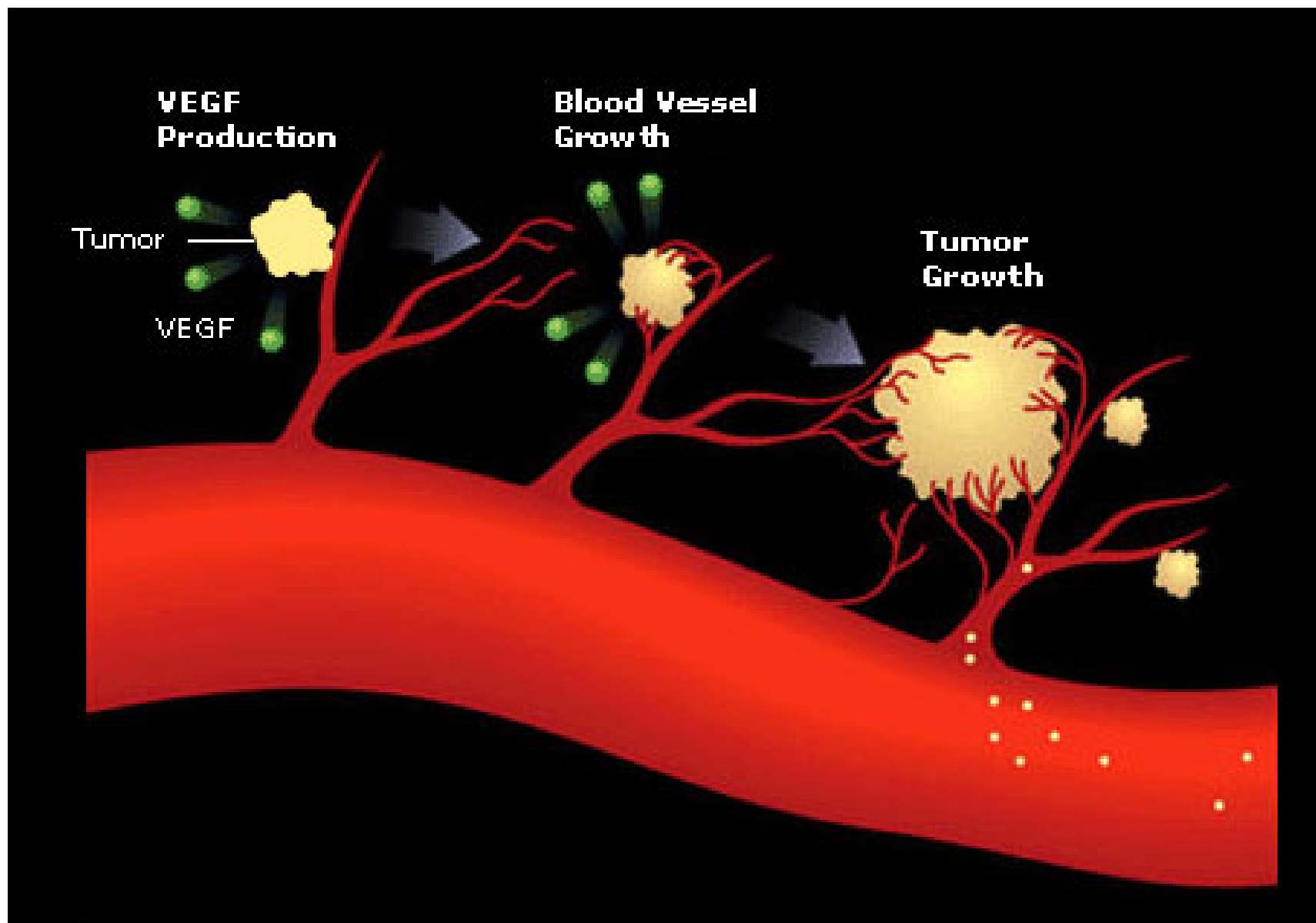












**Table 9.1.** Angiogenic Growth Factors

Factor	Properties	Receptor
Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF)	Endothelial mitogen, survival factor, and permeability inducer produced by many types of tumor cells	Flk-1/KDR Flt-1 (both present on activated endothelium)
Placental growth factor (PIGF)	Weak endothelial mitogen	Flt-1
Basic fibroblast growth factor (bFGF/FGF-2)	Endothelial mitogen, angiogenesis inducer, and survival factor; inducer of Flk-1 expression	FGFR1-4
Acidic fibroblast growth factor (aFGF/FGF-1)	Endothelial mitogen and angiogenesis inducer	FGFR1-4
Fibroblast growth factor 3 (FGF-3/Int-2)	Endothelial mitogen and angiogenesis inducer	FGFR1-4
Fibroblast growth factor 4 (FGF-4/Hst/K-FGF)	Endothelial mitogen and angiogenesis inducer	FGFR1-4
Transforming growth factor $\alpha$ (TGF- $\alpha$ )	Endothelial mitogen and angiogenesis inducer; inducer of VEGF expression	EGFR
Epidermal growth factor (EGF)	Weak endothelial mitogen; inducer of VEGF expression	EGFR
Hepatocyte growth factor/scatter factor (HGF/SF)	Endothelial mitogen and angiogenesis inducer	c-MET
Transforming growth factor $\beta$ (TGF- $\beta$ )	In vivo acting angiogenesis inducer or endothelial growth inhibitor; inducer of VEGF expression	TGF- $\beta$ R I, II, III
Tumor necrosis factor $\alpha$ (TNF- $\alpha$ )	In vivo acting angiogenesis inducer. Endothelial mitogen (low concentrations) or inhibitor (high concentrations); inducer of VEGF expression	TNFR-55 (TNFR-75?)
Platelet-derived growth factor (PDGF)	Mitogen and motility factor for endothelial cells and fibroblasts; in vivo angiogenesis inducer	PDGFR
Granulocyte colony-stimulating factor (G-CSF)	In vivo acting angiogenesis-inducing factor with some mitogenic activity for endothelial cells	G-CSFR
Pleiotrophin	Angiogenesis inducing pleiotrophic growth factor	proteoglycan
Thymidine phosphorylase (tP)/platelet-derived endothelial cell growth factor (PD-ECGF)	In vivo acting angiogenesis factor	The mode of action remains unclear
Angiogenin	In vivo acting angiogenesis inducer with RNase activity	170-kDa angiogenin receptor
