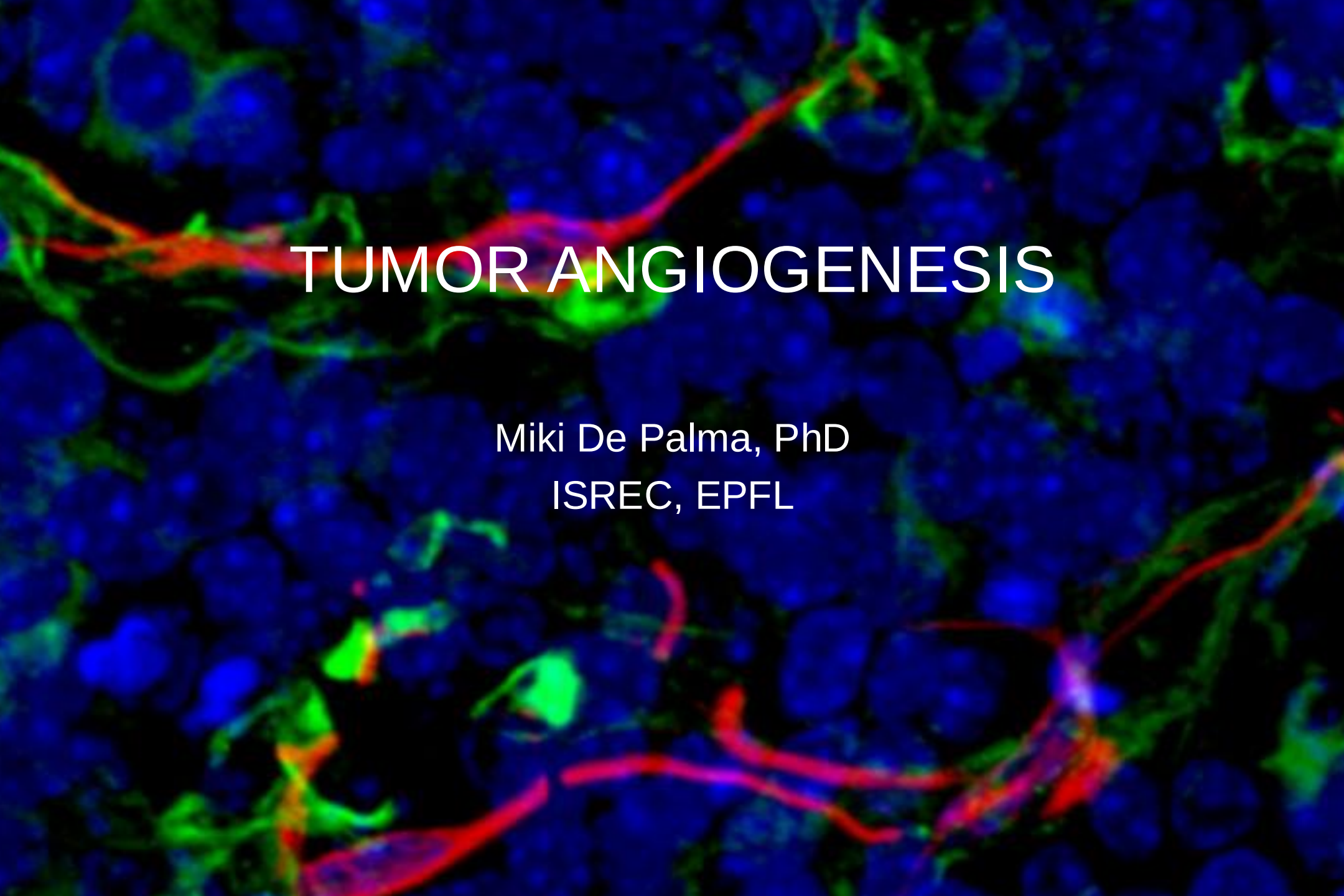


TUMOR ANGIOGENESIS

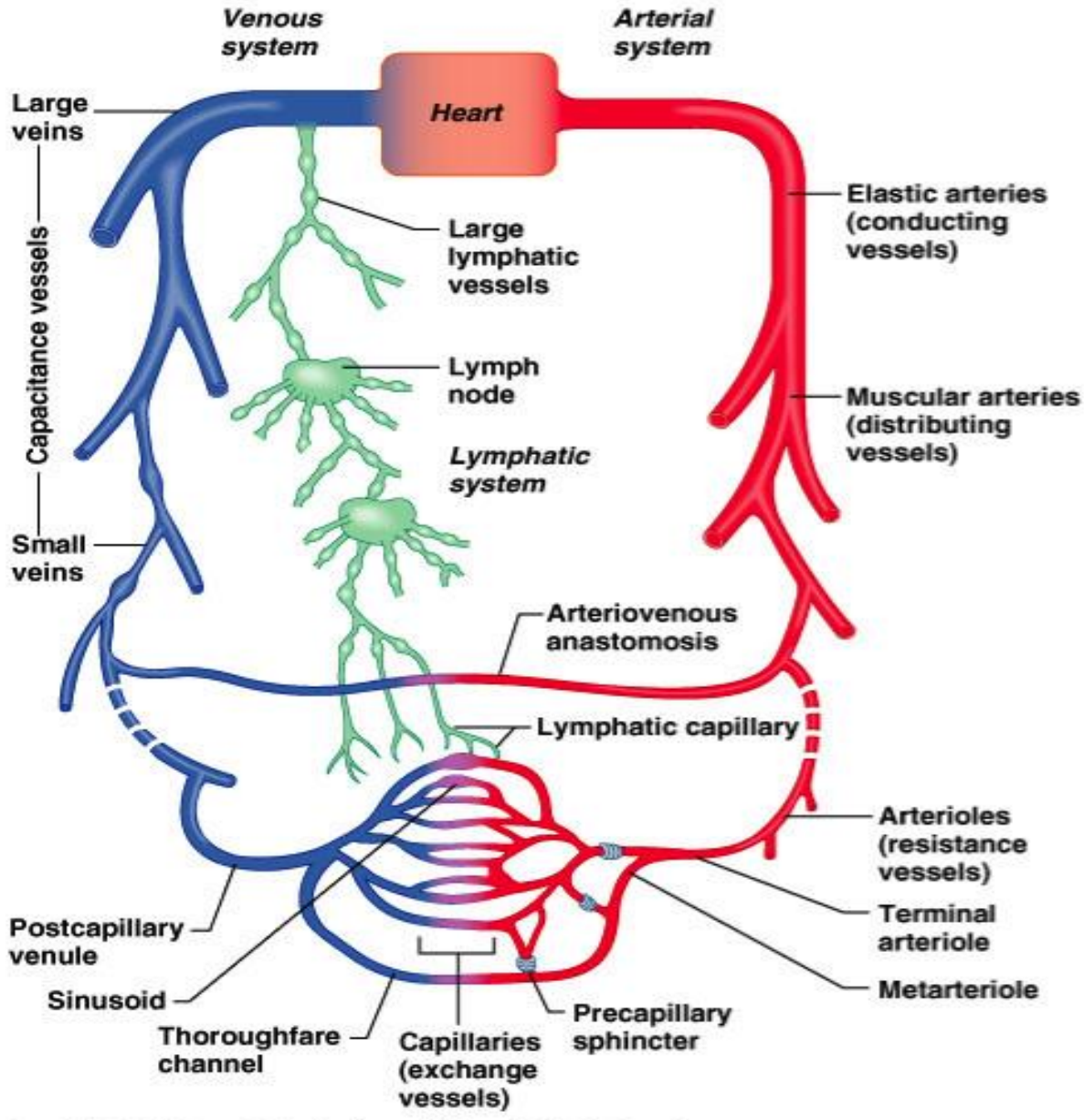
Miki De Palma, PhD
ISREC, EPFL

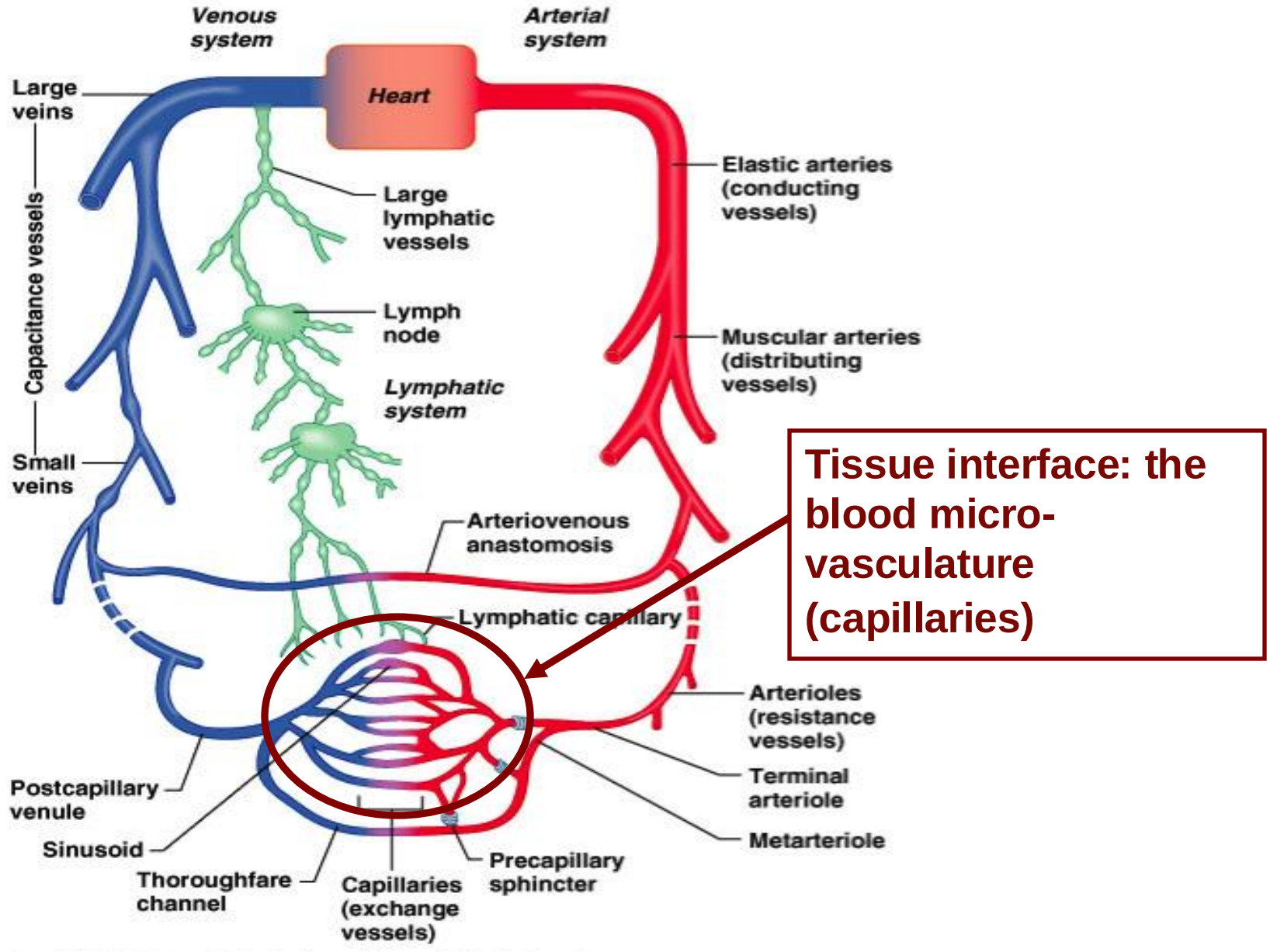
1. An overview of the circulatory system, composed of blood and lymphatic vessels

The circulatory system has two basic components:

-- The blood vascular system, which circulates blood from the heart throughout the body and back through the kidney, liver and lung for cleaning and re-oxygenation.

-- The lymphatic vascular system, which collects fluid (e.g. leaked blood) from tissues and recycles it onto the bloodstream, after the fluid first flows through a series of lymph nodes involved in immune surveillance of tissues.


Venous system
Arterial system
Heart
Large veins
Capacitance vessels
Small veins
Large lymphatic vessels
Lymph node
Lymphatic system
Elastic arteries (conducting vessels)
Muscular arteries (distributing vessels)
Arteriovenous anastomosis
Lymphatic capillary
Arterioles (resistance vessels)
Terminal arteriole
Metarteriole
Precapillary sphincter
Capillaries (exchange vessels)
Thoroughfare channel
Sinusoid
Postcapillary venule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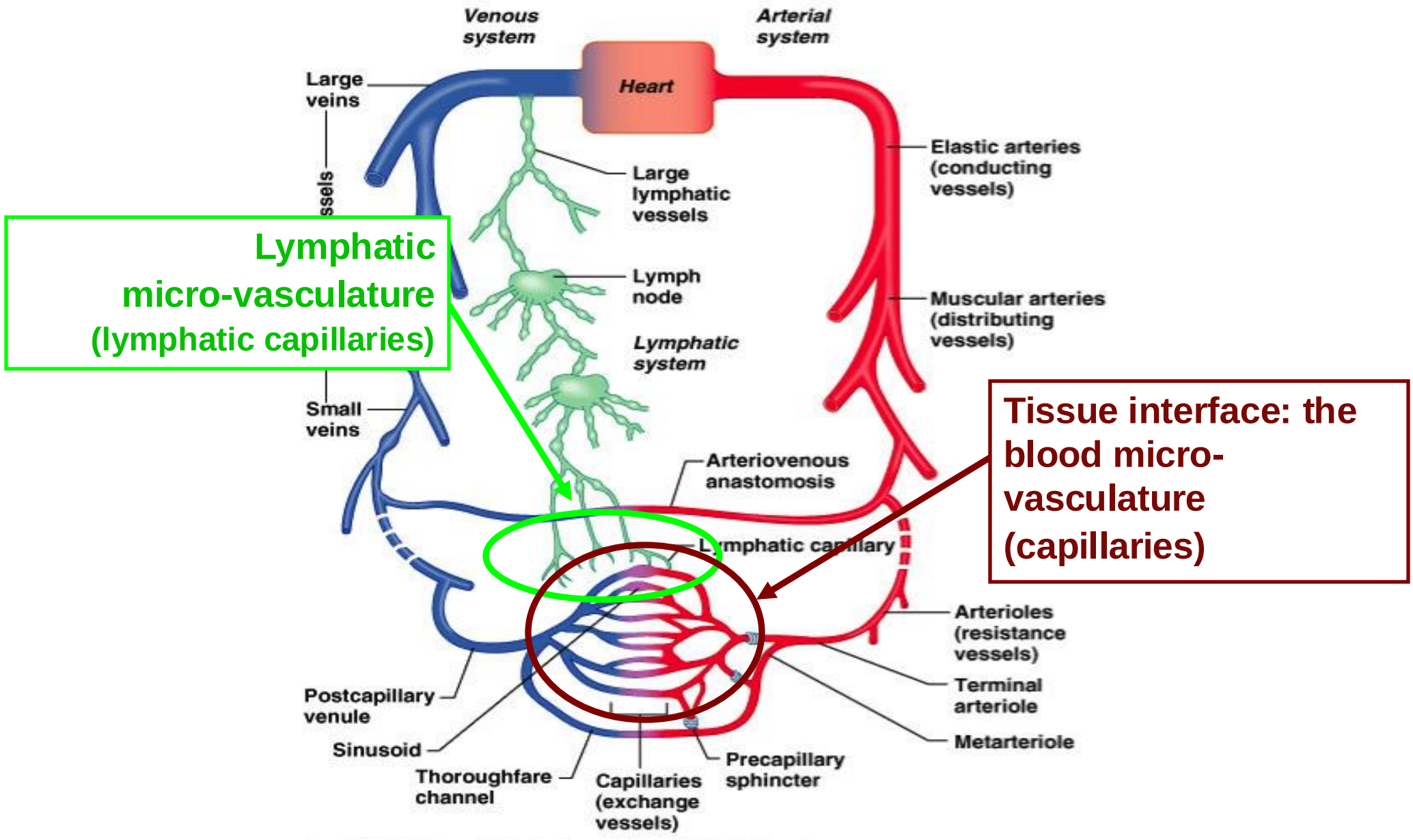
Tissue interface: the blood micro-vasculature (capillaries)


Lymphatic micro-vasculature (lymphatic capillaries)
Tissue interface: the blood micro-vasculature (capillaries)
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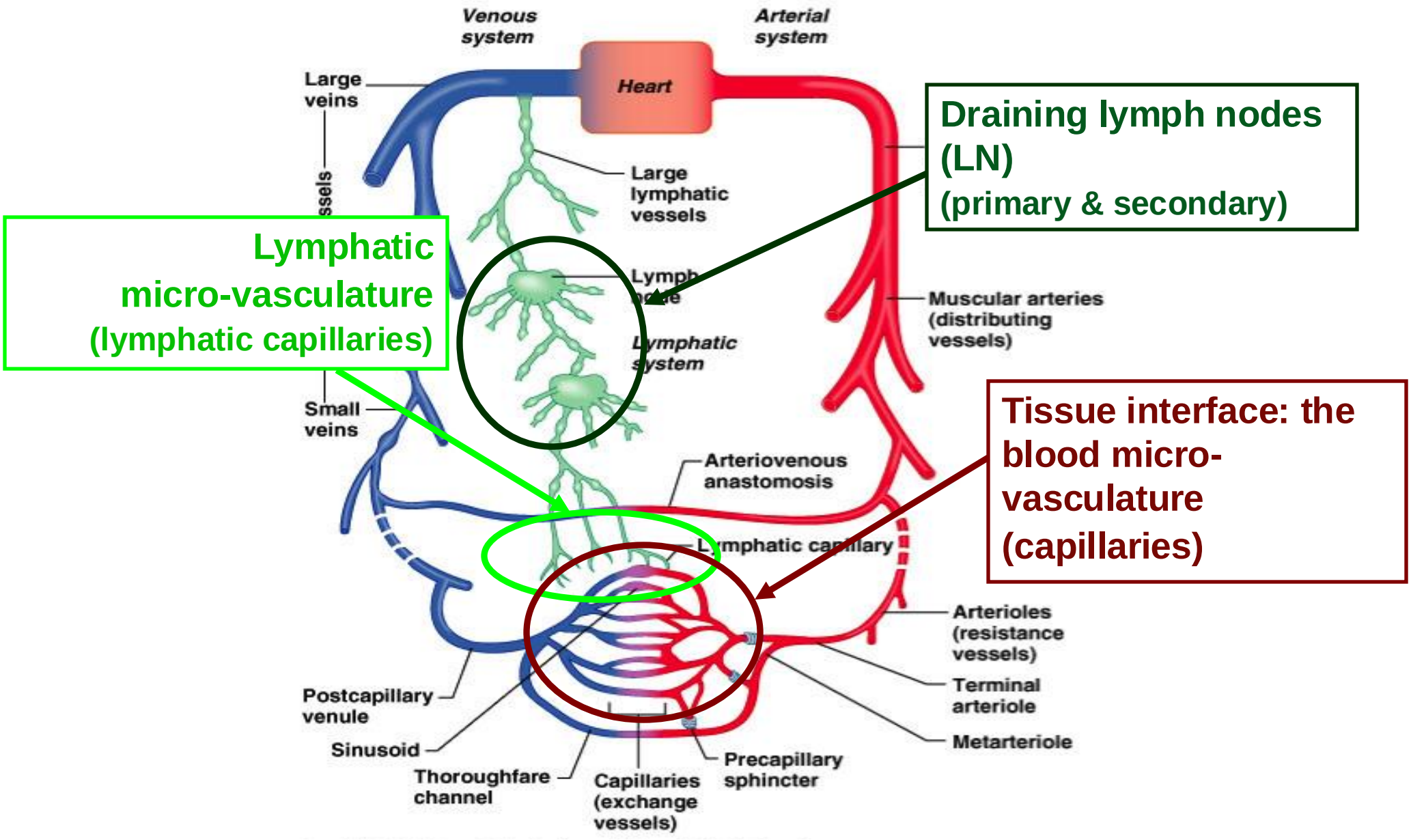
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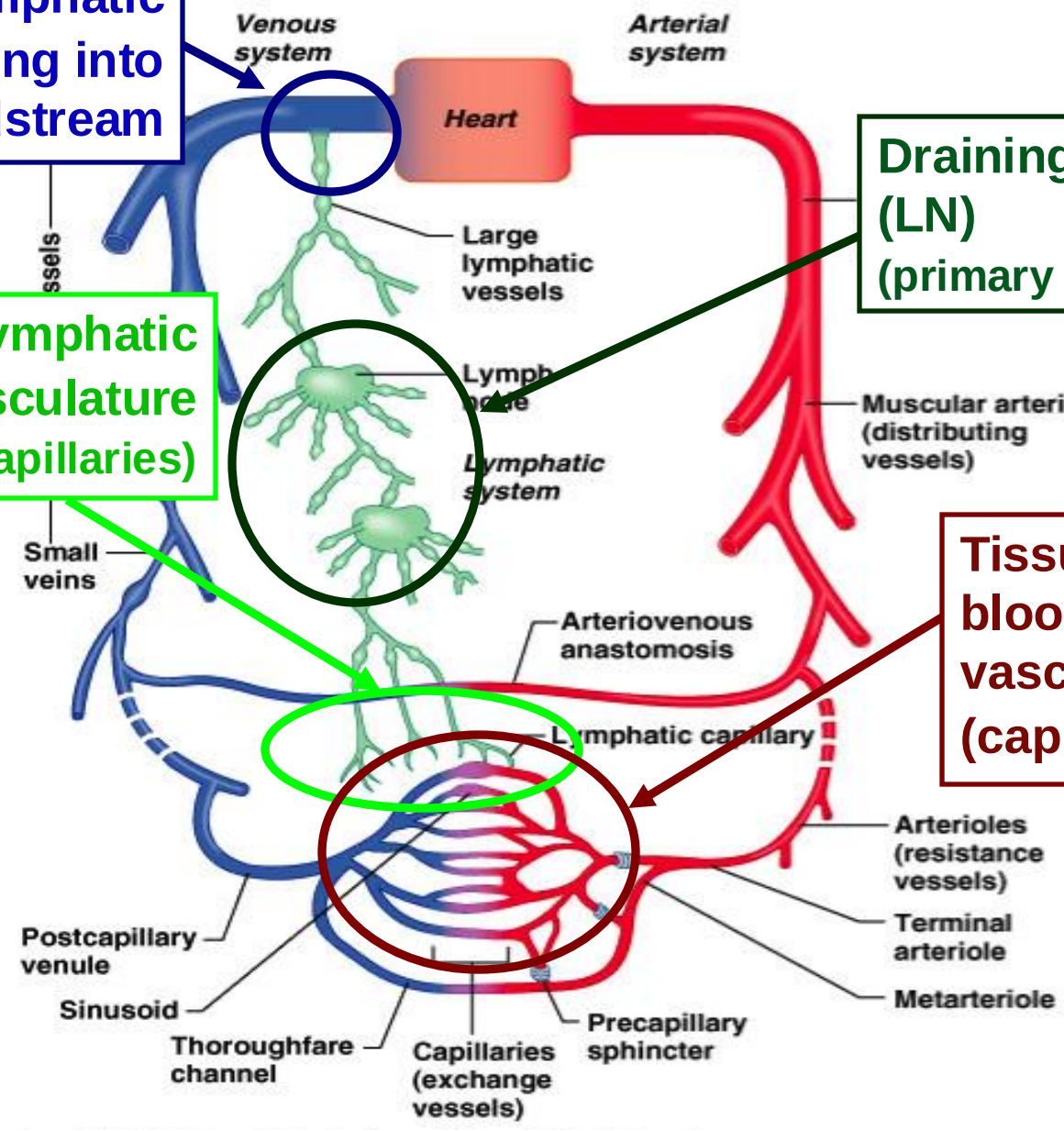
Thoroughfare channel
Sinusoid
Postcapillary venule
Draining lymph nodes (LN) (primary & secondary)
Lymphatic micro-vasculature (lymphatic capillaries)
Tissue interface: the blood micro-vasculature (capillaries)

