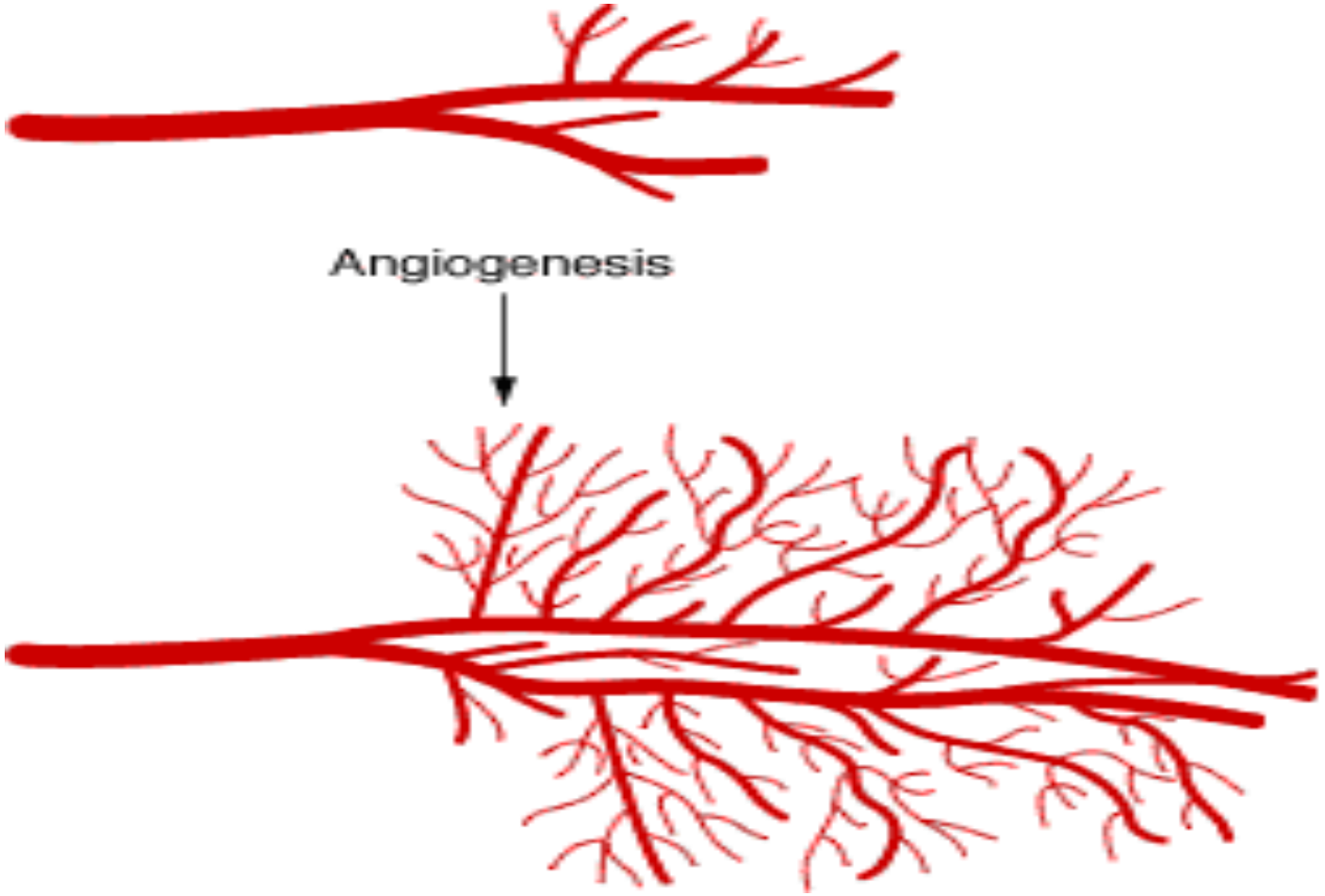
Angiogenesis and Metastasis

(RPN 530 11/3 lecture)

Tariq Bhat

(Immunology)

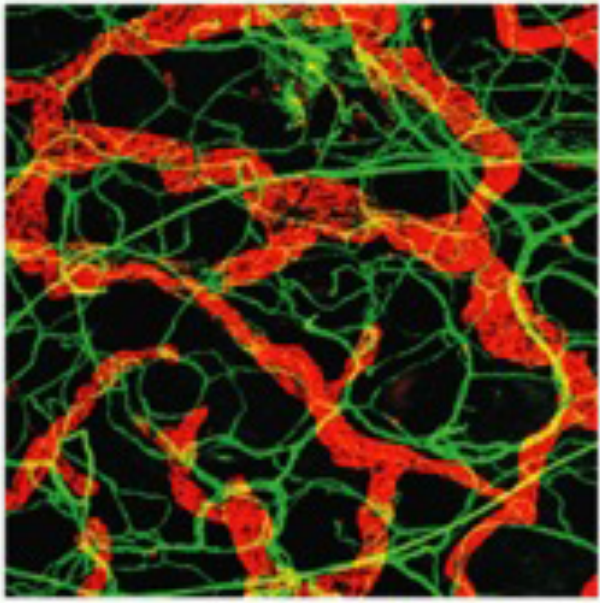
Angiogenesis



- Blood vessels in the body- composed of macro (artery/vein) and micro-vessels.

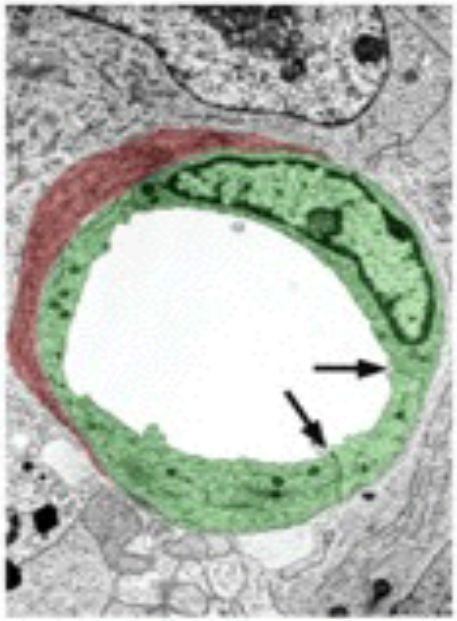
- Blood vessels- supply oxygen and nutrition, and removal of wastes.

- Lymphatic vessel- drain the tissue fluid to blood circulation and protect from germs by immunity at lymph nodes



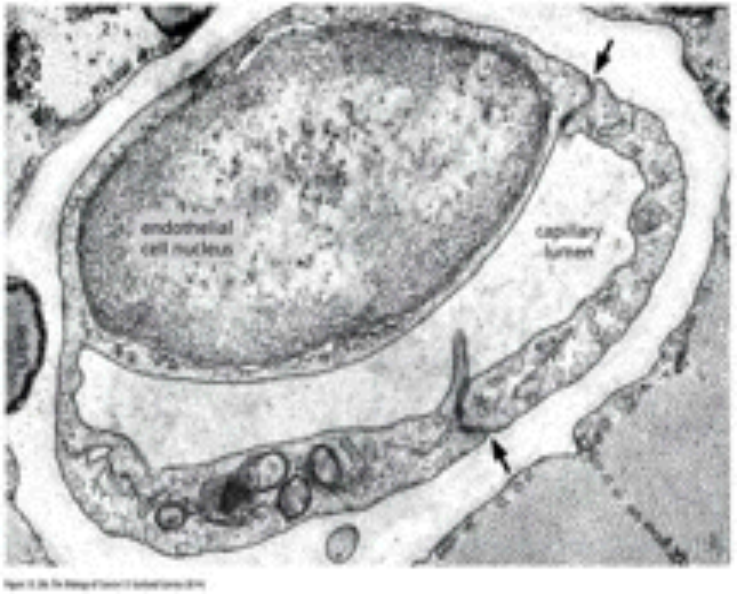
Capillaries Lymph ducts

Macro-vessel



Endothelial cells Pericyte

Micro-vessel



Angiogenesis

The formation of new blood vessels out of pre-existing capillaries.

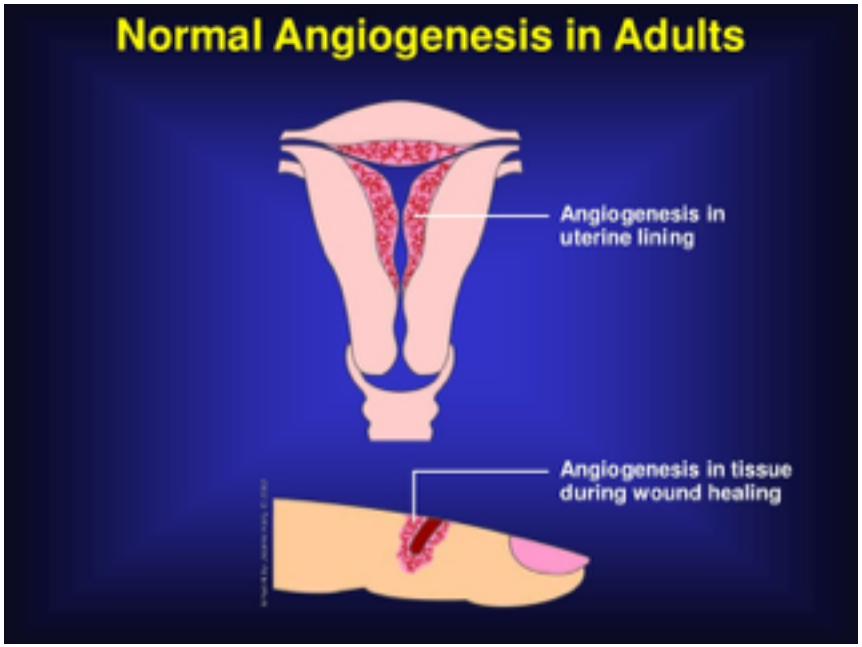
INVOLVES : Sprouting
Splitting
Remodeling of the existing vessels

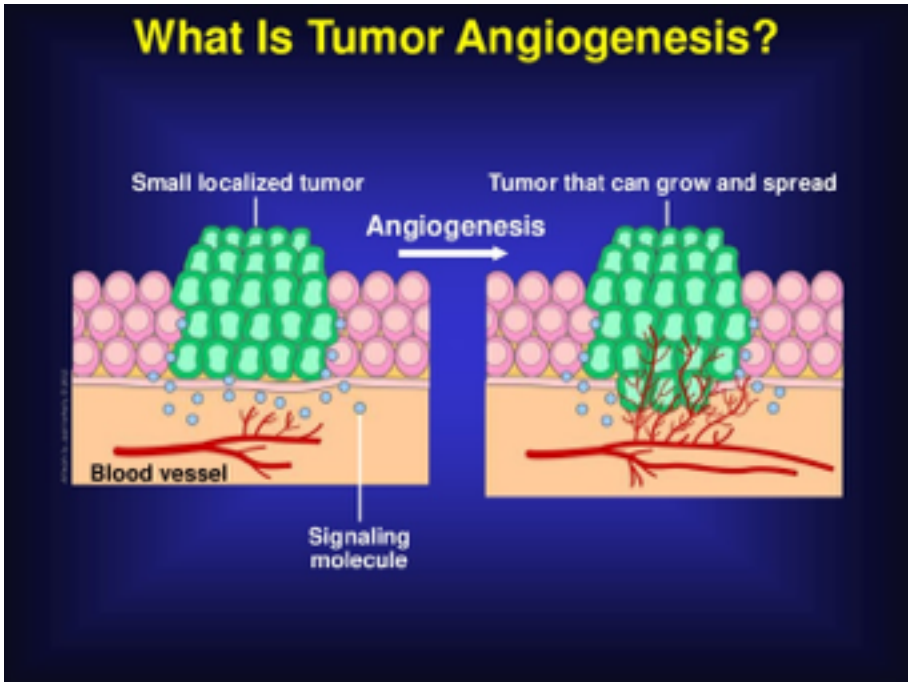
WHY IT IS IMPORTANT?

- Supply of oxygen and nutrients
- Removal of waste products

ANGIOGENESIS	VASCULOGENESIS
❑ New blood vessels mainly emerge from pre-existing ones.	❑ New endothelial cells differentiate from stem cells.
❑ Can be seen in adult life also.	❑ Seen during embryonic development(for primary vasculature).
❑ Physiologic stimuli during wound healing and the reproductive cycle in women lead to angiogenesis.	❑ Vasculogenesis is absent even in presence of physiologic stimuli.


Normal Angiogenesis in Children


Normal Angiogenesis in Adults
Angiogenesis in uterine lining
Angiogenesis in tissue during wound healing


What Is Tumor Angiogenesis?
Small localized tumor
Tumor that can grow and spread
Angiogenesis
Blood vessel
Signaling molecule

Definitions

Vasculogenesis - Angiogenesis - Arteriogenesis

Vasculogenesis
Formation of blood vessels by differentiation from (hem)angioblasts

Sprouting angiogenesis
Sprouting of cells from mature endothelial cells of the vessel wall

Arteriogenesis
growth of large arteries from pre-existing small vessels/capillaries

Lymphangiogenesis
Formation of the lymphatic vasculature

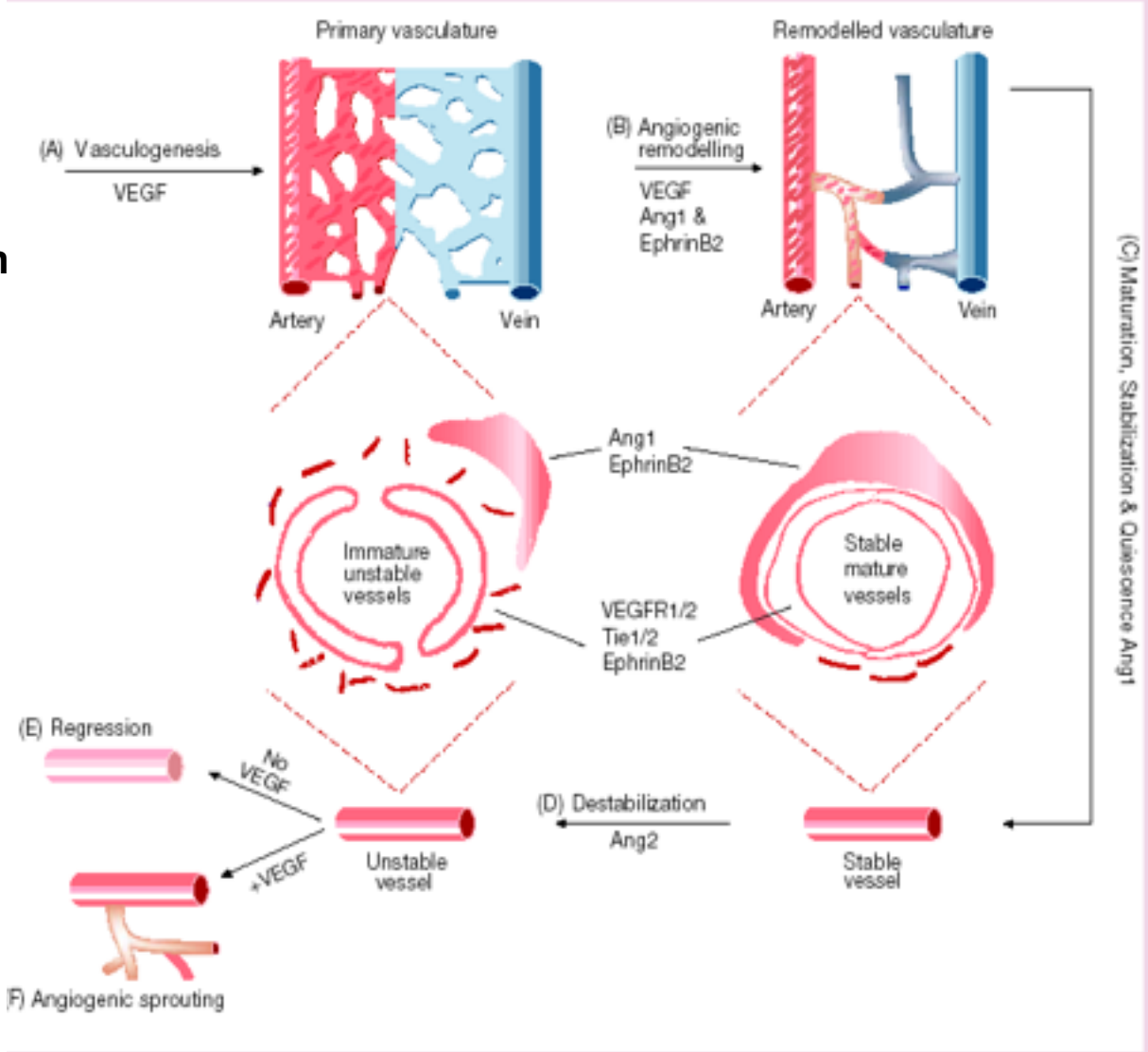
Vasculogenesis

Formation of vessels by differentiation of cells from angioblasts in the yolk sac of the embryo:

Is differentiation and proliferation of endothelial cells in a non-vascularized tissue

Leads to formation of a primitive tubular network

Has to undergo angiogenic remodeling to stable vascular system



Postnatal vasculogenesis

Hemangioblast → Angioblast → EC

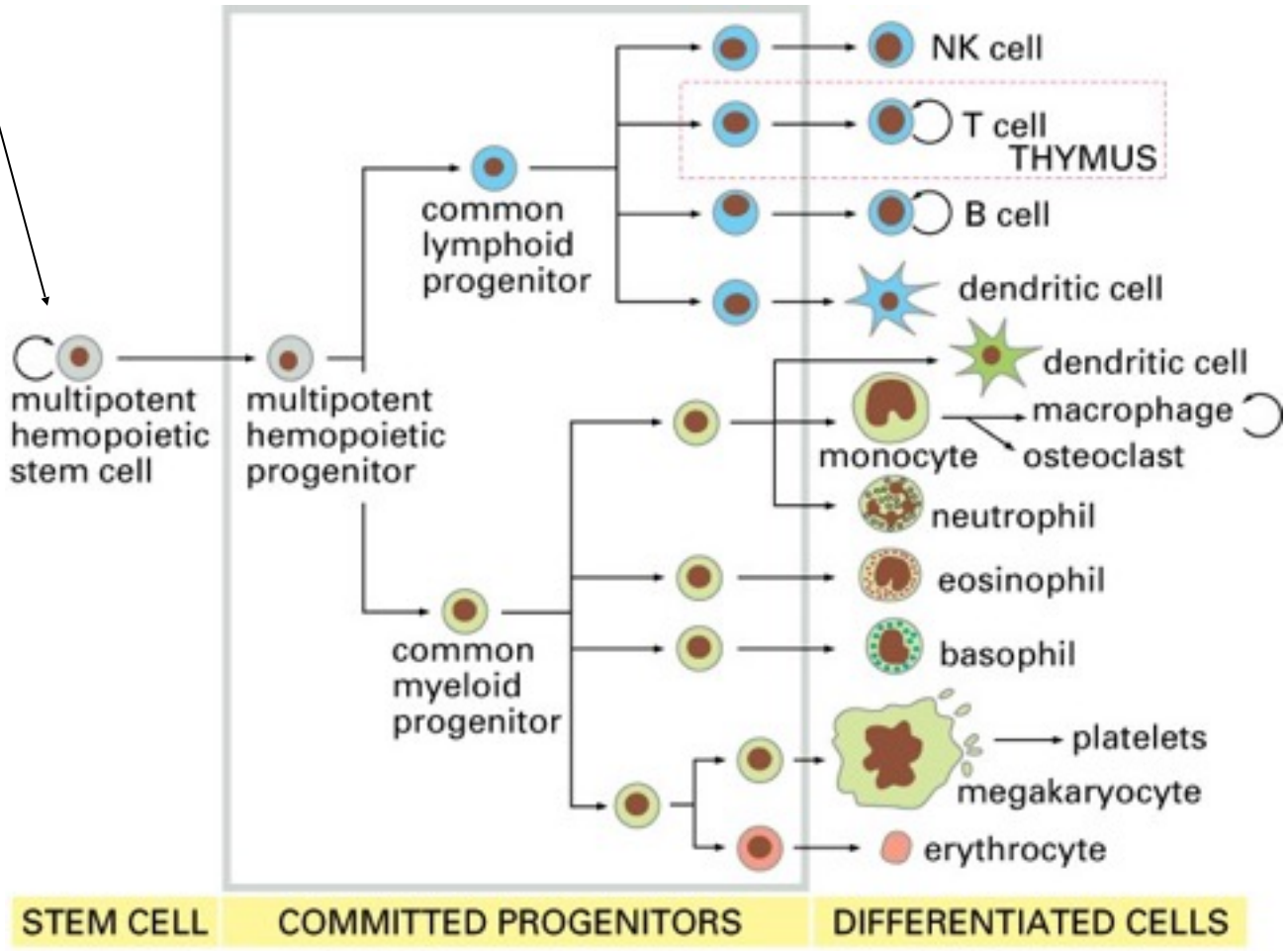
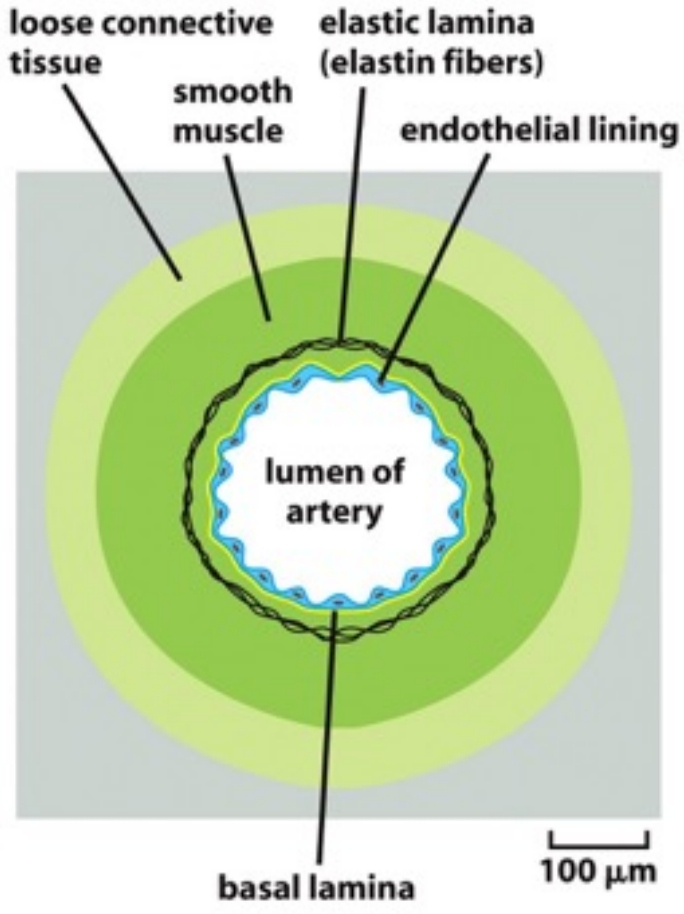


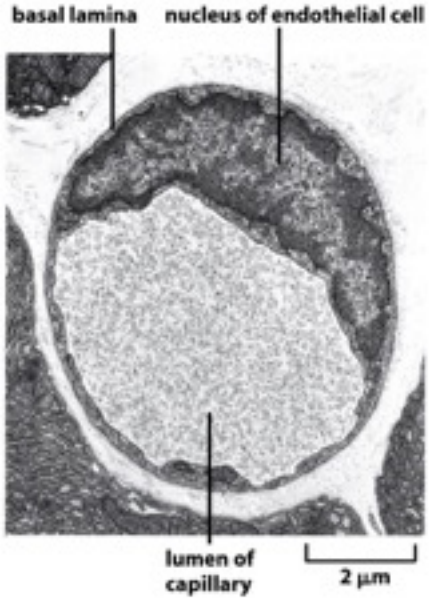
Figure 22–35. Molecular Biology of the Cell, 4th Edition.