Proliferin	35-kDa angiogenesis-inducing protein in mouse	unknown

**Table 9.2.** Key Molecular Regulators of Blood Vessel Formation as Defined by Gene Knockout Studies

Molecule	Function	Gene Knockout Phenotype	Relevance to Tumor angiogenesis
VEGF/VPF	Polypeptide growth factor that mediates survival and differentiation of endothelial cells	Embryonic lethal (both homo- and heterozygotes) Impaired endothelial cell differentiation and angiogenesis	Elevated levels expressed in tumor parenchyma
Flt-1 (VEGFR-1)	VEGF receptor tyrosine kinase	Embryonic lethal Disorganized assembly of endothelial cells	Expression induced in tumor blood vessels. Flt-1 antagonists inhibit tumor angiogenesis
Flk-1/KDR (VEGFR-2)	VEGF receptor tyrosine kinase	Embryonic lethal Lack of endothelial cell differentiation and angiogenesis	Expression induced in tumor blood vessels. Flk-1 antagonists inhibit tumor angiogenesis
Angiopoietin-1	Polypeptide factor that maintains capillary integrity	Embryonic lethal Defective complexity of the vasculature, poor interaction between endothelial cells and vessel wall	Unknown
Angiopoietin-2	Physiologic antagonist of angiopoietin-1	Unknown	Unknown
Tek/Tie-2	Angiopoietin-1/2 receptor tyrosine kinase	Embryonic lethal Defective blood vessel complexity and structure	Expression induced in tumor blood vessels. Tie-2 antagonists inhibit tumor angiogenesis
Tie-1	Receptor tyrosine kinase (ligand unknown)	Embryonic lethal Impaired integrity of capillaries, edema, hemorrhage	Expression elevated in tumor blood vessels
TGF- $\beta$ 1	Polypeptide growth factor	Defect in angiogenesis in 50% of homozygotes	Expressed in tumors
PDGFR- $\beta$	Receptor tyrosine kinase	Perinatal hemorrhage, lack of pericytes (similar phenotype in the case of PDGF-B ligand)	Expressed in tumors
Tissue factor	Polypeptide active in the coagulation cascade	Embryonic lethal Vascular abnormalities, deficient recruitment of pericytes and smooth muscle cells	Expressed in tumors
RasGAP	Signaling cofactor of Ras (activating GTPase activity)	Embryonic lethal Abnormal vessel branching, edema	May regulate responses of endothelial cells to tumor angiogenic factors

