Teaching Unit 02

Tumor angiogenesis

Mechanisms of neoangiogenesis

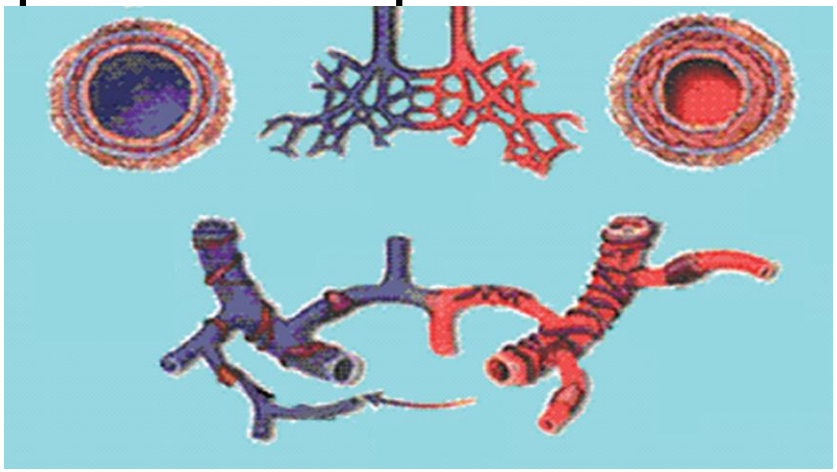
Tumor blood vessels

Mediators of angiogenesis

Microvascular network consists of the smallest blood vessels.

The main functions of the microcirculation are:

1. **the delivery of oxygen and nutrients to tissues,**
2. **and the removal of carbon dioxide (CO2) and other metabolic wastes from tissues**
3. **regulate local blood flow and conducts blood–tissue exchange thereby affecting blood pressure and responses to inflammation which can include edema (swelling).**



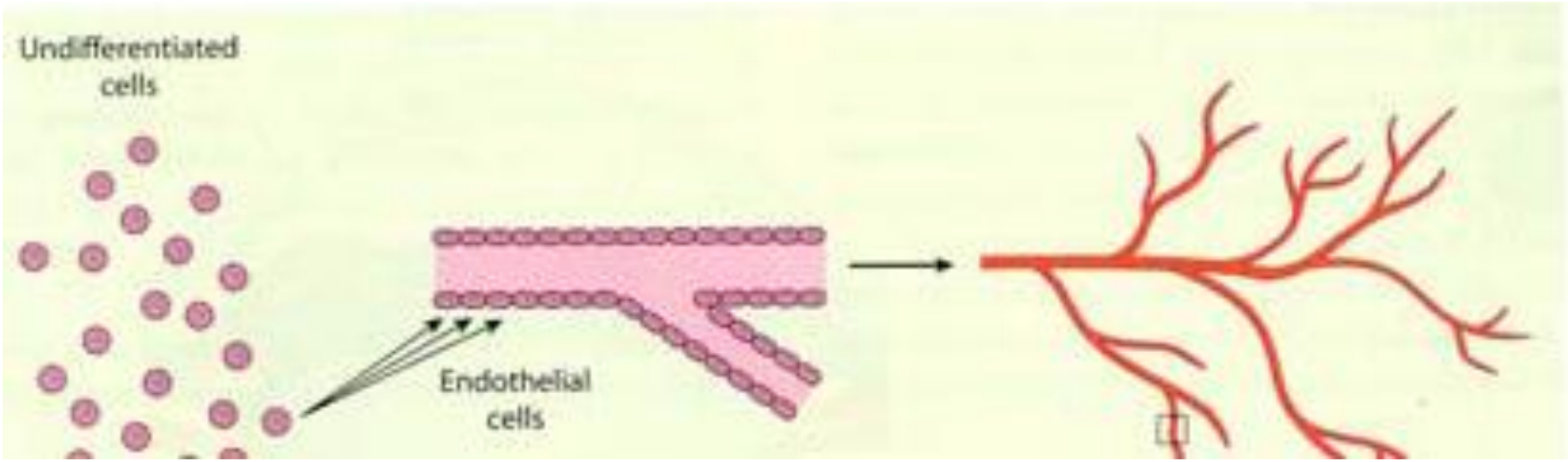
Even in the absence of changes in oxygen demand or other functional requirements, microvascular network structures are dynamic and likely undergo continuous changes.

New blood vessels are formed by two basic processes:

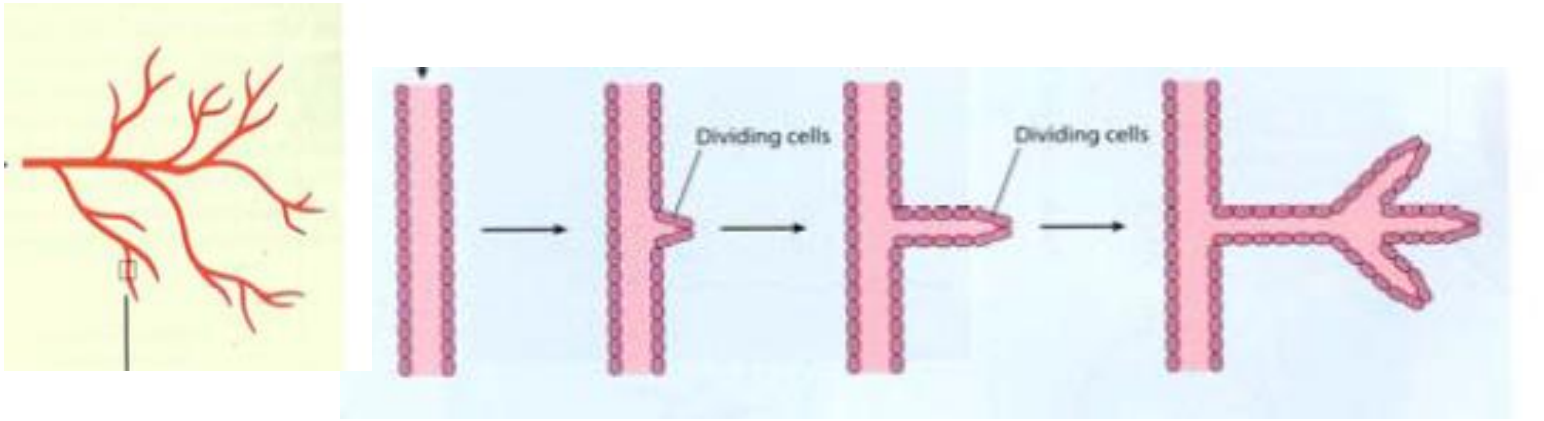
vasculogenesis

angiogenesis.