Lymphatic fluid recycling into bloodstream

Lymphatic micro-vasculature (lymphatic Capillaries)

Draining lymph nodes (LN) (primary & secondary)

Tissue interface: the blood micro-vasculature (capillaries)



2. Cellular composition of the blood vessels

- The tubes of the vascular system are composed of ‘blood endothelial cells’ (BEC)

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- Blood vessels have associated peri-endothelial support cells, of two prominent types:

  -- pericytes

  -- smooth muscle cells (SMC)

2. Cellular composition of the blood vessels

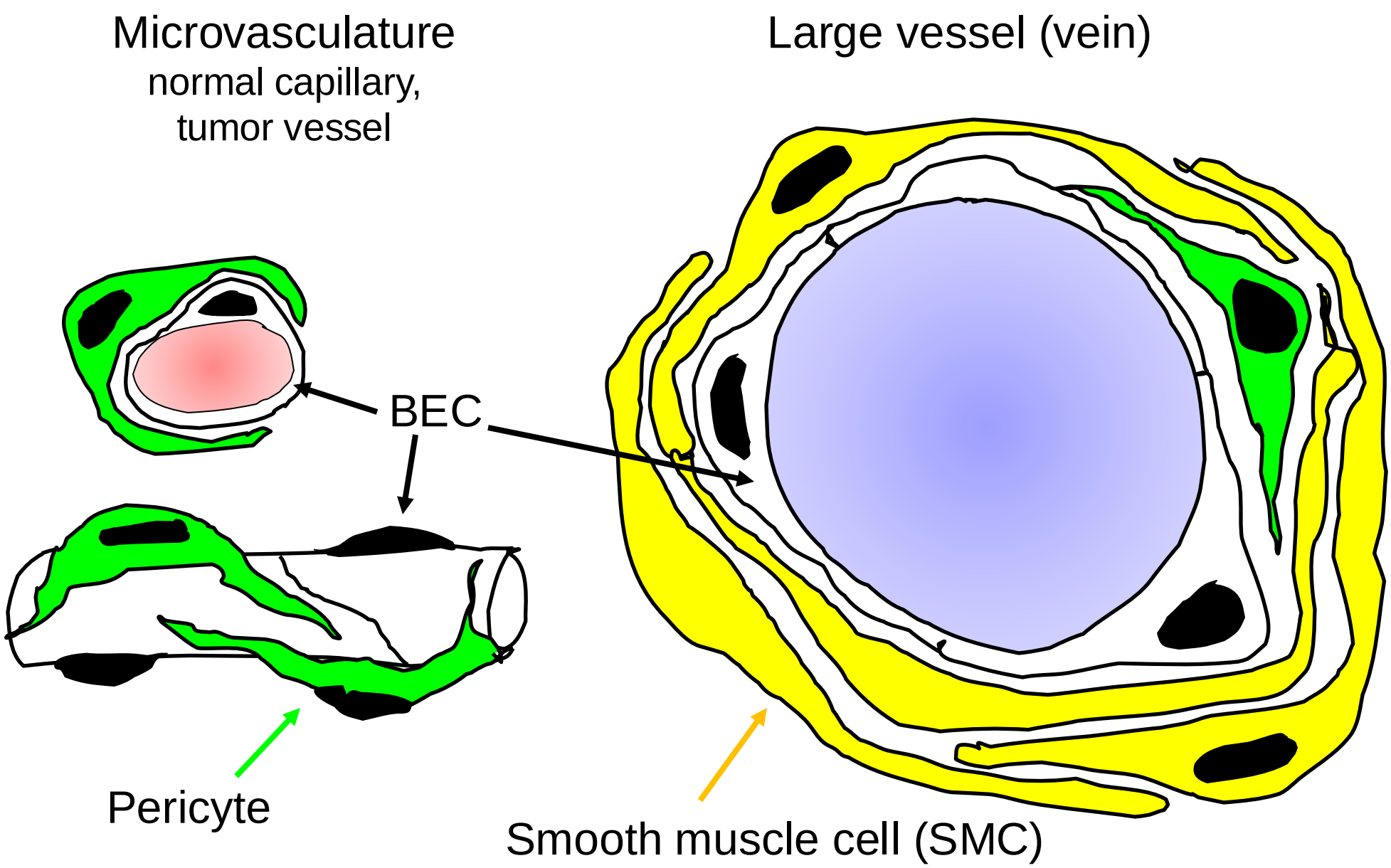
- The tubes of the vascular system are composed of ‘blood endothelial cells’ (BEC)

- Blood vessels have associated peri-endothelial support cells, of two prominent types:

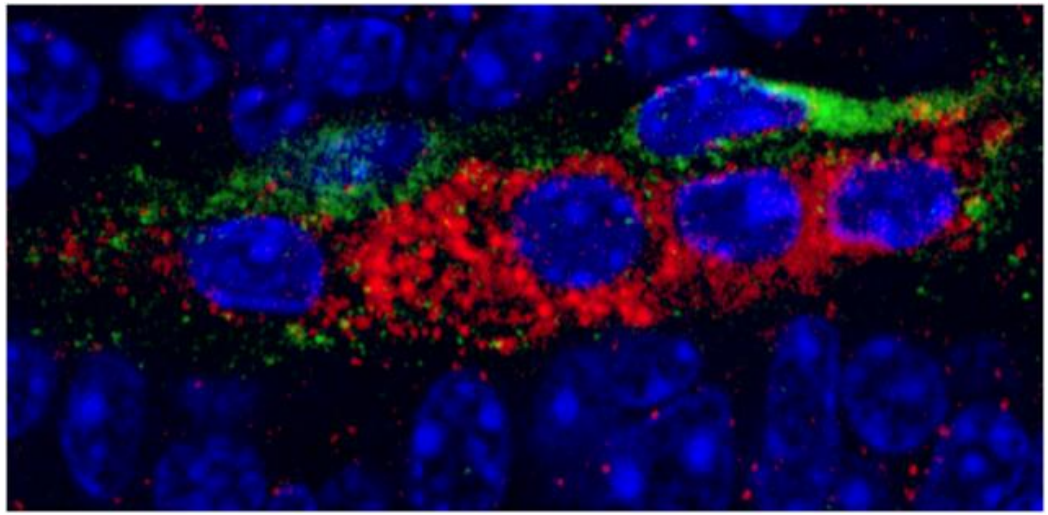
  -- pericytes

  -- smooth muscle cells (SMC)

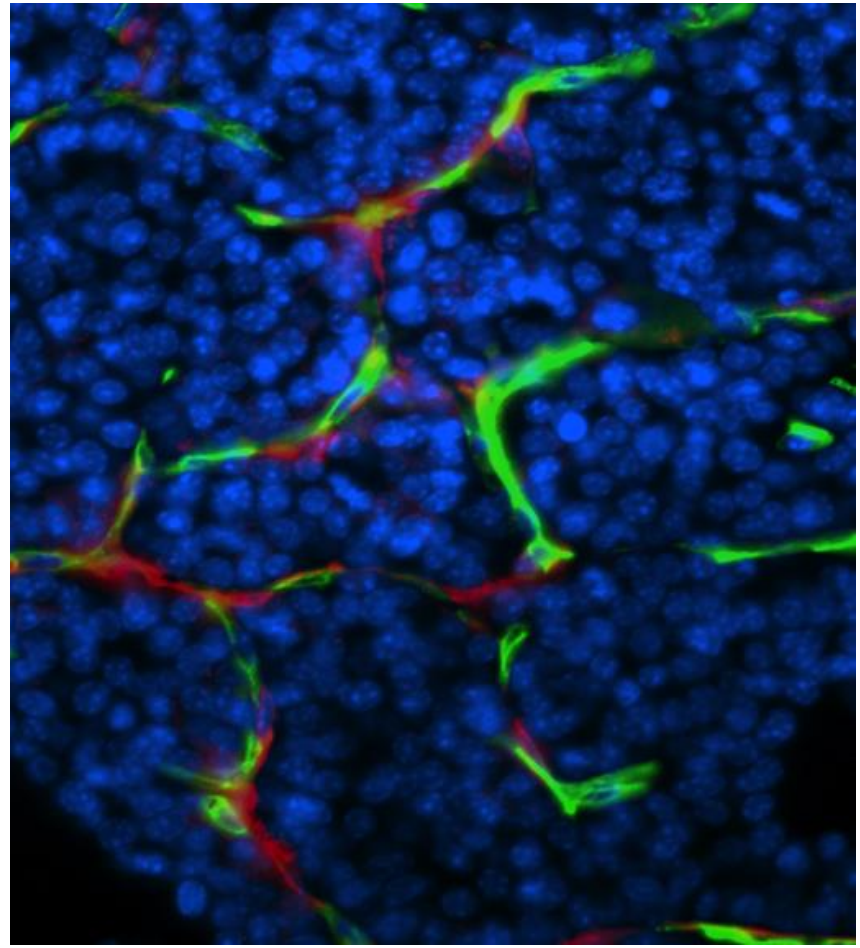
- All blood vessels have pericytes
- Large vessels (arteries and veins) have layers of SMC, either more (arteries) or less (veins).


Microvasculature
normal capillary,
tumor vessel
Large vessel (vein)
BEC
Pericyte
Smooth muscle cell (SMC)

Pericyte and BEC associations in small blood vessels



Pericytes / BECs



Pericytes / BECs

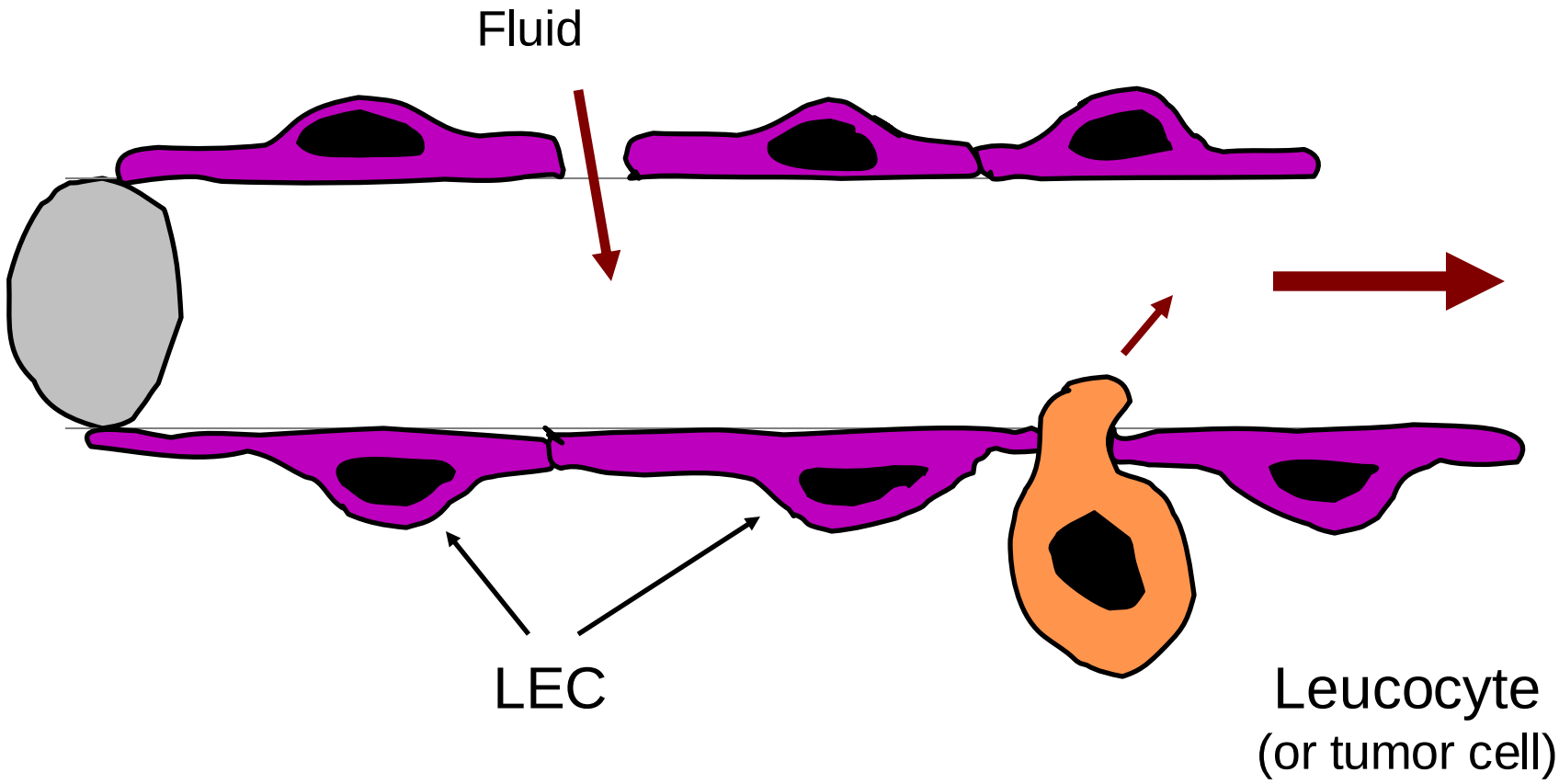
BEC: blood endothelial cell

2. Cellular composition of the lymphatic vessels

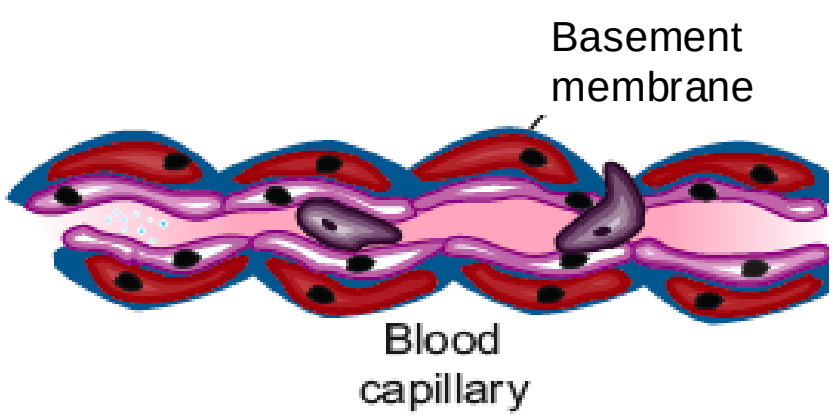
- The tubes of the lymphatic system are composed of a developmentally related but functionally distinctive lymphatic endothelial cell (LEC)

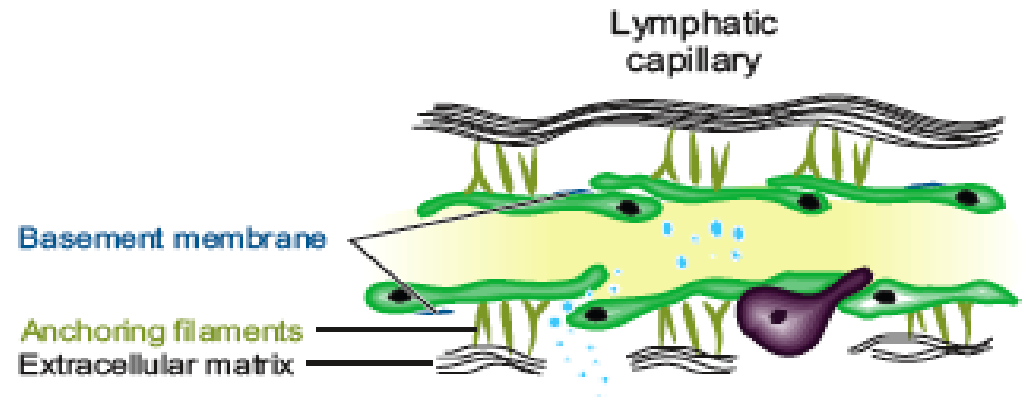
- There are no associated pericytes or smooth muscle support cells

Lymphatic vasculature



Blood vs lymphatic capillaries





Blood capillaries have a continuous basement membrane and associated pericytes, and the BECs form tight and adherence junctions.

Lymphatic vessels are thin walled and have a relatively wide lumen. The endothelial cells of lymphatic capillaries (*green*) lack tight junctions. The LECs partly overlap, forming valve-like openings, which allow easy access for fluid, macromolecules, and cells into the vessel lumen. Lymphatic capillaries lack pericytes and have a fragmentary basement membrane.