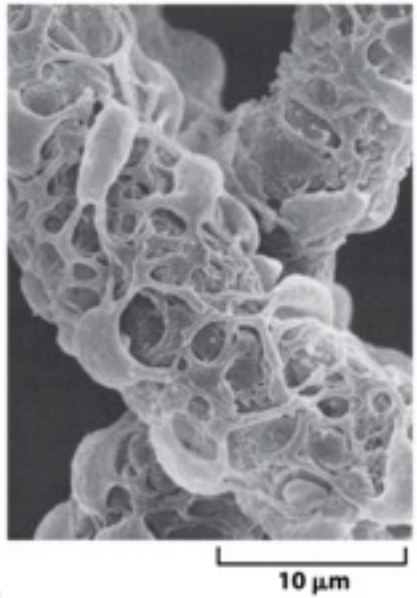
Structure of vessels and capillaries

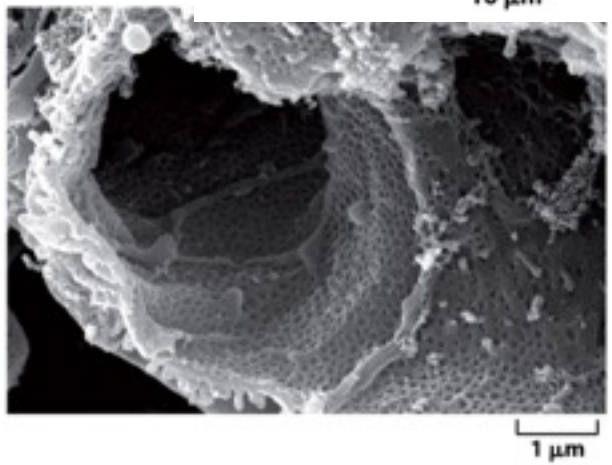
Small artery: Monocellular layer of endothelial cells



Capillary: endothelial cell, basal lamina, pericytes







Angiogenesis is a multi-step process

The 4 major steps of endothelial cells in angiogenesis

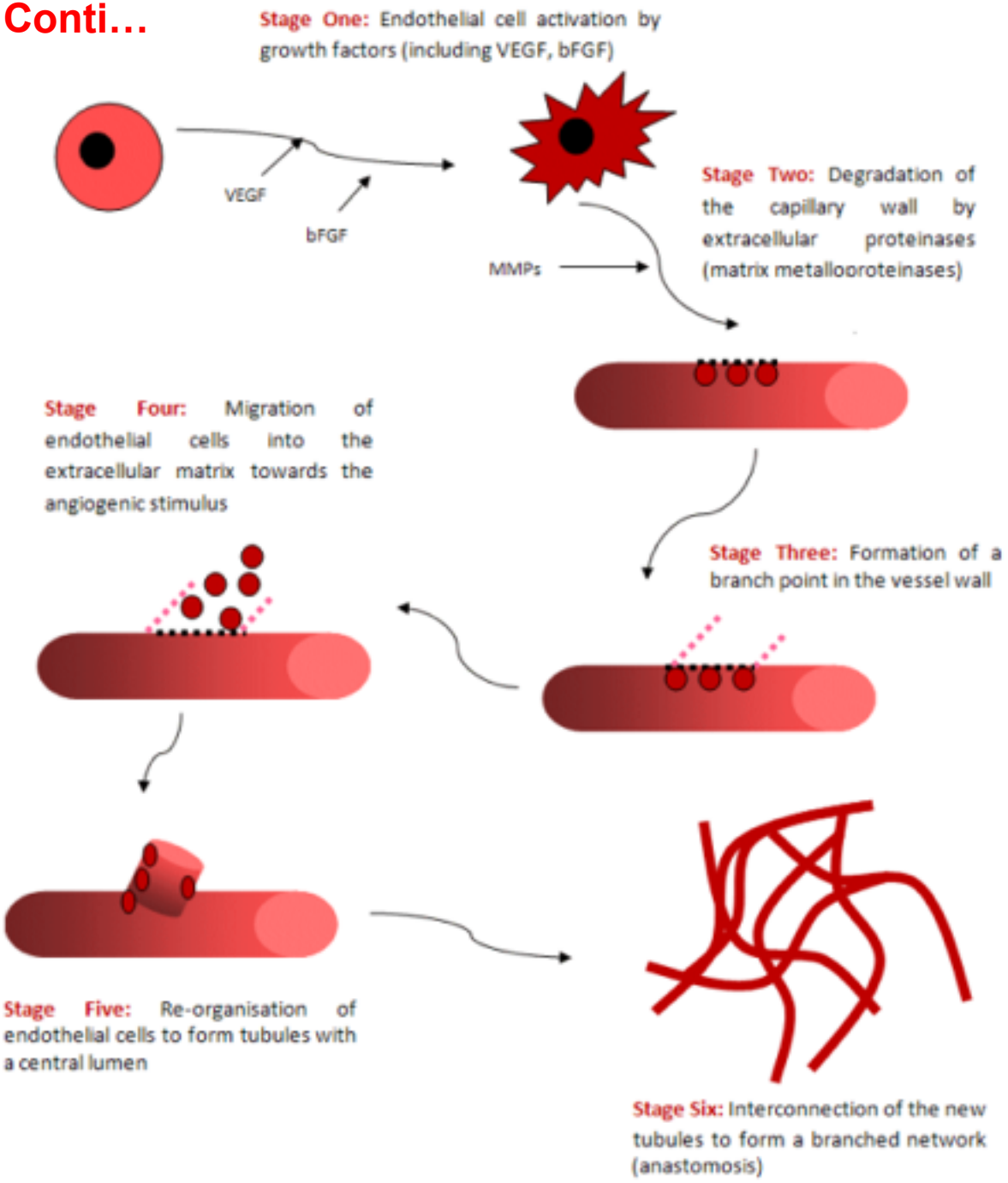
1.Breaking through of the basal lamina that envelops existing blood vessels

2. Migration toward a source signal

3. Proliferation

4. Formation of tubes

Conti…



Key Stage	Markers
Stage One: Endothelial cell activation in response to angiogenic factors.	Basic Fibroblast Growth Factor (bFGF): a potent stimulatory factor for endothelial cell migration and proliferation. Vascular Endothelial Growth Factor (VEGF): initiates cell proliferation and migration.
Stage Two: Degradation of the capillary wall by extracellular proteinases.	Matrix Metalloproteinases (MMPs): MMP1 (a collagenase) and MMP2 are expressed during angiogenesis and act to degrade extracellular matrix components.
Stage Three: Formation of a branch point in the vessel wall.	Integrins: expressed on newly forming vessels.
Stage Four: Migration of endothelial cells into the extracellular matrix towards the angiogenic stimulus.	Integrins: allow migrating endothelial cells to interact with specific components of the surrounding matrix. MMPs and urokinase: aid migration of endothelial cells into the surrounding matrix.
Stage Five: Re-organisation of endothelial cells to form tubules with a central lumen.	Angiopoietin (Ang 1): produced by surrounding stromal cells; facilitates endothelial cell survival and stabilisation of new capillary tubes.
Stage Six: Interconnection of the new tubules to form a network (anastomosis).	Platelet Derived Growth Factor (PDGF): produced by endothelial cells of the new capillaries; recruits pericytes which stabilize the new vessels.

Life time of endothelial cells (Major Players):

months (lung, liver) to years (brain, muscle)

Slow repair and renewal of vascular wall

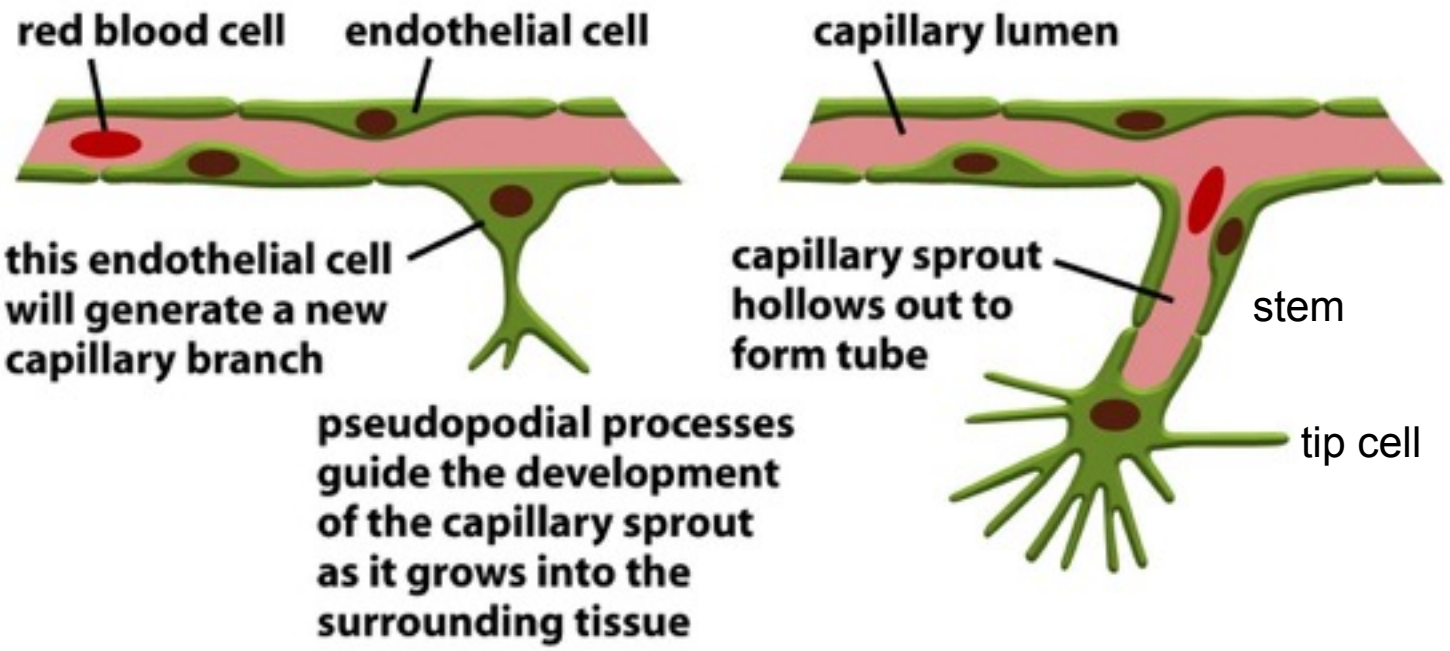
New vessel formation:

Embryo, growth
In uterus, during menstruation cycle
Wound repair

Angiogenesis:

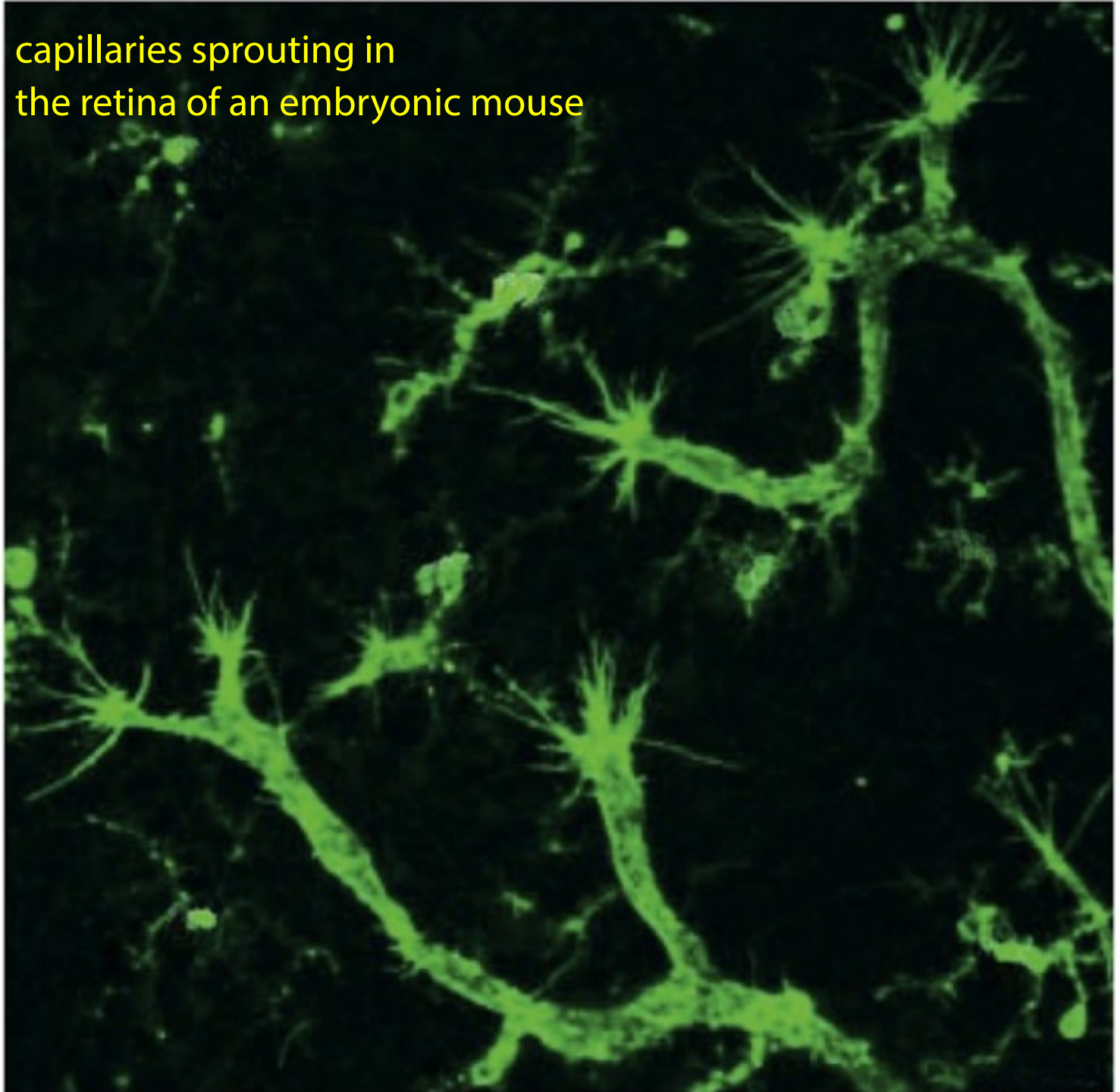
Sprouting of cells from mature endothelial cells of the vessel wall

Angiogenesis is involved in formation, maturation and differentiation of blood vessels from pre-existing vessels. Angiogenesis can be observed in Physiological and pathological conditions including growth, injury, inflammation and **cancer**. Occasionally, angiogenesis is called neovascularization. (**Extend and expand blood vessels)**

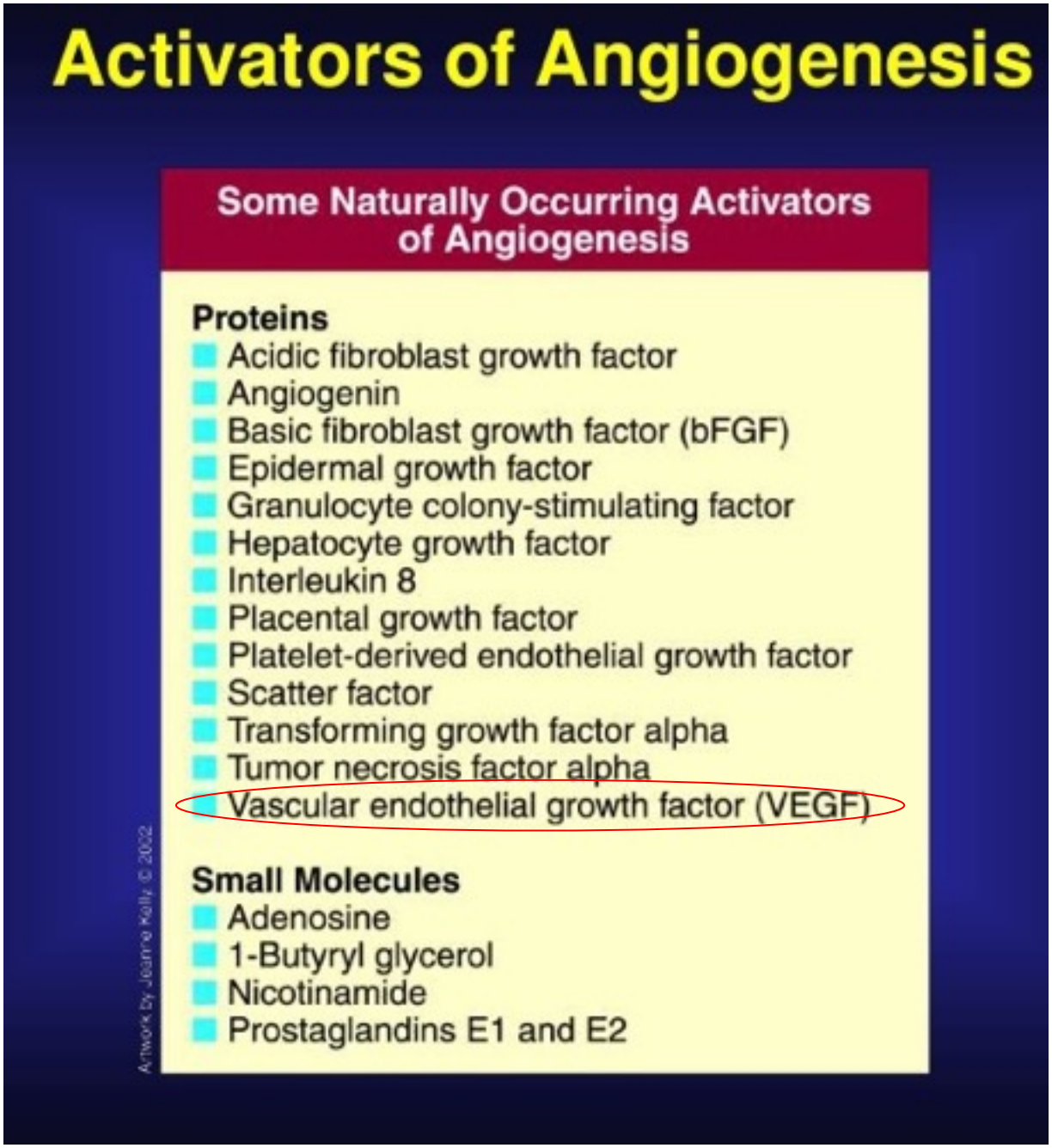


secretion of proteases, resolution of basal lamina, migration towards chemotactic gradient, proliferation, tube formation

VEGF is factor largely specific for endothelial cells,
bFGF can also induce,
not specific for EC


capillaries sprouting in
the retina of an embryonic mouse

Activators of Angiogenesis



ROLE OF VEGF

- VEGF production is under control of :
  hypoxia inducible factor (HIF)
- VEGF receptor expression is up-regulated under :
  hypoxic or ischemic conditions.

❖ So, early involvement of VEGF in this process.

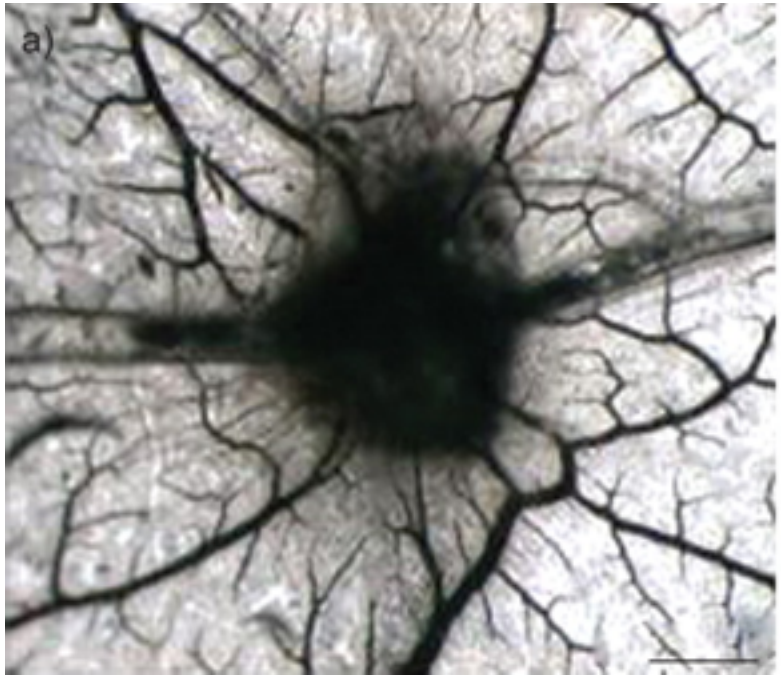
- VEGF is a major player in angiogenesis initiation because: i) it induces vasodilatation via endothelial NO production
  ii)it increases endothelial cell permeability

VEGF conti..

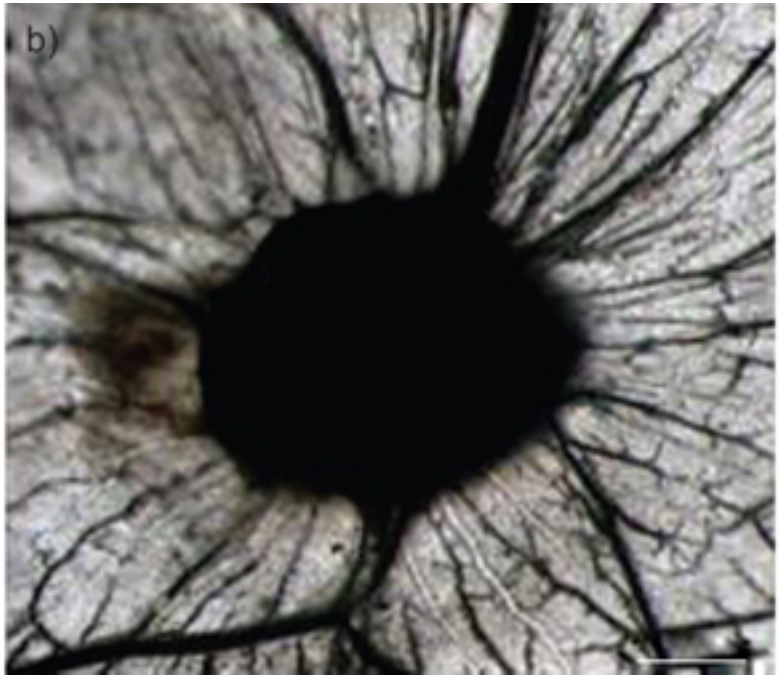
1. vasodilatation
2. increased vascular permeability
3. can induce the expression of proteases and receptors important in cellular invasion and tissue remodeling
4. prevent endothelial cell apoptosis

But angiogenesis is not completely dependent on VEGF production. Recently shown by : Hansen-Algenstaedt et al.

Chorioallantoic Membrane Assay (CAM)

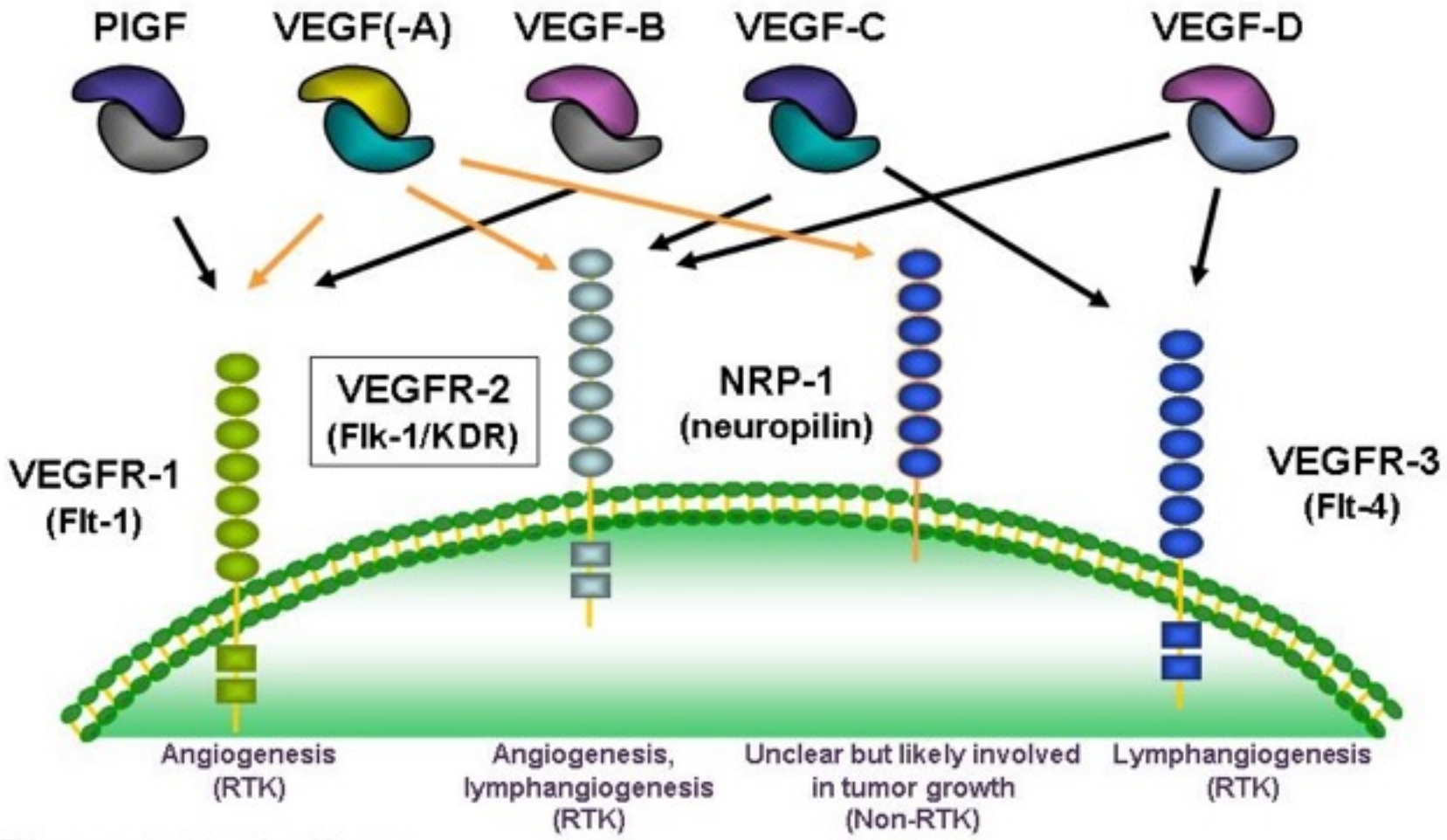


Serum free-media



Serum free-media plus VEGF

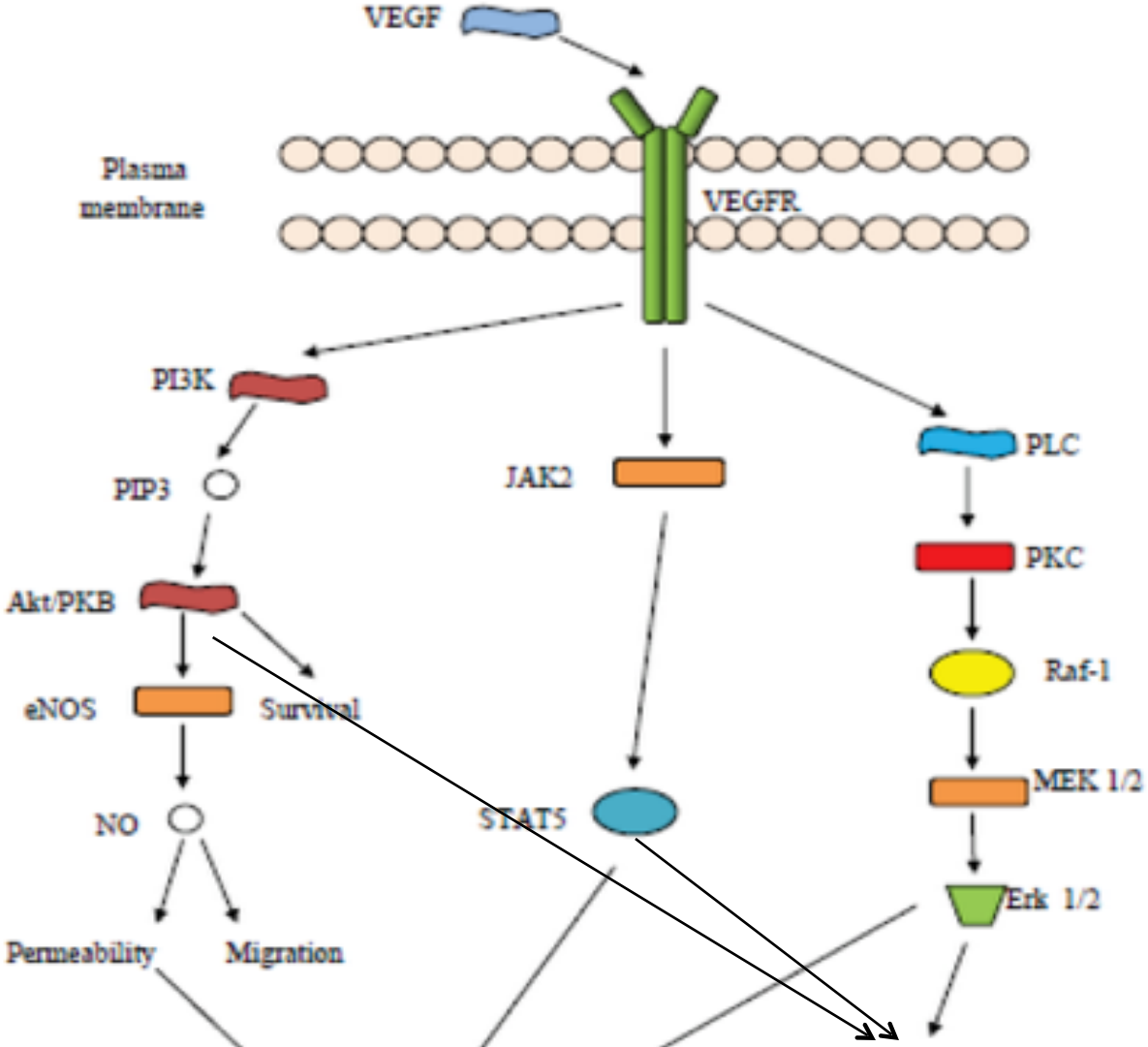
The VEGF Family and Its Receptors



RTK = receptor tyrosine kinase.