**Table 9.3.** Inhibitors of Angiogenesis

Inhibitor	Characteristics and Mode of Action
Endogenous Inhibitors of Angiogenesis	
Angiostatin	Systemically acting angiogenesis inhibitor. Plasminogen fragment generated by proteolytic action of elastase. Inhibits endothelial cell proliferation by unknown mechanism.
Endostatin	Systemically acting angiogenesis inhibitor. Proteolytic fragment of collagen XVIII, a component of perivascular extracellular matrix. Inhibits endothelial cell proliferation and angiogenesis by unknown mechanism.
Platelet factor 4 (PF4)	Inhibits angiogenesis by interference with bFGF receptor. An active fragment can be generated by N-terminal cleavage. In clinical trials as an antiangiogenic agent.
Prolactin (16-kDa fragment)	Inhibits angiogenesis, by bFGF and VEGF induced proliferation of endothelial cell (S-phase arrest).
EGF fragment	Inhibits EGF and laminin–dependent endothelial cell motility and angiogenesis.
Thrombospondin-1 (TSP-1)	TSP-1 (fragment) binds to CD36 on endothelial cells and inhibits angiogenesis by a mechanism possibly involving proliferation, migration, and cell survival.
Interferon $\alpha$ (IFN- $\alpha$ )	Inhibits angiogenesis by antimitotic and antimigratory effect on endothelial cells and in part by blockade of bFGF production by parenchymal cells. In clinical trials as an antiangiogenic agent.
Interferon $\beta$ (IFN- $\beta$ )	Inhibits endothelial cell migration and proliferation.
Interferon $\gamma$ (IFN- $\gamma$ )	Inhibits endothelial cell proliferation and migration.
Interleukin 12 (IL-12)	Inhibits angiogenesis by stimulation of IFN- $\gamma$ and interferon-inducible-protein-10 (IP-10) production. In clinical trials as an antiangiogenic agent
Tissue inhibitors of metalloproteinases (TIMP1-3, CDI)	Block dissolution of the extracellular matrix and endothelial cell invasion by inhibiting metalloproteinases.
Proliferin-related protein (PRP)	Inhibits directed migration of mouse endothelial cells.
2-Methoxy estradiol	Inhibits endothelial proliferation and angiogenesis by unknown mechanism.

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### Exogenous Inhibitors of Angiogenesis

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TNP-470 (AGM1470)	Synthetic analog of fumagillin; fungal inhibitor of endothelial cell proliferation and angiogenesis. In clinical trials as an antiangiogenic agent.
Pentosan polysulfate	Inhibits paracrine action of heparin-binding angiogenic growth factors (bFGF, VEGF). In clinical trials as an antiangiogenic agent.
Tecogalan	Sulfated bacterial polysaccharide peptidoglycan complex that is inhibitory for endothelial cell proliferation, particularly in combination with tamoxifen.
Batimastat (BB-94)	Synthetic inhibitor of metalloproteinases. Inhibits endothelial cell invasion. In clinical trials.
Marimastat (BB2516)	Synthetic, oral inhibitor of metalloproteinases. Inhibits endothelial cell invasion. In clinical trials.
Suramin	Interferes with multiple endothelial functions and bFGFR activity.
CM101	Streptococcal polysaccharide; binds specifically to activated endothelial cells and induces vascular inflammation.
Vitaxin	Humanized anti $\alpha v \beta 3$ antibody. Prevents ligation of $\alpha v \beta 3$ integrin causing endothelial apoptosis.
Vitamin D3 analogues	Inhibit angiogenesis by unknown mechanism.
Thalidomide	Originally used as a sedative and to treat leprosy. Teratogenic, antiangiogenic (epoxide metabolite). In clinical trials as an antiangiogenic agent.
Minocycline	A tetracycline with antiangiogenic activity.