1. Vasculogenesis means the *de novo* formation of initial vascular networks by the differentiation of endothelial progenitor cells (EPCs). At early stages of development blood vessels are formed from angioblasts, which differentiate into endothelial cells.

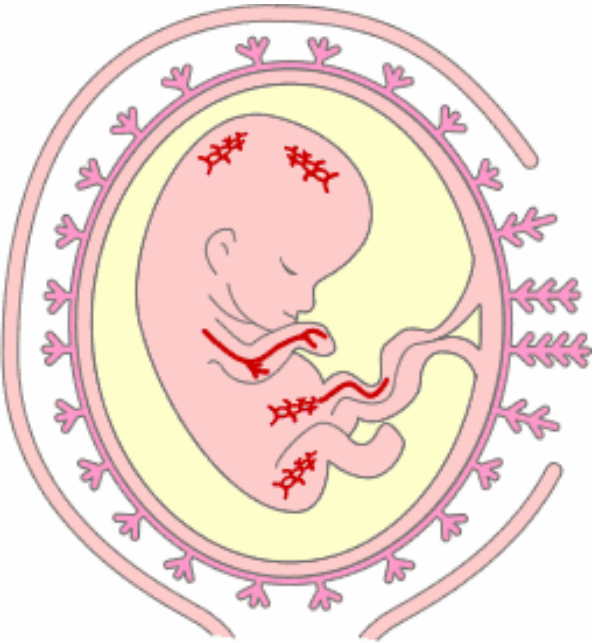


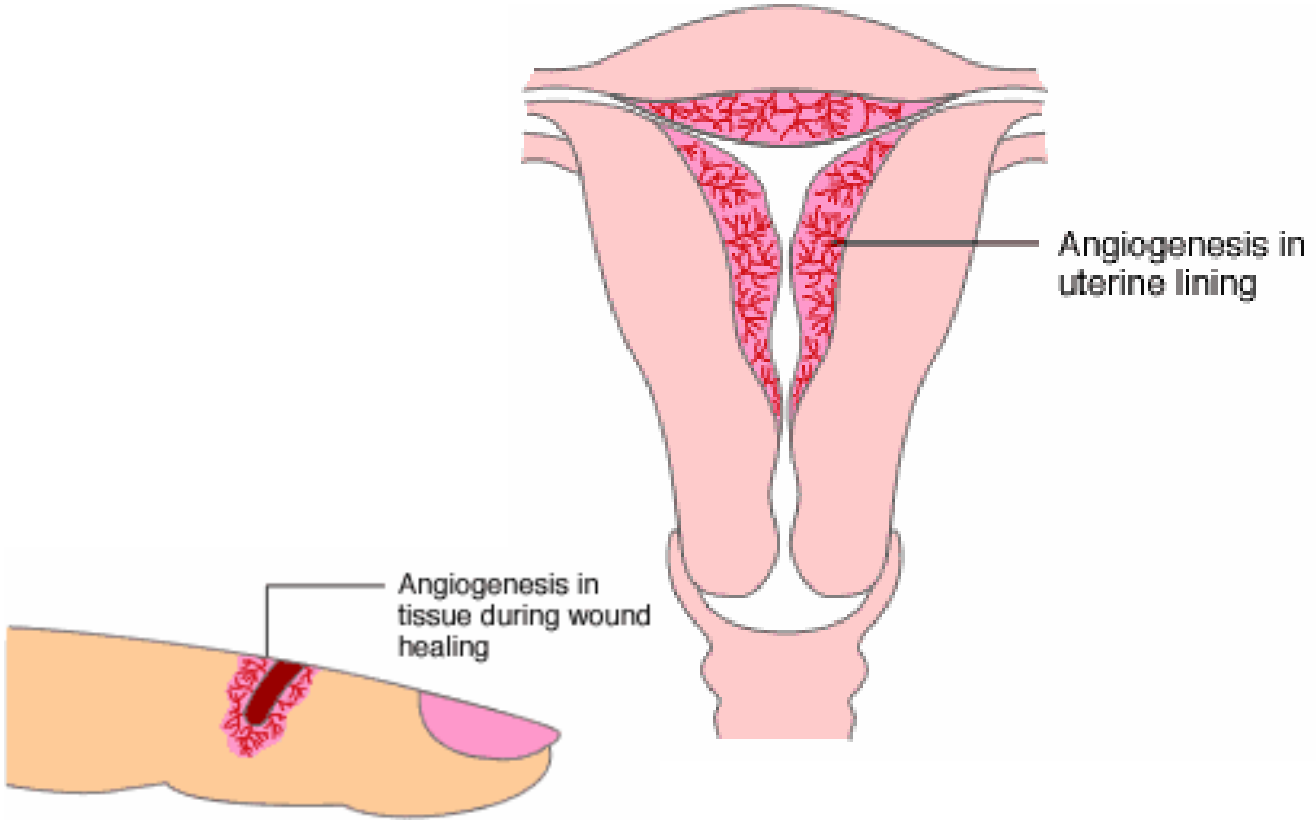
2. Angiogenesis is the process of forming new blood vessels from pre-existing blood vessels, whereby the endothelial cells of the existing blood vessels proliferate and migrate, thus building an initial capillary tube that subsequently expand and form new blood vessel structures.



Angiogenesis is a tightly controlled program. Some molecules act as **proangiogenic factors** that stimulate angiogenesis processes, while other molecules are **antiangiogenic factors** and they inhibit angiogenesis.

... <u>Physiological angiogenesis</u> occurs during embryonic development, wound healing, during the formation of the corpus luteum and ovarian follicles, as well as during the growth of the uterine endometrium.