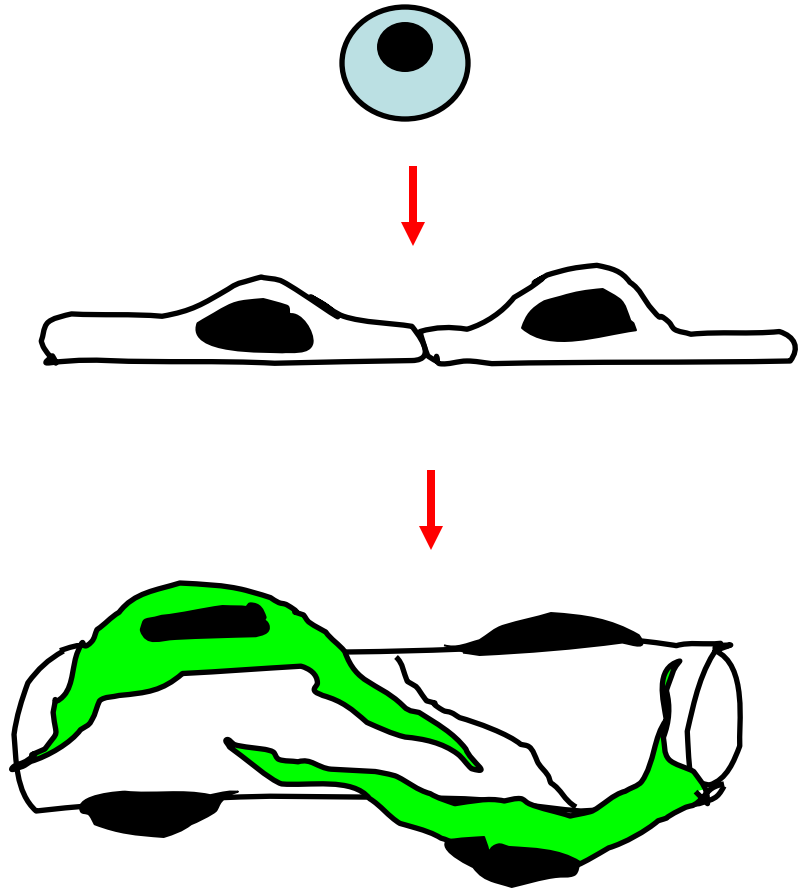
3. Vascular development: vasculogenesis, angiogenesis, and lymphangiogenesis

Vessels are produced during embryonic development by two mechanisms:

Vasculogenesis, the birth of new endothelial cells from yolk-sac-derived stem cells, and their assembly into tubes;

Angiogenesis, the sprouting of new vessels from preexisting vessels

Vasculogenesis (only during development)

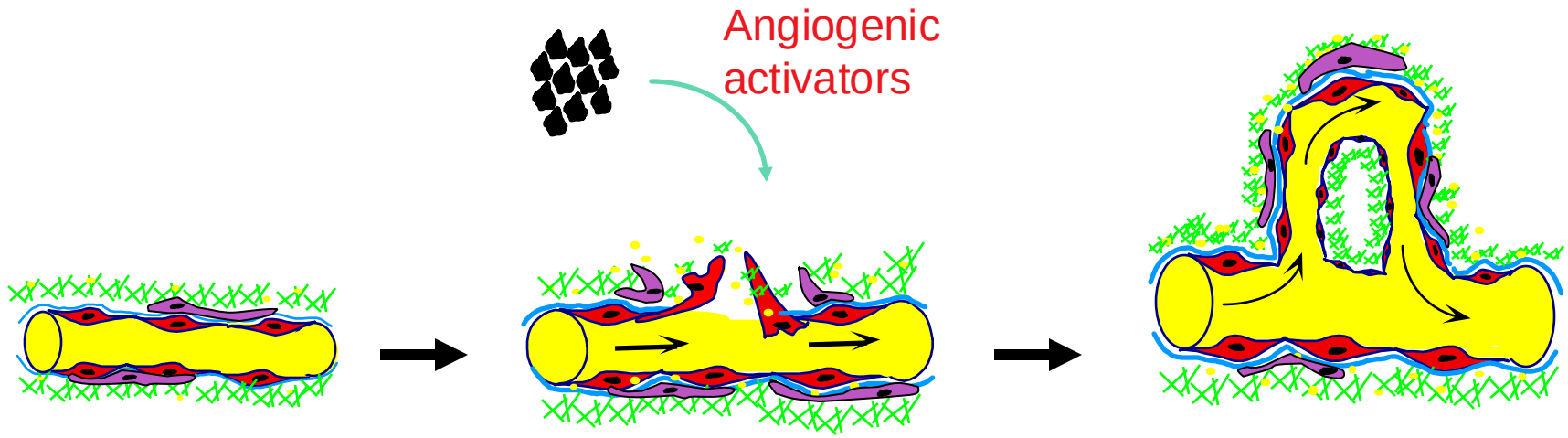


Hemato-angioblast progenitor

Endothelial cells

New blood vessel

Angiogenesis (development and adult life)



Quiescent, normal blood vessel

Basement membrane & ECM degradation

Pericyte detachment

BEC sprouting, proliferation & migration

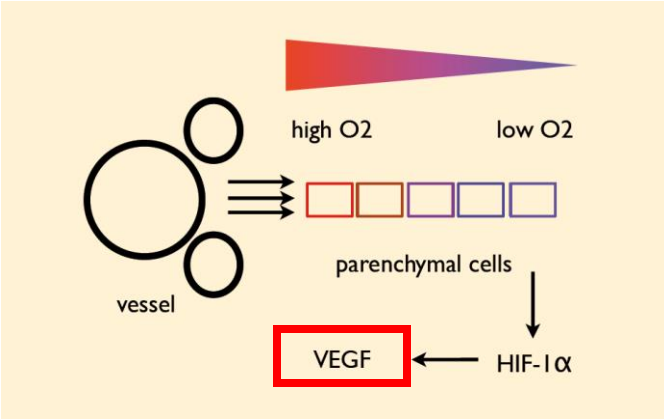
Formation & closure of capillary tube

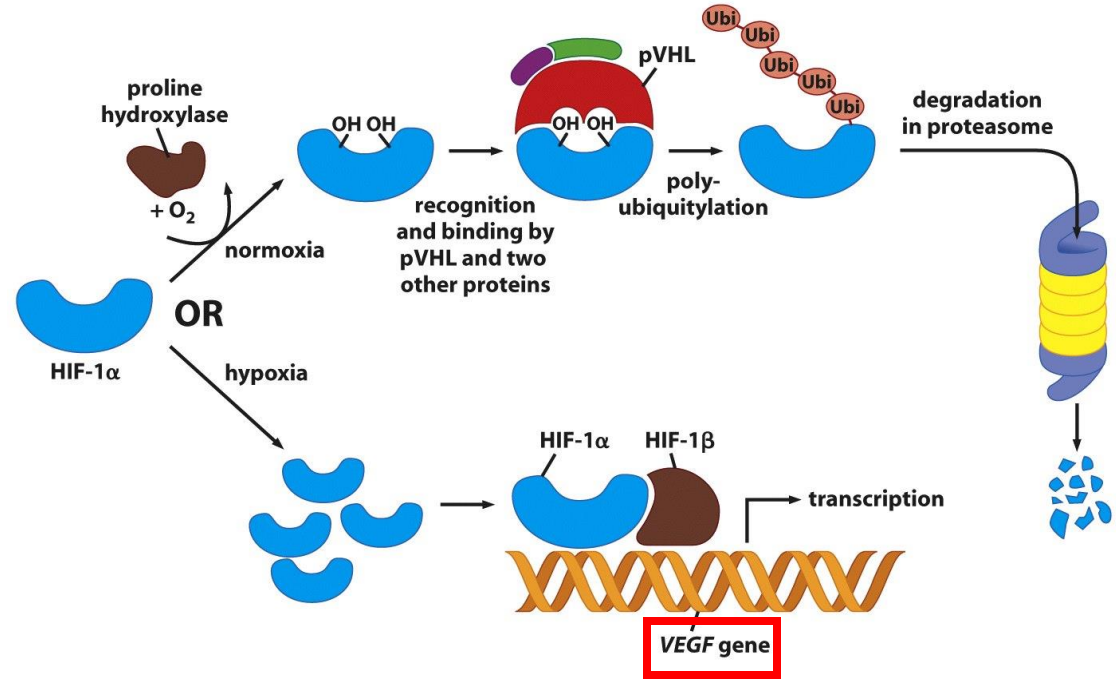
Pericyte attachment

$\Rightarrow$ A functional new blood vessel

4. Vascular regulatory signals

Hypoxia (low oxygen) stimulates angiogenesis through stabilization of HIF1a, which induces VEGFA transcription



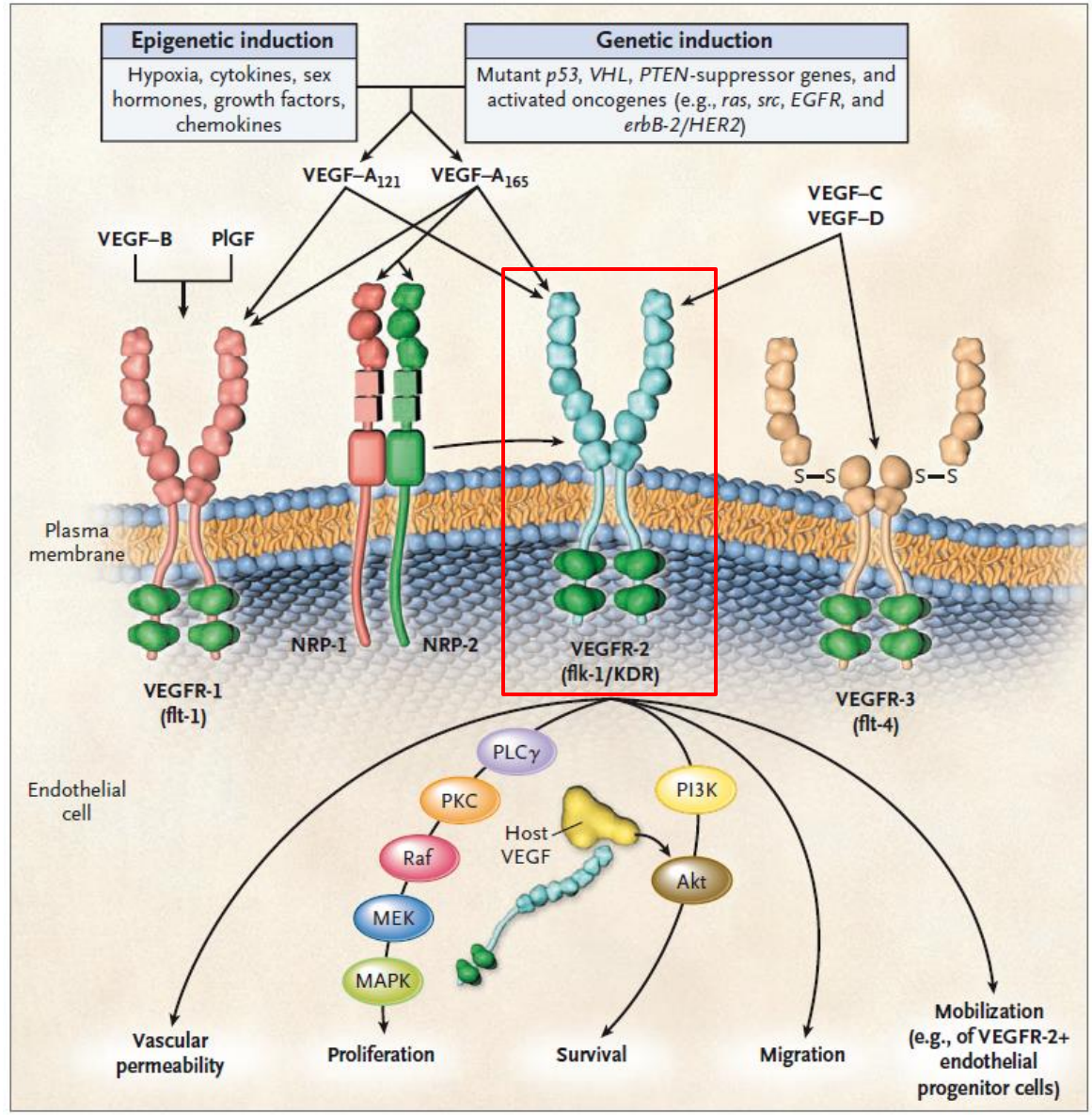


HIF1a (hypoxia-inducible factor 1a) is constitutively expressed but its stability and activity are regulated by oxygen levels

Prolyl hydroxylases (PHDs): hydroxylate HIF in the presence of oxygen

pVHL: Von Hippel-Lindau (a tumor suppressor mutated in sporadic kideny cancer), promotes HIF1alpha degradation in the presence of oxygen

VEGFA is the master regulator of angiogenesis



VEGFR2 is the key receptor for VEGFA in ECs

Kerbel, NEJM 2008

VEGFA induces directional growth of blood vessels

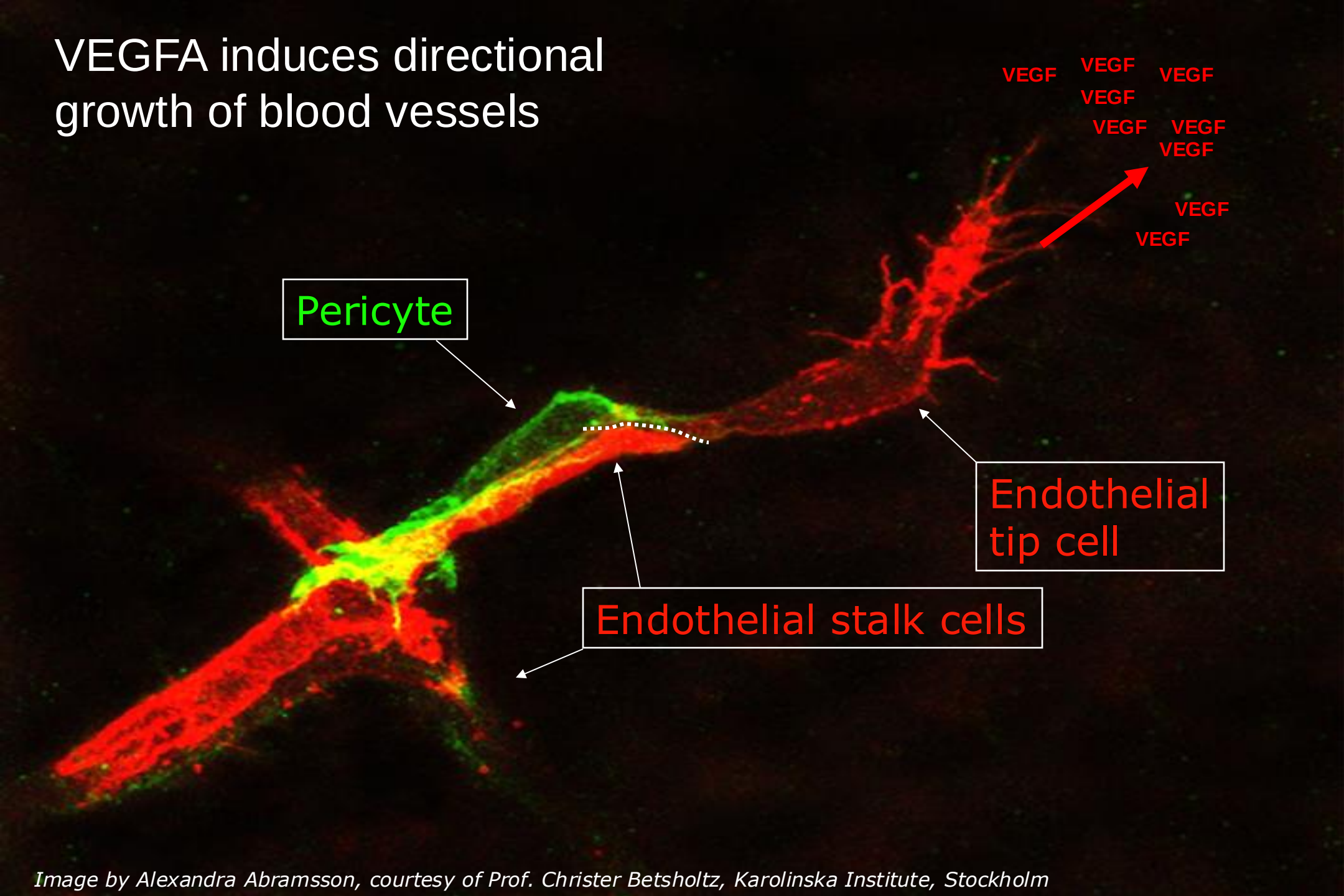
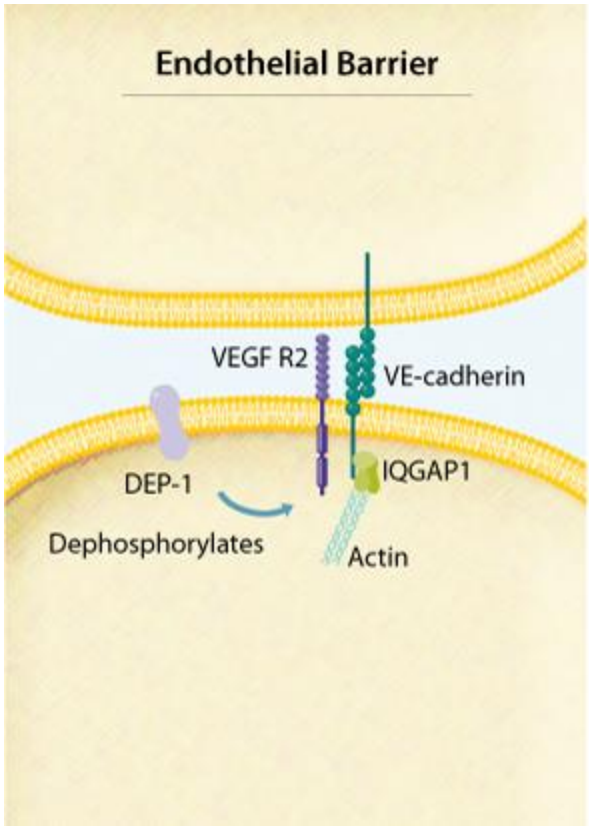
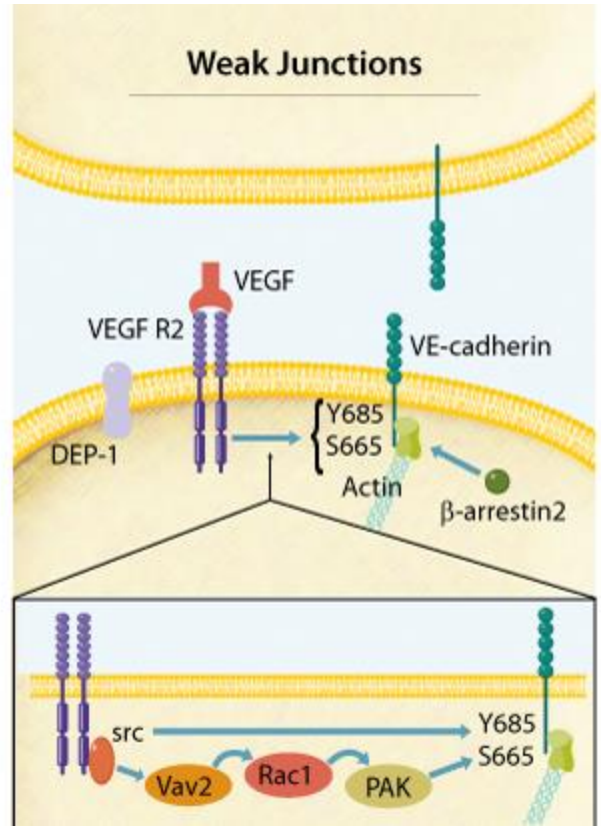
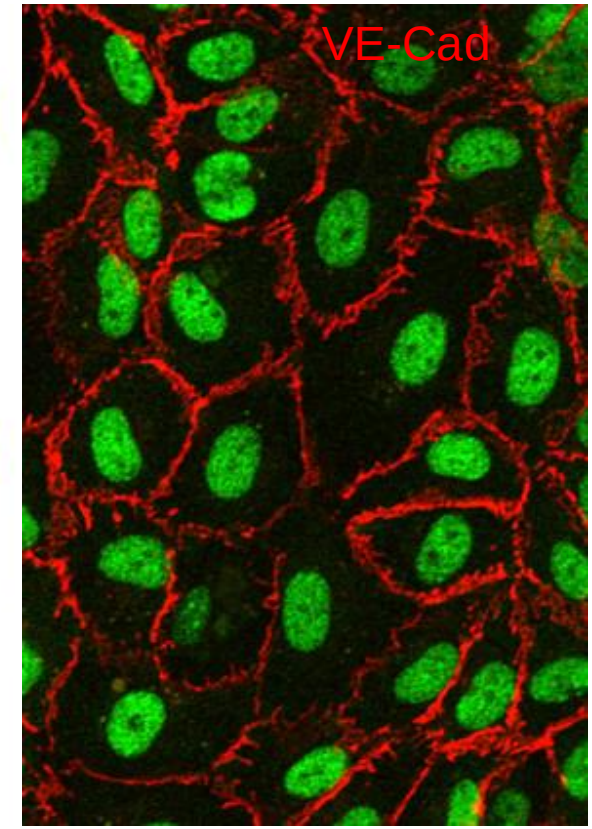