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## Developmental Angiogenesis

## Tumor Angiogenesis

Hemangioblast

Angioblast

**Vasculogenesis**

**VEGF**

Primitive  
plexus

**Angiogenesis**

**VEGF  
Ang-1**

Mature  
vascular  
system

**Angiogenic  
vessel**

Circulating  
EC progenitors

**EC  
proliferation  
sprouting**

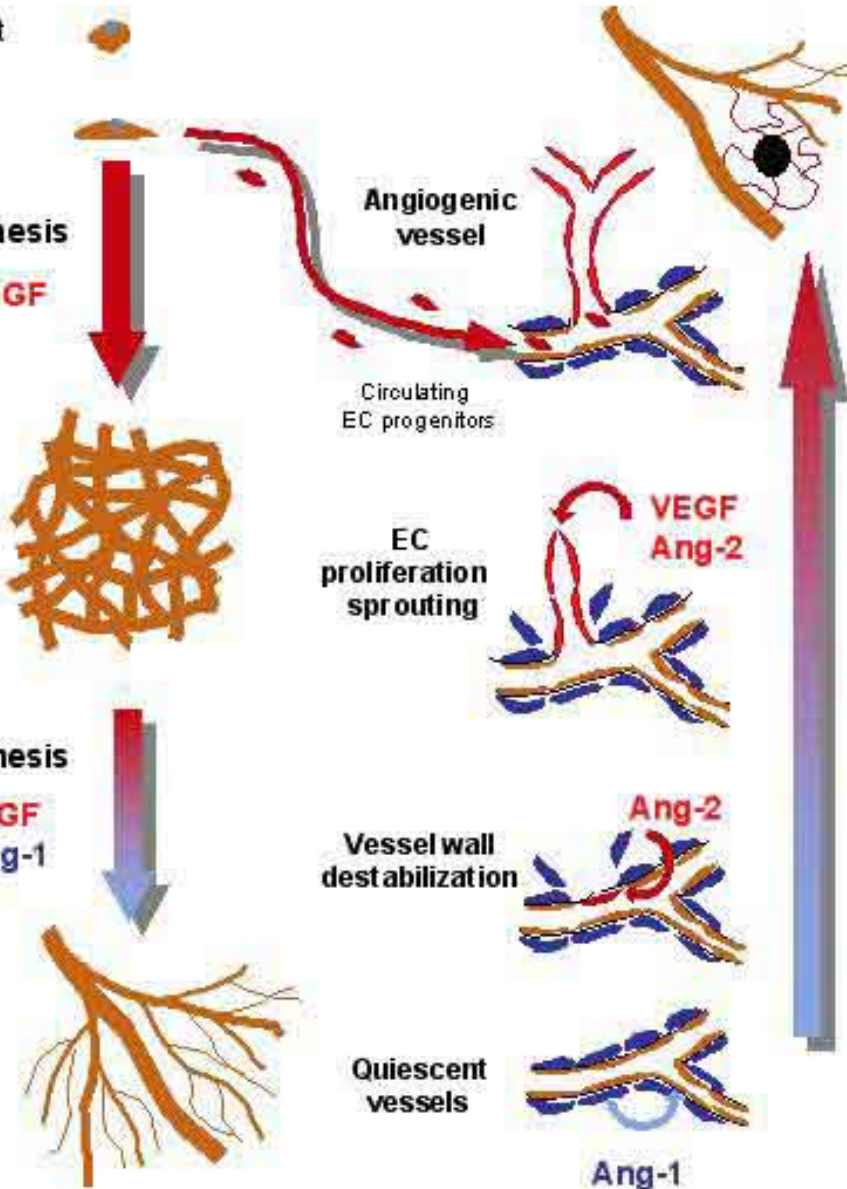
**Vessel wall  
destabilization**