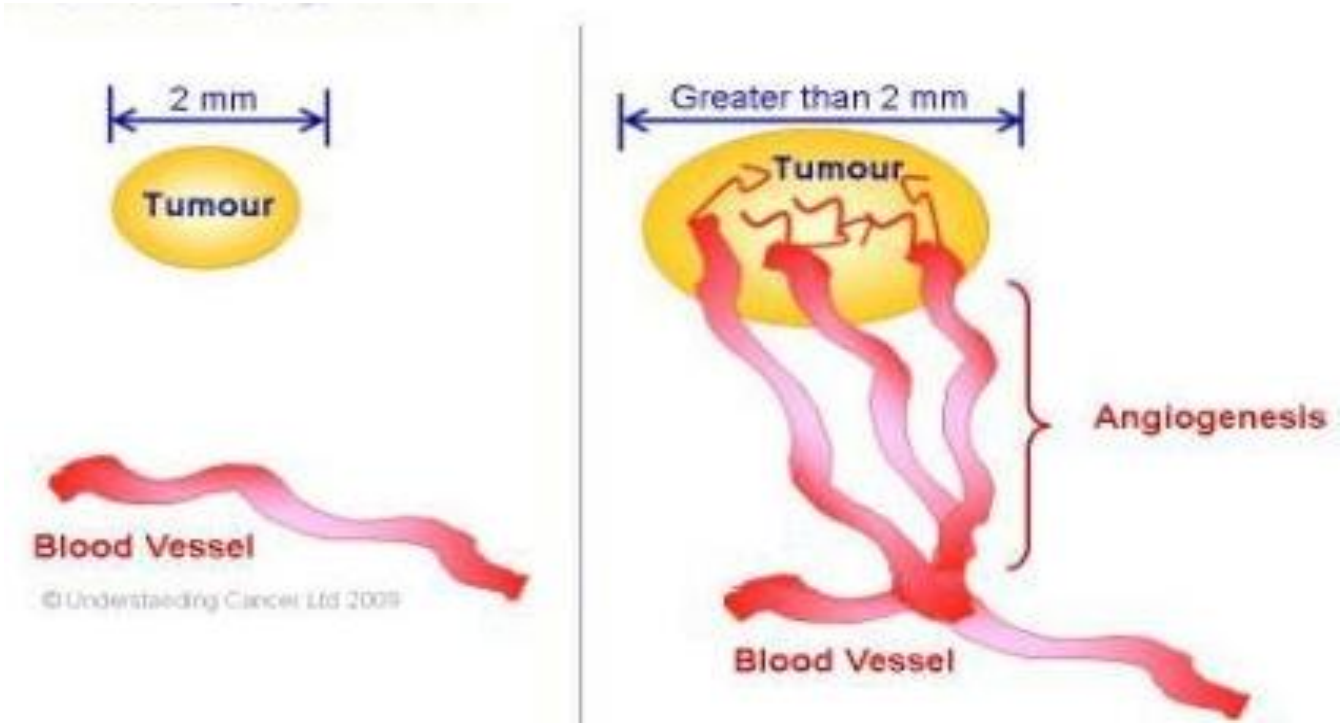
... **Abnormal (pathological or uncontoled) angiogenesis** can be involved in the pathogenesis of various diseases:

- diabetic retinopathy
- atherosclerosis
- psoriasis
- rheumatoid arthritis
- **tumor**

Tumor angiogenesis

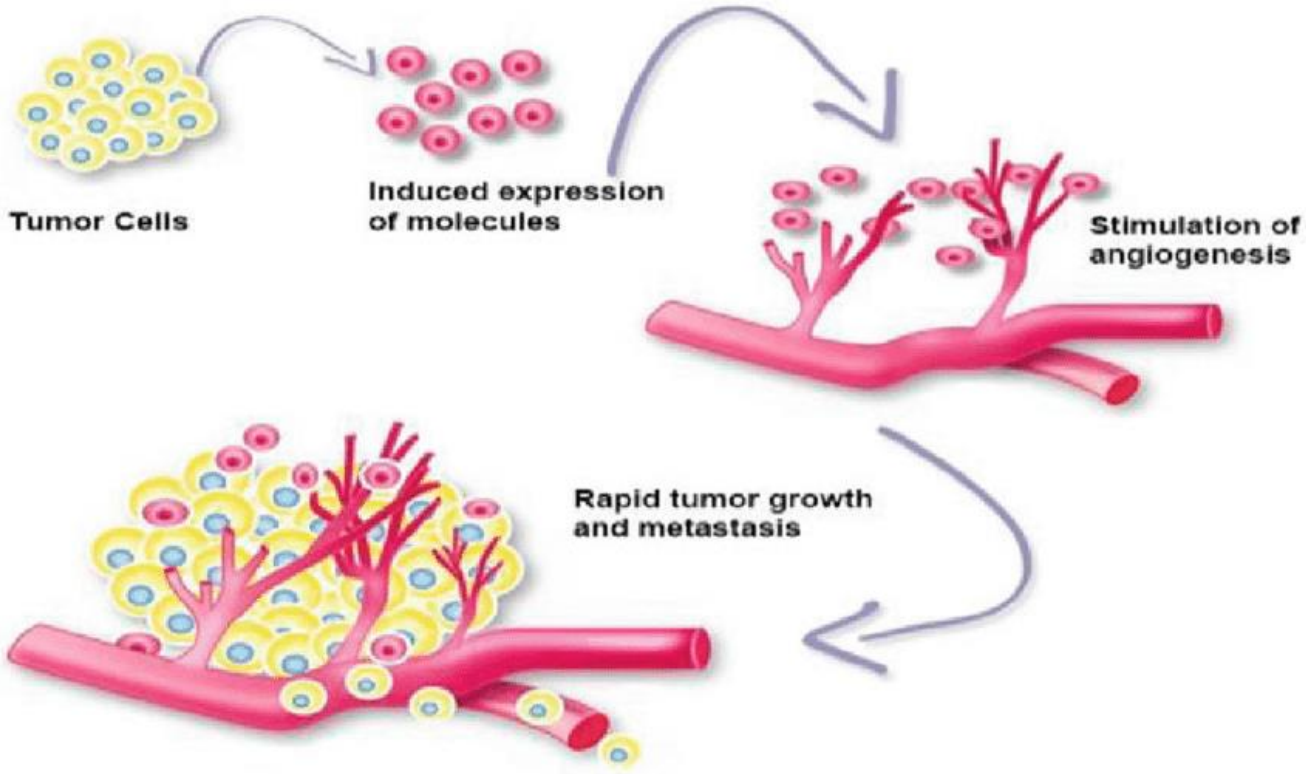
-Mechanisms of angiogenesis

Solid tumors cannot grow more than any 2-3 millimeters in diameter without an angiogenesis.



The increased interstitial pressure in the tumor tissue inhibits the diffusion of metabolites and nutrients necessary for the multiplication and survival of malignant cells.