Image by Alexandra Abramsson, courtesy of Prof. Christer Betsholtz, Karolinska Institute, Stockholm

VEGFA promotes disruption of endothelial cell junctions by targeting VE-Cadherin

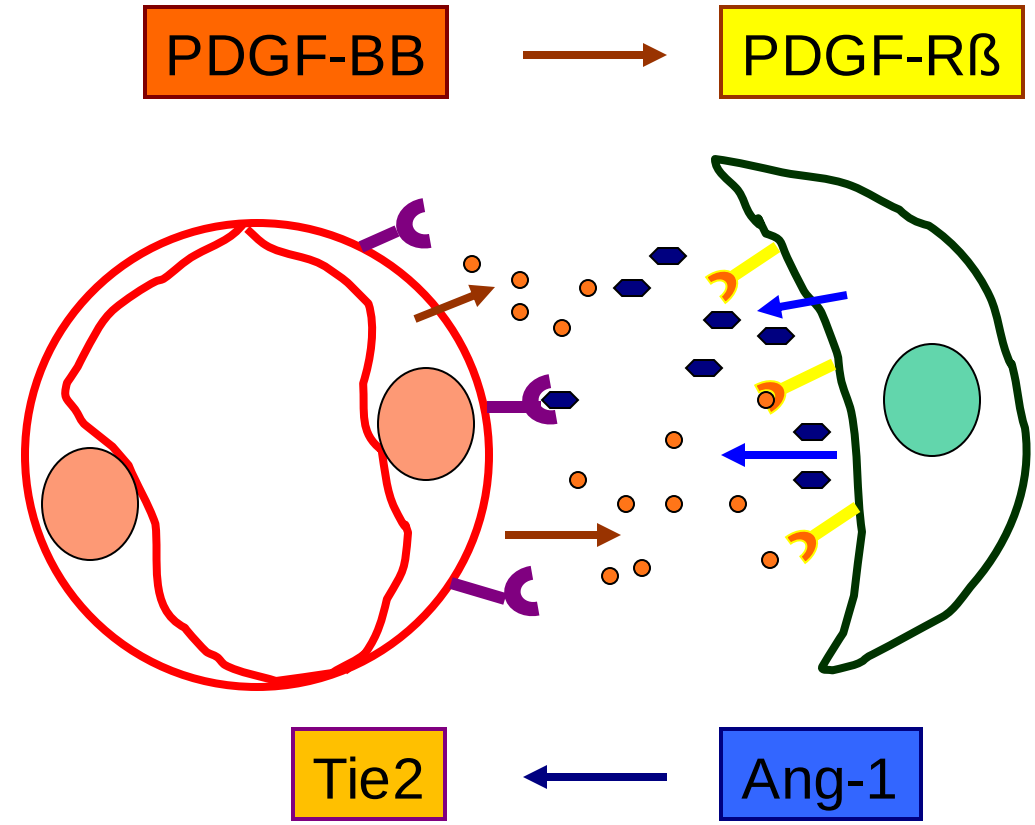




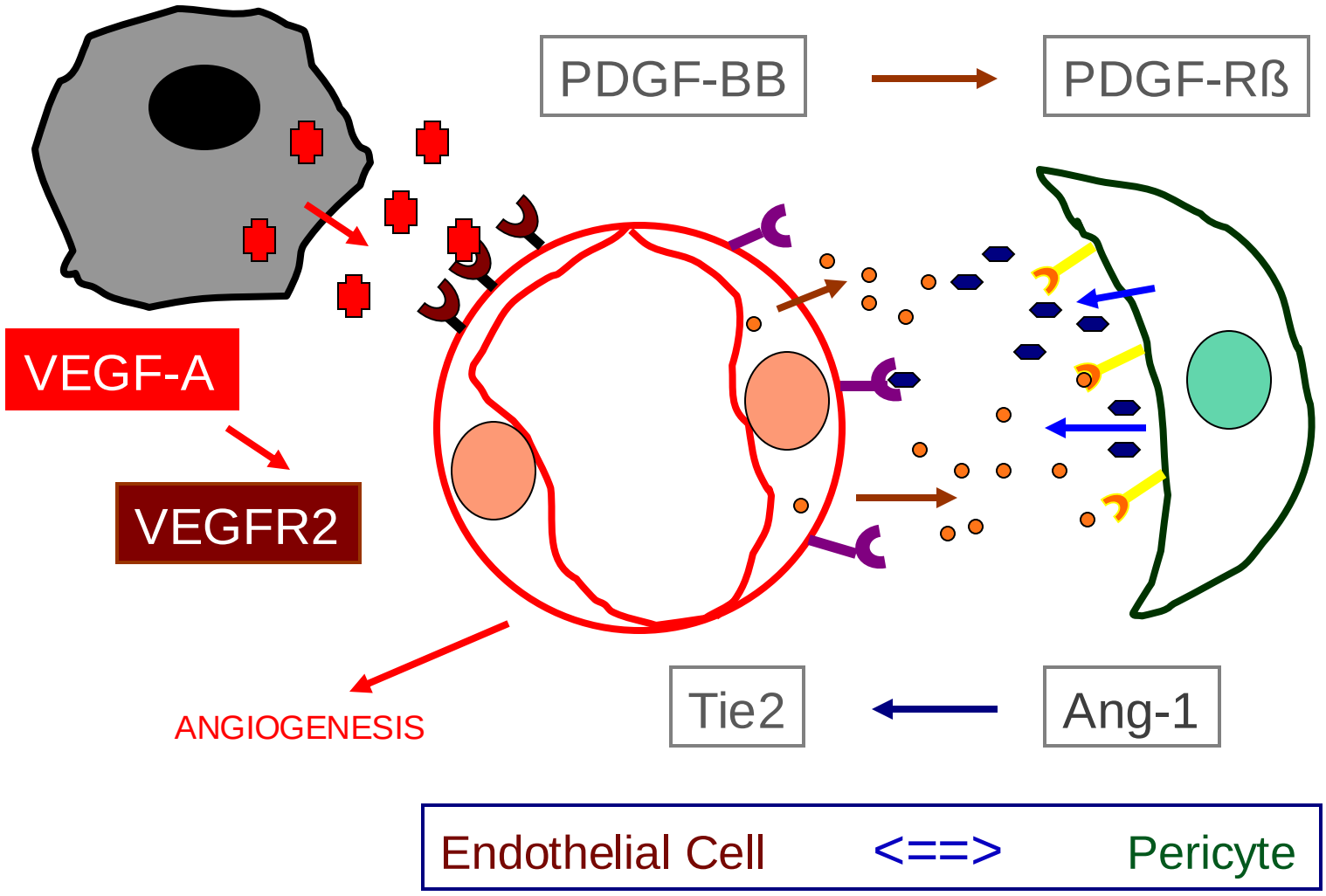


In adherens junctions between quiescent ECs, VEGFR2 is maintained in an inactive state by protein tyrosine phosphatases. VEGF binding activates VEGFR2, initiating the sequential activation of the Src-Vav2-Rac1-PAK pathway, which results in the phosphorylation of **VE-Cadherin** at Ser665 by PAK. The subsequent binding of beta-arrestin2 to serine-phosphorylated VE-Cadherin promotes the internalization of VE-Cadherin. This disrupts the architecture of endothelial junctions and allows for the passage of molecules and cells. In addition, direct phosphorylation of VE-Cadherin by Src at Tyr685 may contribute to the disassembly of adherens junctions.

Endothelial cells and pericytes associate via reciprocal paracrine interactions of regulatory ligands binding signaling receptors



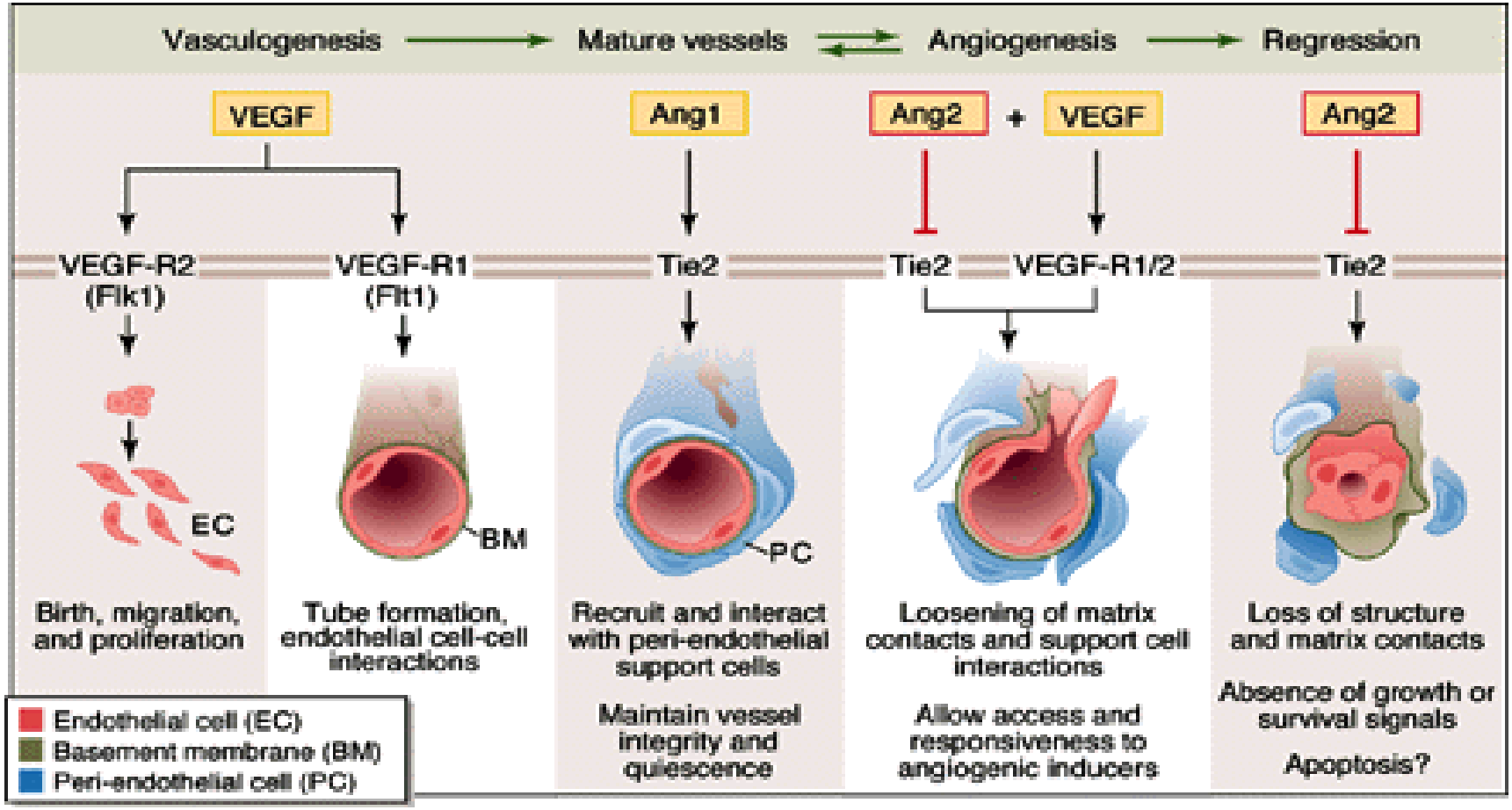
A pro-angiogenic factor such as VEGFA can overrule the quiescence of pericyte-endothelial interactions to induce angiogenesis



Angiopoietins intersect with VEGFA signaling

Ang1 / Tie2 signaling is implicated in recruiting pericytes and enabling vessel maturation; Ang-2 blocks this function.

In the presence of Ang2 + VEGFA, angiogenesis occurs while in the absence of VEGFA, Ang2 can *in some cases* promote vessel regression.



Hanahan, Science 1997

Key points about neovascularization in the adult

• **The vasculature is quiescent in the adult**. Endothelial cells are amongst the most quiescent cells of the body. Turnover times of ECs are measured in hundreds of days. In contrast, bone marrow cells have a turnover time of 2-5 days.

• In the adult, **physiological angiogenesis** (repair and reproductive) occurs as brief bursts of capillary blood vessel growth that usually last only days or weeks. During angiogenesis, ECs can divide as rapidly as bone marrow cells.

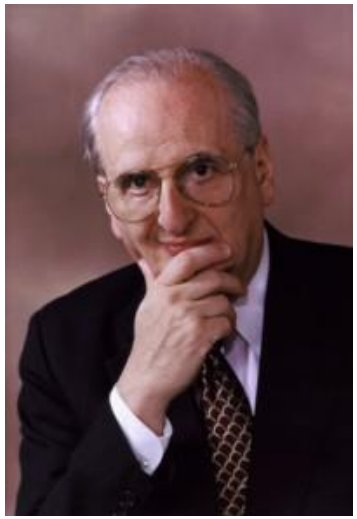
• During **pathological angiogenesis**, blood vessel growth is persistent and can continue for months or years supporting the progression of neoplastic and non-neoplastic diseases.

5. Tumor angiogenesis

A principle has been established from the work of Judah Folkman and many others during the past 50+ years:

Cells in tissues must be within 100 microns of a capillary in order to receive oxygen and nutrients by diffusion;

Nests of tumor cells cannot grow beyond a few hundred microns in diameter without inducing angiogenesis and becoming vascularized (or otherwise achieving it, e.g. by co-opting normal tissue vessels).

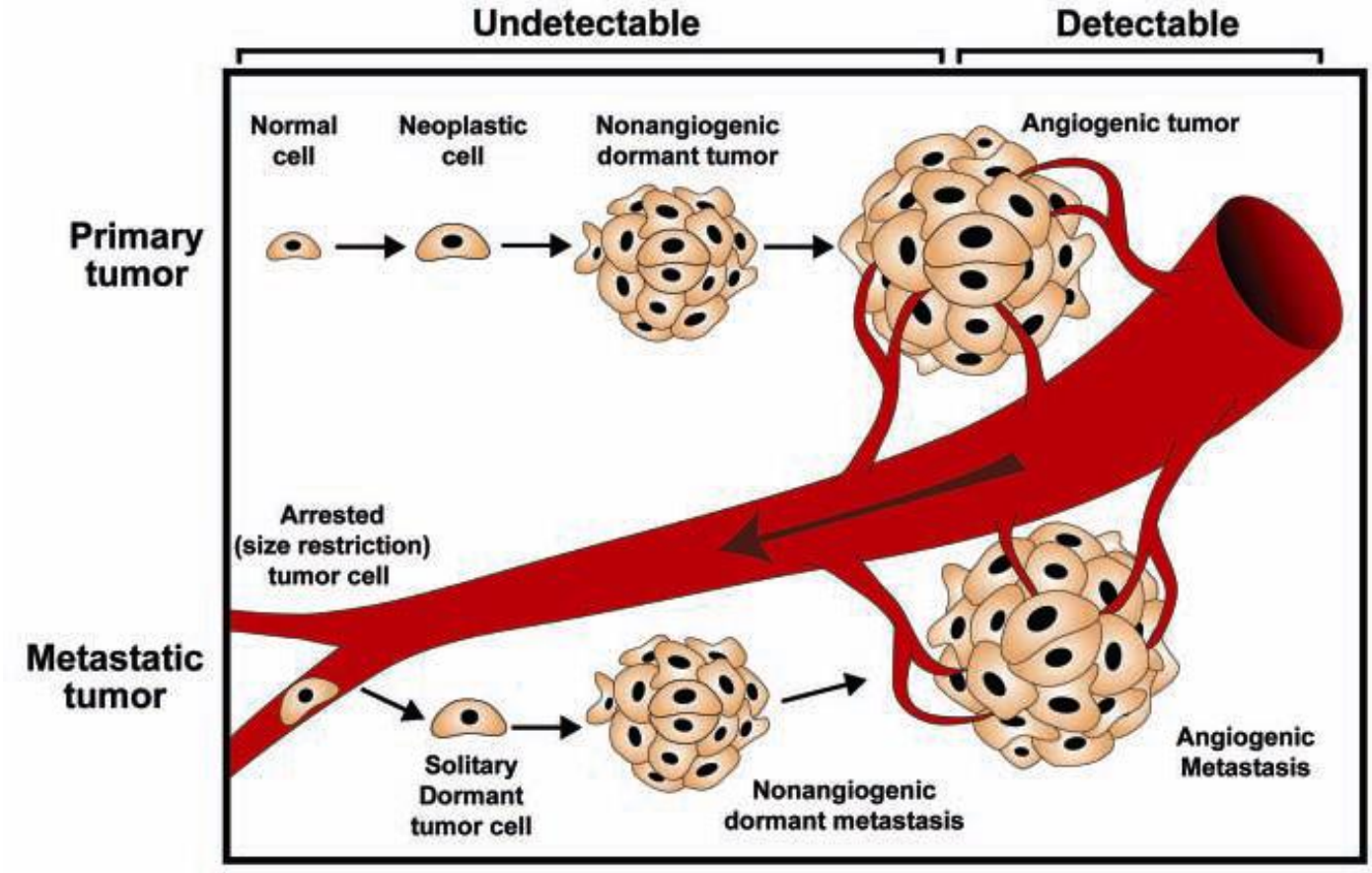


Dr. Judah Folkman

"Every tumor is dependent on a sufficient blood supply"

"Tumor growth is angiogenesis dependent".

"Therefore inhibition of angiogenesis should inhibit tumor growth." (1971)



Evidence for tumor angiogenesis and its potential requirement for tumor growth

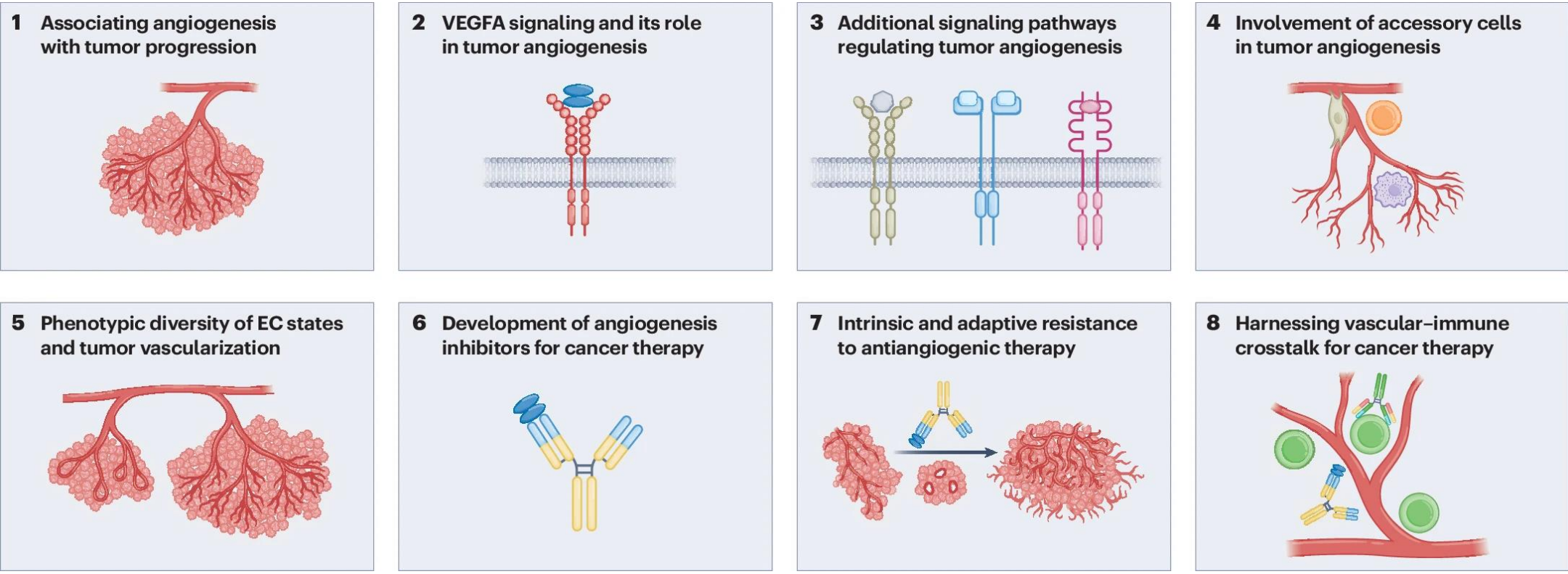
In support of the principle, virtually all tumors analyzed in humans and in animal models:

a) are highly vascularized by vessels with aberrant morphology

b) have evidence of ongoing angiogenesis (at least partly)

A variety of histological characteristics mark the angiogenic phenotype in tumors

Eight conceptual milestones for tumor vascularization and its therapeutic targeting



De Palma & Hanahan, Nature Cancer, 2024

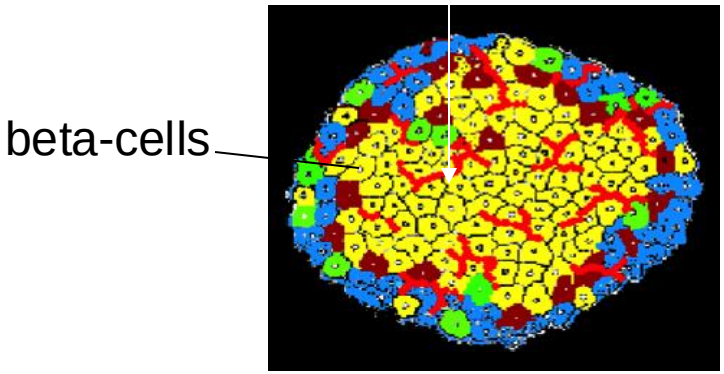
Histological signs of tumor angiogenesis

- Endothelial cell proliferation (i.e. cell cycle progression, being rare in normal vessels);
- Endothelial cell elongation and sprouting from established vessels;
- Increased branching of blood vessels;
- Dilation of vessels;
- Micro-hemorrhaging (from the tips of new sprouts, and due to poor cell-cell adhesion, which can produce the blood red color typical of some tumor types);
- Variable and less intimate coverage by pericytes.