Flt- FMS-like tyrosine kinase
KDR- Kinase insert domain receptor (*KDR*, a type III receptor tyrosine kinase)

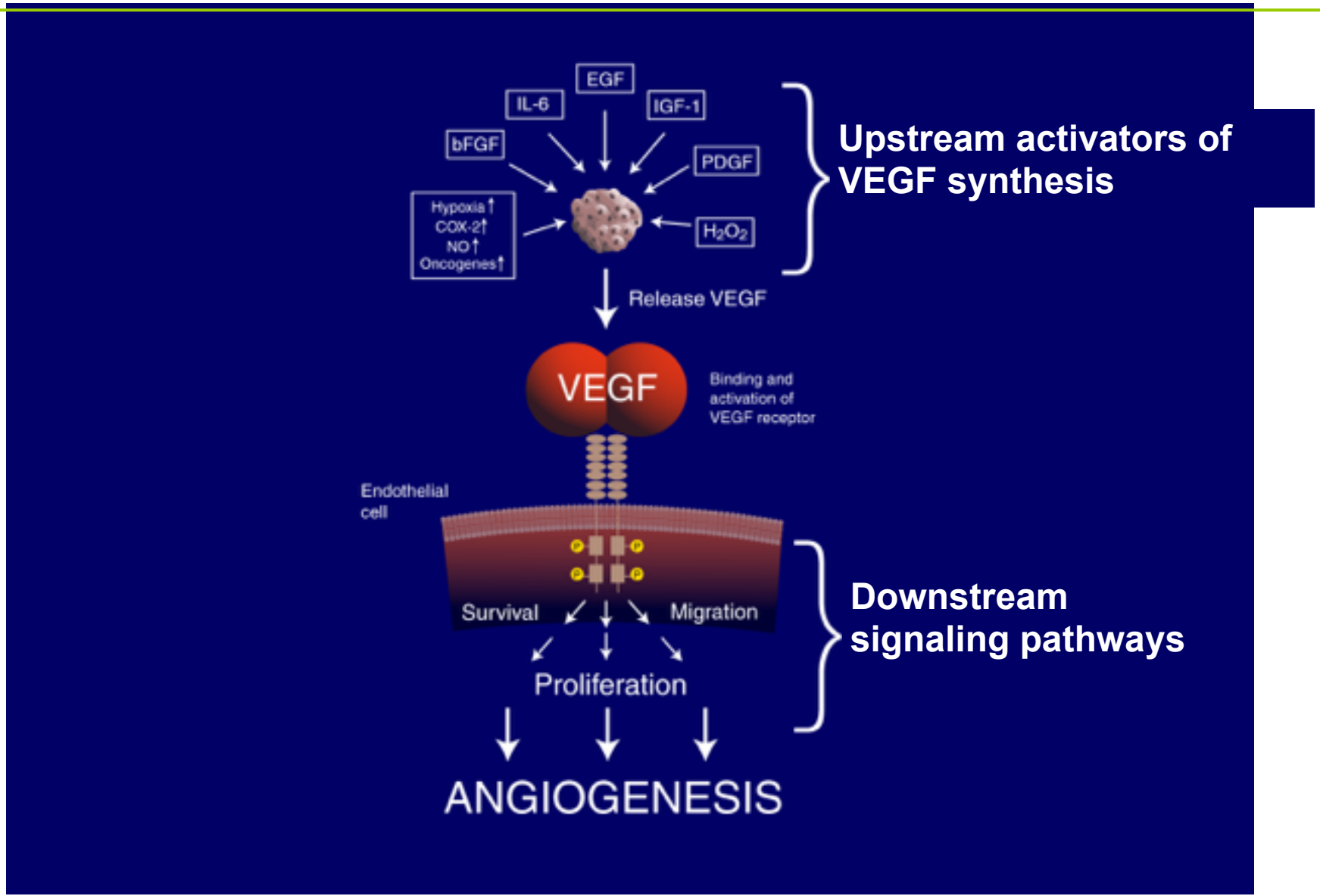
VEGF-VEGFR signaling



Angiogenesis

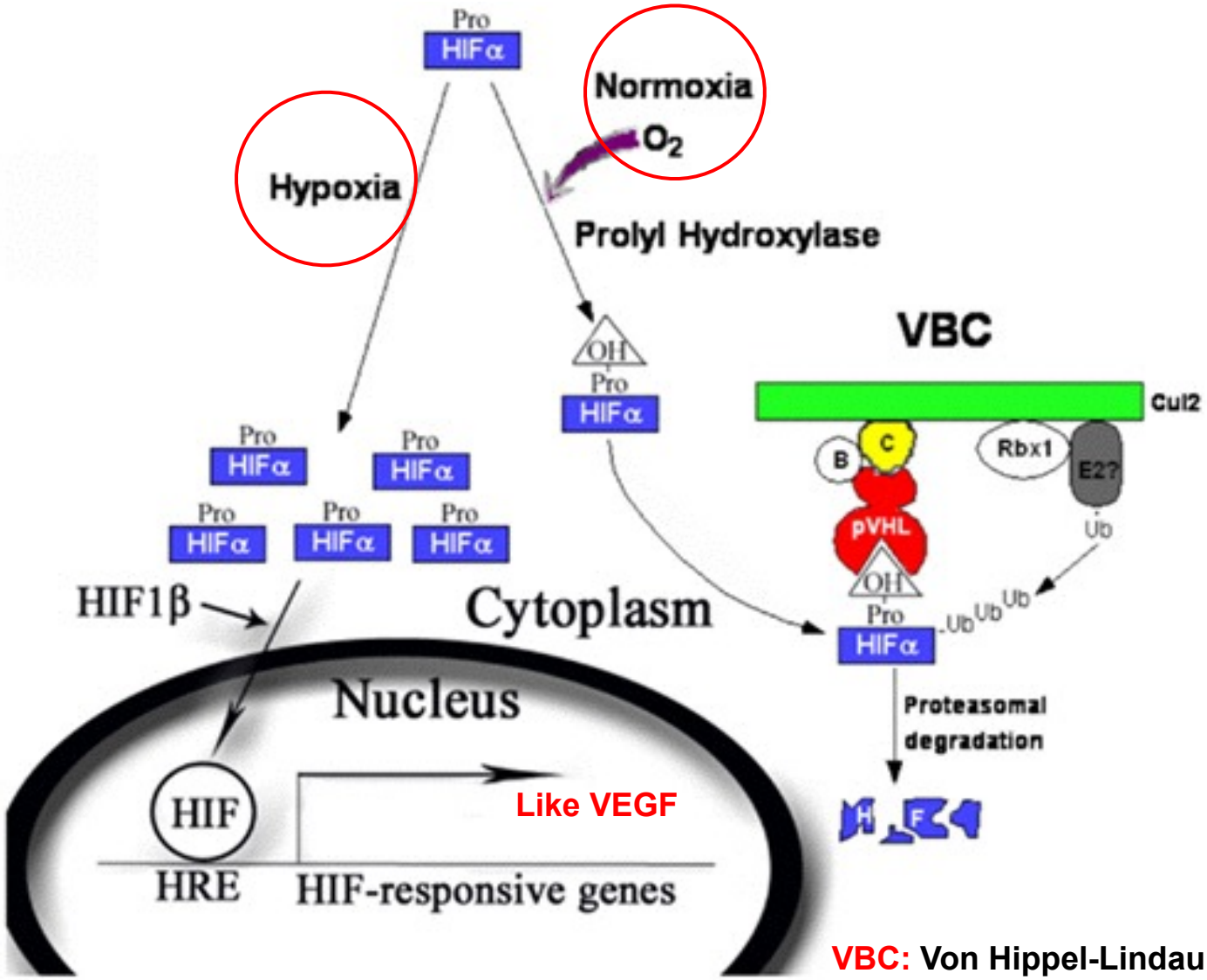
EC proliferation, survival, migration and invasion

VEGF Is a Key Mediator of Angiogenesis



Role of hypoxia in angiogenesis:

(Hypoxia - HIF – VEGF module)

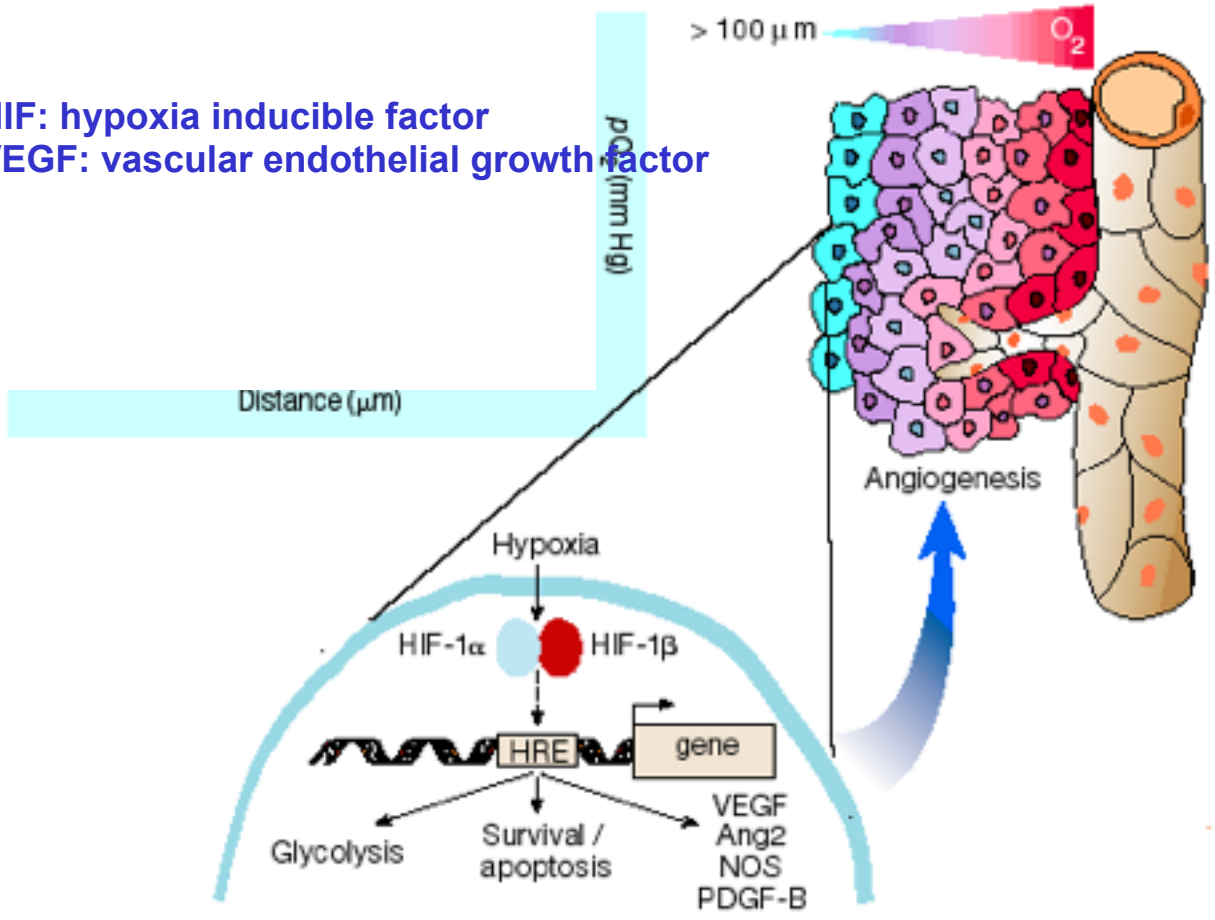


VBC: Von Hippel-Lindau (VHL)-containing VHL-elongin BC

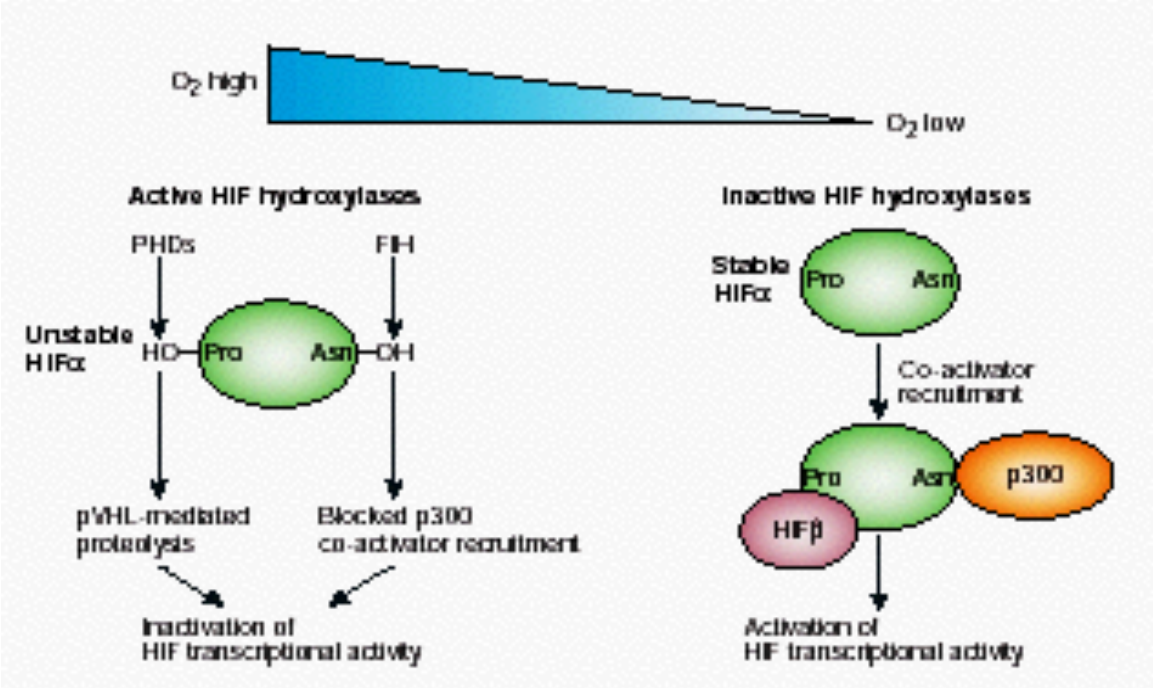
Role of hypoxia in angiogenesis:

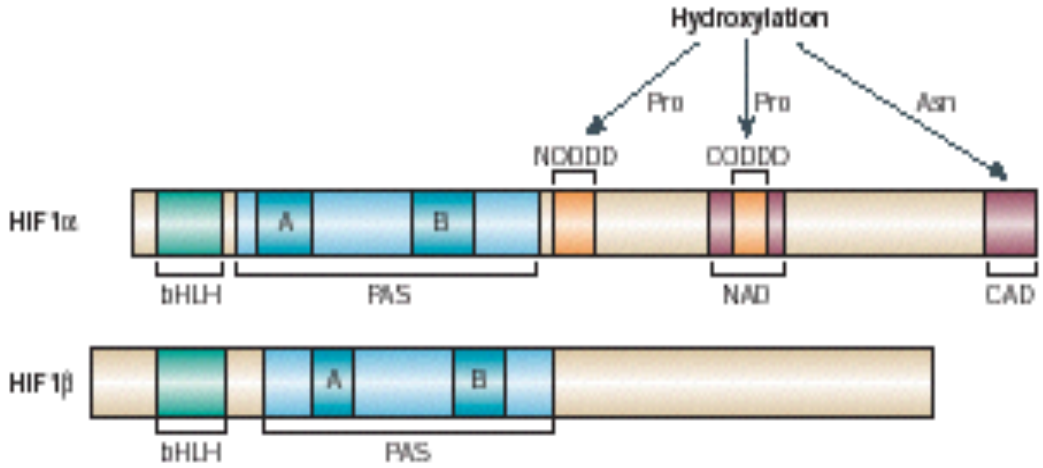
(Hypoxia - HIF – VEGF module) conti…

HIF: hypoxia inducible factor
VEGF: vascular endothelial growth factor



Von Hippel-Lindau Tumor Suppressor, HIF and VEGF





VEGF-gene expression:
Regulated by HIF,
HIF is continuously produced,
ubiquitinylated,
degraded in proteasome,
therefore low concentration;

Ubiquitinylation is dependent on Hippel-Lindau tumor suppressor (part of an E3 ubiquitin-ligase complex)

HIF1α is modified by a prolyl hydroxylase,
then better interaction with vHL protein, high turnover;
Hydroxylase is regulated by O_2

FIH: Factor inhibiting HIF1α-Aaparaginyl hydroxulation leading to HIF inactivation

Angiogenesis-dependent diseases

Excess:

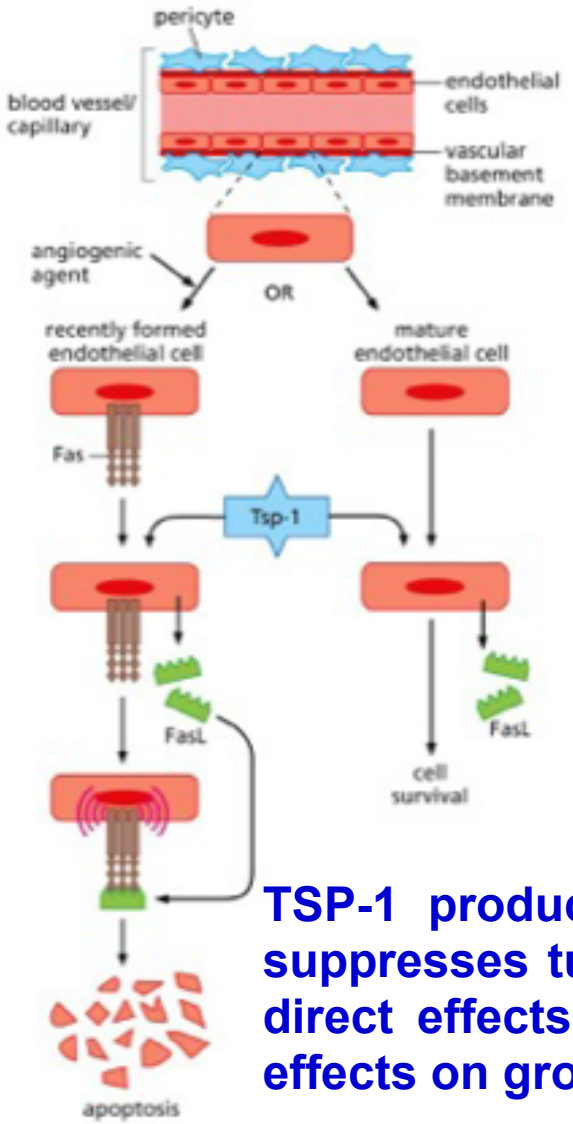
- Cancer
- Infantile hemangiomas
- Autoimmune diseases, chronic inflammatory diseases:
- Rheumatoid arthritis
- Psoriasis
- Age-related macular degeneration
- Atherosclerosis

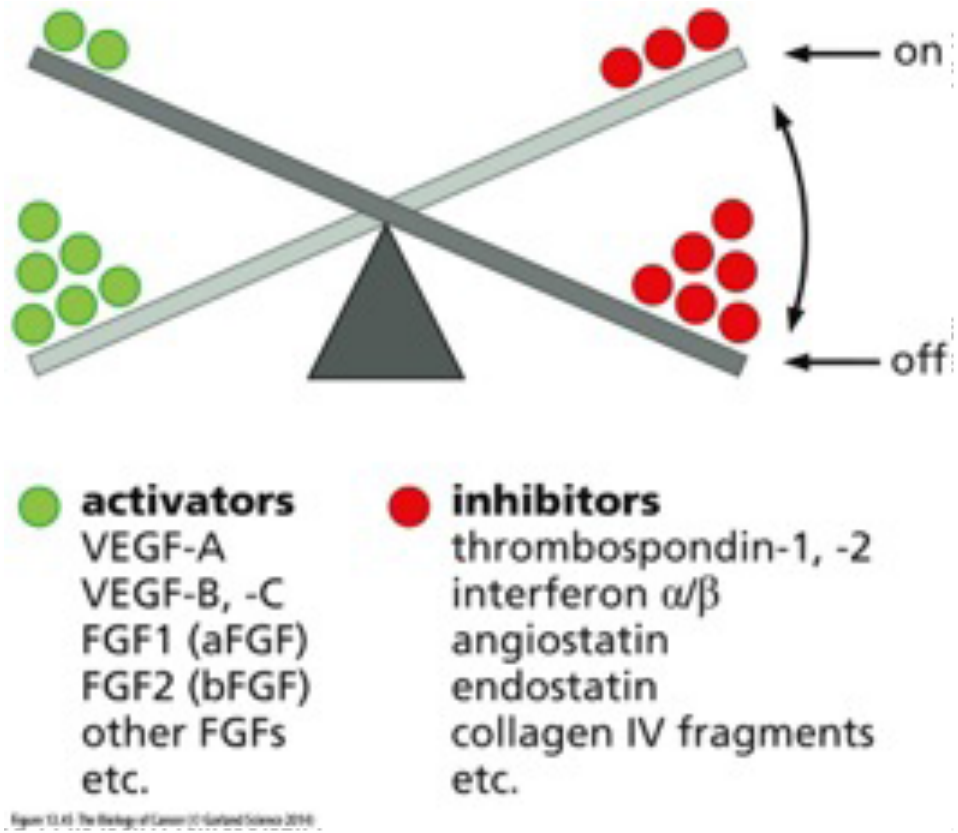
Deficiency:

- Limb ischemia
- Myocardial ischemia

Angiogenic inhibitors:

•During the process of wound healing, the burst of angiogenesis must be shut down once the newly formed capillaries have reached a certain density.





TSP-1 produced by stromal fibroblasts, ECs and immune cells suppresses tumor progression by inhibiting angiogenesis through direct effects on EC migration and survival and through indirect effects on growth factor mobilization.

Inhibitors of Angiogenesis

Angiogenesis Inhibitors

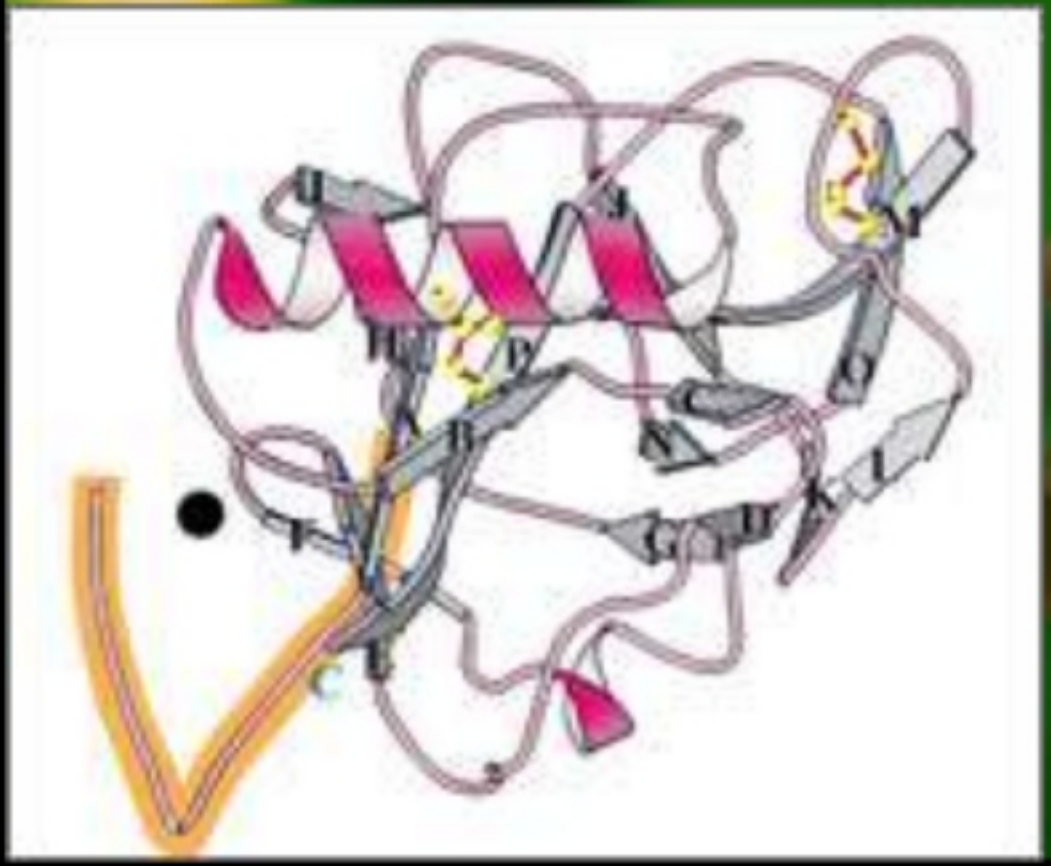
Proteins

- Angiostatin
- Endostatin
- Interferons
- Platelet factor 4
- Prolactin 16Kd fragment
- Thrombospondin
- TIMP-1 (tissue inhibitor of metalloproteinase-1)
- TIMP-2 (tissue inhibitor of metalloproteinase-2)
- TIMP-3 (tissue inhibitor of metalloproteinase-3)

Angiogenesis Inhibitors

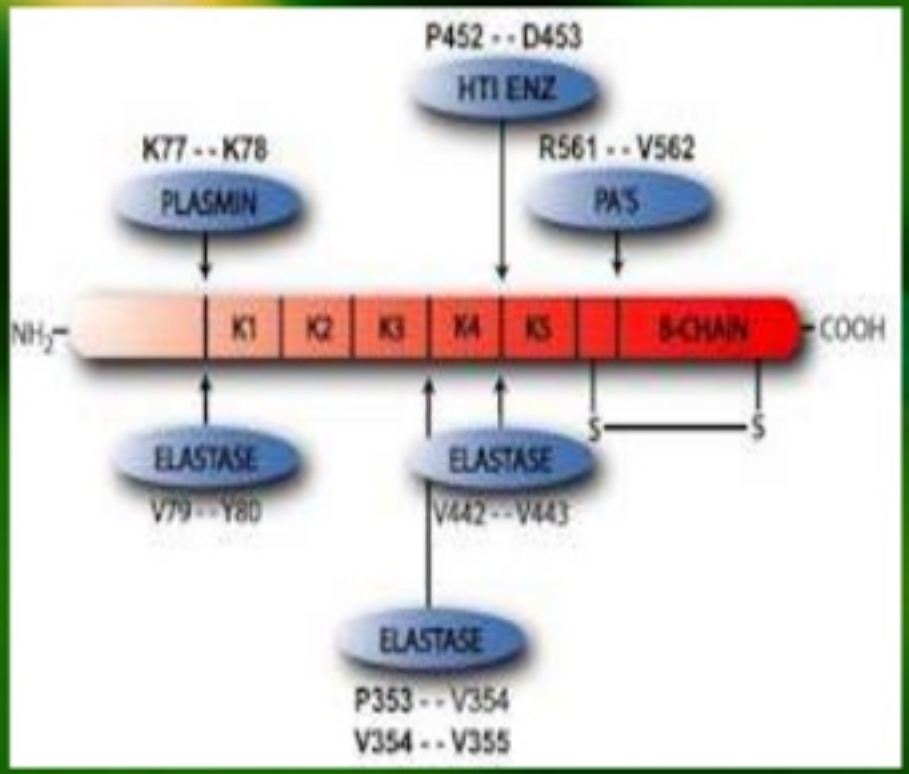
- Other angiogenesis inhibitors have been found in nature - in green tea, soy products, fungi, mushrooms, Chinese cabbage, tree bark, shark tissues, snake venom, red wine, and many other substances.
- Still other angiogenesis inhibitors have been manufactured synthetically in the laboratory.
- Some FDA-approved medicines have also been "re-discovered" to have anti-angiogenic properties.