This environment stimulates the malignant cells to release soluble molecules by which these cells induce the formation of new vessels from pre-existing blood vessels, allowing the tumor to be supplied with oxygen and nutrients.



Moses Judah Folkman

(1933 –2008)

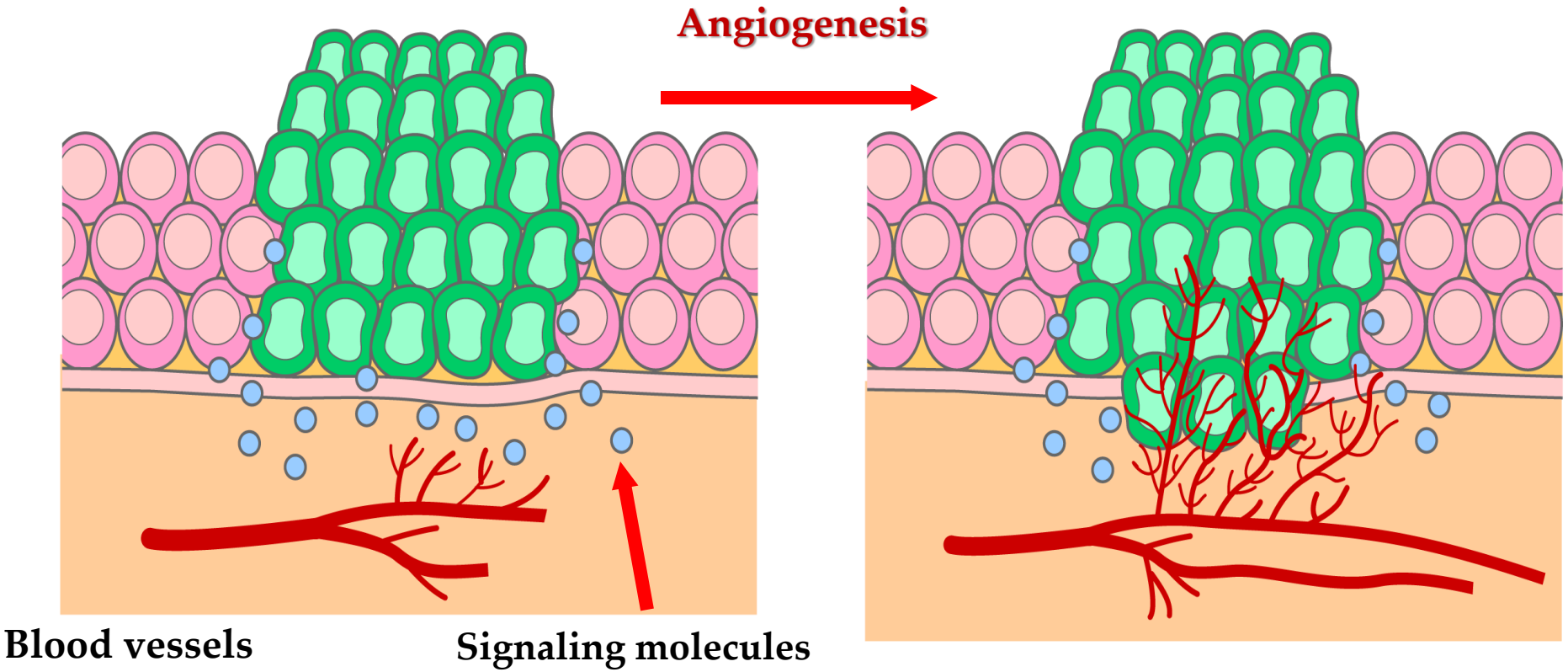
In 1971, M. Judah Folkman proposed a hypothesis that angiogenesis is key players in the tumor progression.

Folkman and colleagues hypothesize that by inhibiting the development of blood vessels in tumors, tumor dormancy can be prolonged and potentially improved the survival of patients with minimal toxicity.

This hypothesis about the essential role of angiogenesis in tumor growth, as well as the potential therapeutic benefit of anti-angiogenic drugs, has been partially confirmed in some tumor types.

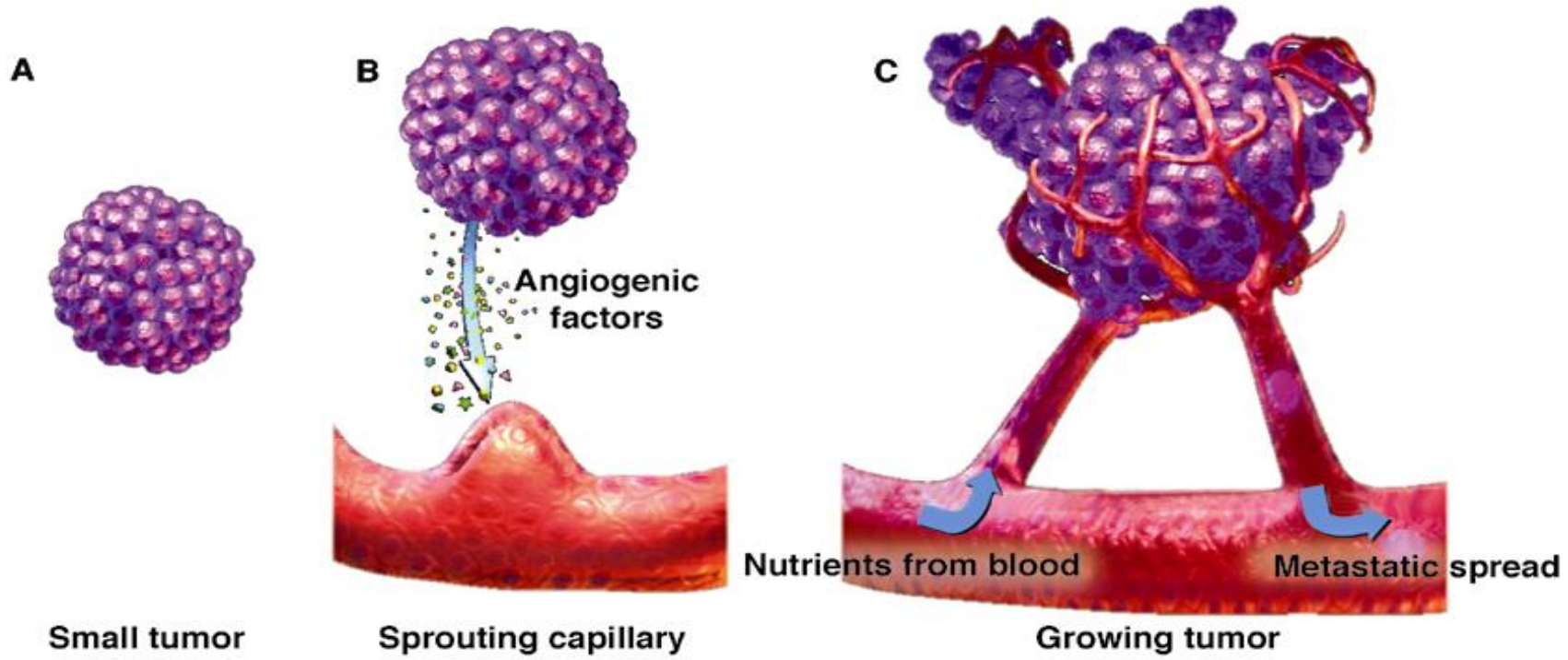
... **Tumor angiogenesis** is a vital process resulting in the formation of new vessels from pre-existing blood vessels in the tumor tissue. The term **"neoangiogenesis"** is often applied to pathological angiogenesis in tumours.