RIP1-Tag2 transgenic mouse model of pancreatic neuroendocrine cancer

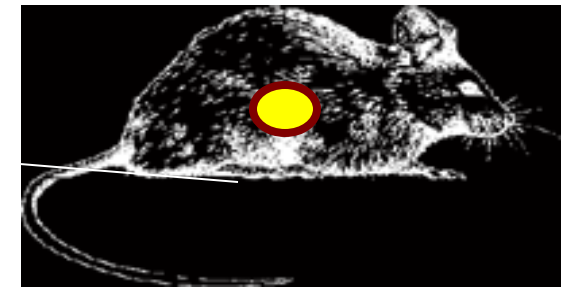
(Rat insulin gene promoter region)

(Potent oncoprotein, inactivates p53 and pRb)



Islet of Langerhans

RIP1-Tag transgenic mice



Hanahan, Nature 1984

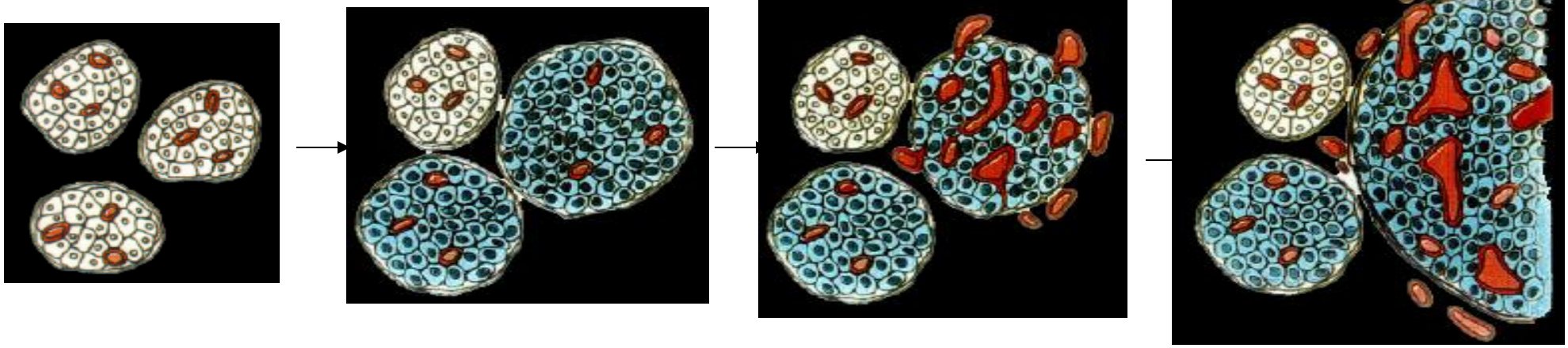
Visualizing the blood vasculature in the RIP-Tag model of multistage pancreatic islet carcinogenesis

Normal stage (onc+)

Hyperplastic/ dysplastic stage

Angiogenic stage (transition to malignancy)

Tumor stage



<5 wks

100%

5-7 wks

~50%

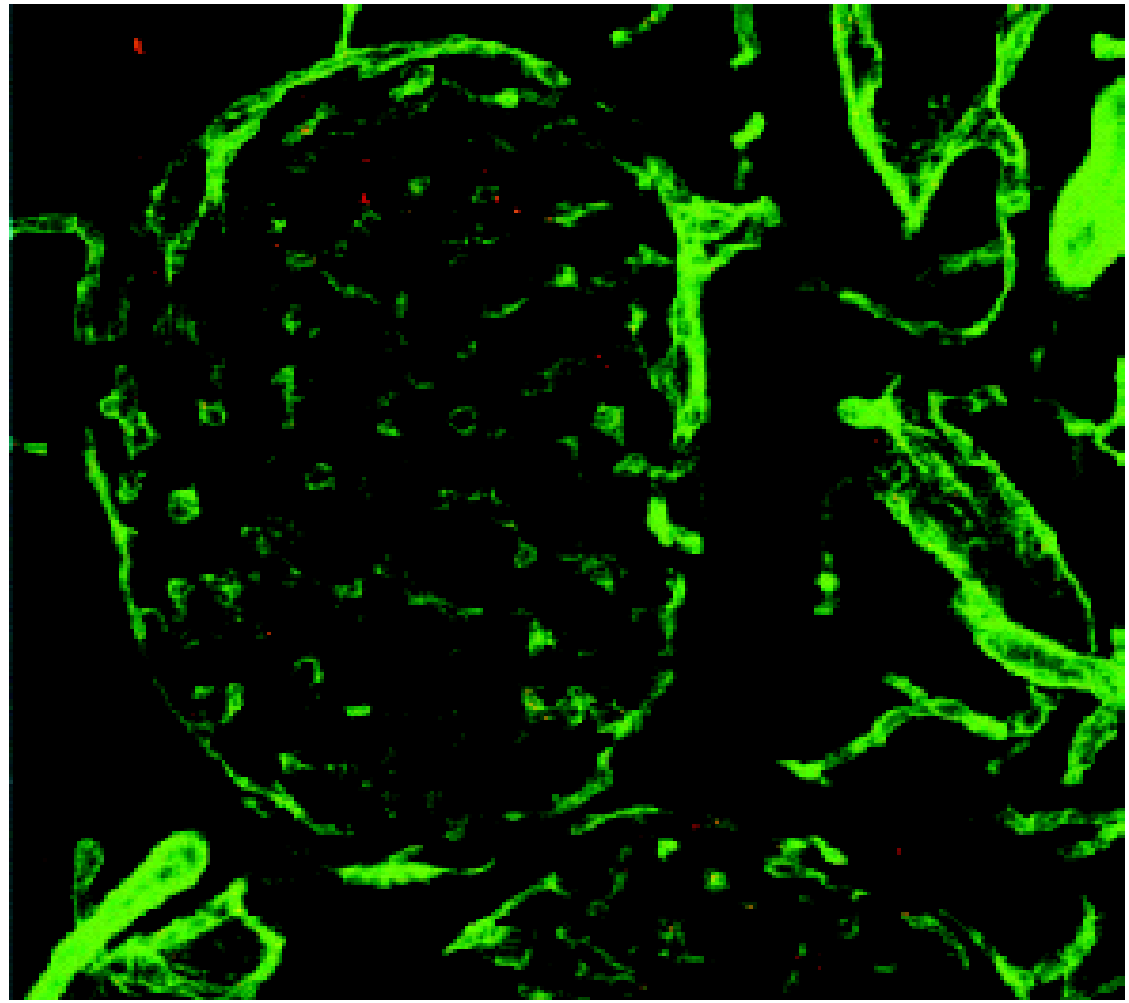
7-12 wks

~10%

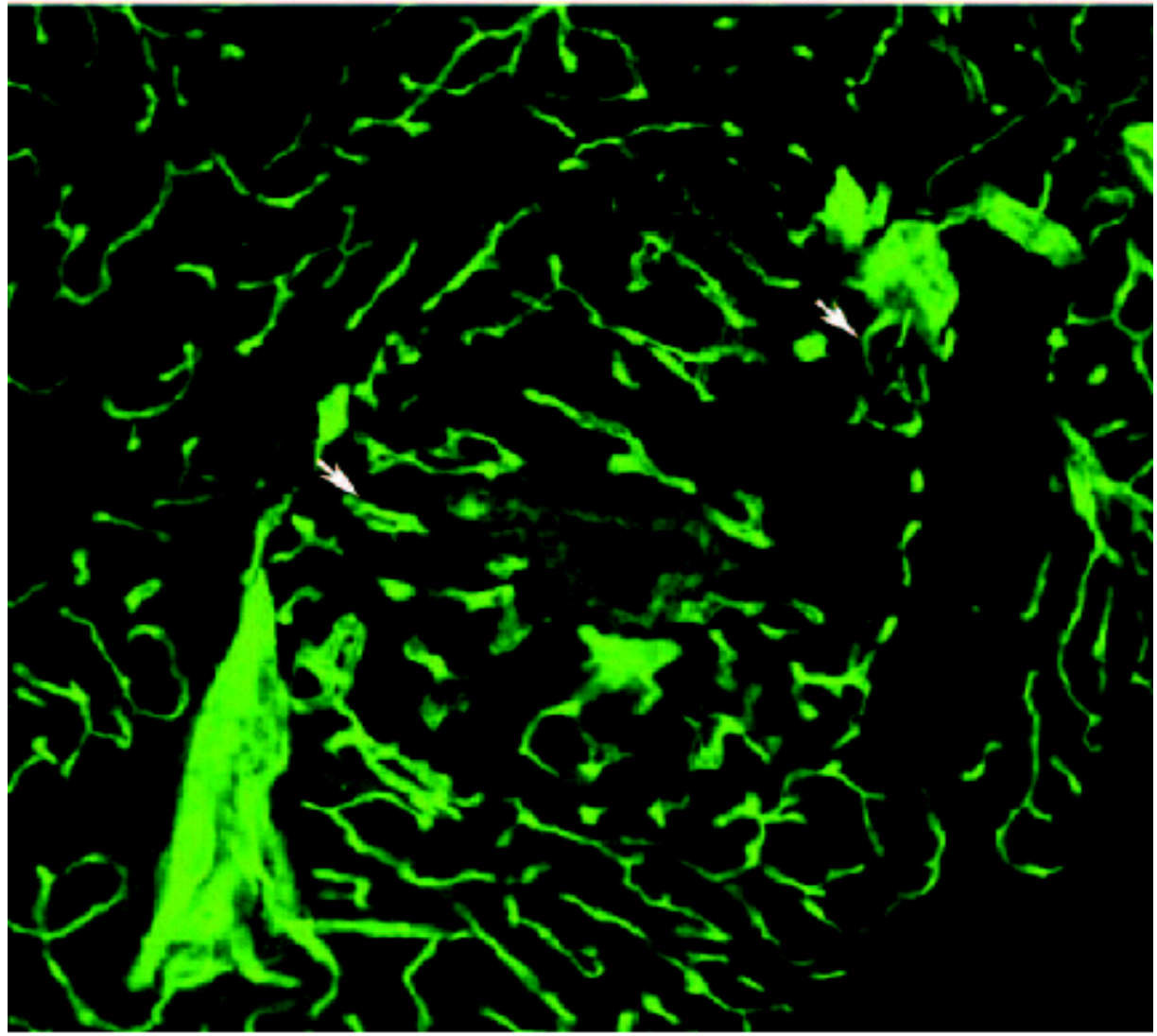
12-14 wks

2-4%

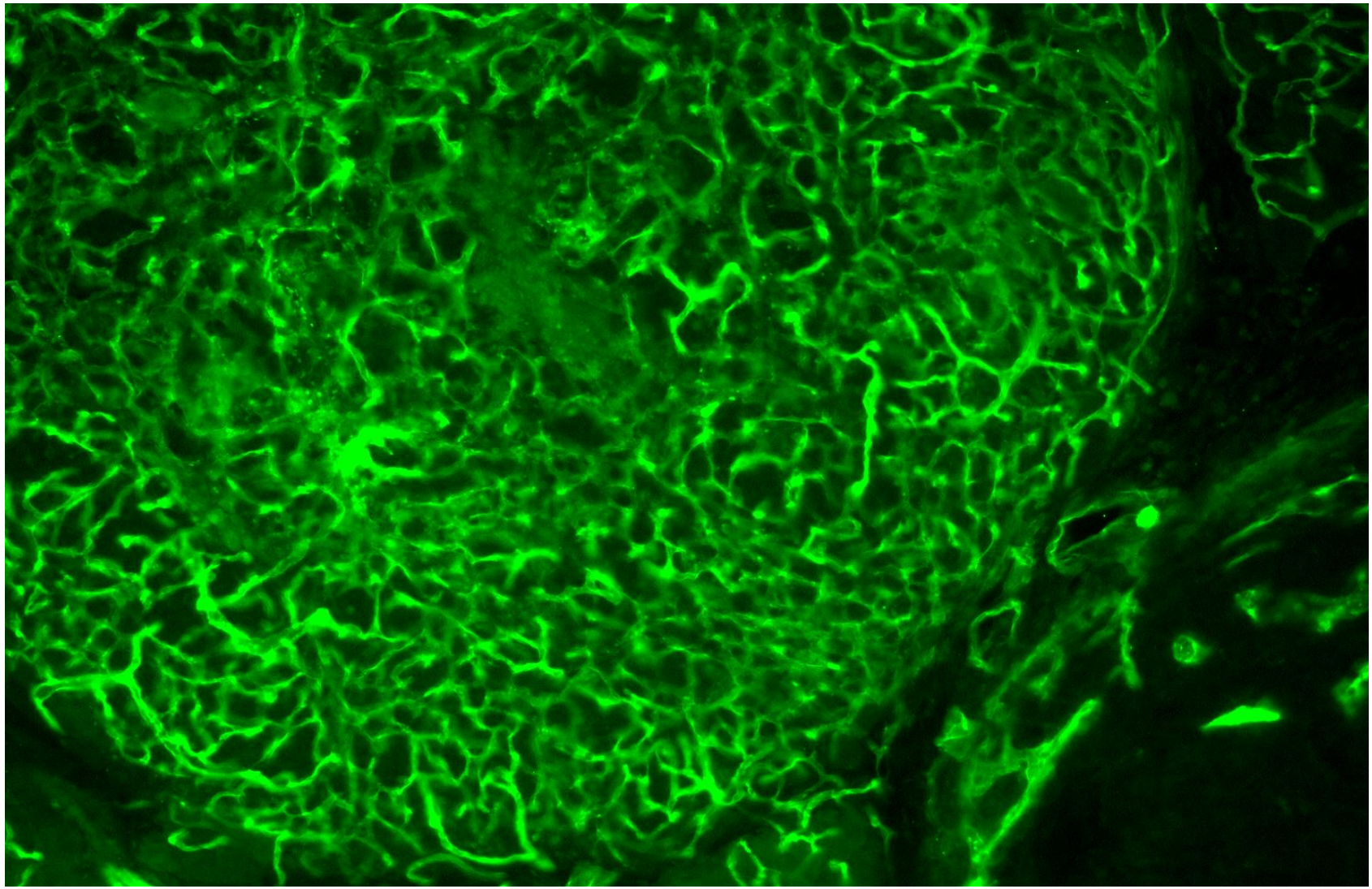
Normal islet



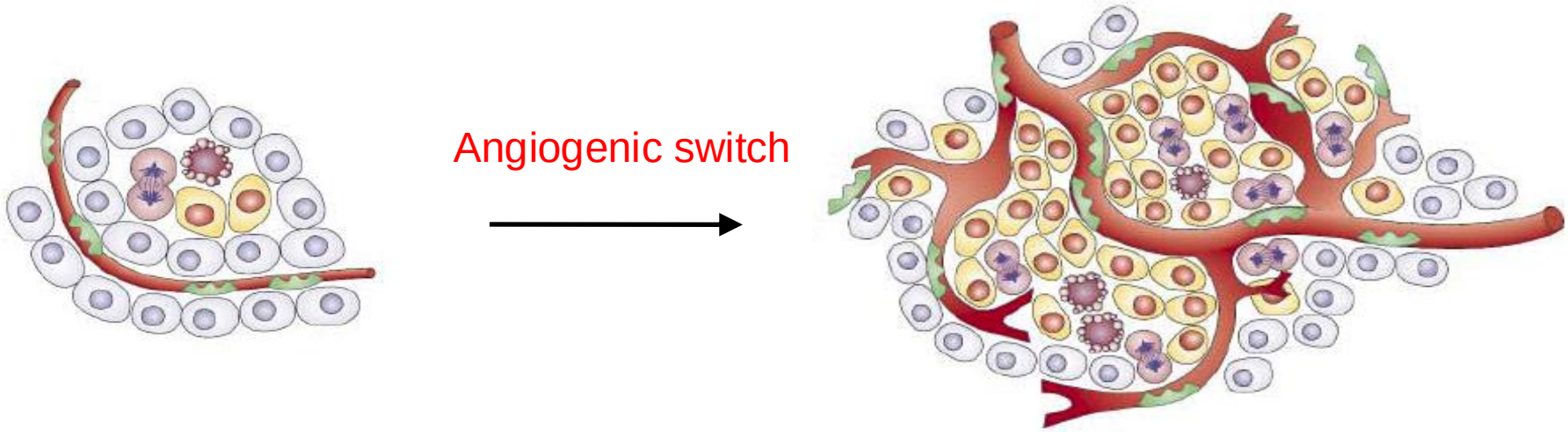
Angiogenic islet (dysplasia)



Solid tumor



The angiogenic switch (induction of angiogenesis) is a discrete step and a hallmark of cancer



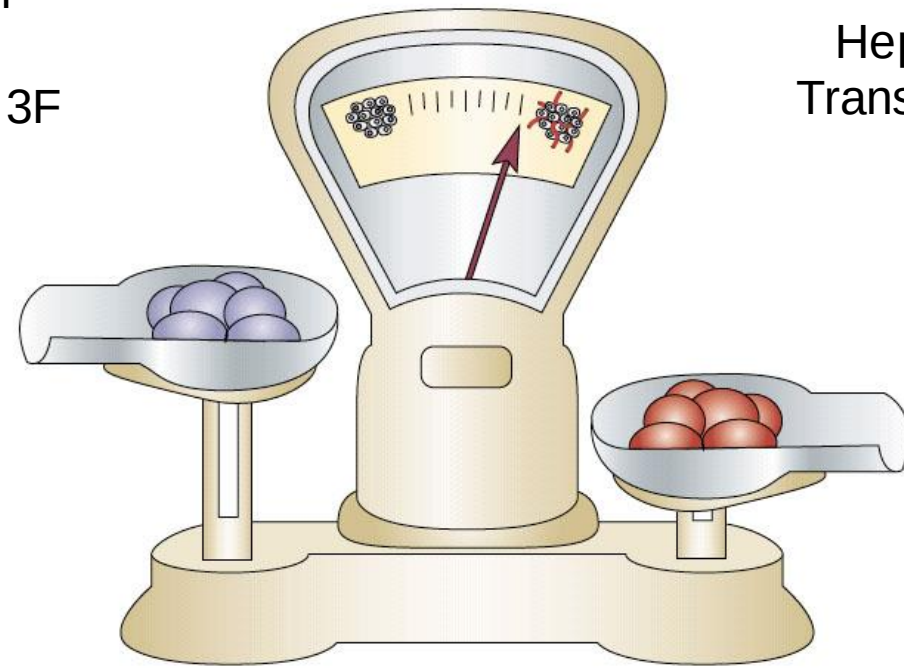
Can occur at distinct stages during tumorigenesis (e.g. premalignant lesions or later stages)

It is dependent on tumor type and the microenvironment

A tilt in the balance between angiogenic activators and inhibitors induces angiogenesis

Inhibitors

α/β-Interferon
Thrombospondin-1
Angiostatin
Endostatin
TIMP-2
Semaphorin 3F
etc



Activators

Vascular endothelial growth factor A
Angiopoietin 2
Basic fibroblast growth factor
Platelet-derived growth factors
Hepatocyte growth factor
Transforming growth factor β
Interleukin 1 and 8
Ephrins
Heparin
etc.