ENDOSTATIN



- It was first discovered in 1995 in Dr. Folkman's lab
- Phase I clinical studies began at M.D. Anderson November 1999
- A naturally-occurring 20-kDa C-terminal fragment derived from type XVIII collagen.
- Interfere with the pro-angiogenic action of growth factors such as basic fibroblast growth factor (bFGF/FGF-2) and vascular endothelial growth factor (VEGF)

ANGIOSTATIN



- Naturally occurring protein found in several animal species, including humans.
- It is an endogenous angiogenesis inhibitor
- Angiostatin is produced by autoproteolytic cleavage of plasminogen,
- Can be cleaved from plasminogen by different metalloproteinases (MMPs), elastase, prostata-specific antigen (PSA), 13 KD serine protease, or 24KD endopeptidase.

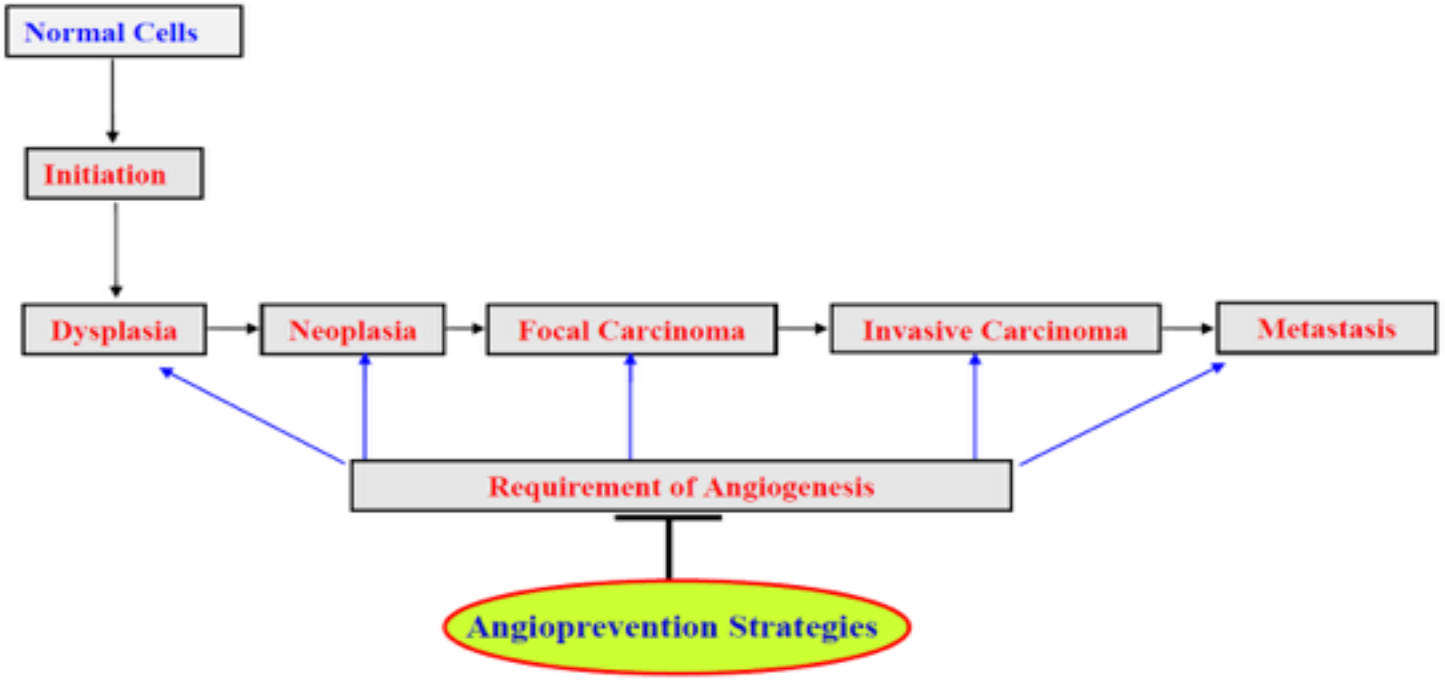
Inhibits endothelial cell migration, proliferation and induces EC apoptosis

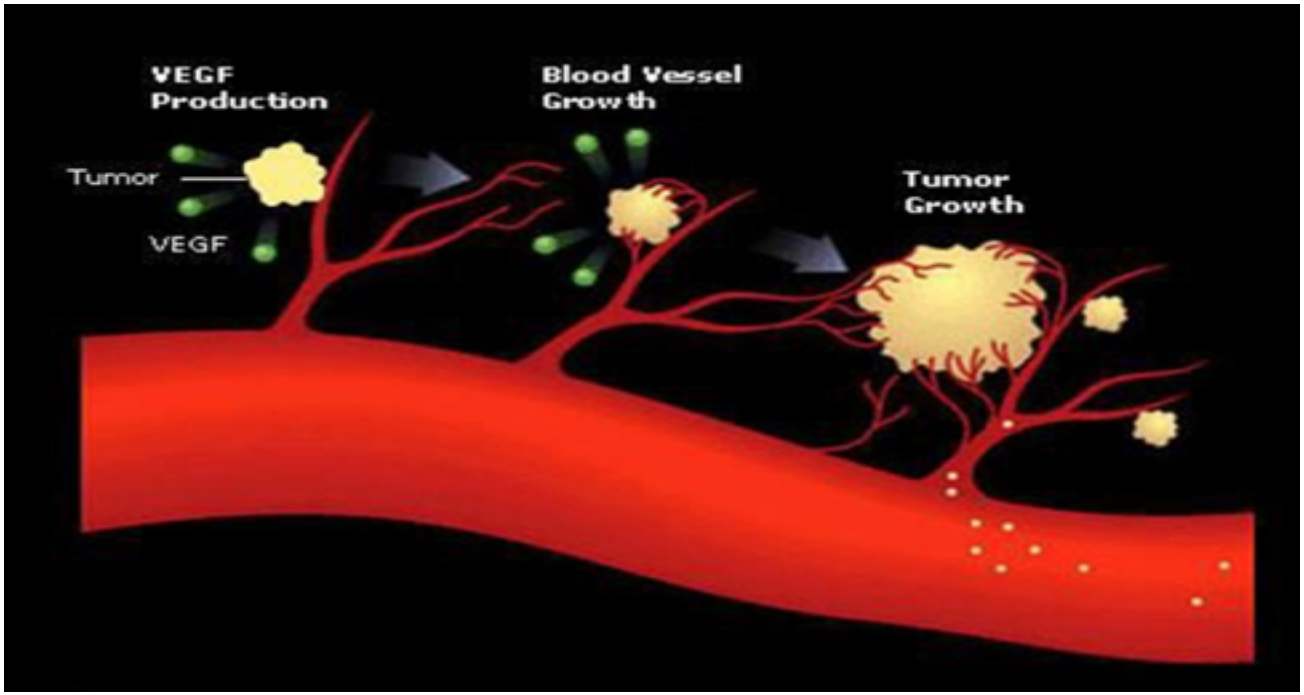
Tumor growth and angiogenesis

Solid tumors can grow in size up to ~1-2 mm diameter by simple diffusion of nutrients and gaseous exchange. However, beyond this size limit they require active supply of such components for tumor growth and progression.

Angiogenic Switch

- Hypoxia
- Stabilization of HIF-alpha
- HR-gene expression (VGF)
- Growth, proliferation, survival and migration of ECs
- Sprouting, tube formation and tumor vasculature formation
- Tumor growth and metastatic progression


Normal Cells
Initiation
Dysplasia
Neoplasia
Focal Carcinoma
Invasive Carcinoma
Metastasis
Requirement of Angiogenesis
Angioprevention Strategies


VEGF Production
Blood Vessel Growth
Tumor
VEGF
Tumor Growth

Features of tumor angiogenesis

- Extreme and chaotic expression of angiogenic factors

↓

- Disorganized vascular structure Low adhesion and pericyte coverage

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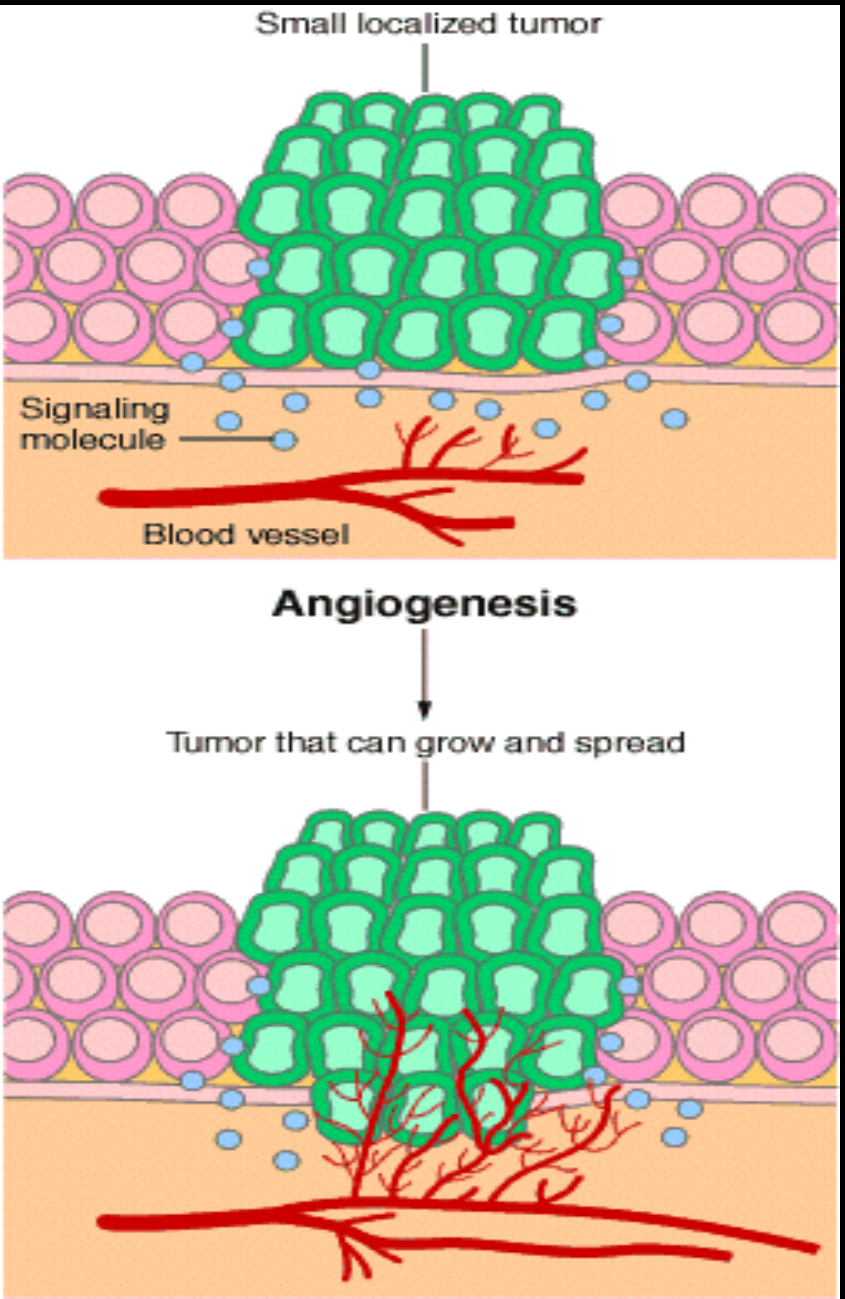
- **Hypoxic stress, metabolic changes, cancer cell intravasation and less effect of chemotherapy**

What Is Tumor Angiogenesis?



Tumor angiogenesis

Proliferation of a network of blood vessels that penetrates into cancerous growths.

Function

Supplying nutrients and oxygen and removing waste products.

Mechanism

Cance cells releas molecules that send signals to surrounding normal host tissue.

This signaling activates certain genes in the host tissue that, in turn, make proteins to encourage growth of new blood vessels.

Stroma contributes to tumor angiogenesis

Tumor Microenvironment (Tumor-associated stroma): induced by cytokines and chemokines secreted from tumor cells

- Macrophage: Tumor-−Associated Macrophages (TAMs)
- Fibroblast: Carcinoma-−Associated Fibroblasts (CAFs)
- Myeloid cell: Bone Marrow Derived Cells (BMDCs)
- Extracellular matrix (ECM)

Tumor microenvironment complexity and degree of infiltration of various components correlates with the tumor angiogenesis and invasiveness

Macrophage and tumor angiogenesis

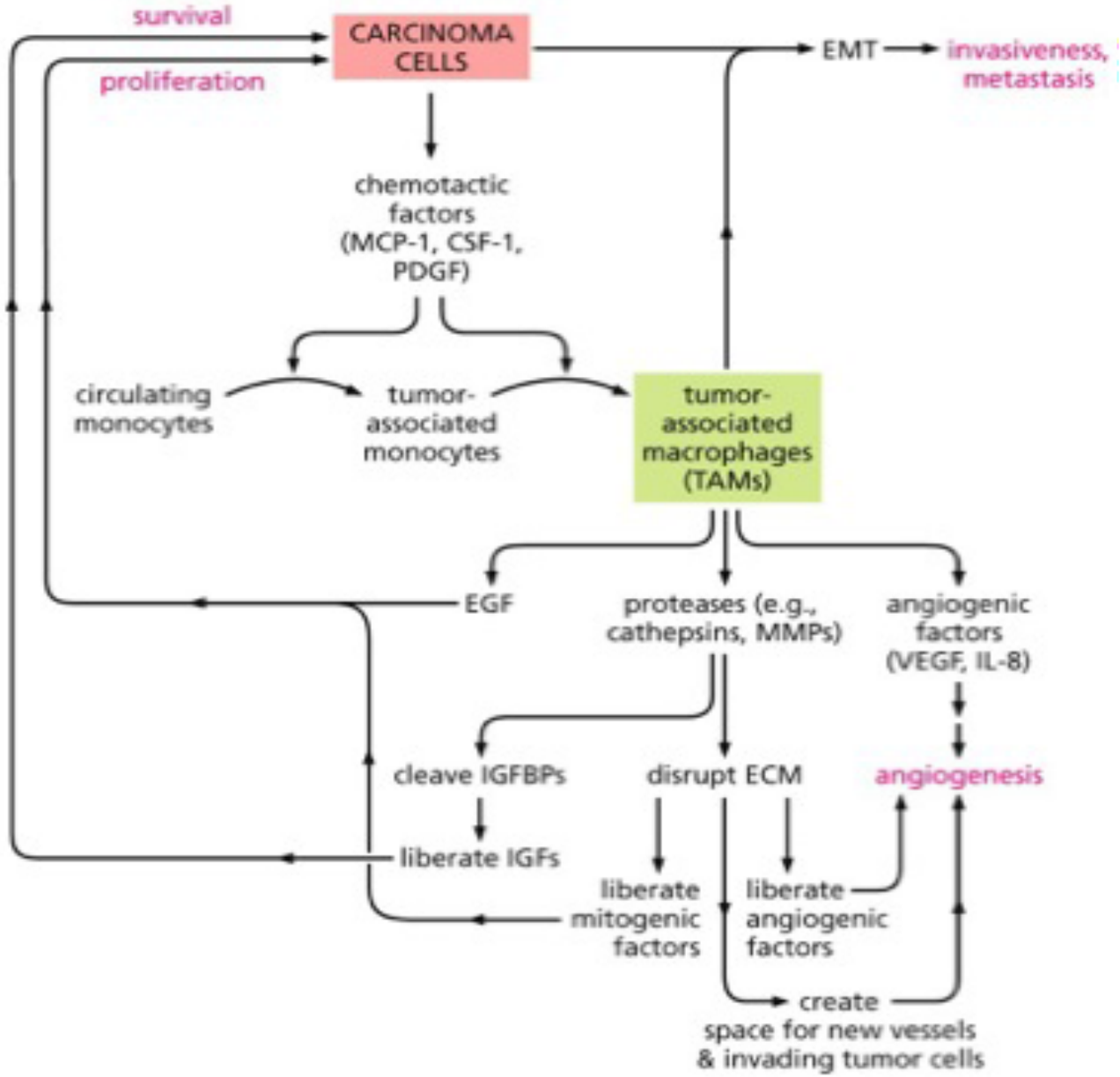
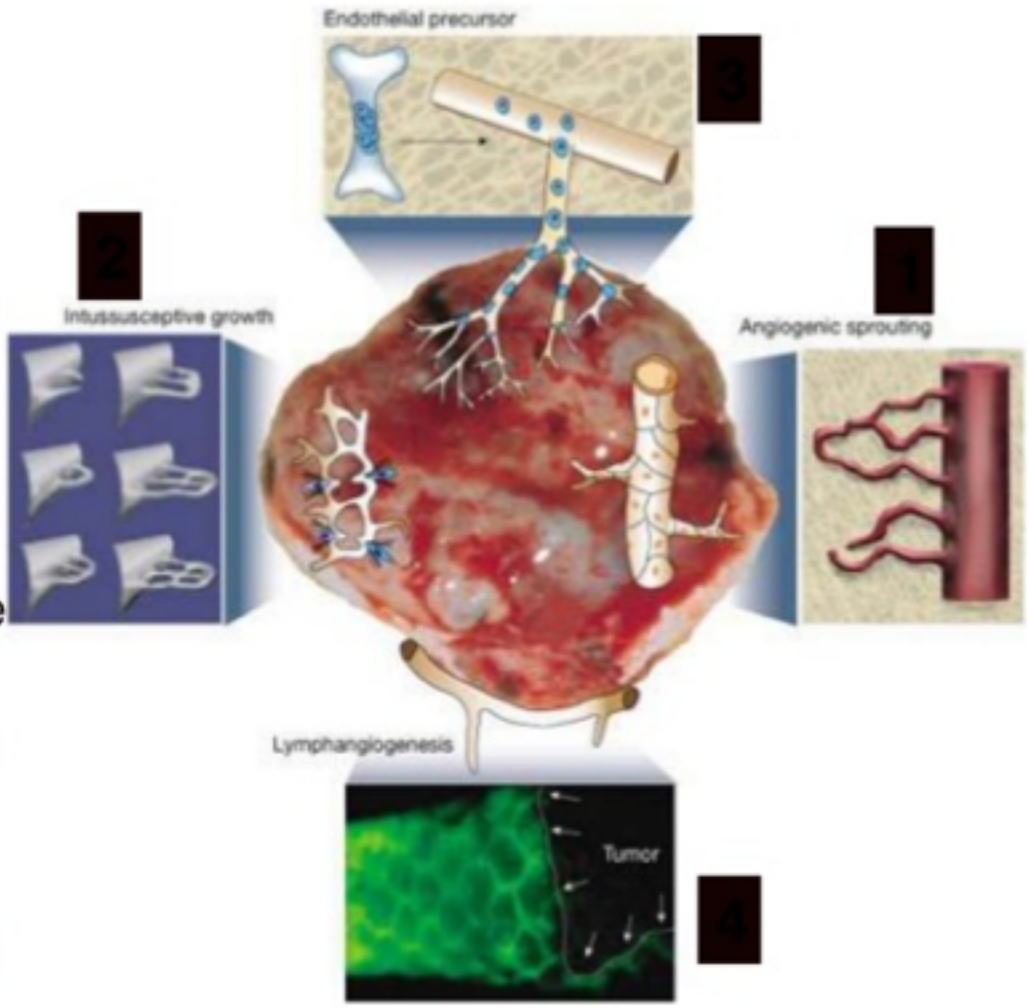


Figure 13.25e The Biology of Cancer (© Garland Science 2014)

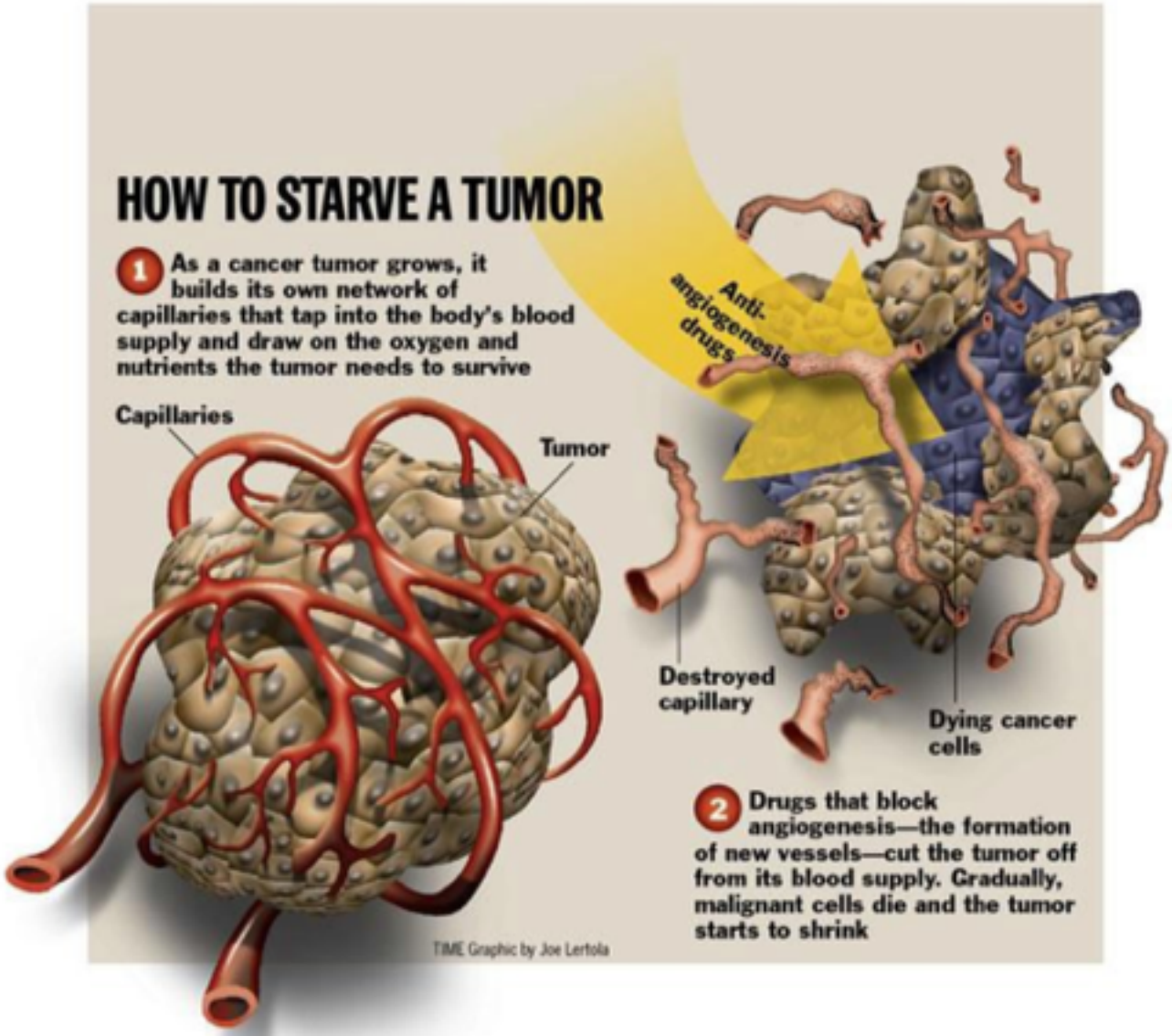
Cellular mechanisms of tumour angiogenesis

1. host vascular network expands by budding of endothelial sprouts or formation of bridges (angiogenesis);
2. tumour vessels remodel and expand by the insertion of interstitial tissue columns into the lumen of pre-existing vessels (intussusception); and
3. endothelial cell precursors (angioblasts) home from the bone marrow or peripheral blood into tumours and contribute to the endothelial lining of tumour vessels (vasculogenesis)
4. Lymphatic vessels around tumours drain the interstitial fluid and provide a gateway for metastasizing tumour cells.