Tumor growth and metastasis depend on angiogenesis, as the newly formed blood vessels allow the delivery of oxygen, nutrients and growth factors.



We should remember...

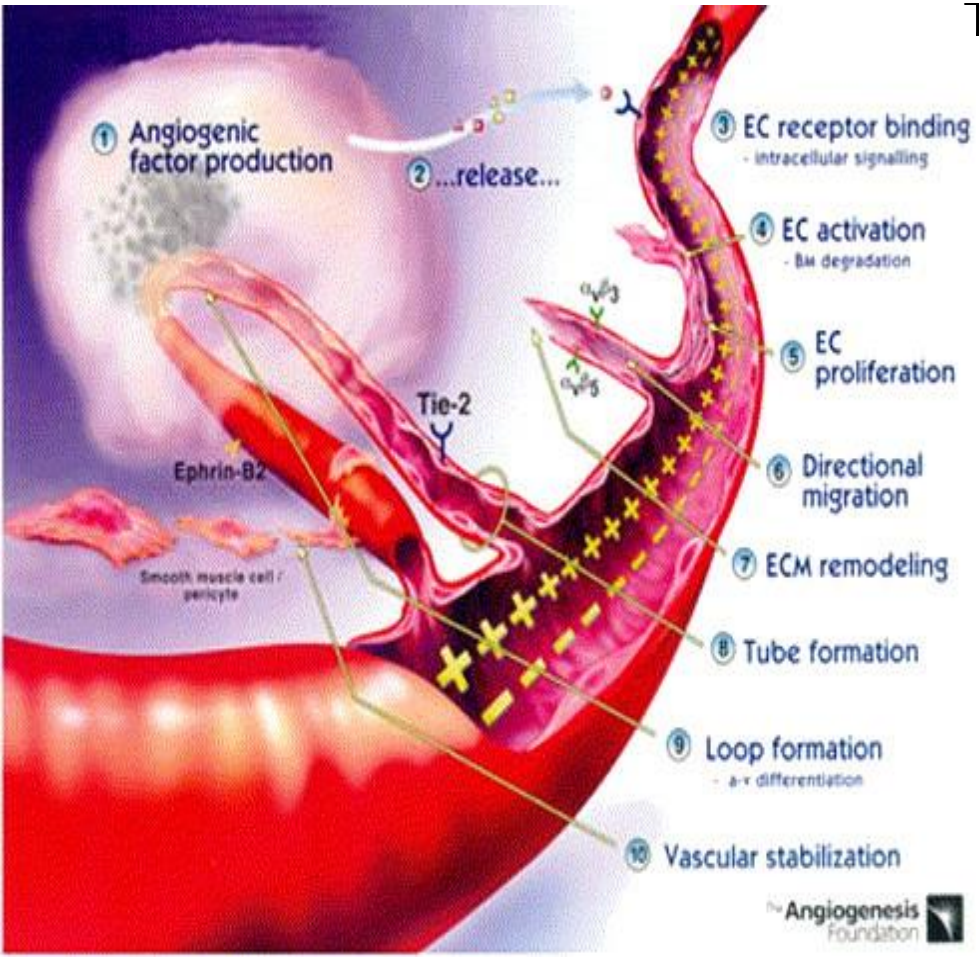
The new blood vessels **supply** the malignant cells with **oxygen and nutrients** and thus **allow the tumor to grow** and invade the surrounding structures....



... Since these blood vessels are in direct contact with the tumor, they **represent the entry point** of malignant cells into the circulation, from where they spread in the body and **enable the creation of new tumor foci (metastases).**

The main steps of tumor blood vessel development

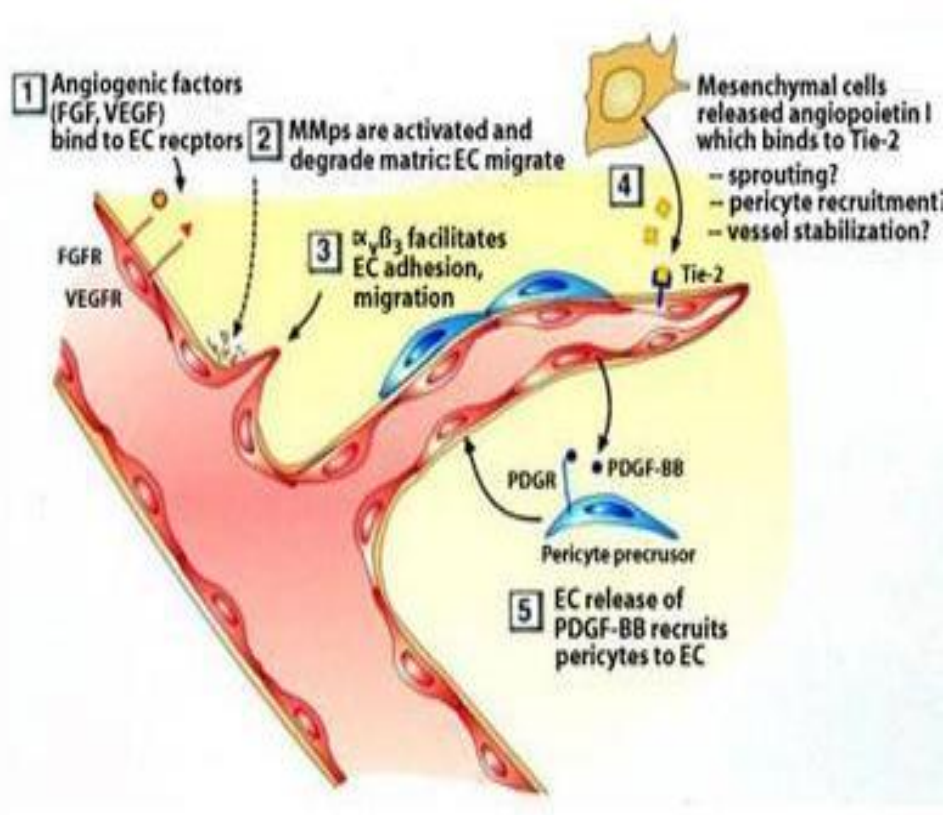
The angiogenesis is a complex multi-step process, involving extensive interplay between cells, soluble factors, and extracellular matrix (ECM) components