The angiogenic switch was first demonstrated in 1989 in the RipTag mouse model of multi-step endocrine pancreatic tumorigenesis

~ 50%

~10%

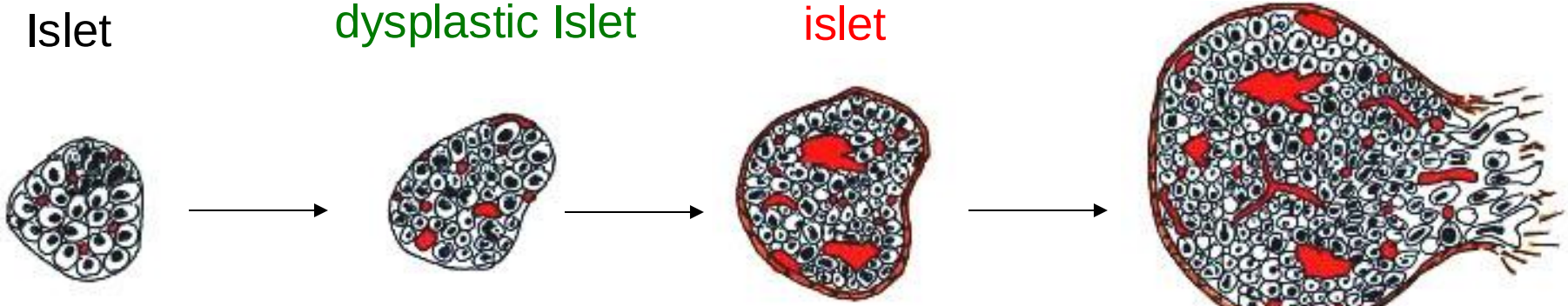
~1-2%

Normal Islet

Hyperplastic/ dysplastic Islet

Angiogenic islet

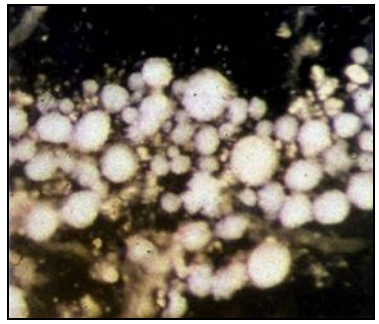
Tumors/ carcinomas

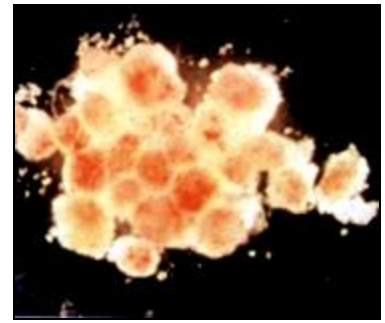


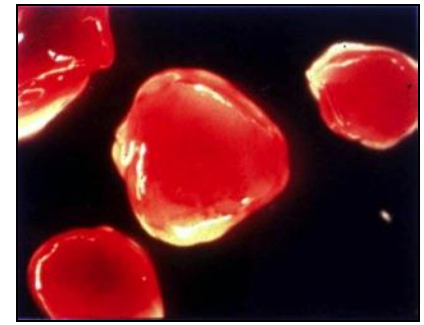
4-5wks

7wks

10wks



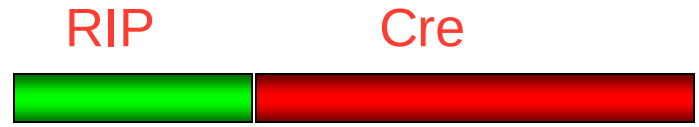


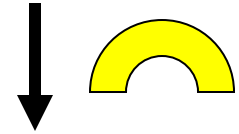


Is VEGFA signaling functionally important?

A genetic test: knocking out expression of VEGFA in tumor cells

Rip-Cre transgene (ß cell selective)





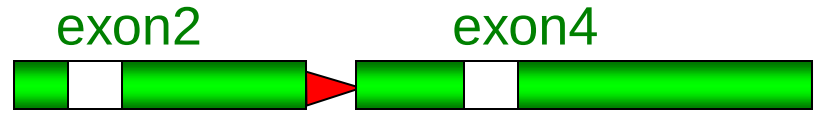
Cre recombinase protein

VEGF gene (functional)

= loxP sites flanking exon 3

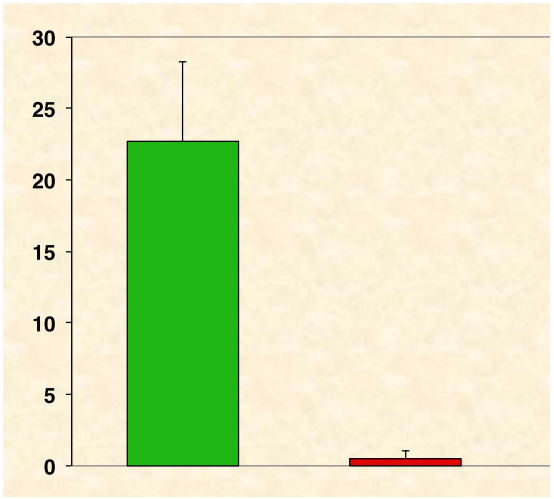
Delete essential exon #3

VEGFA KO (ß cell selective)



Loss of VEGFA impairs the angiogenic switch in hyperplastic-dysplastic islets as well as tumor formation/growth

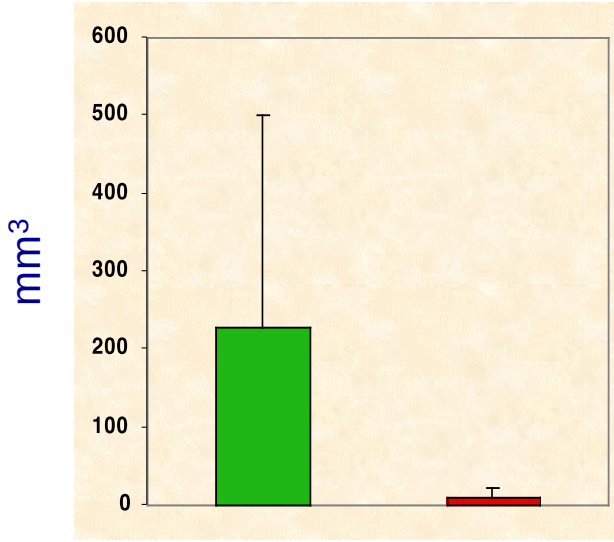
Number of angiogenic Islets



w.t. ko/ko

(10 W)

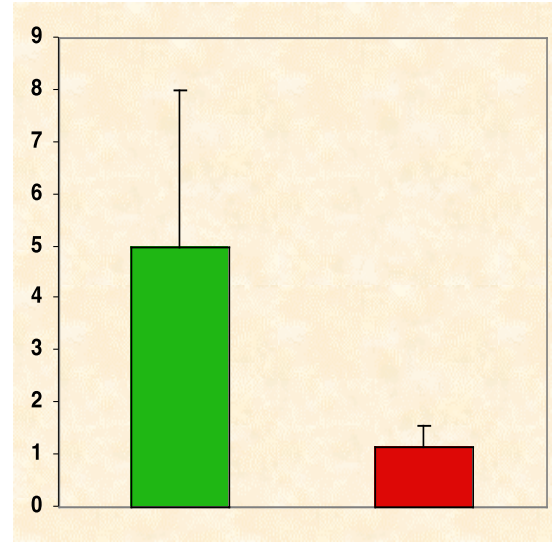
Tumor burden



w.t. ko/ko

(14W) (16W)

Number of tumors



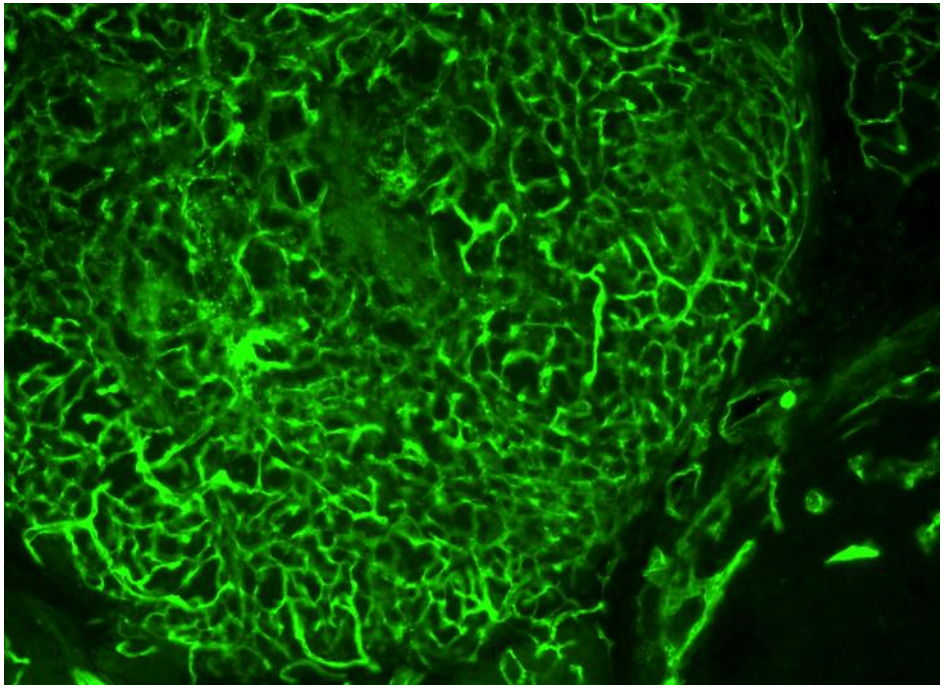
w.t. ko/ko

(14W) (16W)

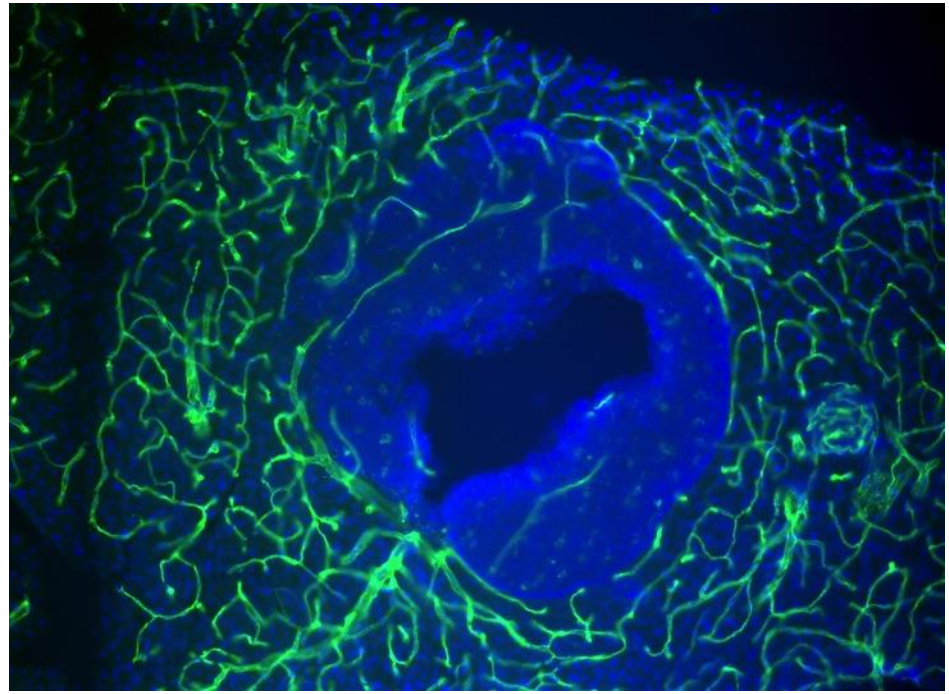
Inoue, M., Hager, J., Ferrara, N., Gerber. H.-P., & Hanahan, D. (2001). VEGF-A has a critical, non-redundant role in angiogenic switching and pancreatic ß-cell carcinogenesis. Cancer Cell, 1: 193 – 202.

Tumorigenesis and the neovasculature are severely disrupted in the absence of VEGFA expression in the cancer cells

Rip1Tag2



Rip1Tag2-
RipCre-VEGF-lox/lox



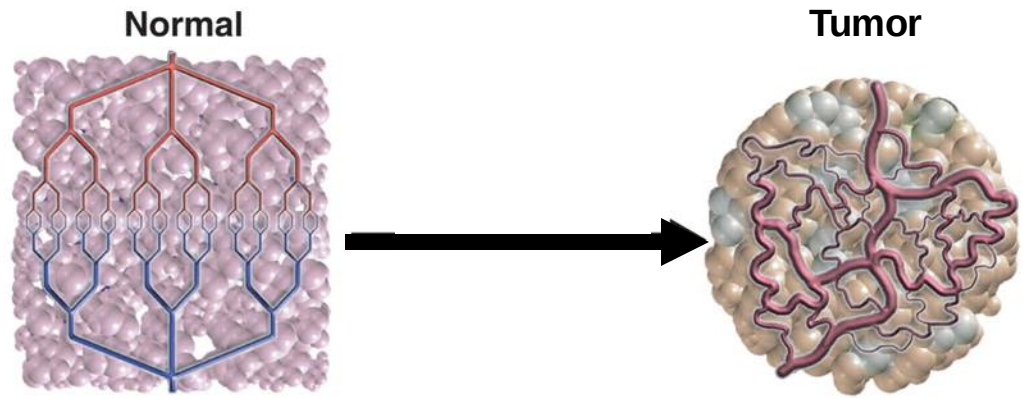
i.v. lectin staining to visualize the vasculature

Hallmarks of the tumor vasculature

- Irregularly shaped, tortuous, dead ends
- Chaotic organization (no defined arterioles, venules etc.)
- Leaky, hemorrhagic
- Pericytes less abundant and more loosely associated with the vasculature
- Cancer cells can be integrated in the vessel wall

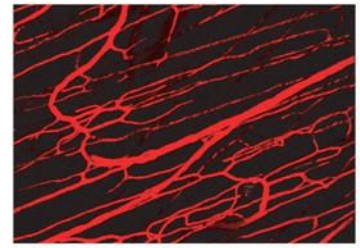
Features of tumor blood vessels

Organization in arteries, veins and capillaries



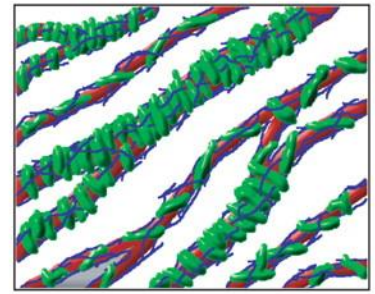
No distinct arteries and veines

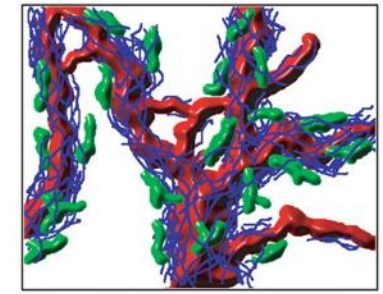
Regular morphology



Irregular morphology

High and even pericyte coverage (maturation; stability)

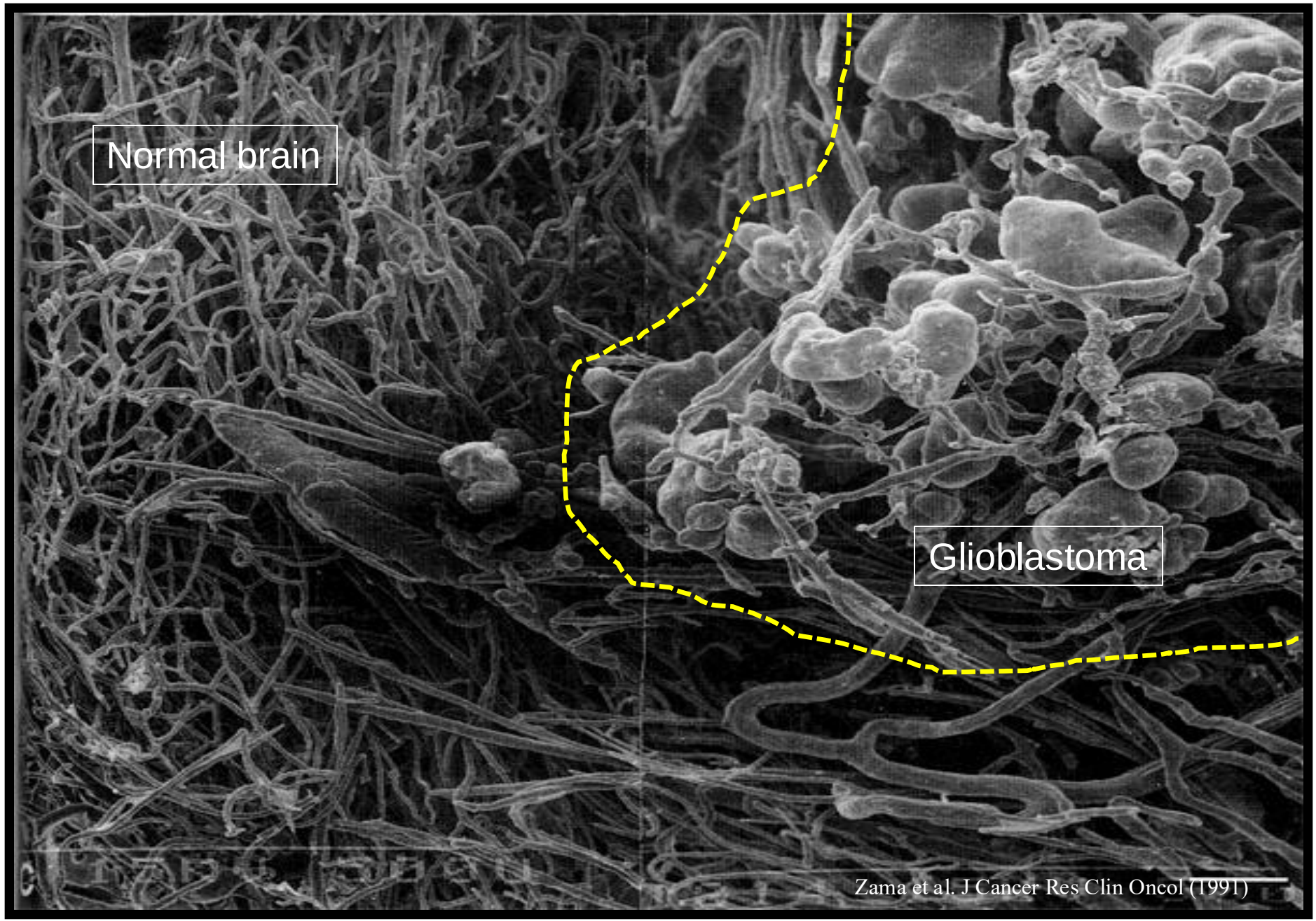




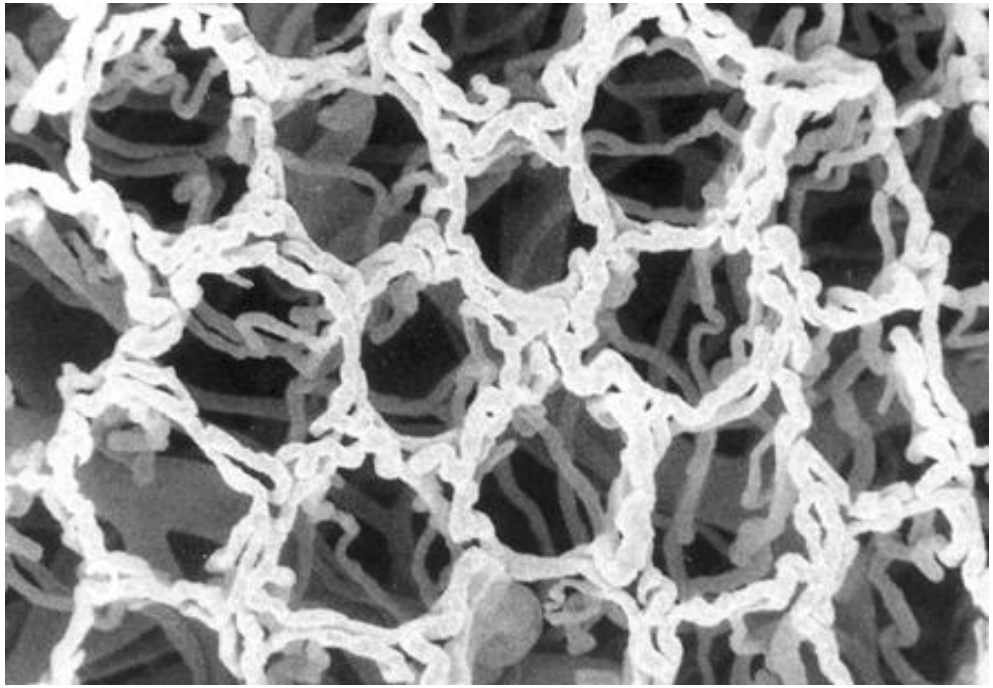
Uneven pericyte coverage (immature, unstable, leaky)