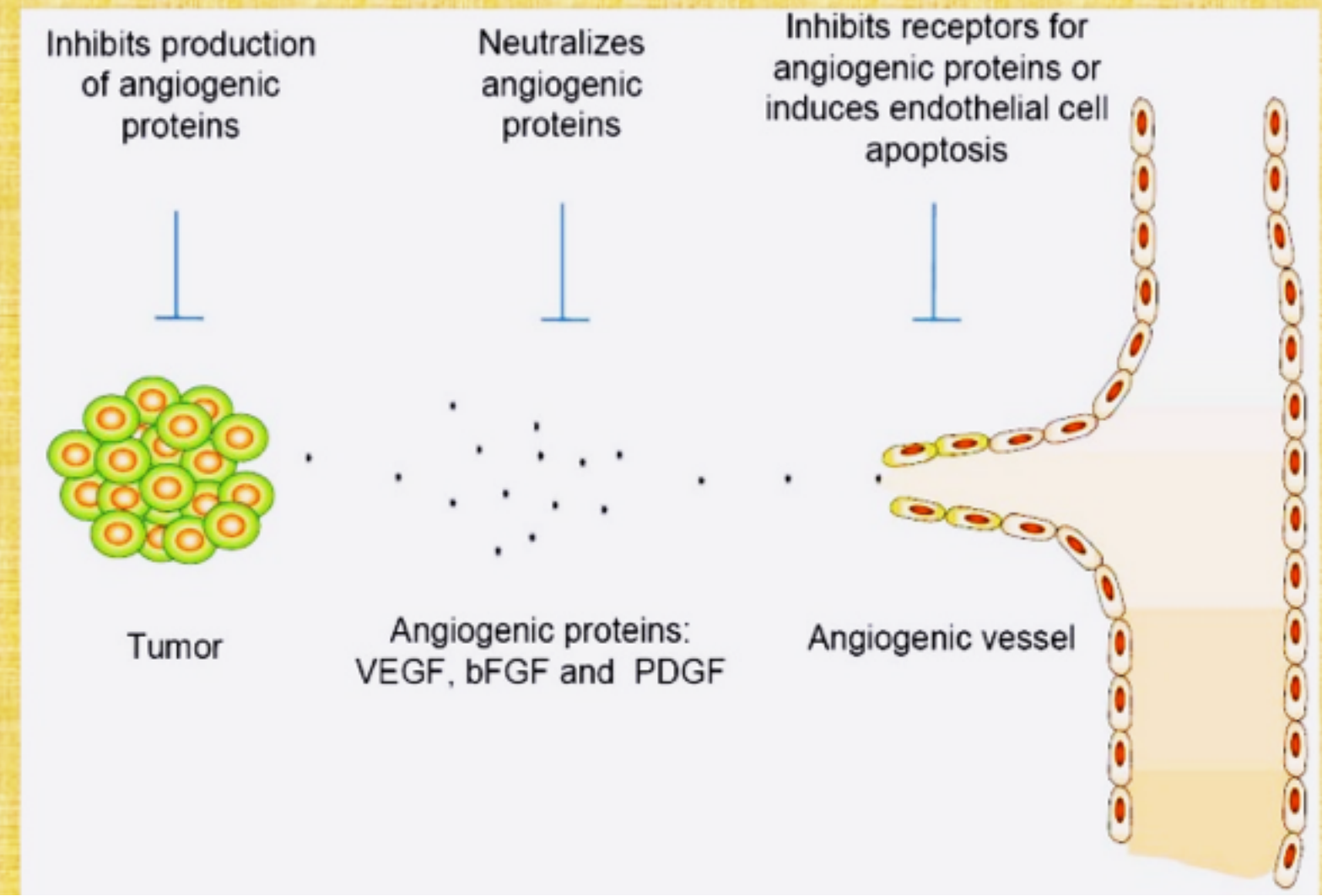


Anti-angiogenic therapy

Dr. Judah Folkman proposed the concept of anti-angiogenic therapy (NEJM.1971).



Strategies for inhibition of tumor growth by anti-angiogenic drugs



Antiangiogenic Therapies: Potential Targets

- Block pro-angiogenic molecules (e.g., VEGF)
- Add anti-angiogenic regulators (e.g. angiostatin, endostatin, TSP-1)
- Inhibit stroma-degrading enzymes (e.g., MMPIs)
- Target vascular antigens (e.g., avb3 integrin)
- Attack pericytes

Current Angiogenic Inhibitors in Clinical Use and Clinical Trials

- Bevacizumab (Avastin™)
- Sunitinib (Sutent™)
- Sorafenib (Nexavar™)
- Cederanib (Recentin™ - AZD- 2171)
- Cilengitide
- VEGF-Trap

Many others in development

"Avastin Bevacizumab- Reach Beyond Convention"

- Recombinant, humanized monoclonal antibody that binds to all isoforms of VEGF-A such that KDR signaling is inhibited
- Developed by Genentech BioOncology
- Not a chemotherapy drug: "Targeted Therapy"

Limitations of Anti-angiogenic therapy

- Resistance: expression other angiogenic factors such as bFGF and PDGF
- Toxicity and dosage (off target effects)
- HIF-can induce EMT and promote invasiveness via cytokine expression
- Normalize disorganized tumor blood vessels
- Side effects (high blood pressure, bleeding and coronary artery disease, etc)

Boxed WARNINGS and Additional Important Safety Information

- Gastrointestinal (GI) perforation
- Wound healing complication
- Hemorrhage
- Neutropenia

Metastasis

When does metastasis begin?

Commitment to the metastatic phenotype:

- How early does it occur?
- Can it be reversed?

Progenitor lesions:

- What are the key progenitor lesions?
- What is the efficiency of transition to invasion?
- Are all metastasis precursors clonal?

What is the role of the host?

- Under what conditions does the host drive or suppress the process?
- Does the transition from pre-invasive to invasive lesions require host participation?
- If so what are the molecular and cellular players that are functionally important?
- The circuitry of the tumor host communication may be the key to prevention of invasion.

Physiologic basis of metastasis

- Is metastasis a normal physiologic program which is disregulated or inappropriately activated?
- Does a physiologic motility and invasion program exist for development, angiogenesis morphogenesis and wound healing?
- Is metastasis colony formation a natural ongoing process conducted by stem cells?

What is the driving force?

- Is the metastatic phenotype pre determined within the primary tumor? Within the host microenvironment?
- Are malignant cells a product of adaptation and selection?
- What is the selection factor? If malignant cells are survival of the fittest, then what is the fitness test?
- Is cell survival in a foreign (non home) tissue the ultimate selection factor?

Metastasis Pre-1900

Hippocrates (460–375 B.C.)
Galen (131–201 A.D.)

Pre-1700: The Greek physician Hippocrates coined "carcinoma" from *karkinos*, the word for crab.

Pre-cellular theory of invasion and metastasis: recognition of malignant tumors and localized versus metastatic disease

LeDran 1757: Noted that malignant tumors begin as localized disease, then spread to regional lymph nodes and then enter the circulation to subsequently appear in the lung

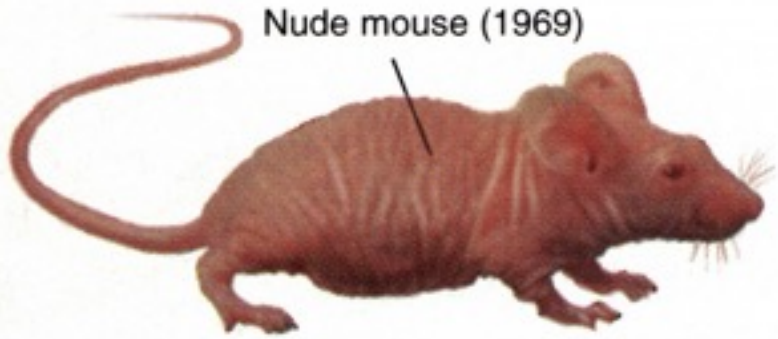
Bichat 1801: Tumors contain both parenchyma and stroma

Recamier 1829 : Used the term "Metastases"

Validation of the cellular theory of cancer metastasis

Takahashi: (1915)

1900–1949: Takahashi found that the cells of various mouse carcinomas and sarcomas produce reproducible patterns of metastases when injected into other mice.



1950–1969: Rygaard and Povlsen showed in 1969 that human tumors can grow in nude mice, which lack a thymus and are T cell deficient. This experimental animal model of human cancer continues to be refined and used today.

Paget
(1889)

1700–1899: Paget proposed that metastases form specifically in organs that are "soil" to a metastatic cell's "seed."

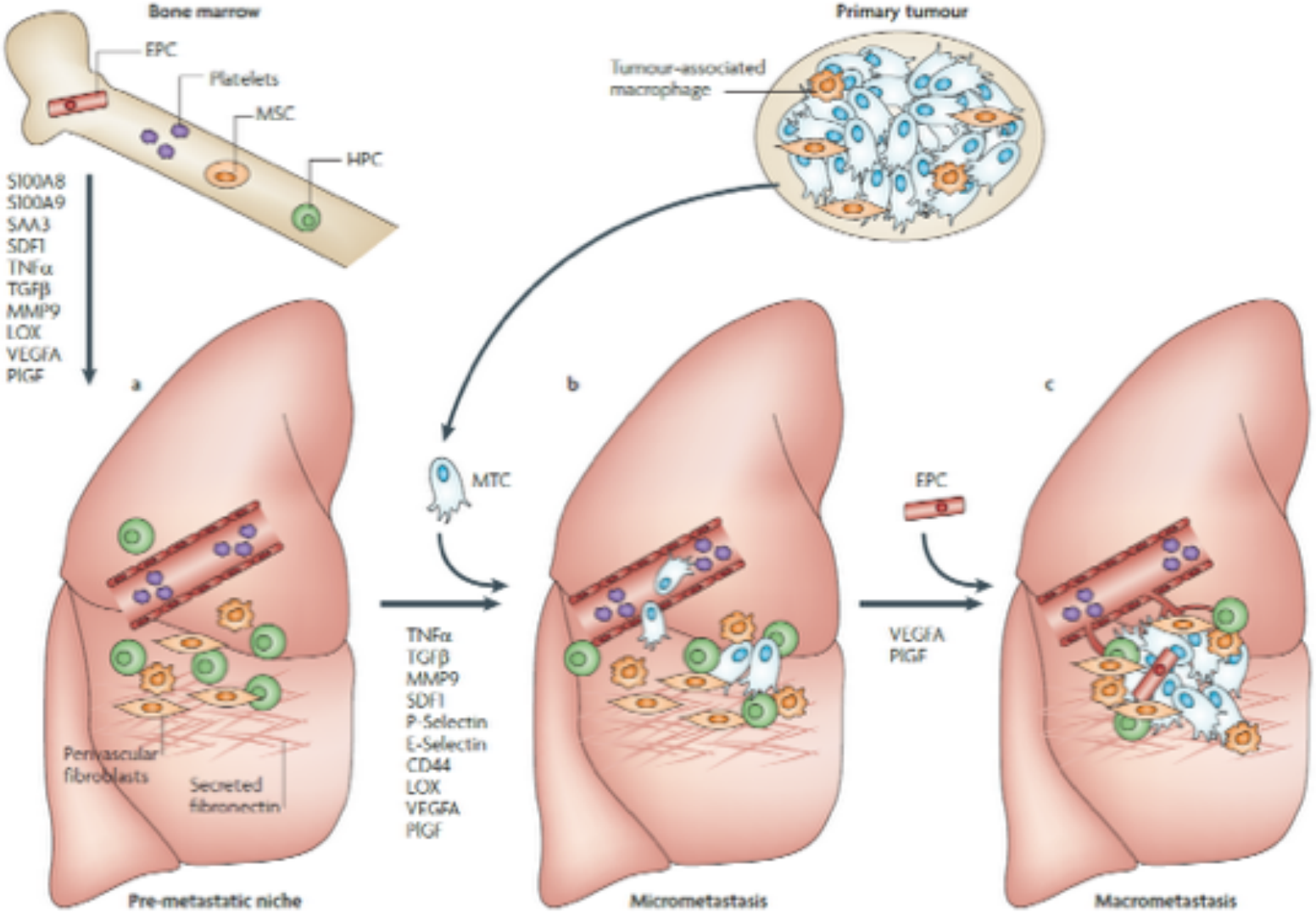
The organ pattern of metastasis is characteristic of the tumor type and tissue of origin. 50-70% of the metastatic pattern can be predicted by the venous drainage blood flow. The remaining 30-50 % may be caused by specific molecular homing mechanisms.

Potential molecular mechanisms:

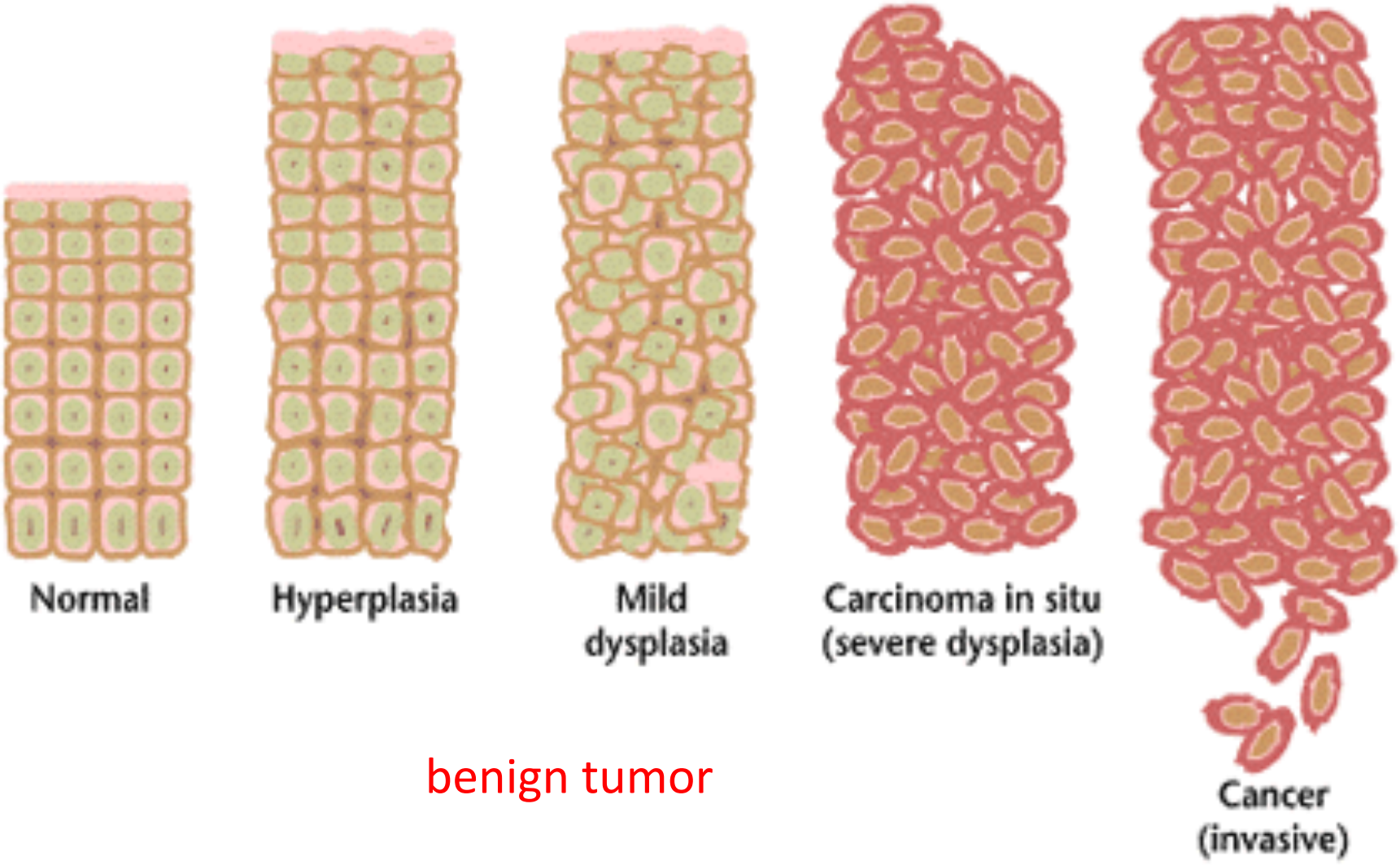
a) **Preferential adhesion in the vessels of the target organ**
b) **Selective extravasation**
c) **Organ attractants**
d) **Organ specific survival and growth**

Pre-metastatic niche formation

Something secreted from primary tumor and changing the behavior of host tissue at distant sites



Cancer develops through gradual changes in cell morphology and properties



benign tumor

malignant tumor

Where do they go?

Metastatic tropism

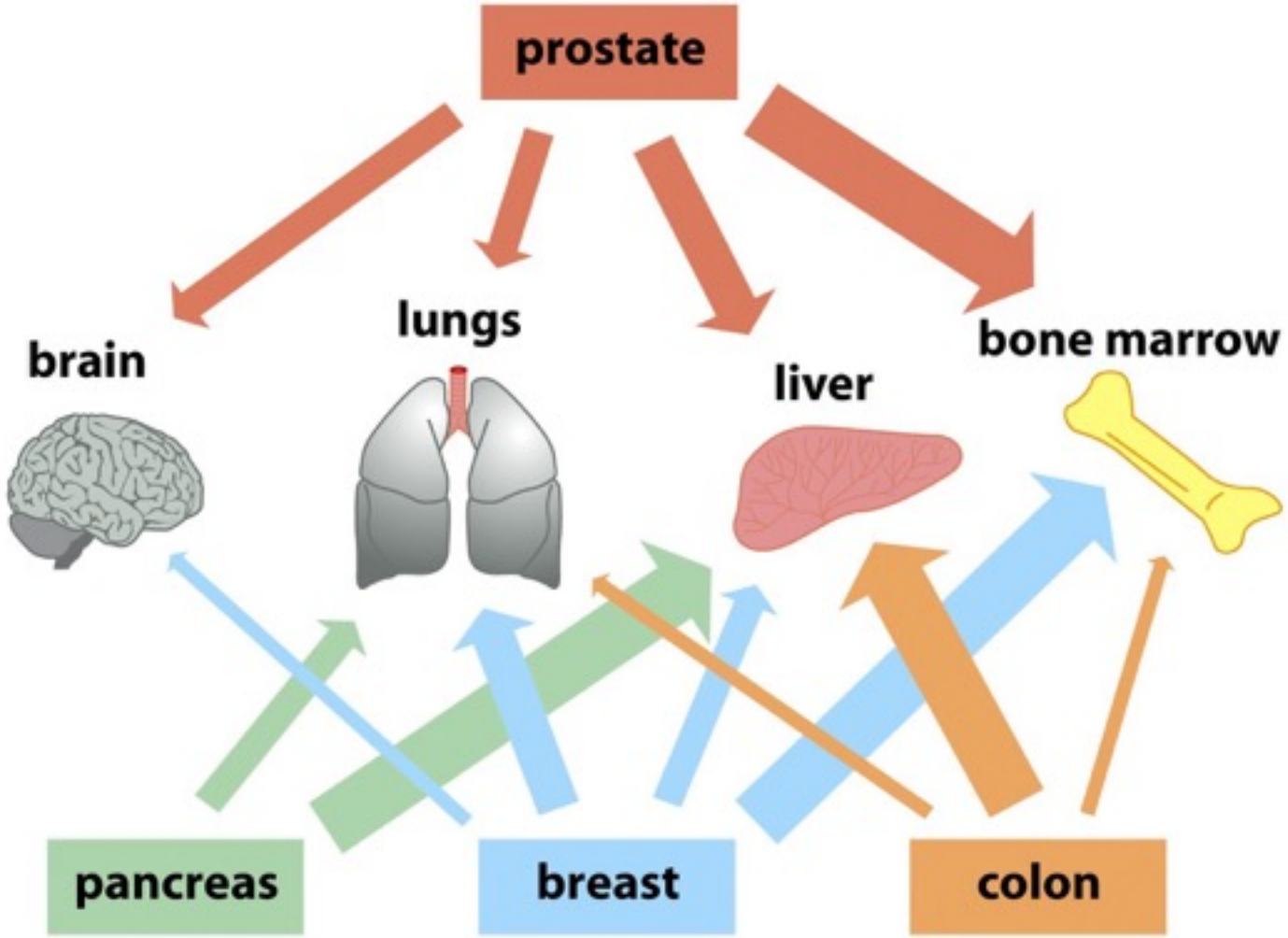


Figure 14.42 *The Biology of Cancer* (© Garland Science 2007)

Metastasis

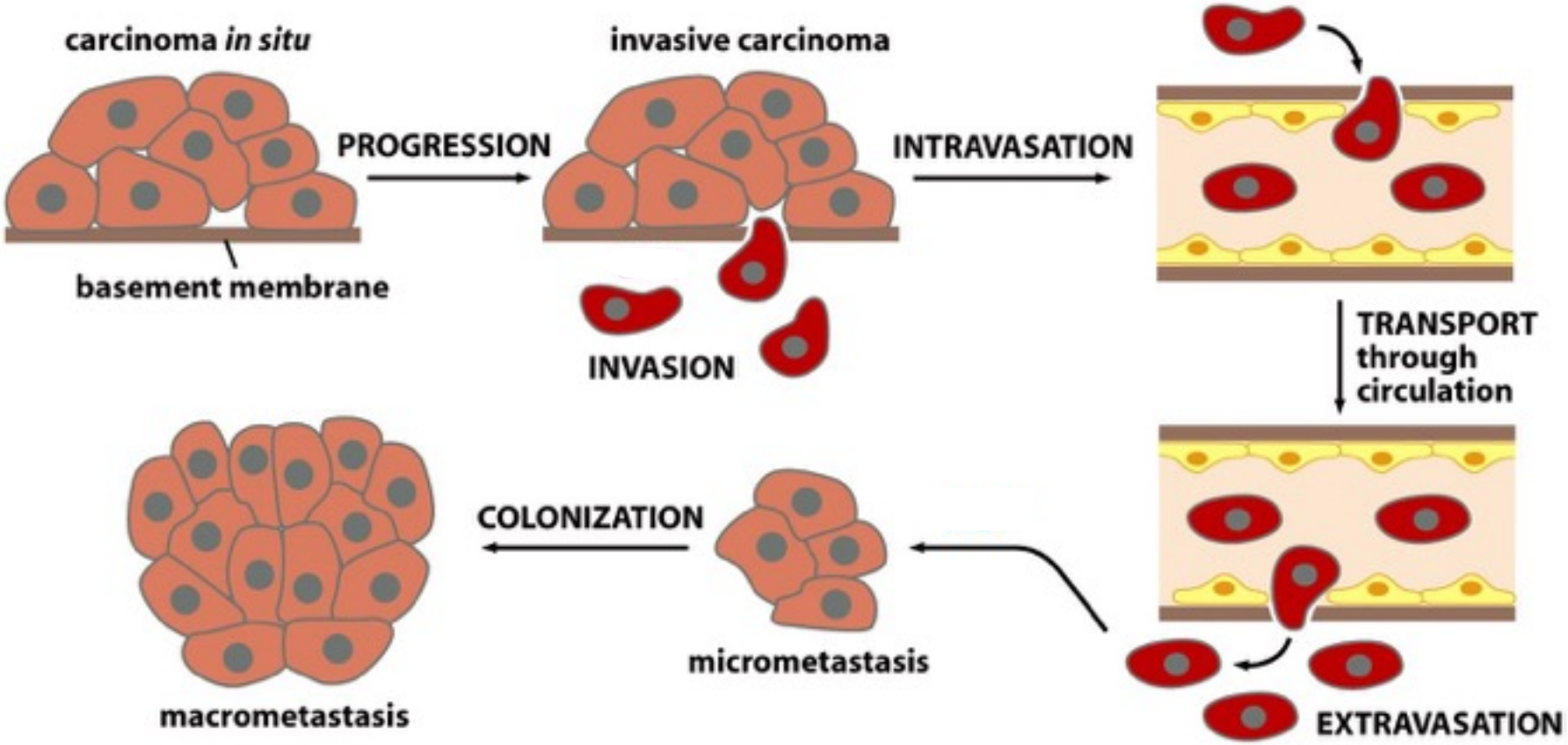
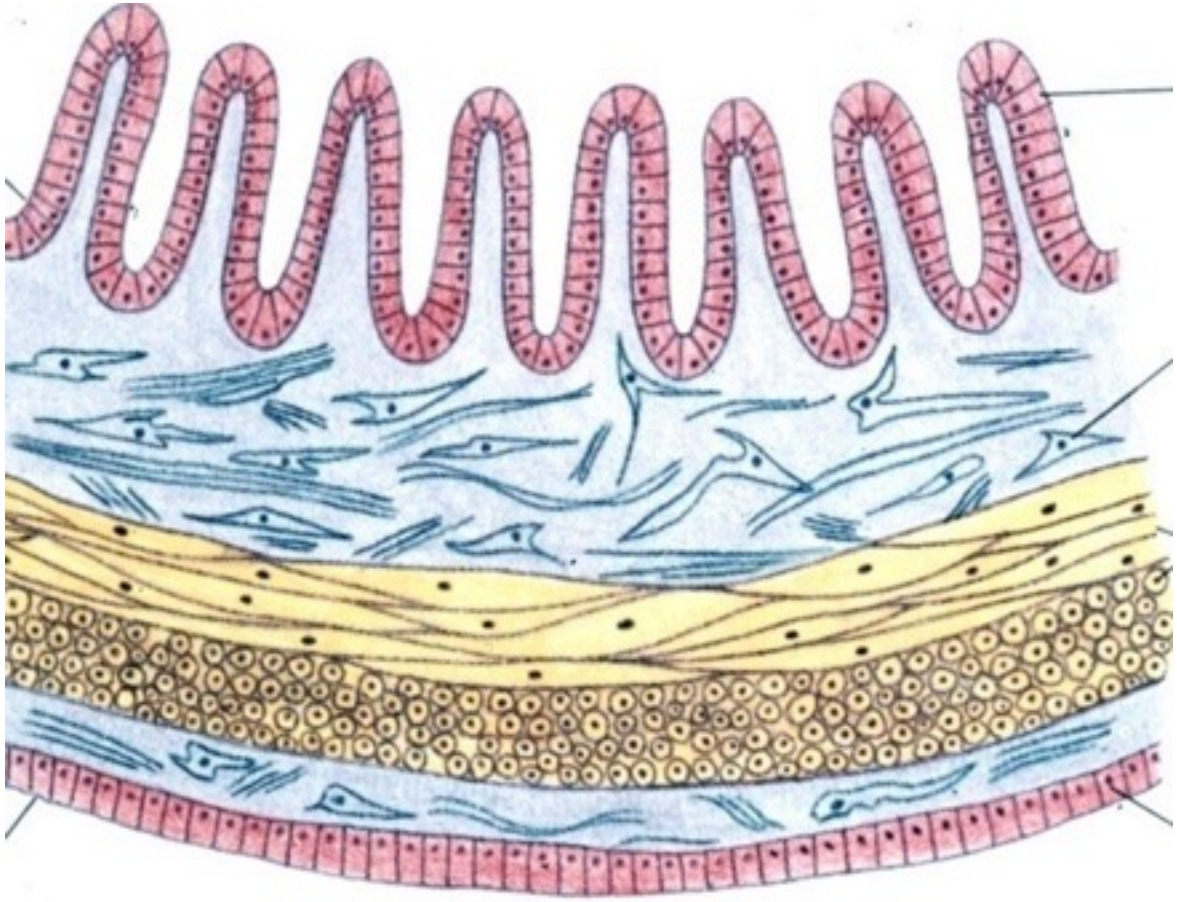