**Quiescent  
vessels**

**VEGF  
Ang-2**

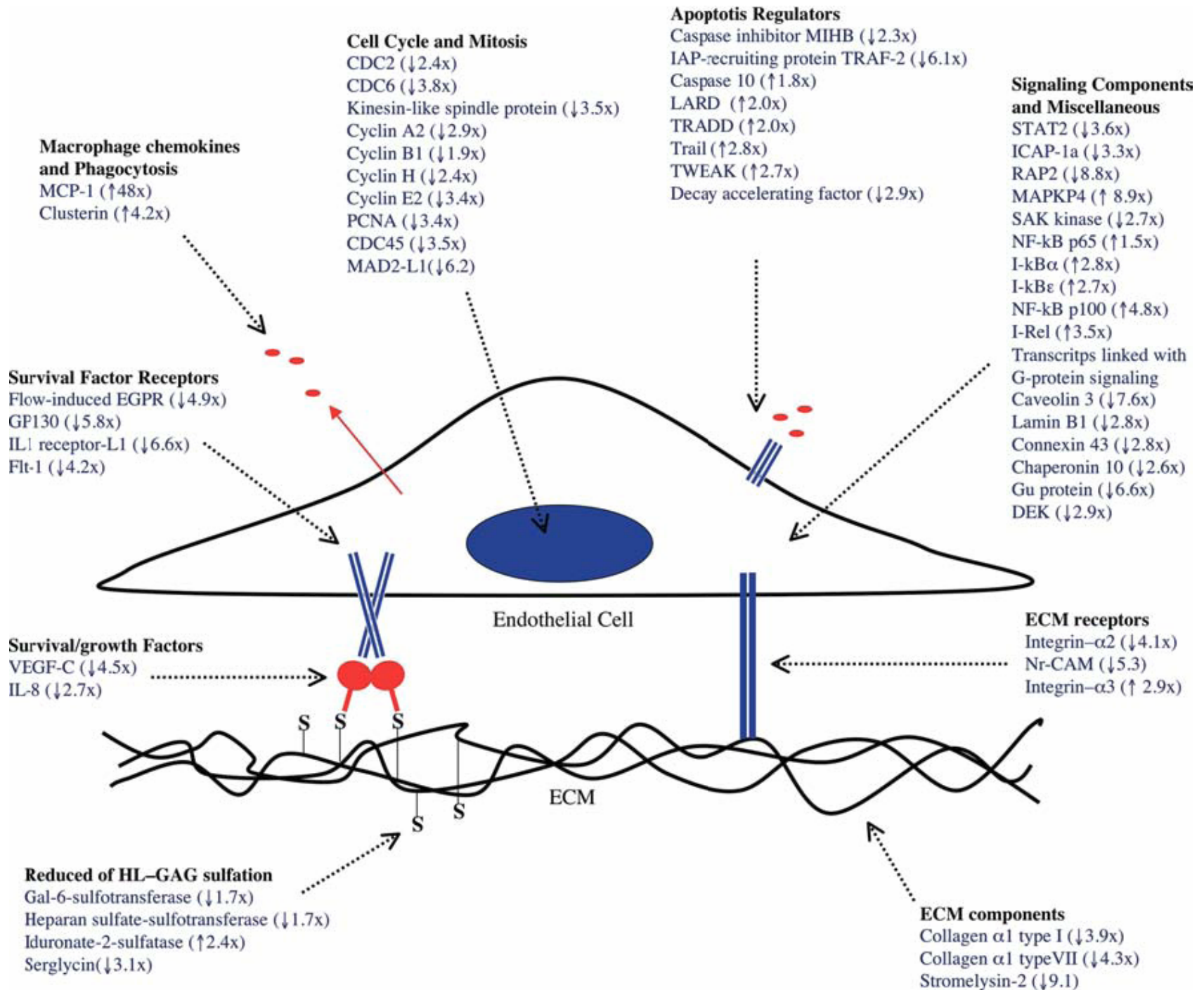
**Ang-2**

**Ang-1**

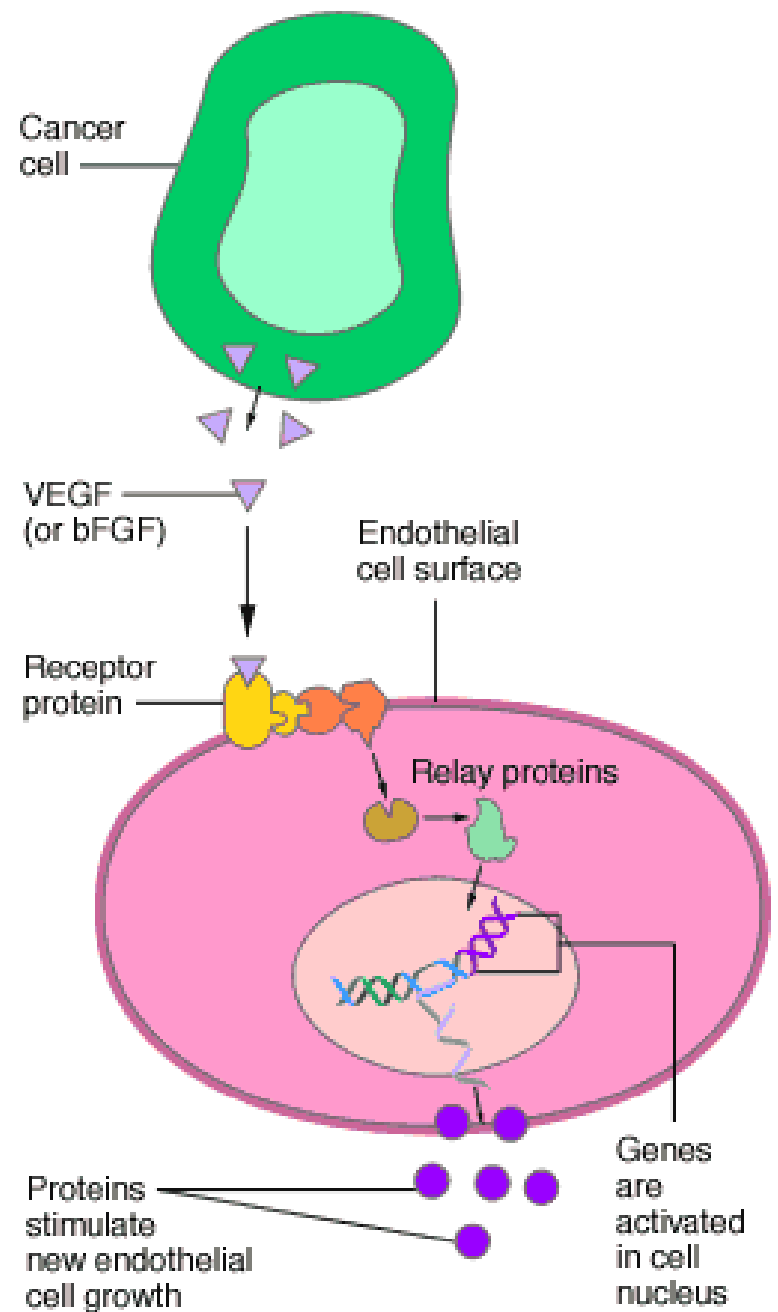
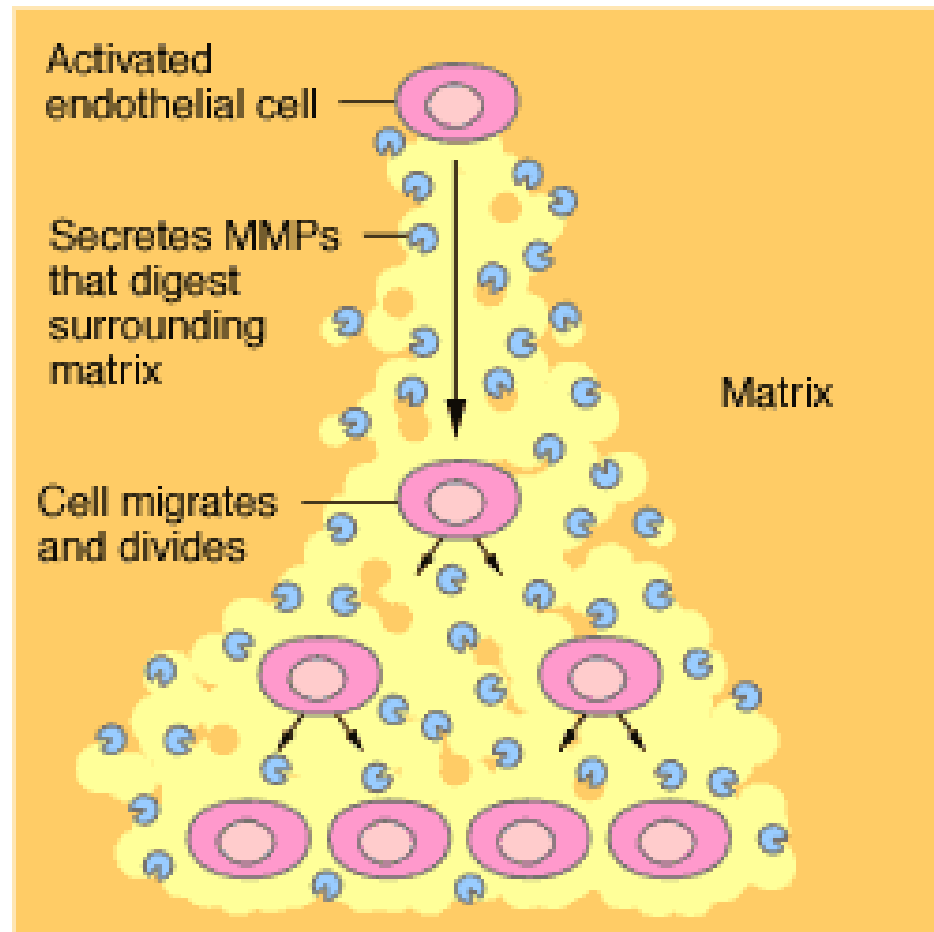