Because of these features, tumor blood vessels are poorly functional, leaky and provide **inadequate** oxygen and nutrient levels to the tumor mass

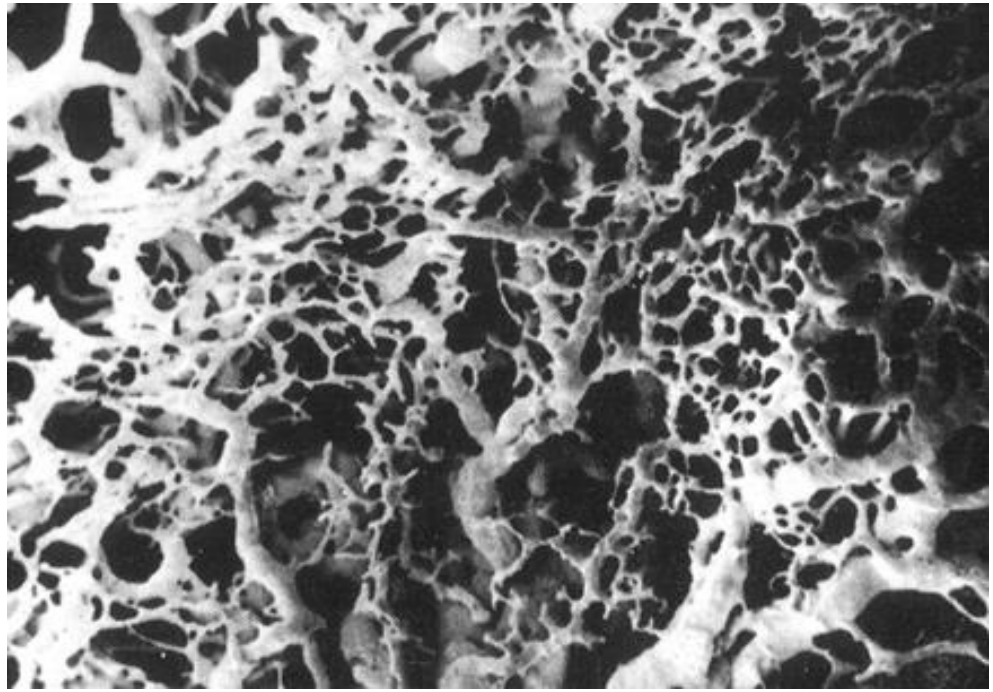
Tumor vessels differ from normal blood vessels



Tumor blood vessels differ from normal vessels



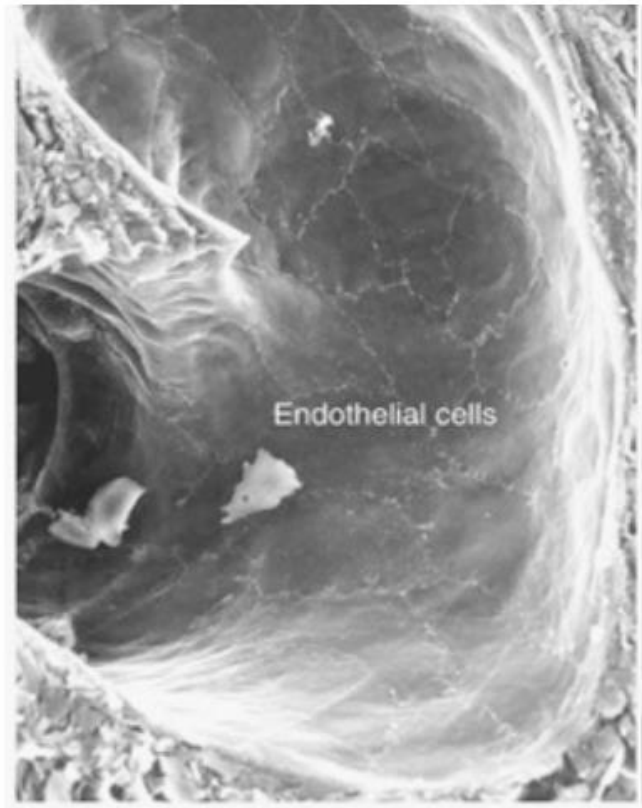
normal colon



colorectal cancer

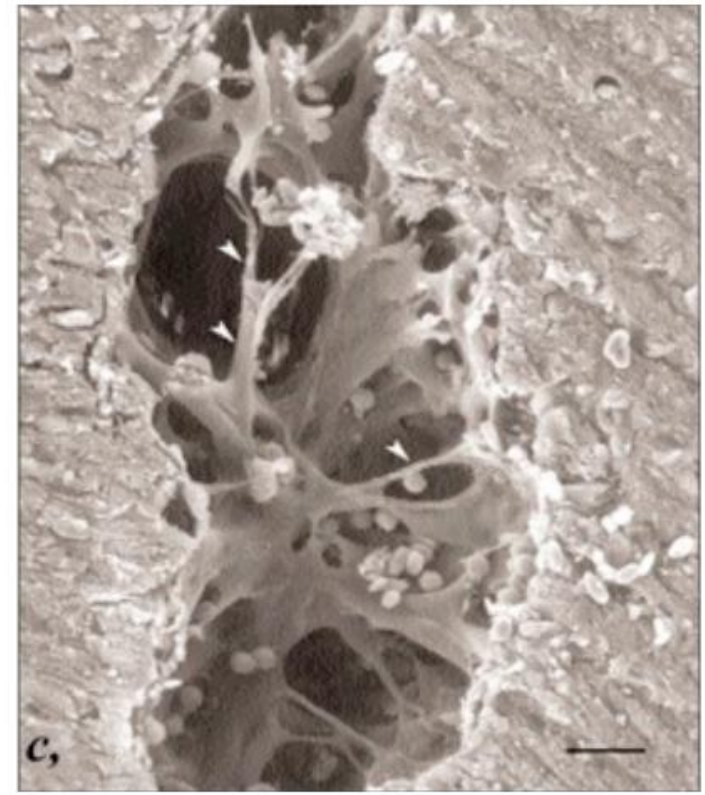
Tumor blood vessels differ from normal vessels

normal



single layer, quiescent, tight barrier, smooth lining, no filopodia or protrusions

tumor



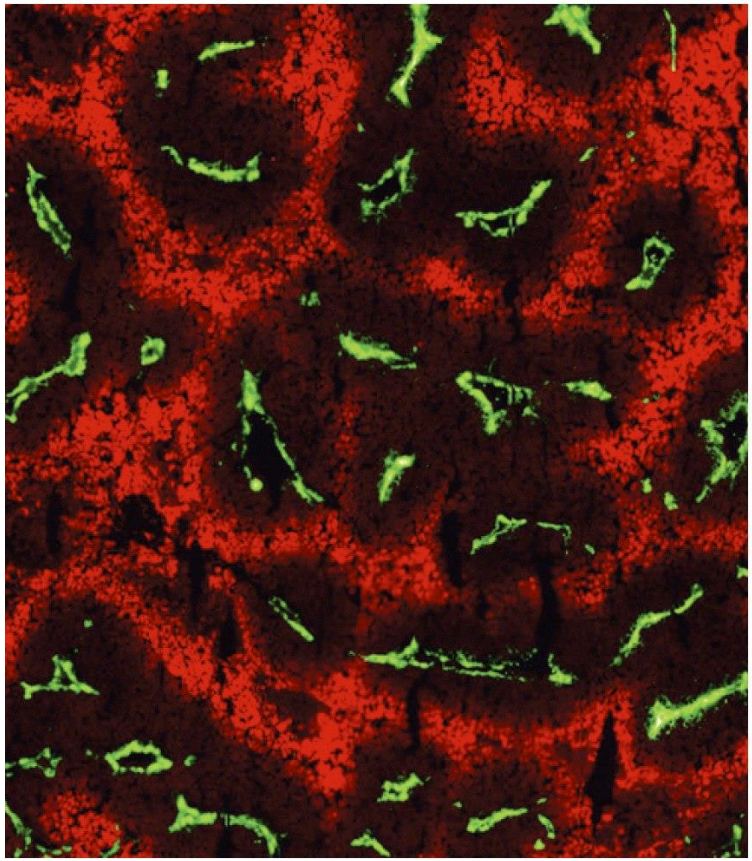
leaky, abnormally aligned, stratification, multilayer, protrusions, non-quiescent, hypoperfused, irregular flow

Courtesy: Max Mazzone, VIB, Leuven (BE)

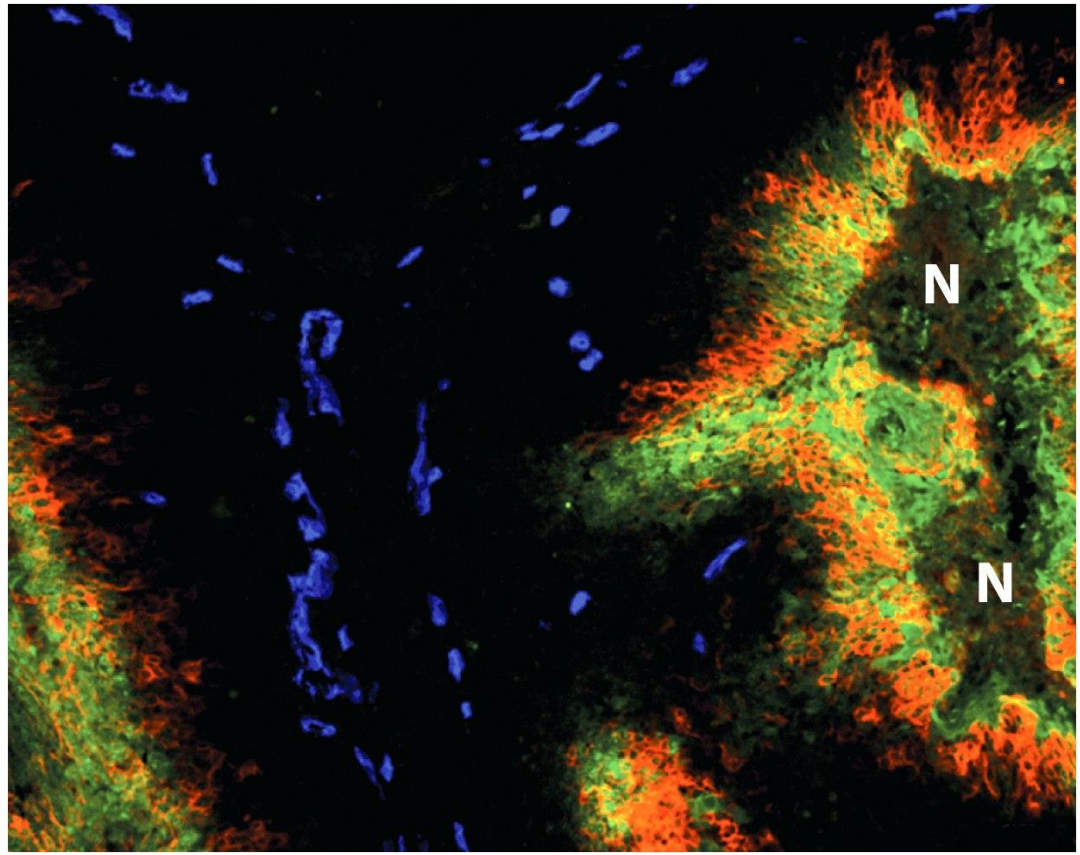
6. Consequences of tumor angiogenesis

The aberrant and dysfunctional tumor vasculature promotes a chronic "wound healing response" that sustains tumor progression

Tumor-associated blood vessels are dysfunctional and lead to variegate tumor hypoxia and necrosis



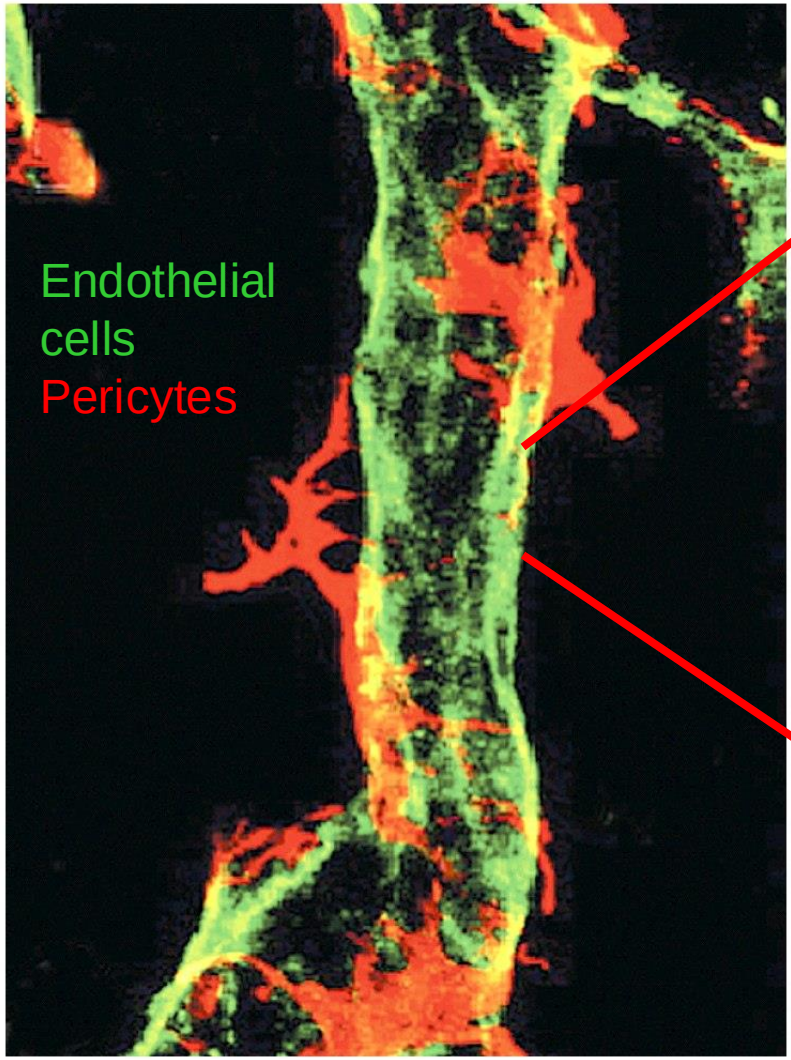
Blood vessels
Hypoxic cells (low oxygen)



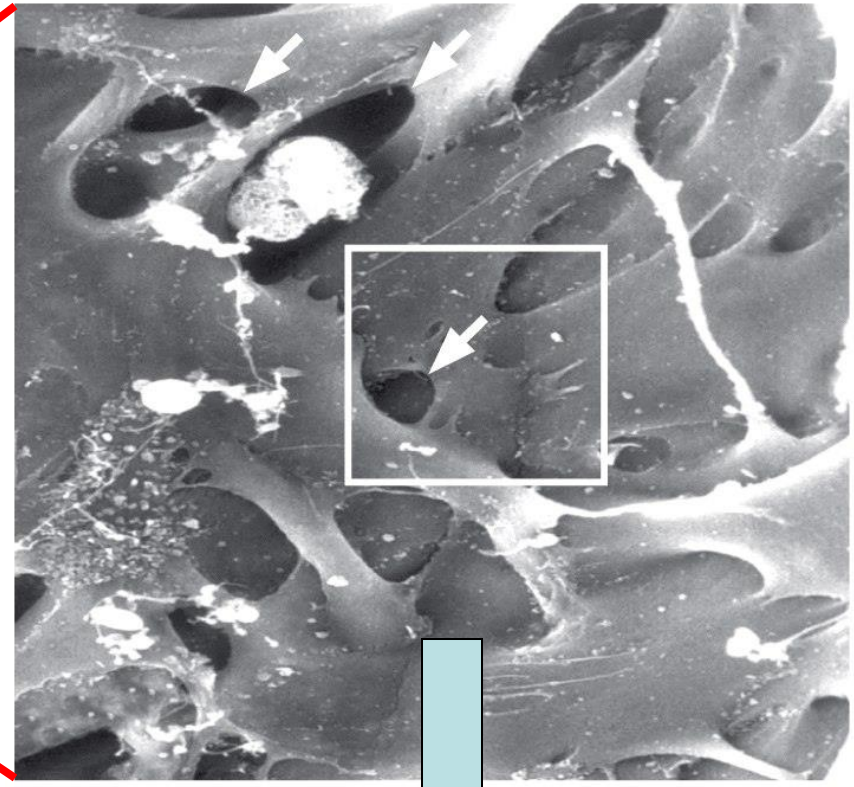
Blood vessels
Carbonic anhydrase (low oxygen)
Pimonidazole adducts (very low oxygen)

Figure 13.27a *The Biology of Cancer* (© Garland Science 2007)

Uneven pericyte coating, endothelial gaps and labile endothelial junctions cause significant accumulation of fluid in the tumor and promote inflammation



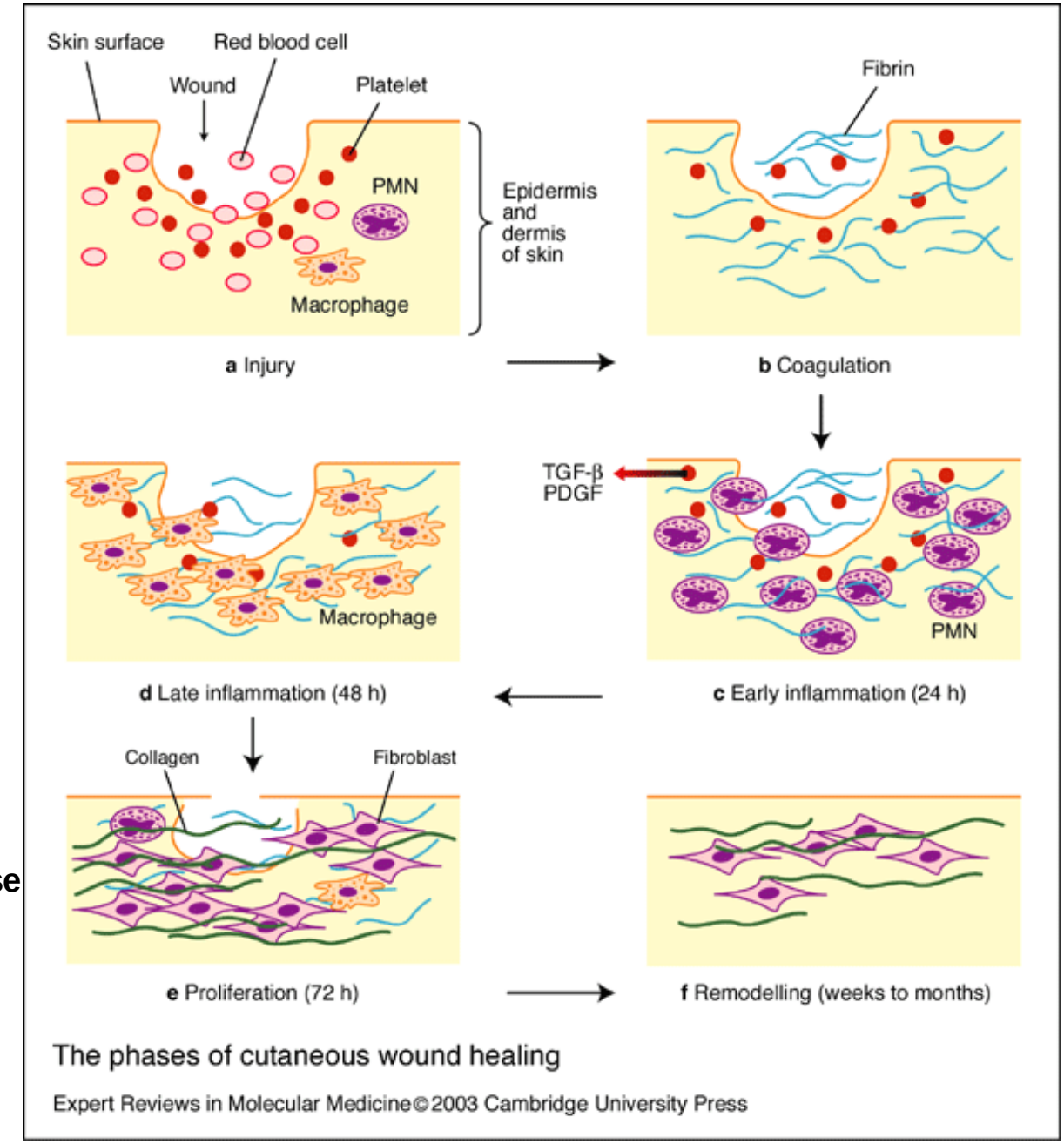
Lumen (inner surface) of a tumor vessel



Leakage of plasma within the tumor mass:

wound healing response

Wound healing (normal tissues)