Figure 14.17b *The Biology of Cancer* (© Garland Science 2007)

An organ is composed of several tissues

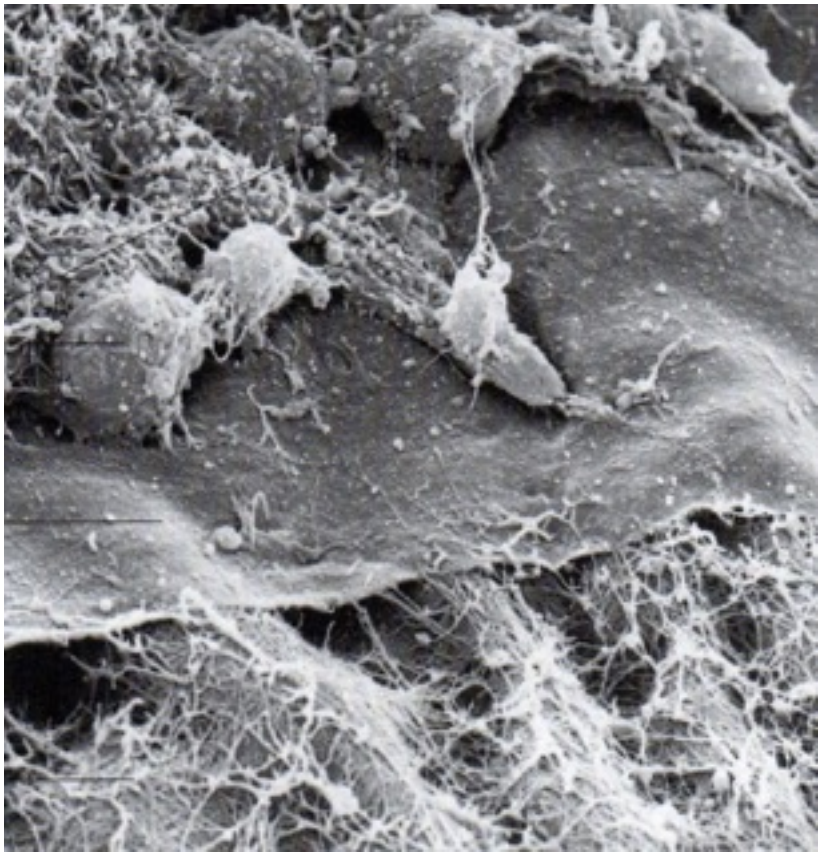


Epithelial cells

Connective tissue

Muscle tissue

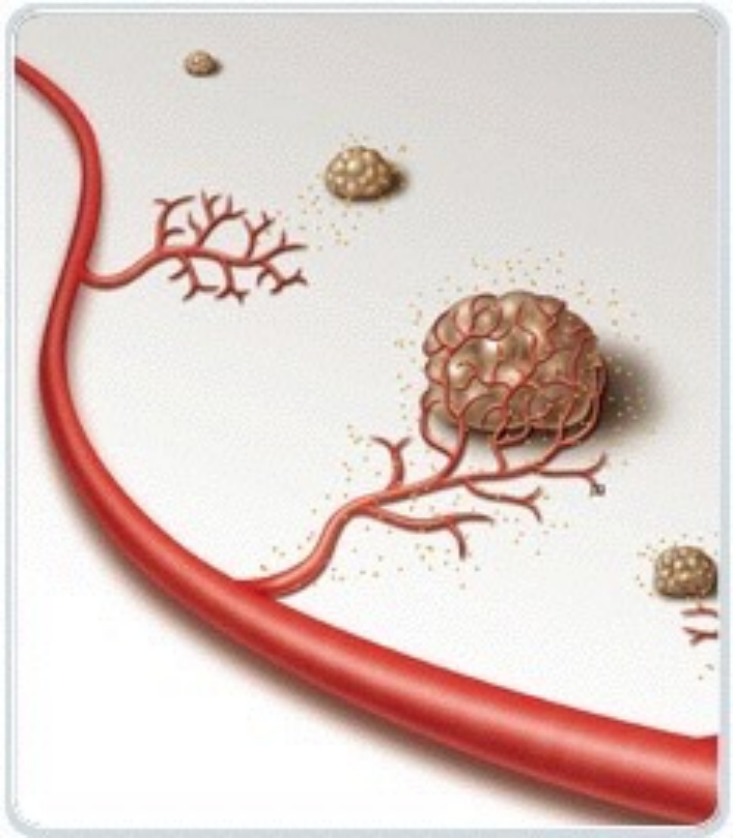
Cancer cells need to change their epithelial properties, to lose their adhesion and to penetrate through potent physical barriers

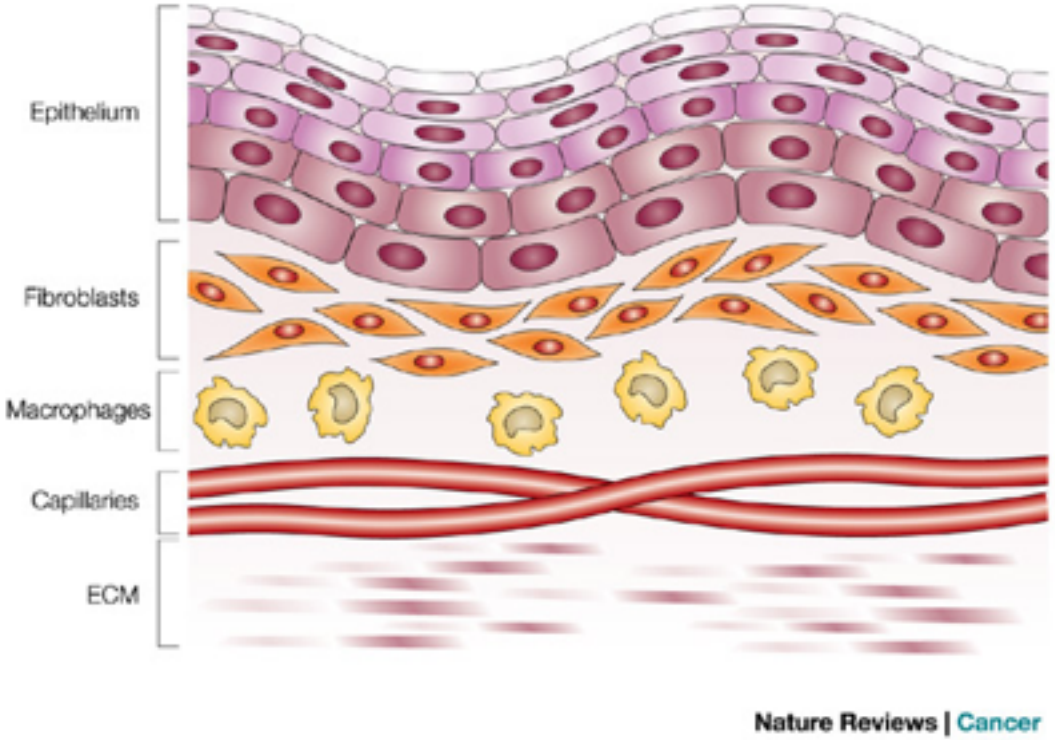


basal lamina

connective tissue

Intravasation





Once lodged in the blood vessels of various tissues, cancer cells must escape from the lumina of these vessels and penetrate into the surrounding tissue- the step termed **extravasation.**

Platelet-mediated tumor cell extravasation

Formation of microthrombus (attachment of platelets) and Proliferation in the lumen of the capillary

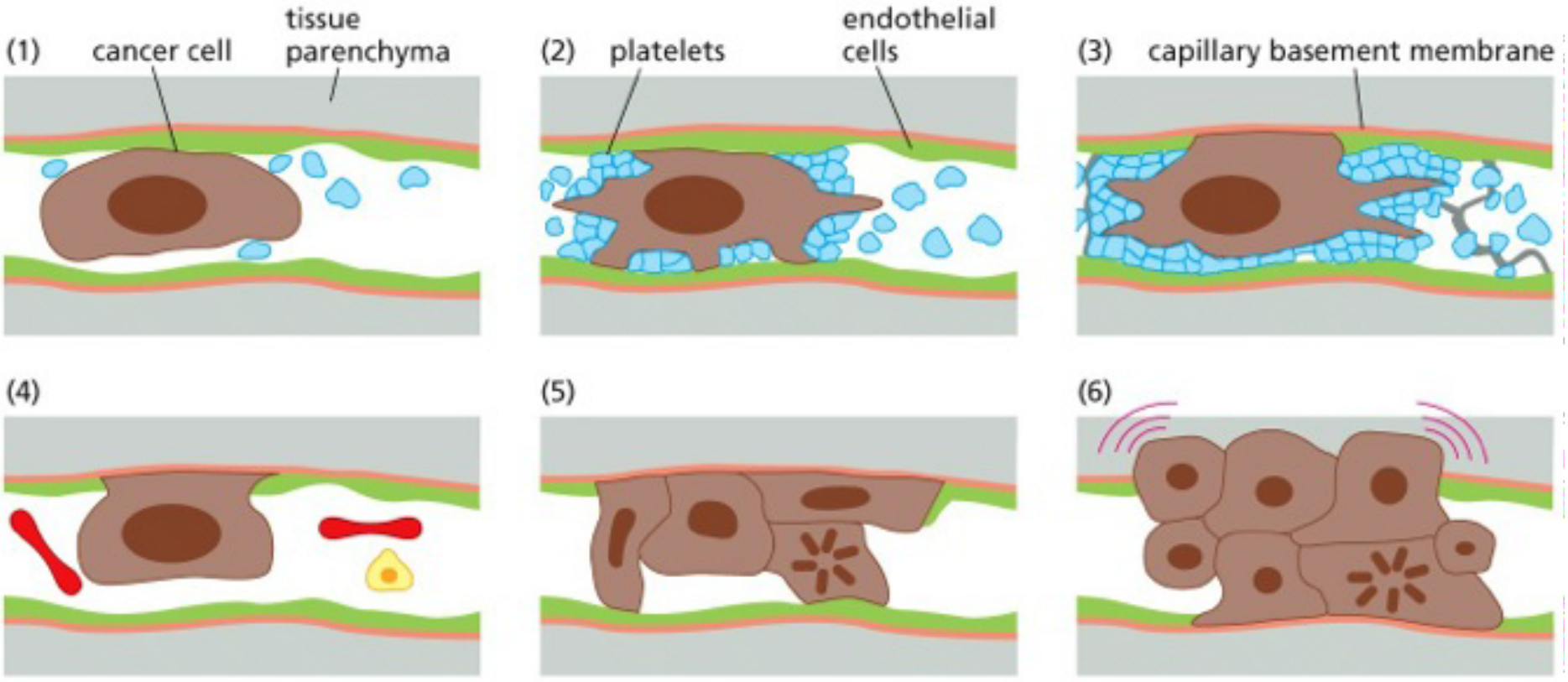
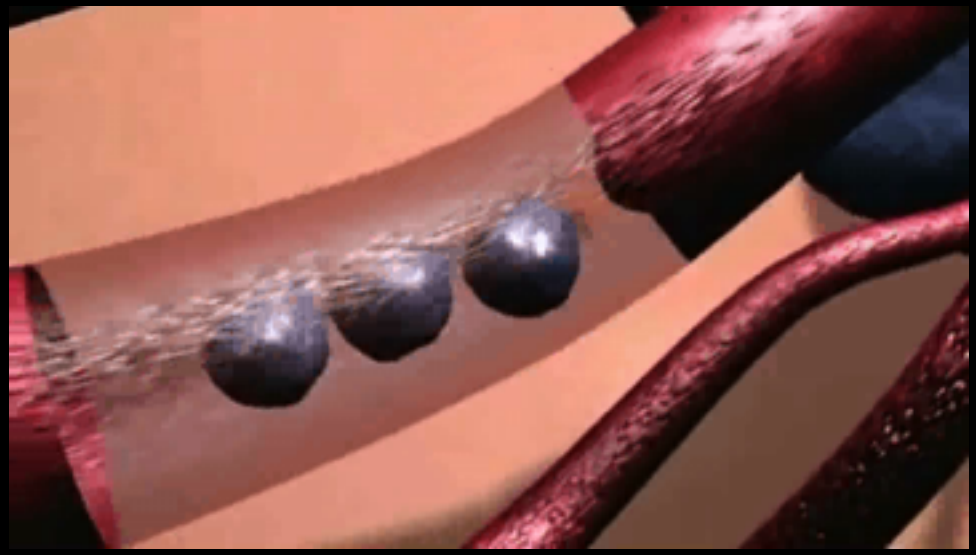
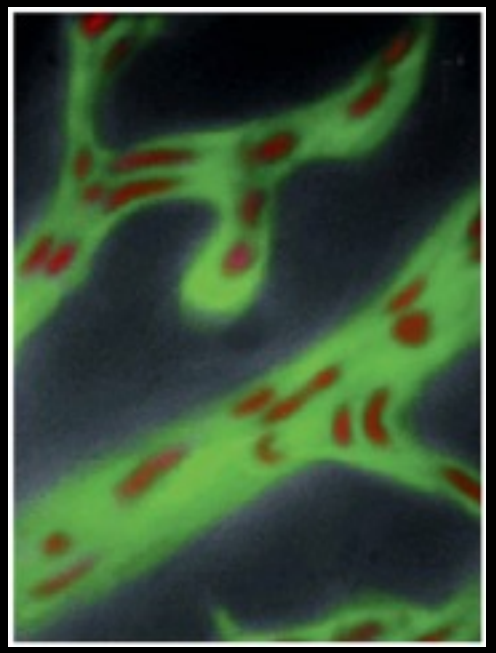


Figure 14.9d The Biology of Cancer (© Garland Science 2014)

The blood: a hostile environment

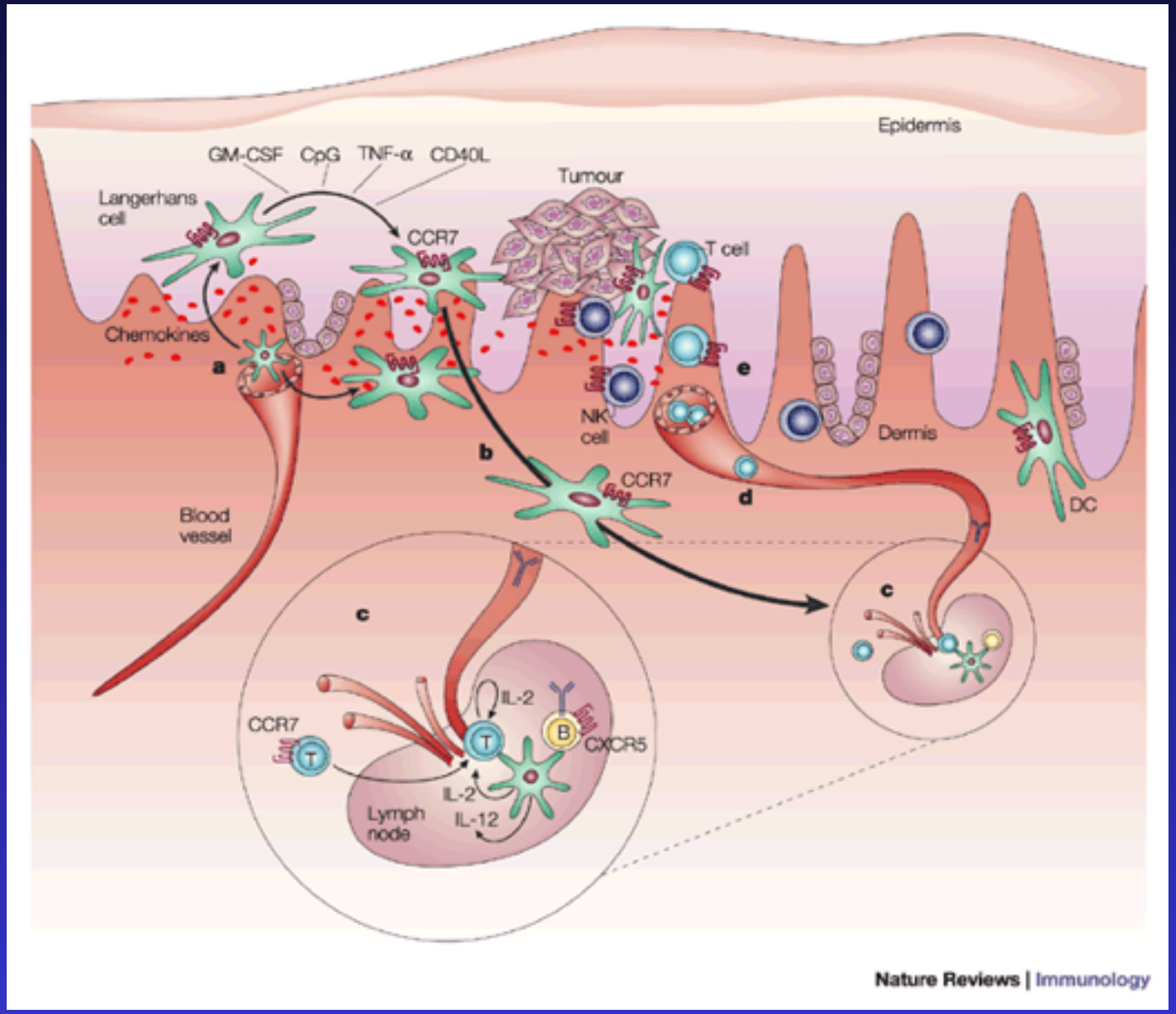
- Cells are normally anchorage-dependent (anoikis)
- Shear forces tear cells apart



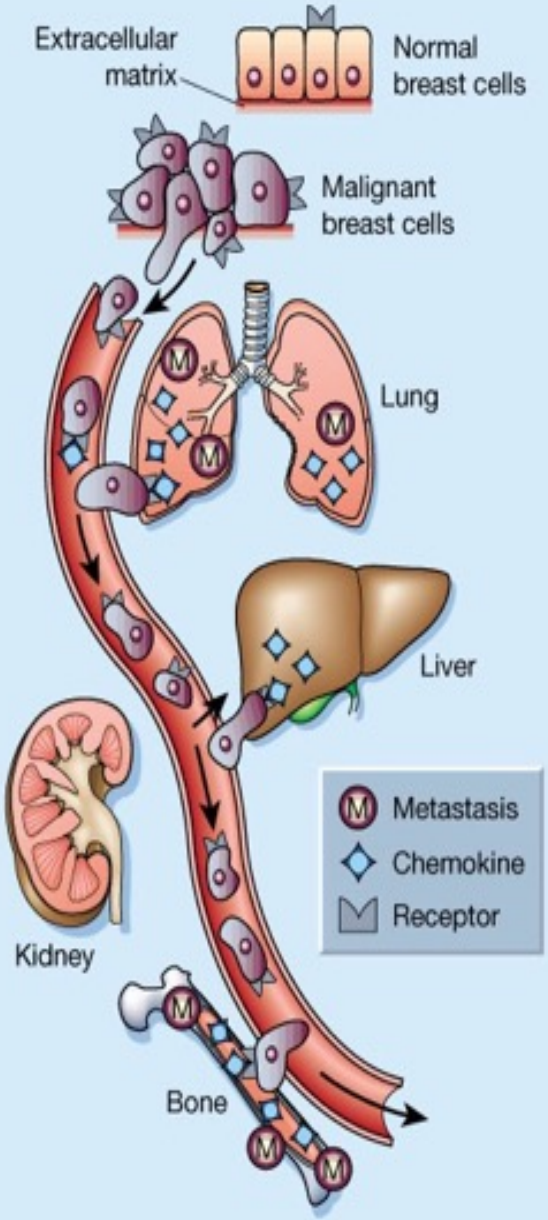


http://www.cancerquest.org/

Chemokines regulate leukocyte recirculation and trafficking to sites of inflammation and infection



Premise: Metastasis homing is dictated by relative abundance of chemokines and cognate receptors on the tumor cell.



Why do the tumor cells express the chemokine receptors in the primary tumor prior to dissemination?

Therapeutic utility is limited because dissemination has already occurred at the time of diagnosis

Colonization

First, micrometasteses

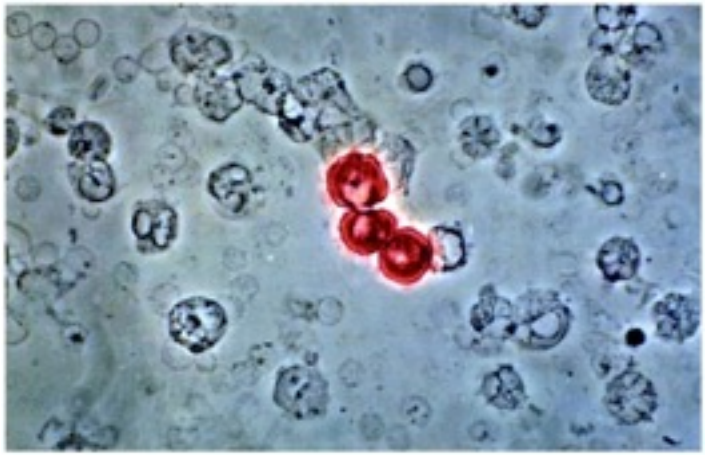
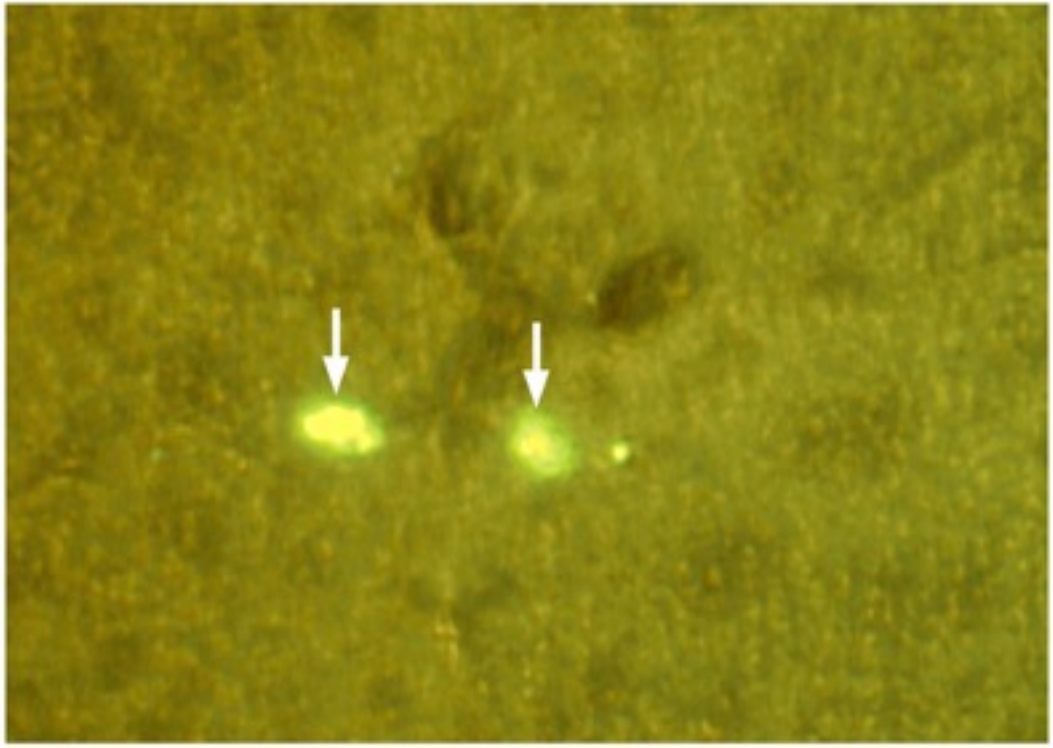


Figure 14.10a *The Biology of Cancer* (© Garland Science 2007)