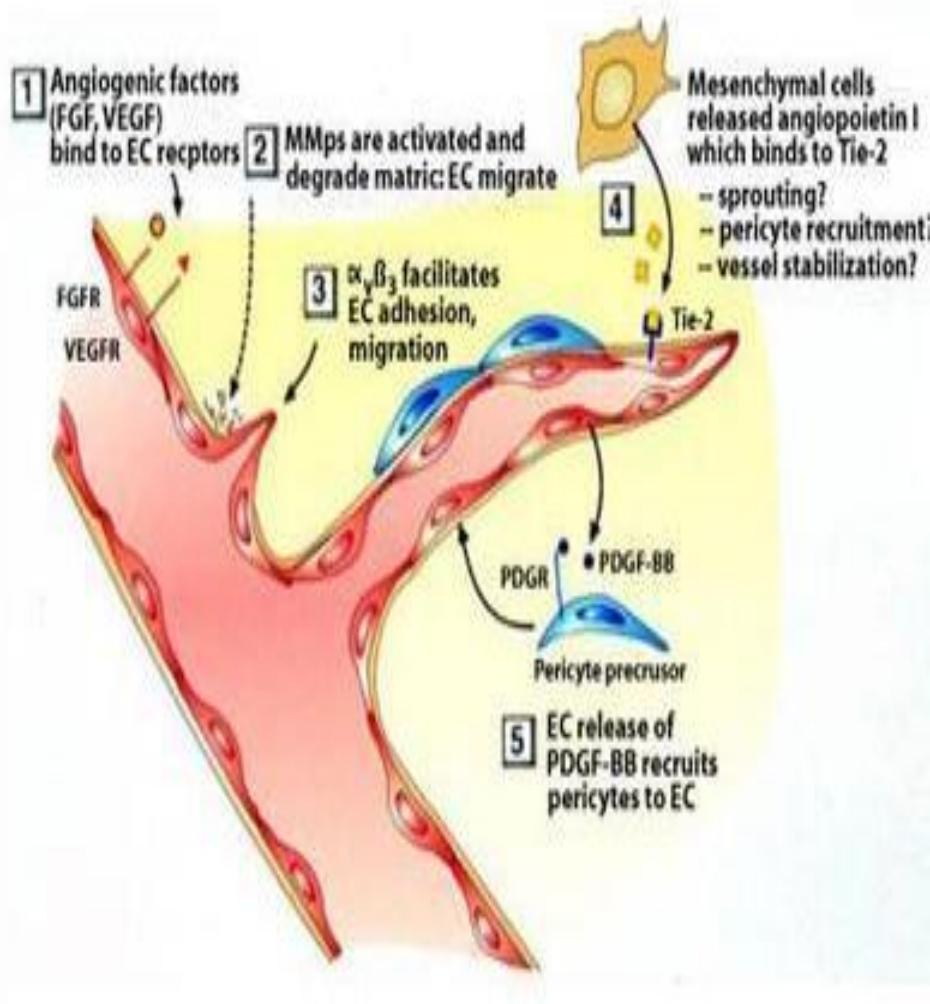
The process of angiogenesis includes:

- ✓ production of angiogenesis factors and endothelial cell (EC) receptors binding
- ✓ EC activation
- ✓ Basement membrane and ECM degradation
- ✓ EC proliferation and migration
- ✓ budding and growth of endothelial bands, resulting in formation of a capillary tube and loop
- ✓ Vascular maturation and stabilization

... **The first step** in the formation of a capillary shoot from an pre-existing blood vessel is the **local degradation of basement membrane** enclosing the postcapillary venule. Local proteolytic degradation of the basement membrane and extracellular matrix is a consequence of the **action of various proangiogenic growth factors**. Under the influence of these factors, the synthesis and release of **numerous proteolytic enzymes** are induced, such as matrix metalloproteinases, cathepsins, and urokinase plasminogen activators.



The next step involves the **direct movement/migration of endothelial cells** toward an tumor cell-derived proangiogenic stimuli. Released proangiogenic factors bind to their receptors on endothelial cells, resulting in the activation of **endothelial cells that acquire an angiogenic phenotype.**



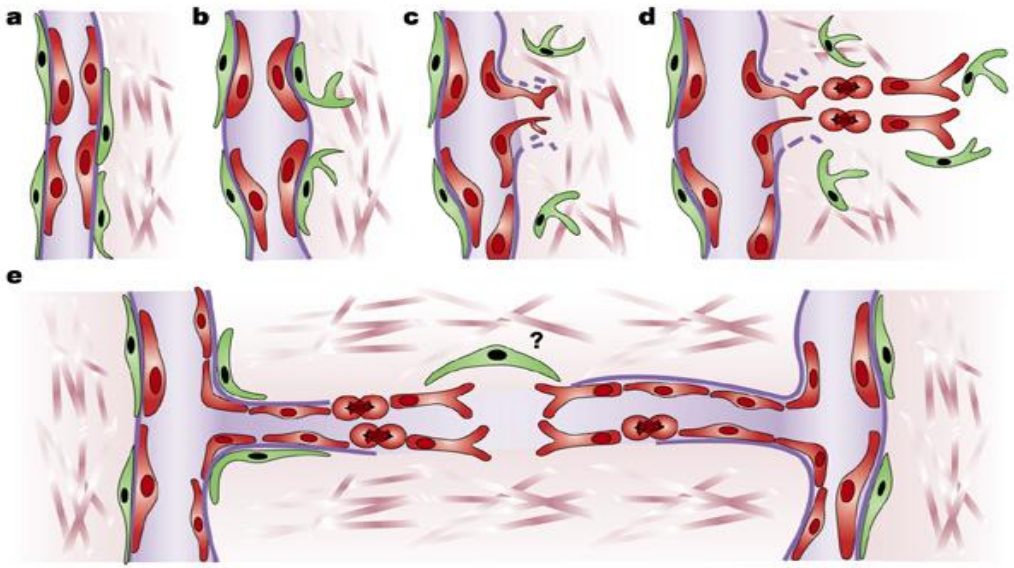
These endothelial cells reorganize their cytoskeleton, change their phenotype, break adhesion, and then migrate through the basement membrane

... **<u>Proliferation of endothelial cells</u>** resulting in the **formation of a capillary bud** that grows as a solid band from pre-existing blood vessels. This is followed by the formation of the lumen, the connection with another capillary tube, the **formation of a capillary loop** and ultimetllly the establishment of circulation.