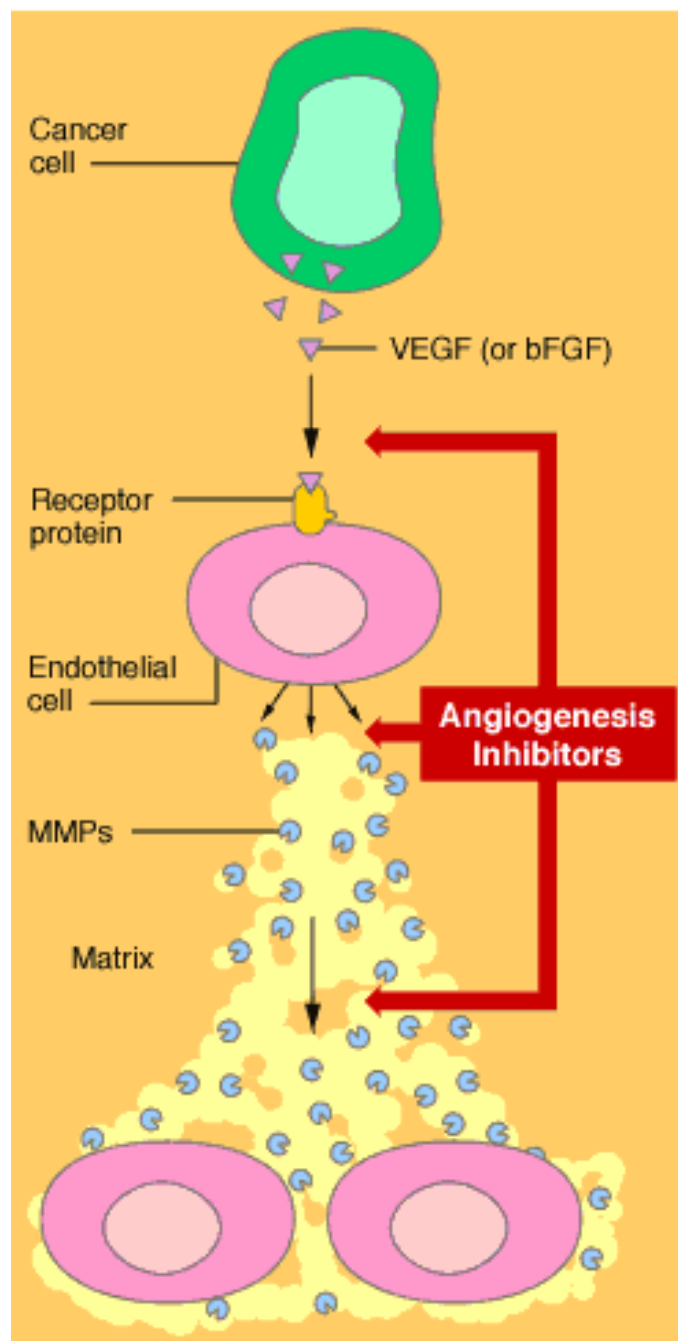
Gene	Phenotype	Refs
Thrombospondin-1	Epithelial and smooth muscle cell hyperplasia and inflammation in the lung; Evidence for less active TGF- $\beta$ 1	[52,67]
Thrombospondin-2	Increased angiogenesis; bleeding diathesis; abnormal collagen fibrils; reduced fibroblast adhesion; increased fragility of skin	[7,20,23,33]
SPARC	Cataracts; accelerated wound healing; increased adipogenesis; increased fibrovascular response to injury; osteopenia; immature collagen fibrils	[8,27••,29,30,35]
Osteopontin	Reduced T-cell-mediated immunity; increased dystrophic calcification; reduced host response to injury; reduced bone resorption	[9,10,68]
Tenascin-C	Subtle neurological/behavioral defects; suppression of hematopoietic activity; abnormal response to injury	[11,69]
Tenascin-X	Reduced collagen content and fibril density in skin; increased tumor invasion and metastasis; increased MMP2 and MMP9	[6,70]