Hemostasis phase

Inflammatory phase

Proliferative phase

Remodeling phase

Flowchart of wound healing (normal tissues)

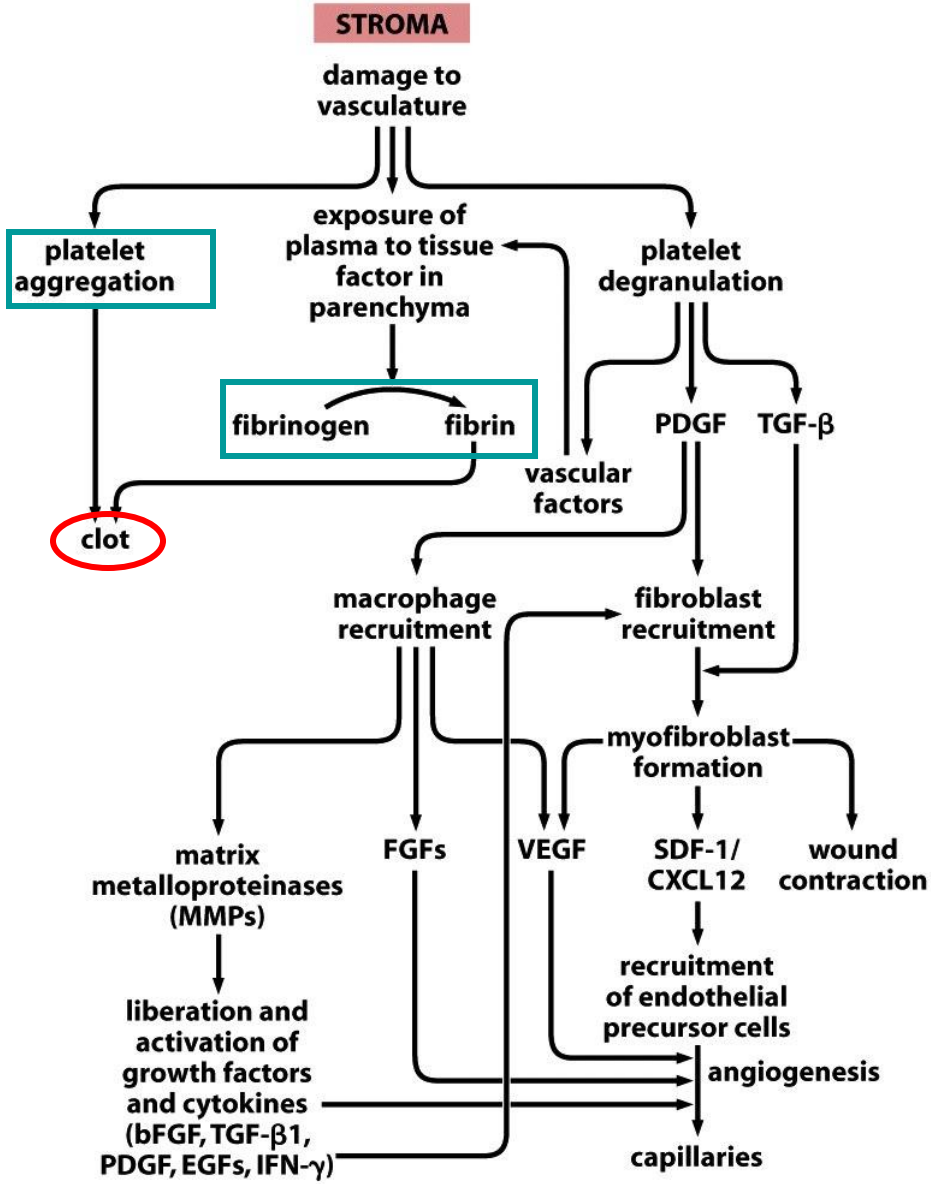


Figure 13.10 *The Biology of Cancer* (© Garland Science 2007)

Wound healing

1) Hemostasis phase

- Vascular injury: endothelium is damaged and blood contacts collagen → activation and aggregation of platelets.

- Tissue factor (TF) initiates a cascade that culminates with the conversion of pro-thrombin into thrombin, which in turn converts fibrinogen into fibrin. Fibrin forms a mesh that traps other platelets ("coagulation"). A "blood clot" is formed.

Flowchart of wound healing (normal tissues)

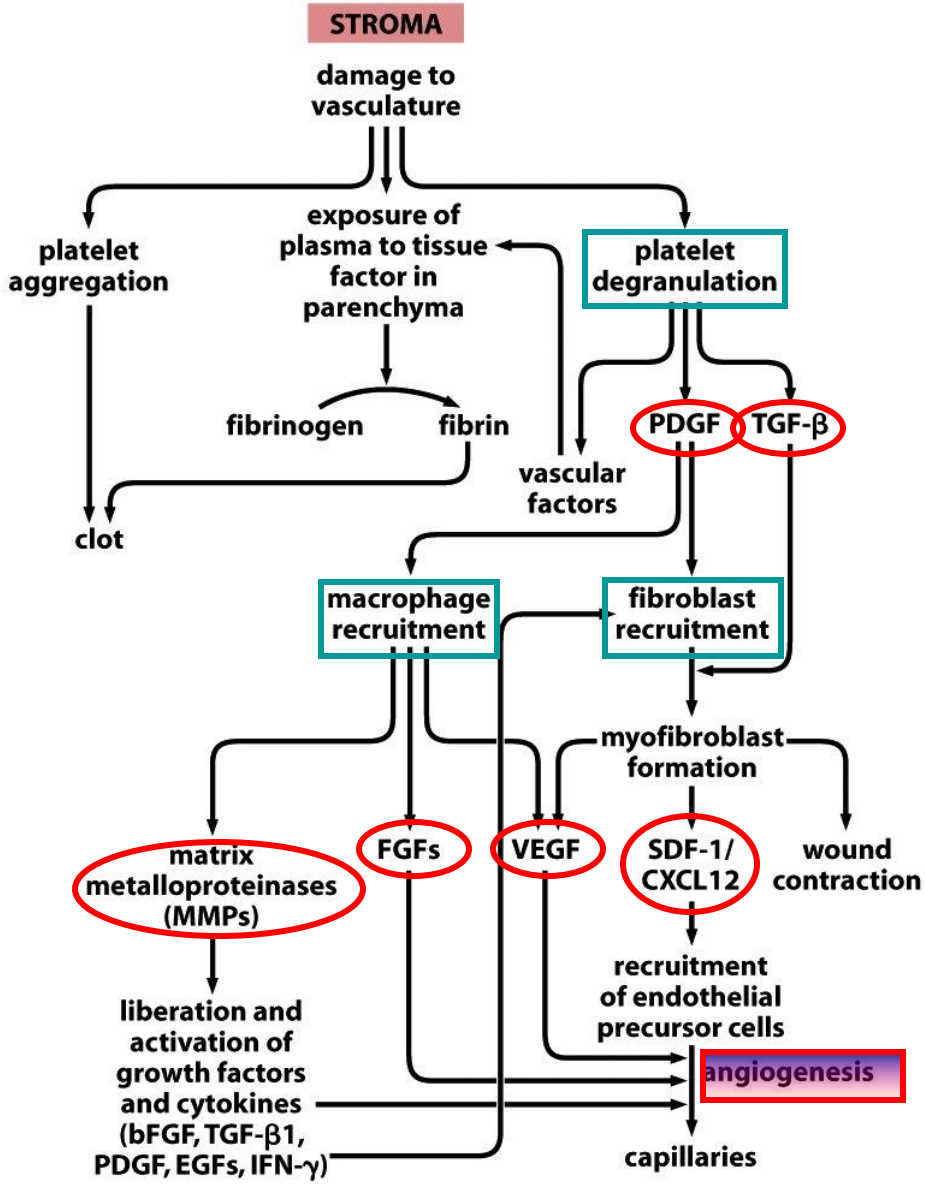


Figure 13.10 *The Biology of Cancer* (© Garland Science 2007)

Wound healing

2) Inflammation and angiogenesis phases

- Growth factors released by activated platelets (PDGF, TGFb, etc.) attract macrophages, which secrete VEGFA and other factors (eg., proteases) that directly or indirectly stimulate angiogenesis.

- PDGF also attracts fibroblasts and stimulates their proliferation; TGFb stimulates them to release proteases. Fibroblasts also secrete VEGFA and CXCL12 that directly promote angiogenesis.

- Matrix-metallo proteinases (MMPs) released by macrophages and fibroblasts (i) digest/remodel the ECM to facilitate cell migration; (ii) release ECM-bound growth factors (e.g. FGF, VEGFA). Both processes facilitate angiogenesis.

Wound healing (normal tissues)

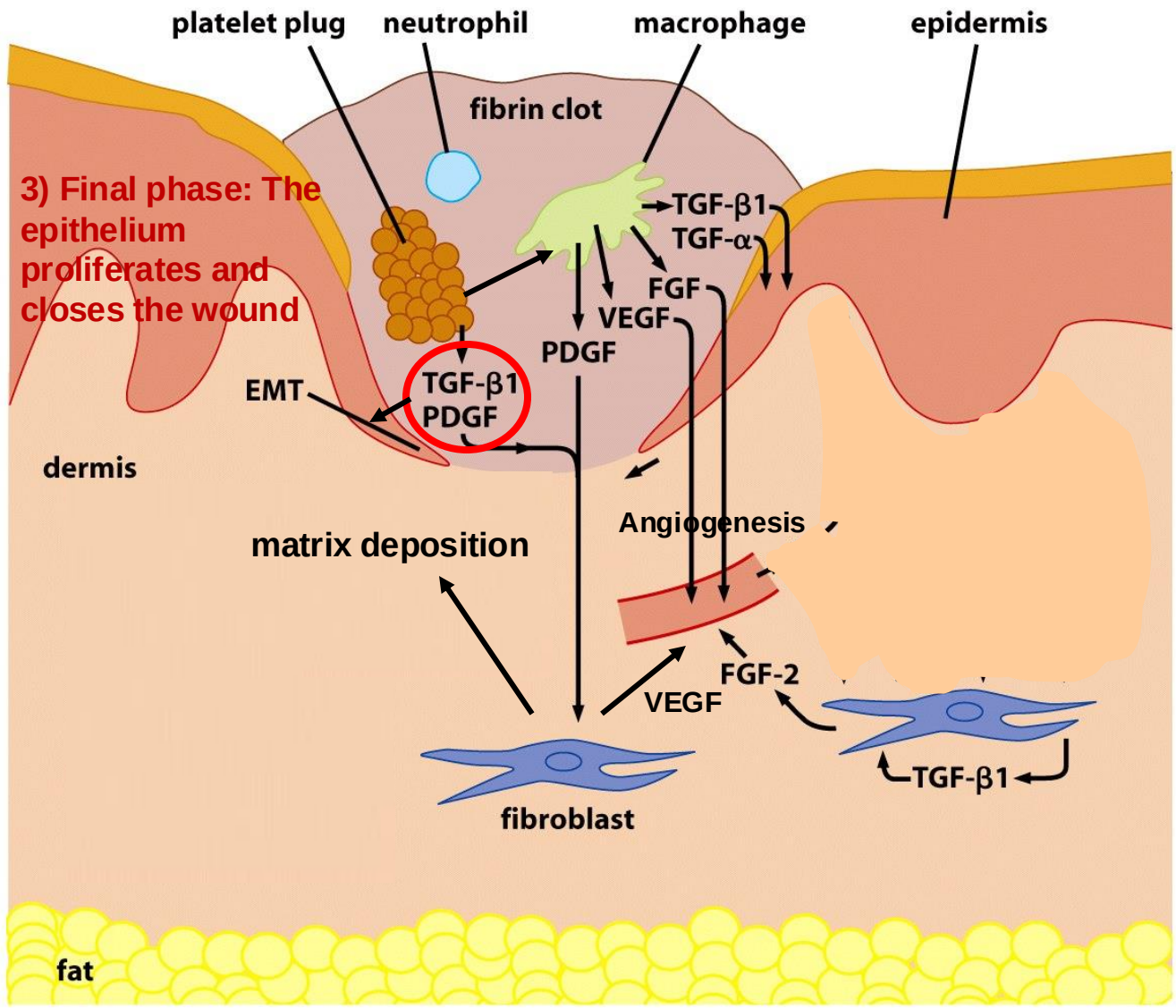
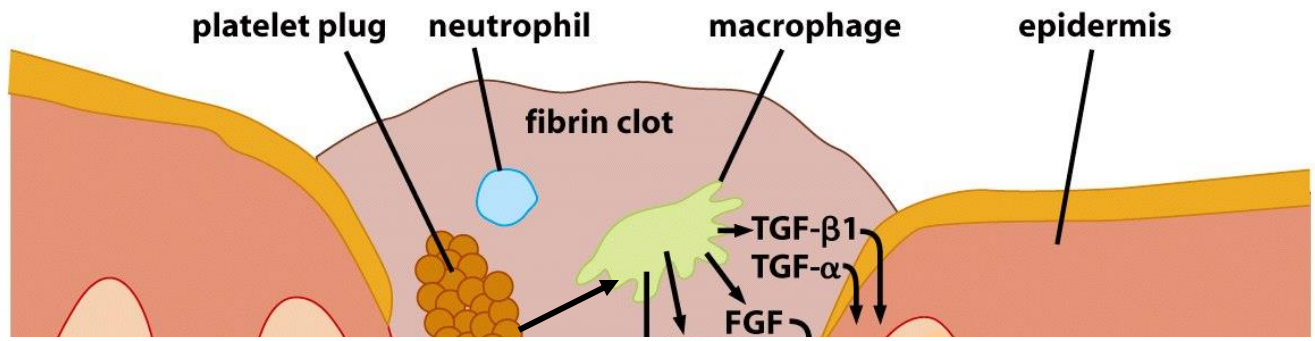


Figure 13.14 *The Biology of Cancer* (© Garland Science 2007)

Wound healing (normal tissues)



Angiogenesis and tissue remodeling subside once the wound has been closed (return to homeostasis)

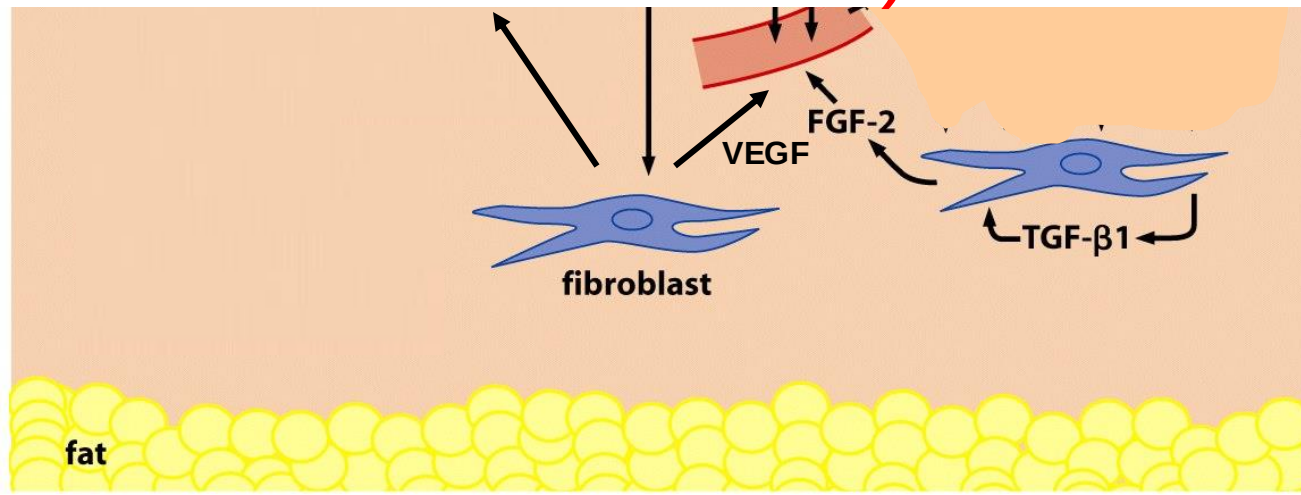
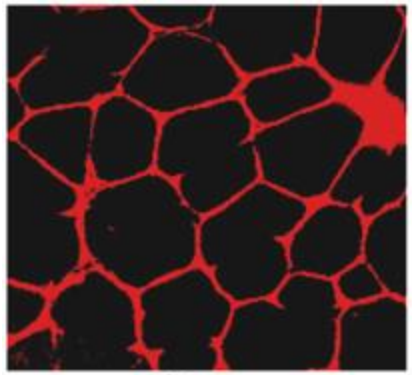


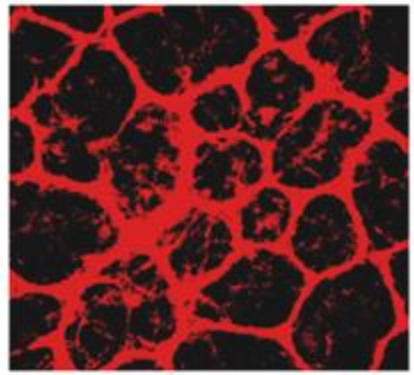
Figure 13.14 *The Biology of Cancer* (© Garland Science 2007)

Owing to incessant cancer cell proliferation, hypoxia and angiogenesis, tumors are like wounds that never heal….

Chronic angiogenesis

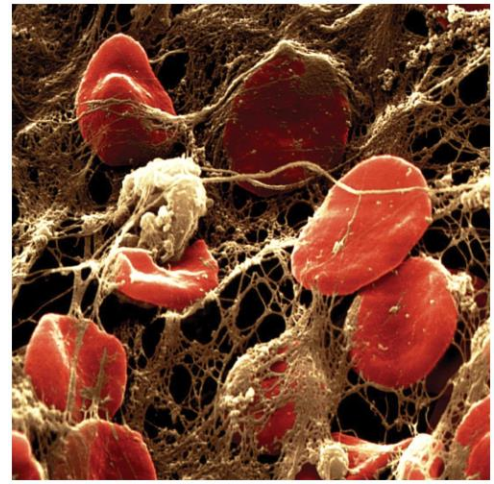


no tumor



tumor

Like in chronic wounds, persistent angiogenesis and vascular leakage at the tumor-host interface generates a peritumoral, “clot-like” tissue.



Platelet degranulation (PDGF, TGFb) and fibrin bundles stimulate **fibroblast proliferation and activation**. These cells secrete abundant ECM and form a **stiff** stromal tissue (**desmoplastic stroma**).

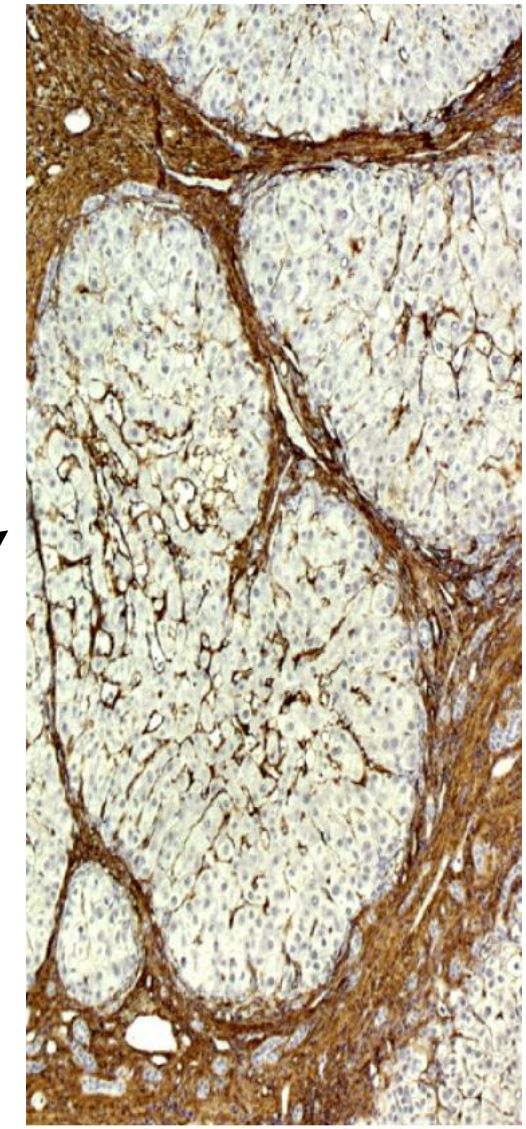


Figure 13.16b *The Biology of Cancer* (© Garland Science 2007)