These unstable nascent capillaries are surrounded by a newly formed basement membrane. Mesenchymal cells are recruited into these capillaries, where they differentiate into pericytes (perivascular wall cells).

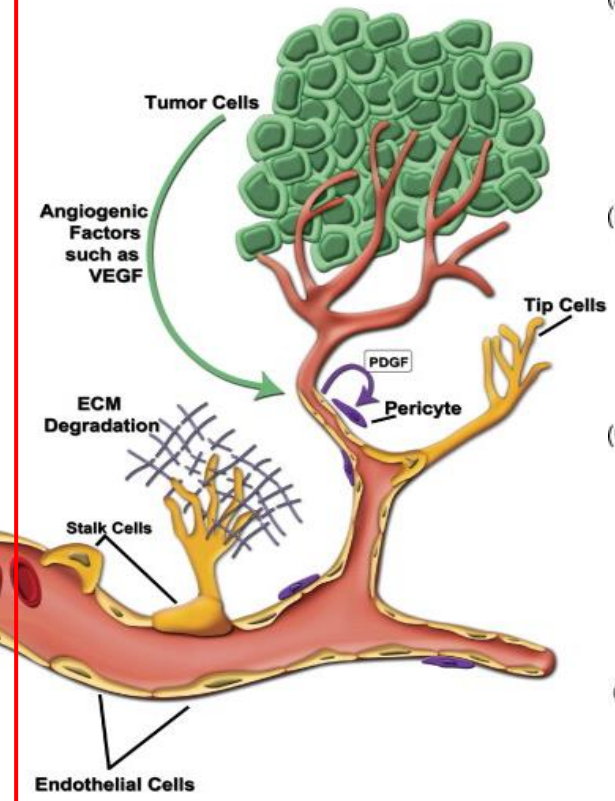
Coordinated activation of pericytes and smooth muscle cells, together called mural cells, results in blood vessel maturation.

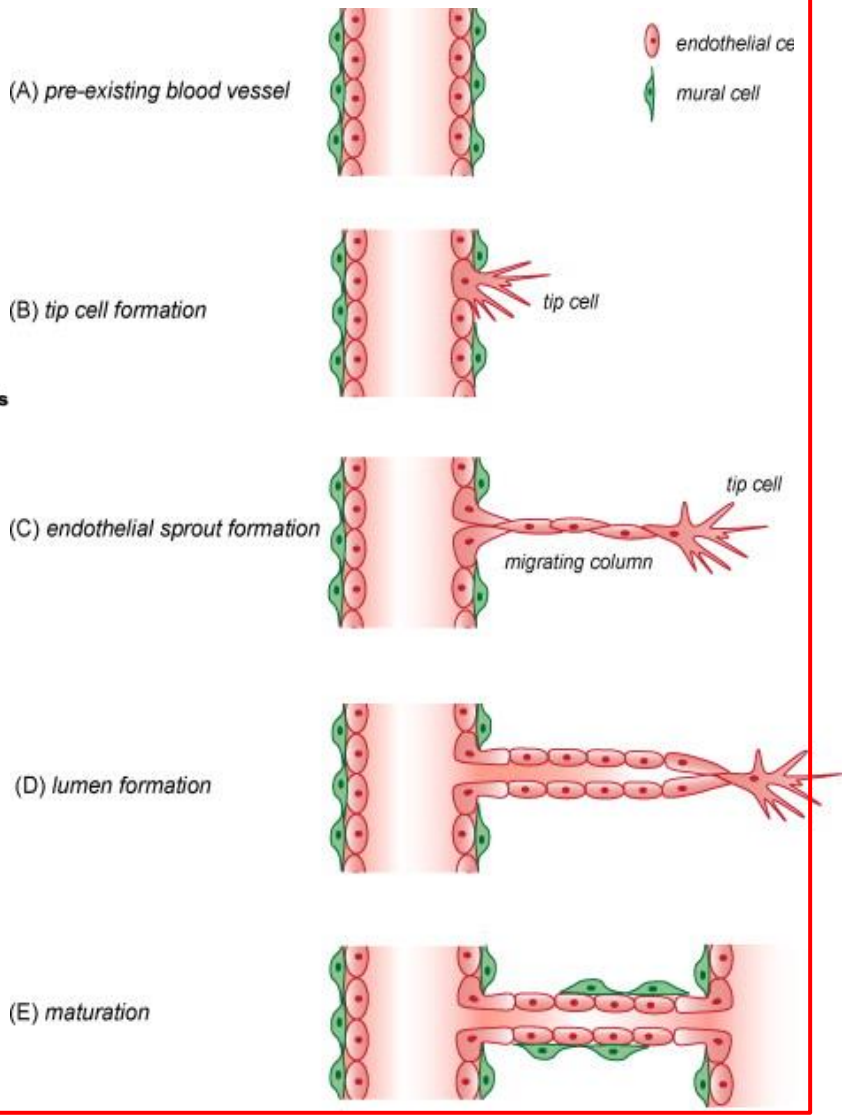
This angiogenic process is called **sprouting angiogenesis.**



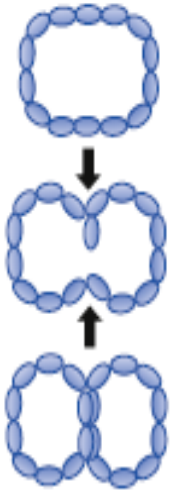
A critical cellular component in this process is the specialized endothelial cells at the ends of newly formed capillaries, called **"tip" cells**, and they fuse with each other to form a new capillary network.