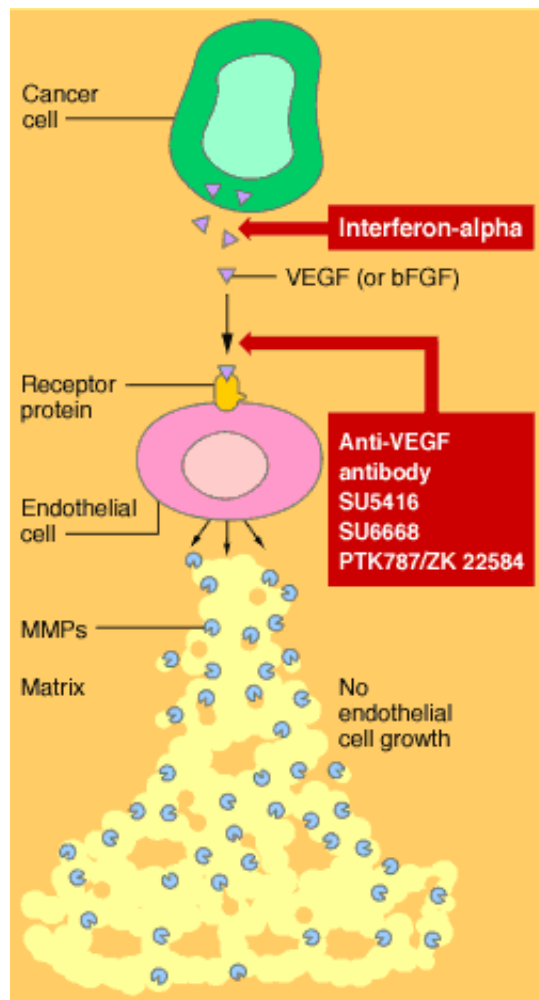




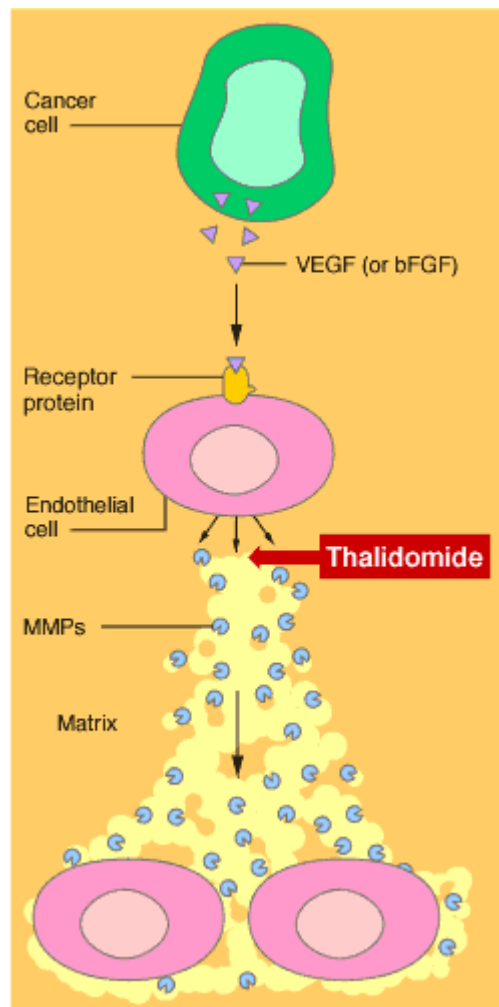




## Cascada de señalización



## Drogas clásicas



## Matriz extracelular