Dormant micrometasteses are viable



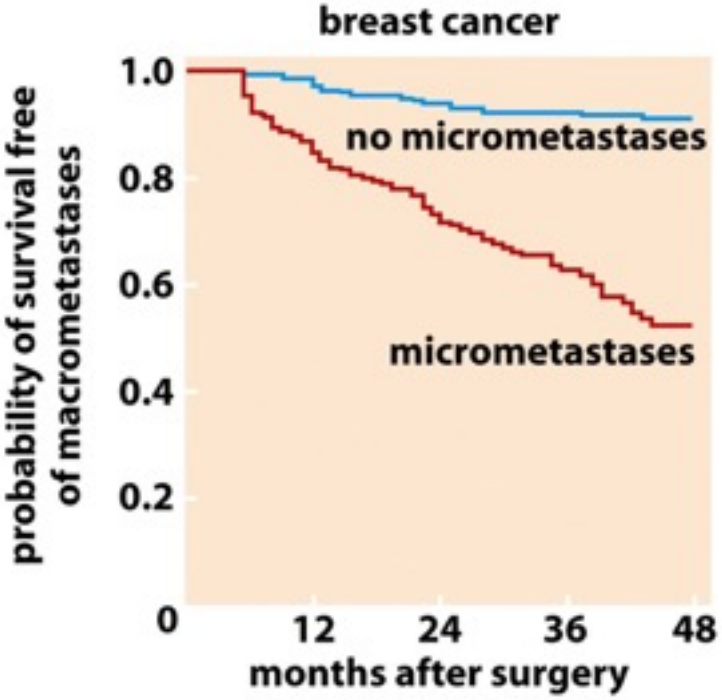
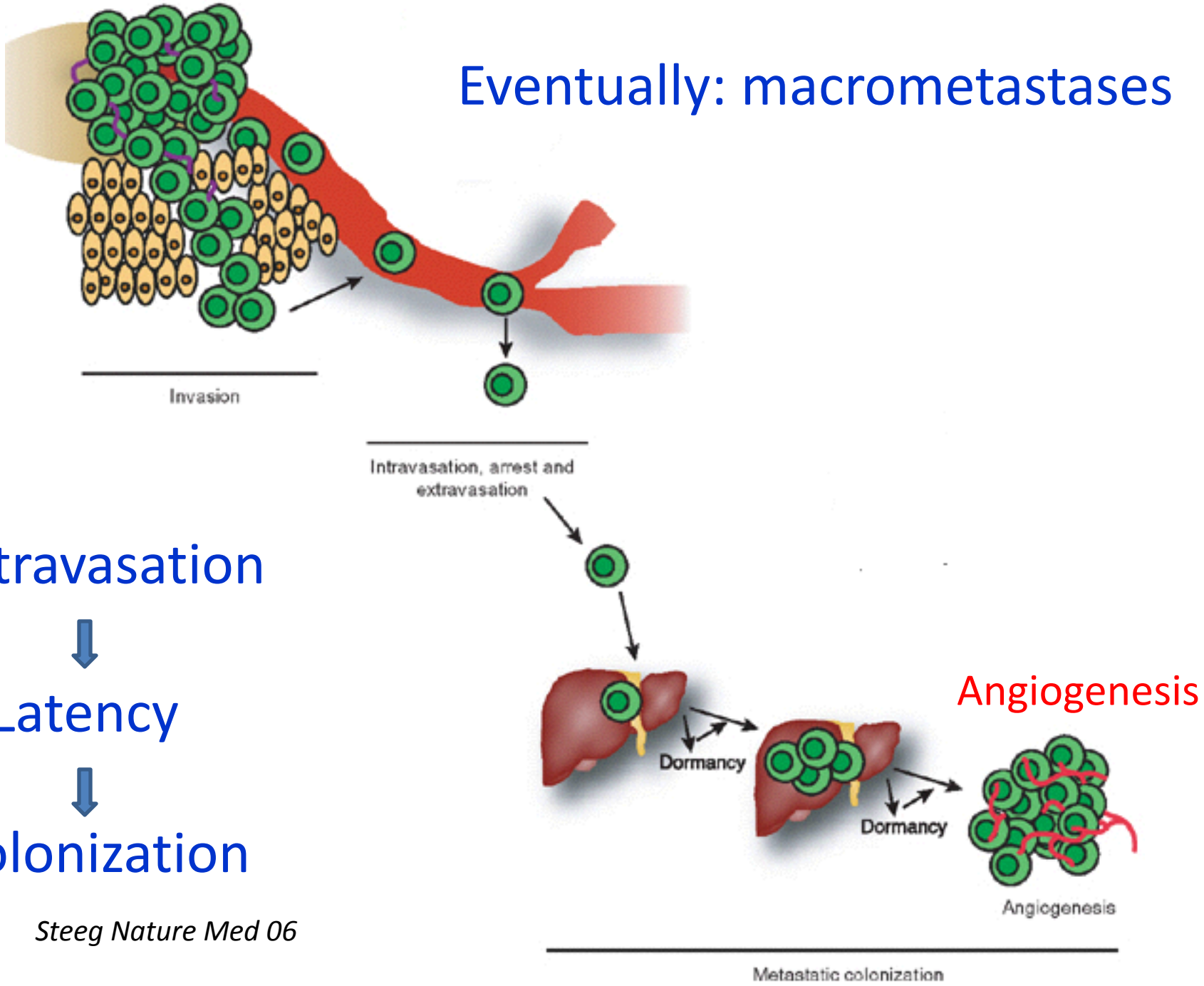


Figure 14.12 *The Biology of Cancer* (© Garland Science 2007)

Figure 14.50a *The Biology of Cancer* (© Garland Science 2007)

Eventually: macrometastases



Intravasation

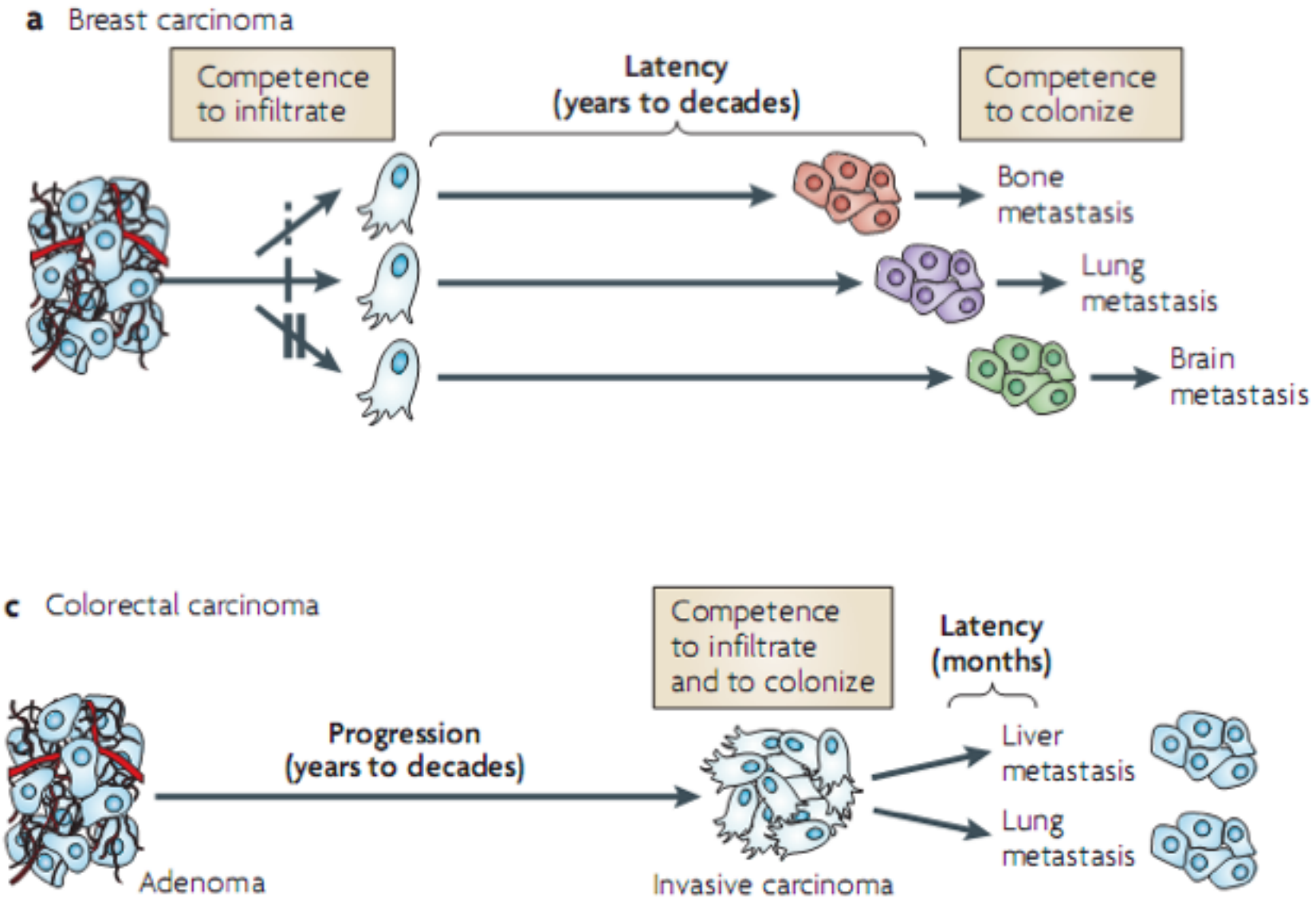
⬇

Latency

⬇

Colonization

Steeg Nature Med 06



Nguyen, Nature Rev. Cancer 2009

Metastatic inefficiency

A sequence of inefficient steps

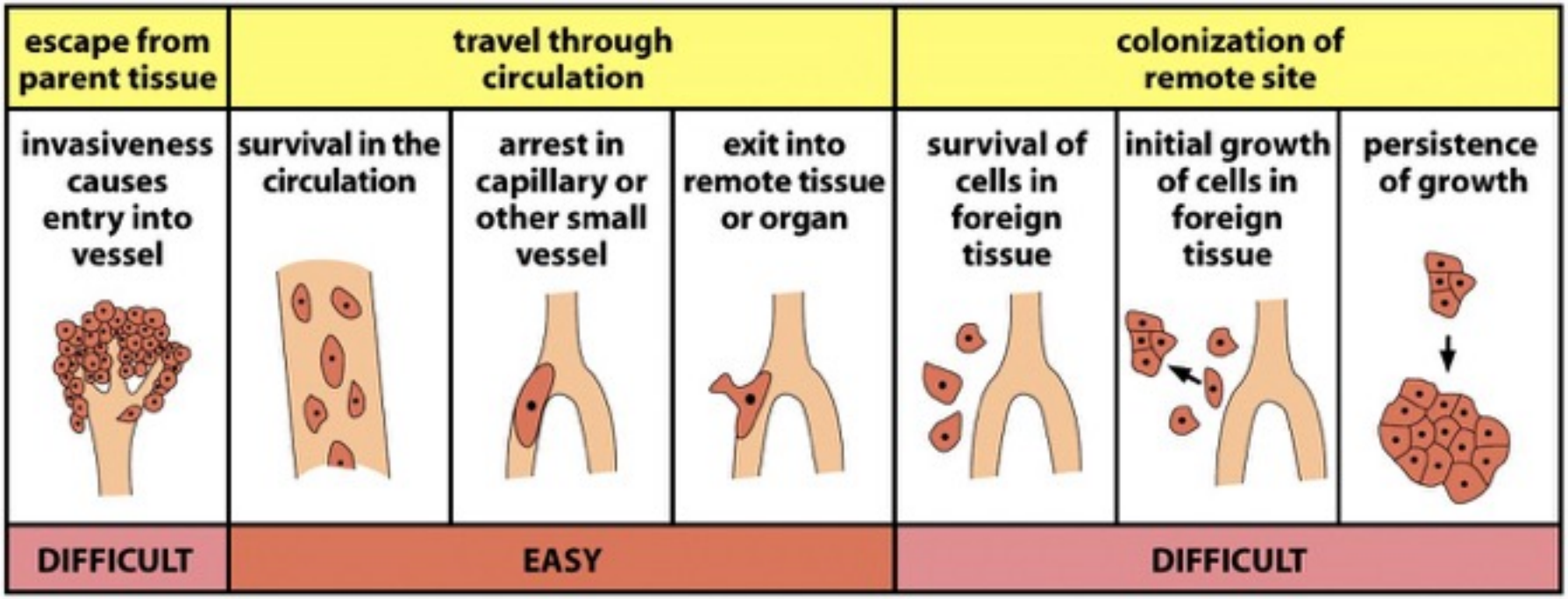


Figure 20-44 *Molecular Biology of the Cell* (© Garland Science 2008)

Metastasis Promoting Genes - I

Gene	Tissue Site	Function
ARM-1	Lymphoma	Promotes adhesion of tumor cells to the endothelium
ATX	Breast, Liver, Lung, Melanoma, Teratocarcinoma	cytoskeletal reorganization and motility; G-protein coupled receptor activation
CD44	Multiple sites	cell-cell interactions; activates HGF/c-Met pathway
Cox2	Breast, Colorectal, Gastric	Prostaglandin synthase; induces VEGF
Cyr61	Breast	Mediates adhesion; Erb-B2/3/4 pathway
Ezrin	Liver, Ovary, Pancreas, Prostate, Uterus	Membrane-cytoskeletal linker; RHO and RAC interactions
HMG-I(Y)	Breast, Cervical, Colorectal, Prostate, Skin, Thyroid, Uterus	Regulated by EGF and MMP-9
Laminin-5	Multiple sites	EGF and TGF-α induce expression of laminin subunits; cell adhesion, motility
c-Met	Multiple sites	Activated by HGF; Modulates Ras and PI3 kinase

Metastasis Promoting Genes - II

Gene	Tissue Site	Function
MTA1	Breast, Cervix, Melanoma, Ovary	Neucleosome remodeling; histone deacetylase complex
Oncostatin M	Lung	Activates PKA-dependent pathway
PP2A	Not determined	Activated by p38/MAPK; inhibits MEK1, MEK2, and MMP-1
RAGE	Gastric, Lung, Pancreatic, Renal	transmembrane receptor; activates p21, MAPKs, NF-6B, cdc42/rac
S100A4	Breast, Colorectal, Prostate	Calcium-binding protein; activates c-erbB-2
S100A9	Colon, Gastric, Skin	Calcium-binding protein; Modulates Mac-1 integrin receptor through G-protein
Semaphorins	Gastric, Leukemia, Lung, Skin	cell-cell interactions; Receptor crosstalk with c-Met binding semaphorin receptor, plexin
Thymosin-β15	Prostate	actin binding; motility
Wnt-5a	Breast, Colon, Lung, Melanoma, Pancreas, Prostate	PKC activation with associated changes in cytoskeleton, cell adhesion, and motility

Metastasis Suppressor Genes - I

Gene	Tissue Site	Function
Annexin7	Prostate	calcium-dependent GTPase; substrate for PKC and other kinases associated with proliferation
BRMS1	Breast, Melanoma	gap-junctional communication
CC3	Colon, Lung	serine/threonine kinase
CEACAM1-4S	Breast, Colon	Bax pathway
CRSP3	Melanoma	transcriptional co-activator
DAP-kinase	Multiple sites	calcium/calmodulin-dependent serine/threonine kinase; pro-apoptotic pathway
E-cadherin	Multiple sites	Wnt signaling; cytoskeleton; cell-cell adhesion
HEPSIN	Ovarian, Prostate, Renal	transmembrane serine protease
HPI$^{HS\alpha}$	Breast	non-histone heterochromatin-associated protein
KAI-1	Breast, Prostate	Transmembrane tetraspondin; role in adhesion, motility, growth regulation, and differentiation; integrin interaction
KiSS1	Breast, Melanoma	Modulates Rho, Rac, and MAPK signaling
Maspin	Breast, Colon, Oral Squamous Cell, Prostate	Serine protease inhibitor; binds collagen and can modulate integrins
Melastatin	Melanoma	Calcium channel protein

Metastasis Suppressor Genes - II

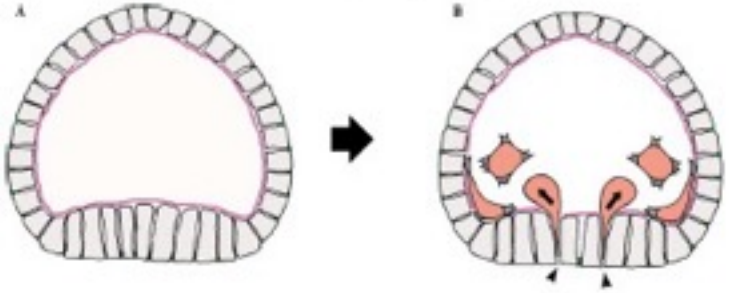
Gene	Tissue Site	Function
MKK4	Ovary, Prostate	MAPK; phosphorylates and activates p38 and JNK kinases
NESH	Lung, Prostate	src homology 3 adapter protein; down regulates p21 pathway
NM23-H1	Breast, Colon, Melanoma, Oral Squamous Cell	histidine kinase; phosphorylates KSR, which might reduce ERK 1/2 activation
PTEN	Multiple sites	phosphatase; growth regulation, cell motility
RhoGD12	Bladder	Inhibits GTP binding; regulates RHO and RAC
SFRP1	Breast, Colorectal	Modulates Wnt signaling pathway
SHPS-1	Breast, Leukemia	glycoprotein; may regulate RAS-MAPK signaling; suppresses anchorage independent growth
Syk	Breast, Colon, Pancreas, Skin	Tyrosine kinase; inhibits PI3 kinase; necessary for MAPK activation
TSP-1	Multiple sites	inhibits endothelial cell proliferation and migration; c-Myc expression inhibits TSP-1
tropomyosins	Breast	interacts with e-cadherin/catenin complex
VDUP1	Melanoma	Thioredoxin inhibitor; upregulates *KiSS1; interacts with CRSPs*

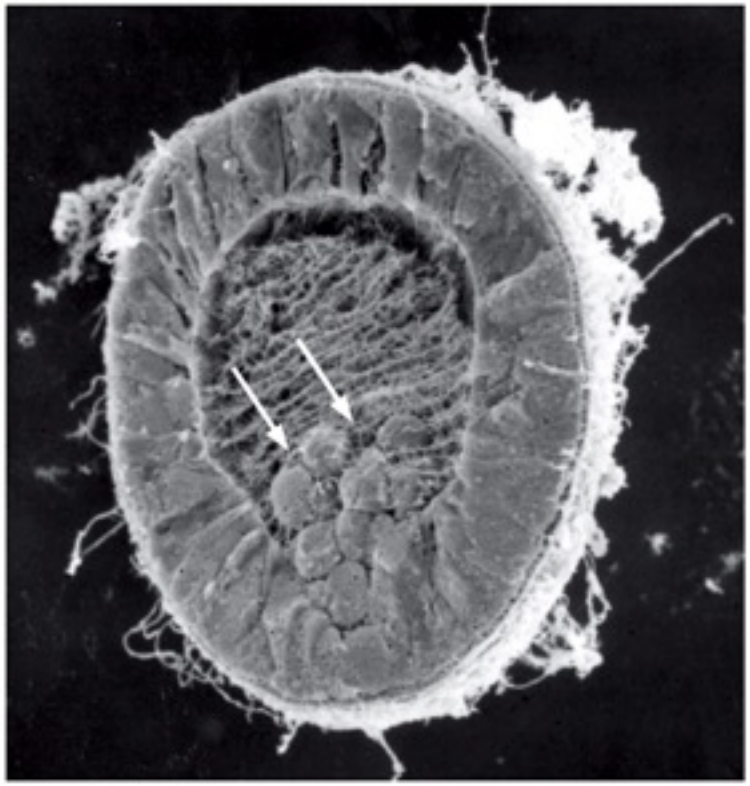
How do cells become invasive???

EMT

Epithelial to Mesenchymal Transition

sea urchin embryo





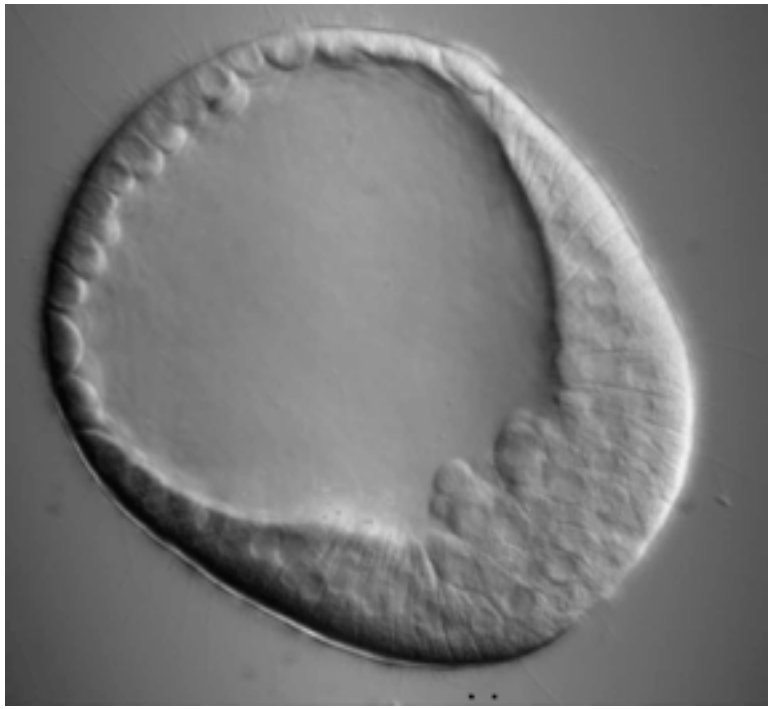
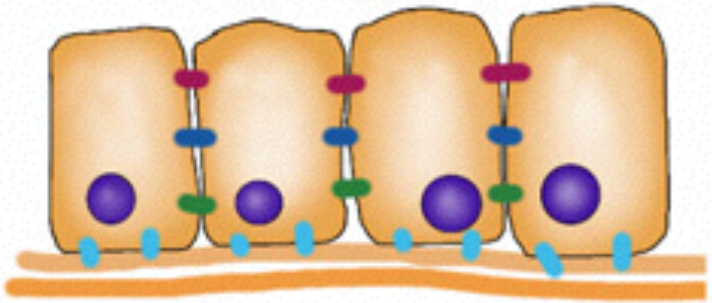


Figure 14.13a *The Biology of Cancer* (© Garland Science 2007)

Major changes during EMT

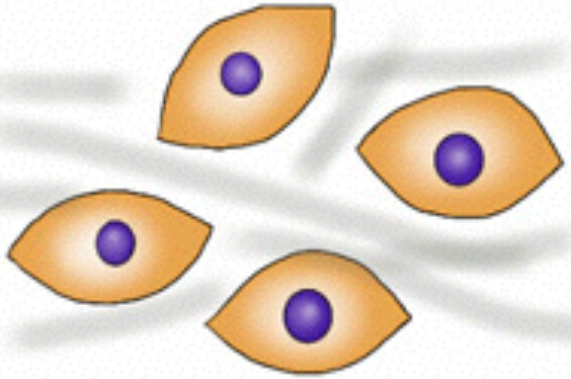
- Loss of E-cadherin
- Cell shape changes driven by Rho GTPases
- MMPs

EPITHELIAL



EMT

MESENCHYMAL



Cell polarity

Cell adhesion (to each other and to Extra Cellular Matrix

Stationary

High level of E-cadherin

Low level of N-cadherin

No cell polarity

Loss of cell adhesion

Ability to migrate and invade

Low level of E-cadherin

High level of N-cadherin

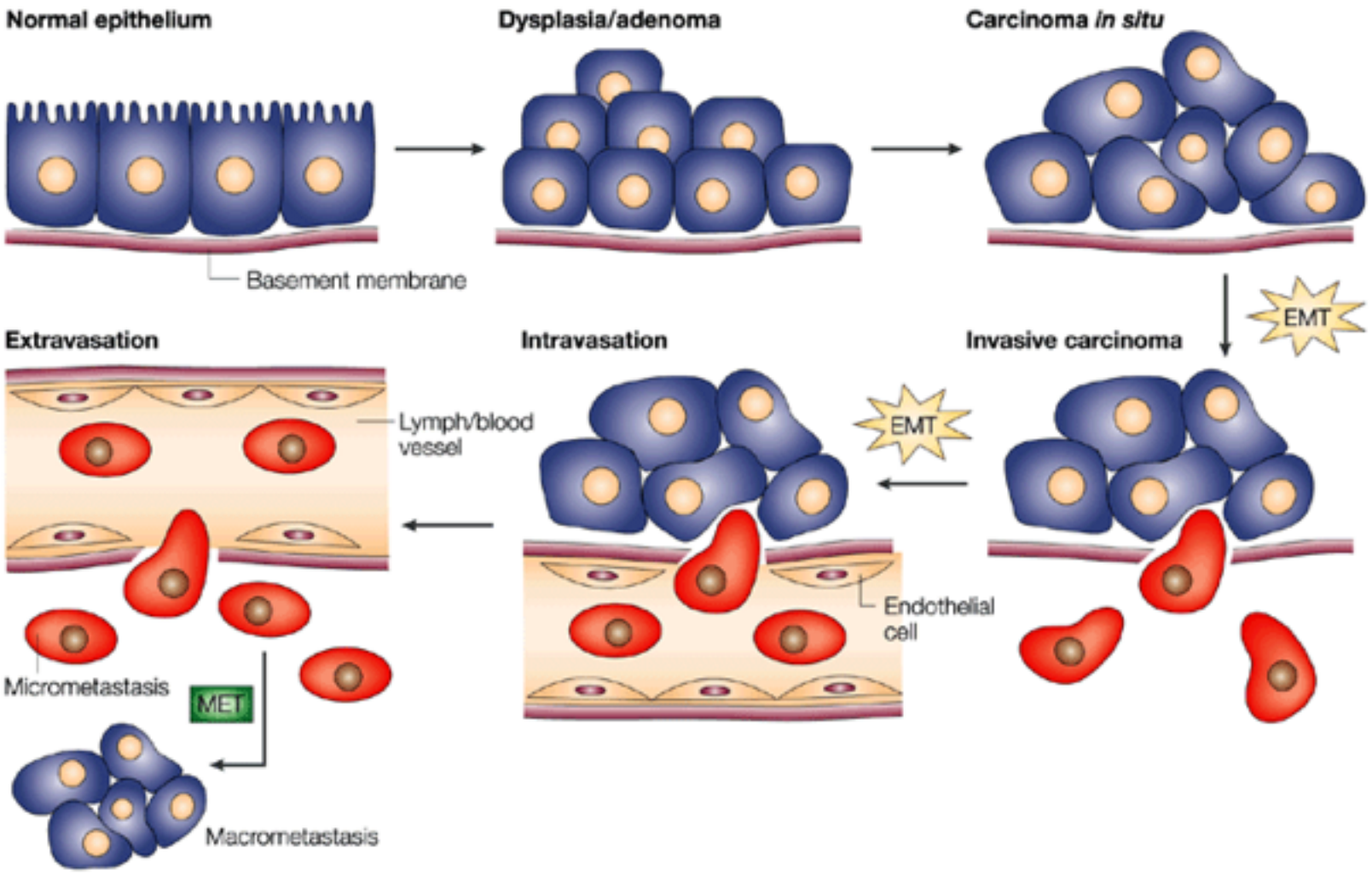
Buddhini Samarasinghe

Table 14.3 Transcription factors orchestrating an EMT

Name	Where first identified	Type of transcription factor	Cancer association
Snail (SNAI1)	mesoderm induction in *Drosophila*; neural crest migration in vertebrates	C2H2-type zinc finger	invasive ductal carcinoma
Slug (SNAI2)	delamination of the neural crest and early mesoderm in chicken	C2H2-type zinc finger	breast cancer cell lines, melanoma
Twist	mesoderm induction in *Drosophila*; emigration from neural crest	bHLH	various carcinomas, high-grade melanoma, neuroblastoma
Goosecoid	gastrulation in frog	paired homeodomain	various carcinomas
FOXC2	mesenchyme formation	winged helix/forkhead	basal-like breast cancer
ZEB1 (δEF1)	postgastrulation mesodermal tissue formation	2-handed zinc finger/ homeodomain	wide variety of cancers
ZEB2 (SIP1)	neurogenesis	2-handed zinc finger/ homeodomain	ovarian, breast, liver carcinomas
E12/E47 (Tcf3)[a]	associated with E-cadherin promoter	bHLH	gastric cancer

[a]It remains unclear whether E12/E47 can function on its own to induce an EMT, or whether this bHLH functions as a subunit of a heterodimeric TF complex formed with other well-validated EMT-TF proteins such as Twist.

EMT in Tumor Progression



Adopting changes typical to EMT

Twist plays an essential role in cancer metastasis. Over-expression of Twist or methylation of its promoter is common in metastatic carcinomas.

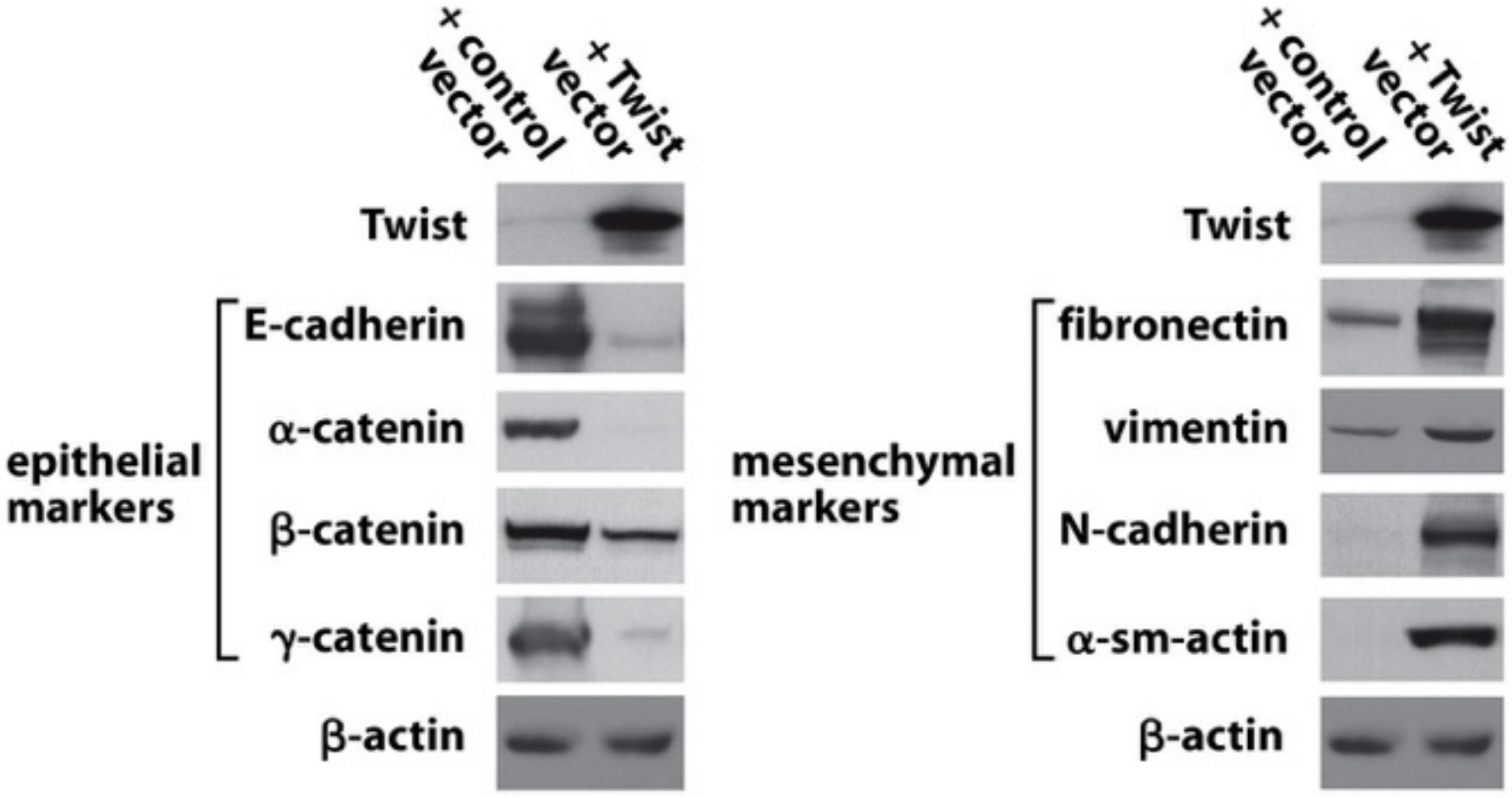
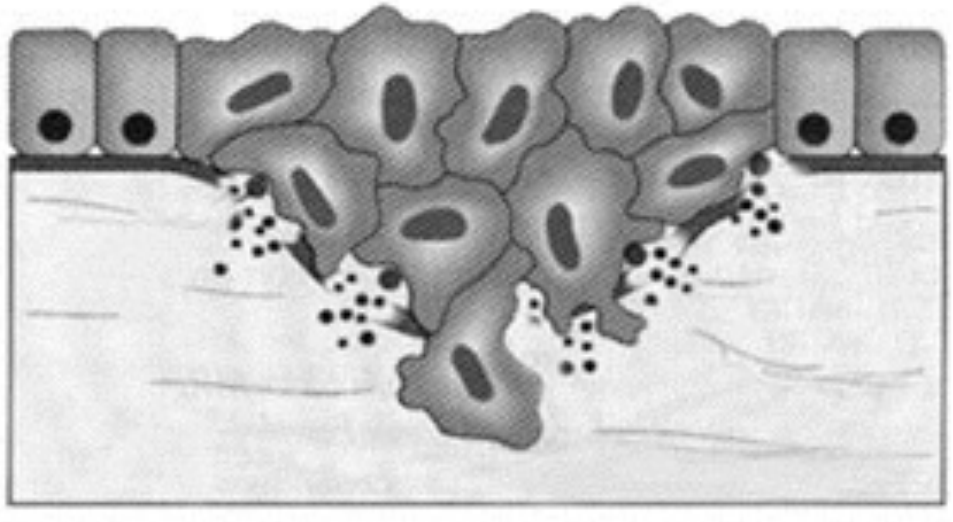


Figure 14.15b *The Biology of Cancer* (© Garland Science 2007)

MMPs (matrix metalloproteinases) help the cancer cells to **invade the ECM**



Signals from stroma controlling EMT

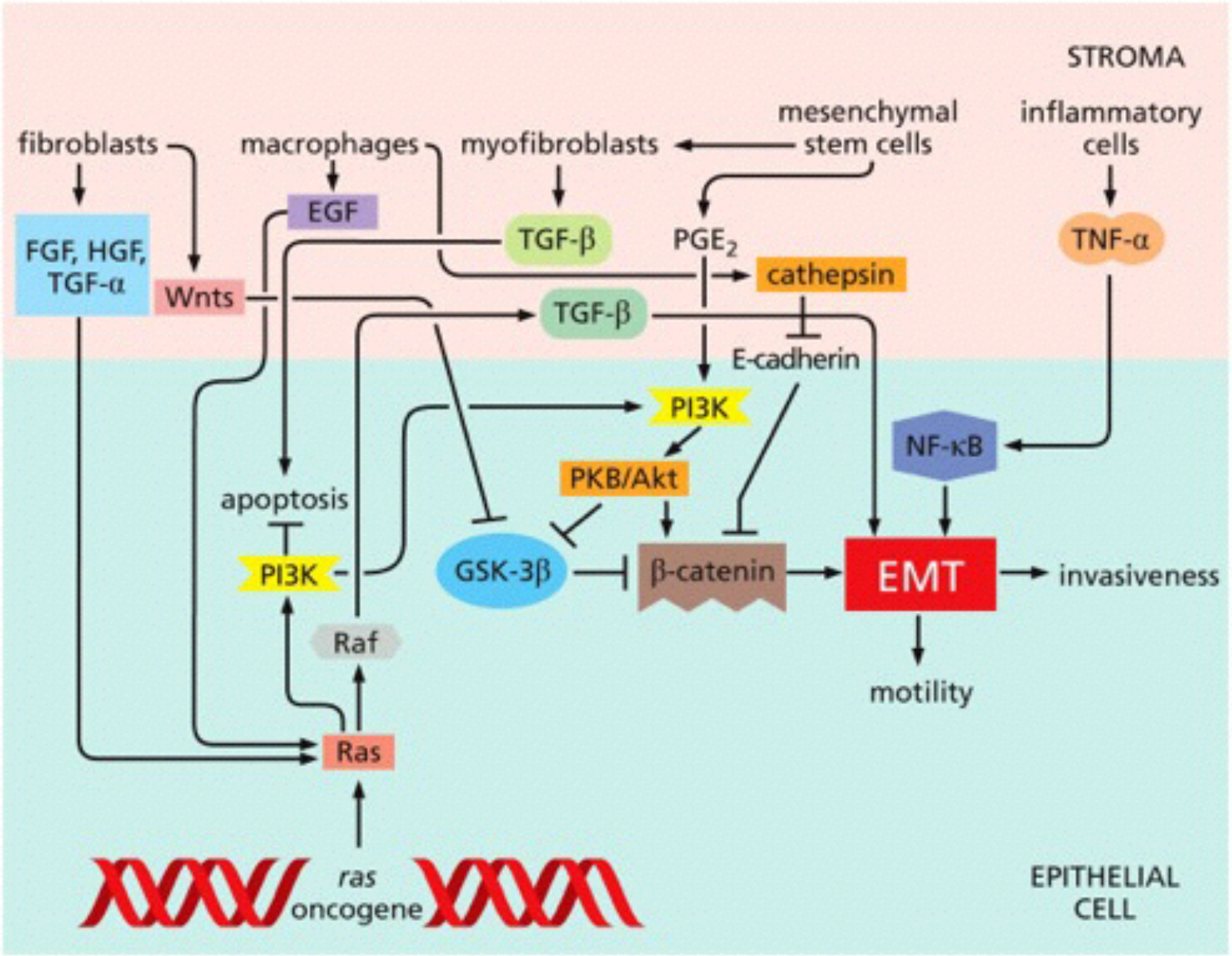
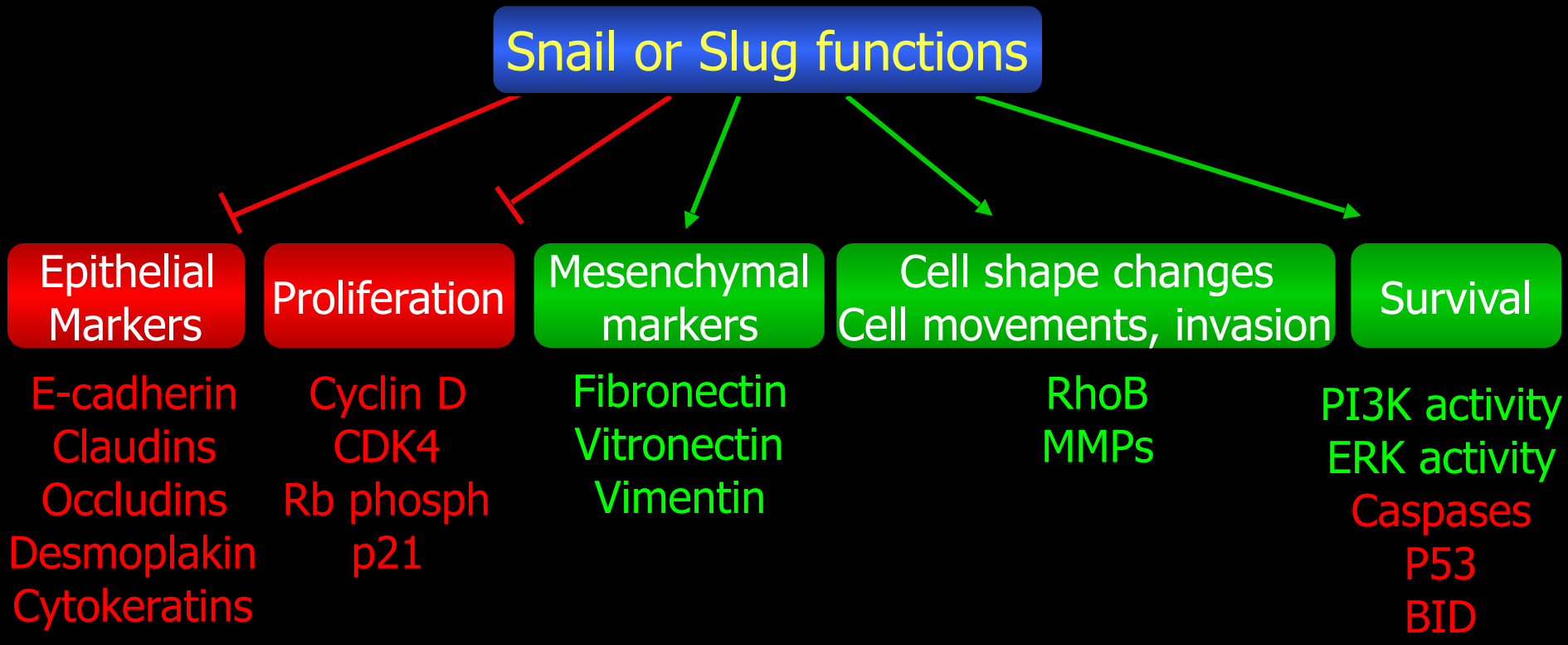


Figure 14.24 The Biology of Cancer (© Garland Science 2014)


Snail or Slug functions
Epithelial Markers
Proliferation
Mesenchymal markers
Cell shape changes Cell movements, invasion
Survival
E-cadherin
Claudins
Occludins
Desmoplakin
Cytokeratins
Cyclin D
CDK4
Rb phosph
p21
Fibronectin
Vitronectin
Vimentin
RhoB
MMPs
PI3K activity
ERK activity
Caspases
P53
BID

Small GTPase family play a key role of cancer cell motility

Cytokines
Chemokines
Extracellular matrix

↓

Rho family
•Rho
•Rac
•Cdc42

↓

Stress fiber
Focal adhesion
Lamellipodia
Filopodia

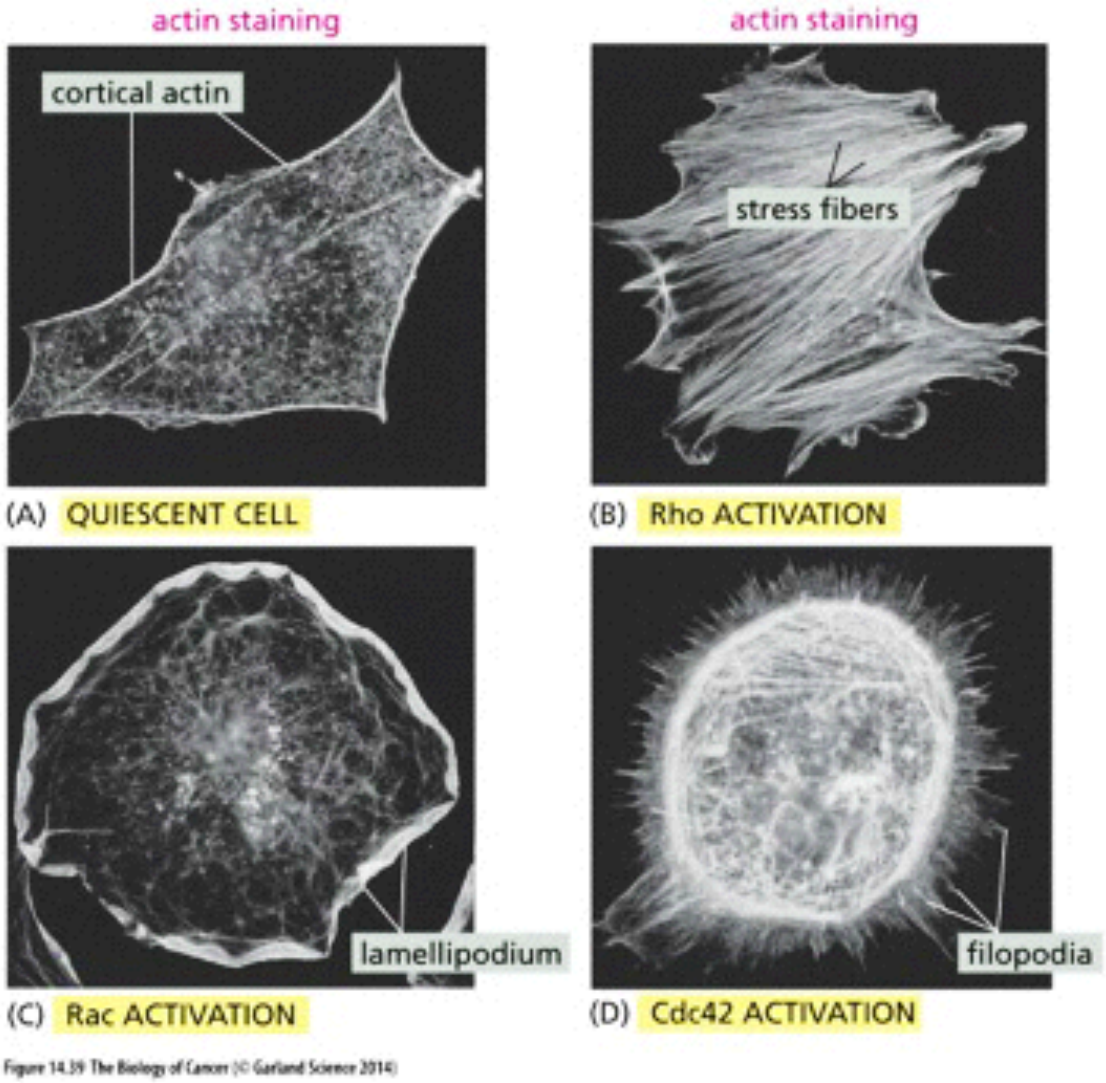
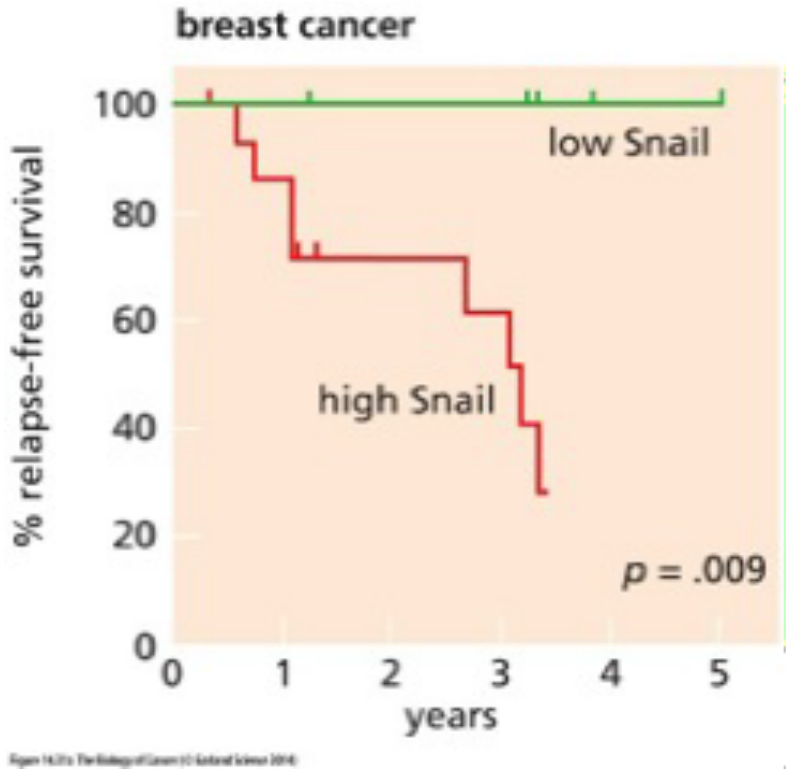
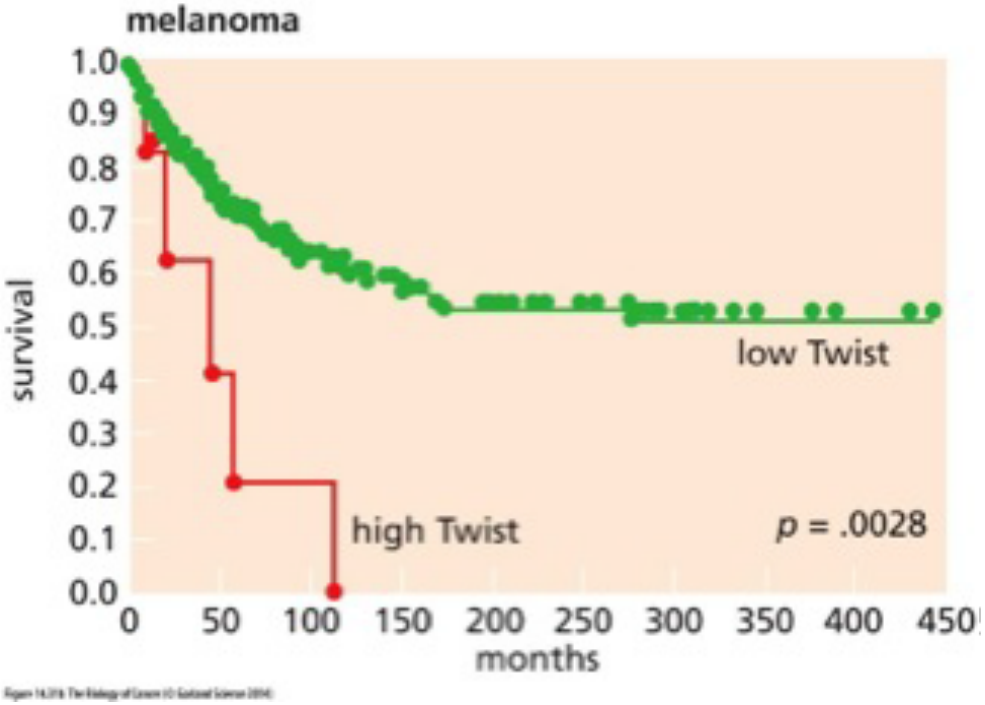


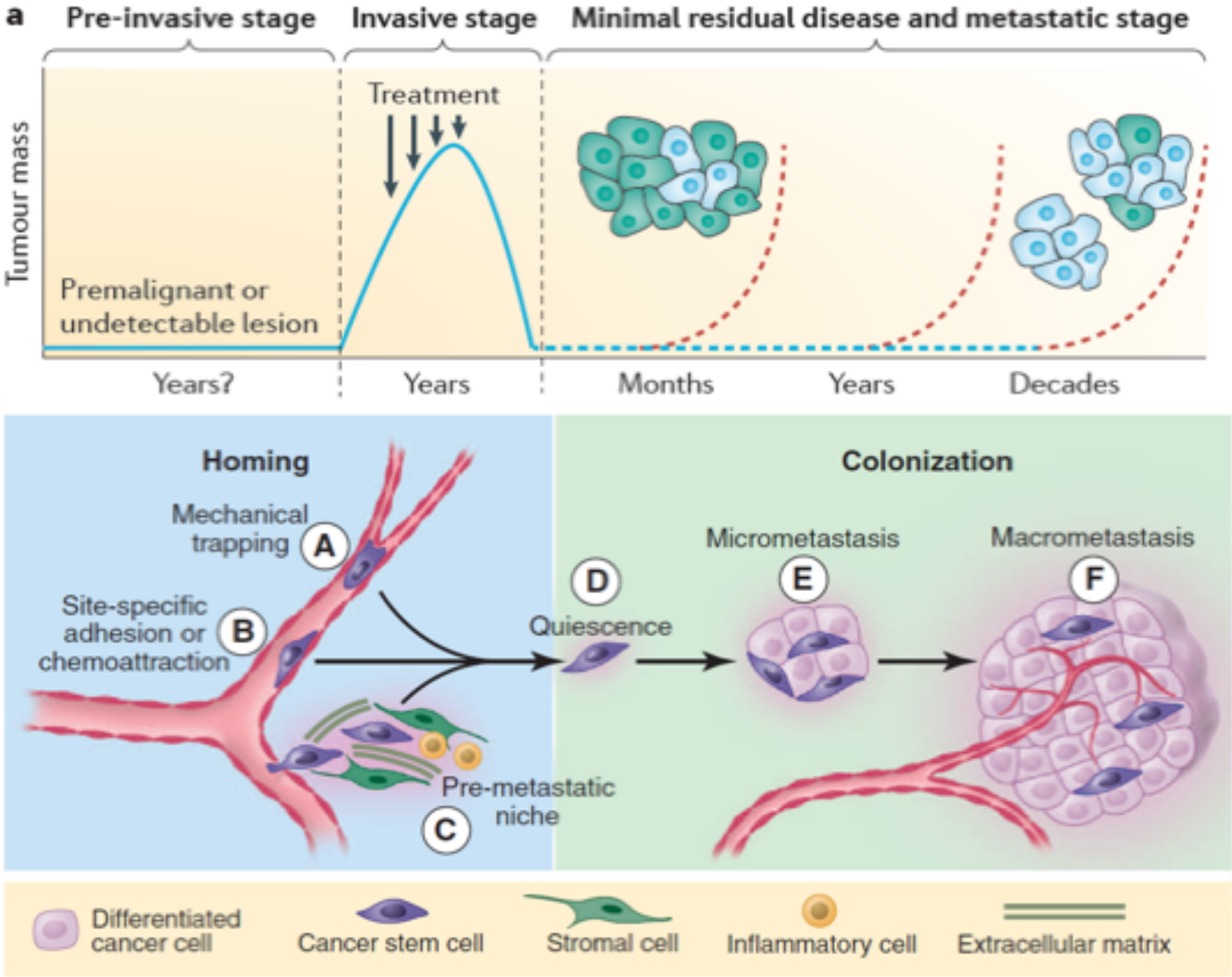
Figure 14.39 The Biology of Cancer (© Garland Science 2014)

EMT and cancer progression

Correlation between EMT inducing TFs with the malignant behavior in cancer patients






a
Pre-invasive stage
Invasive stage
Minimal residual disease and metastatic stage
Tumour mass
Treatment
Premalignant or undetectable lesion
Years?
Years
Months
Years
Decades
Homing
Colonization
Mechanical trapping
A
Site-specific adhesion or chemoattraction
B
Pre-metastatic niche
C
D
Quiescence
Micrometastasis
E
Macrometastasis
F
Differentiated cancer cell
Cancer stem cell
Stromal cell
Inflammatory cell
Extracellular matrix

Thank you

Questions????