(1)



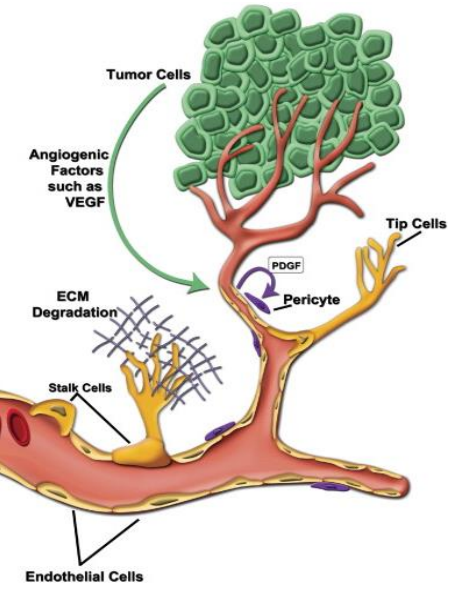


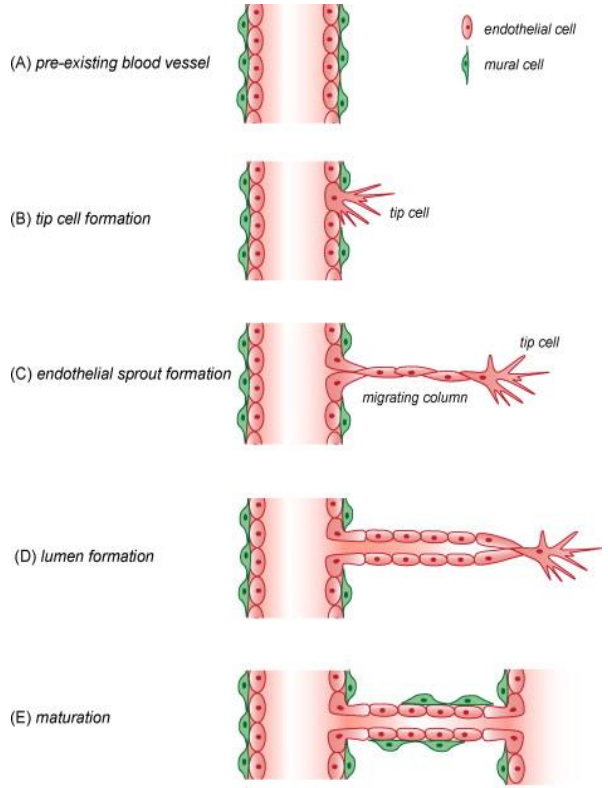
(2) Intussusceptive growth

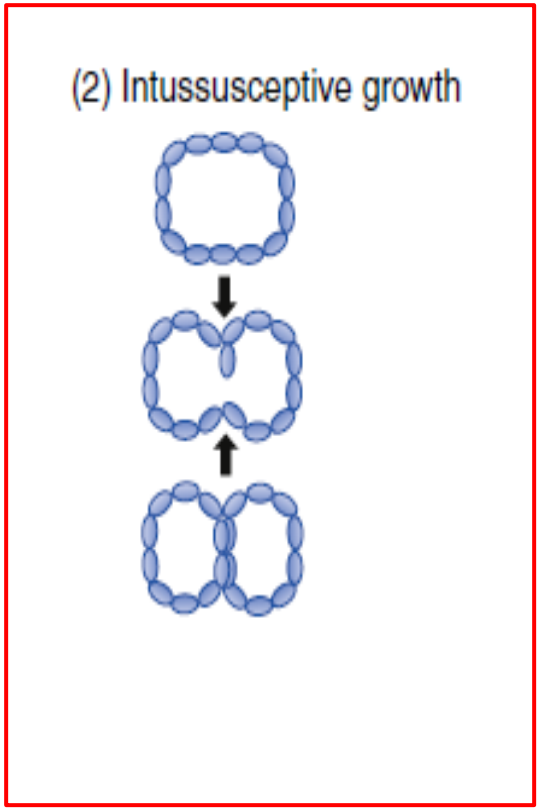


Intussusceptive angiogenesis is forming solid bands of endothelial cells create a barrier inside the lumen of an existing blood vessel. In this way, the existing blood vessel is divided into independent new blood vessels.

(1)





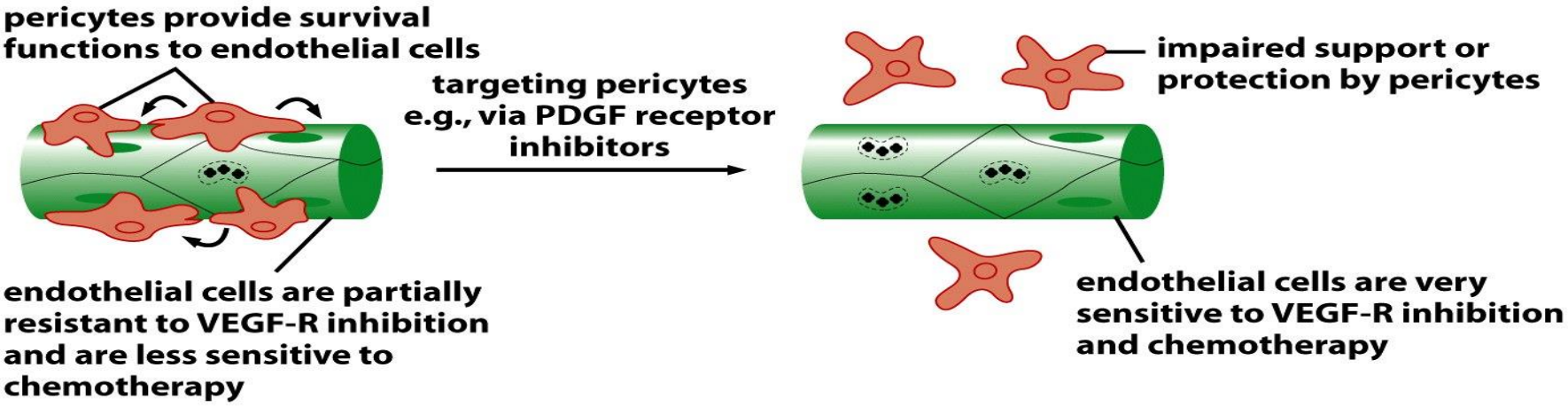


The role of pericytes in angiogenesis

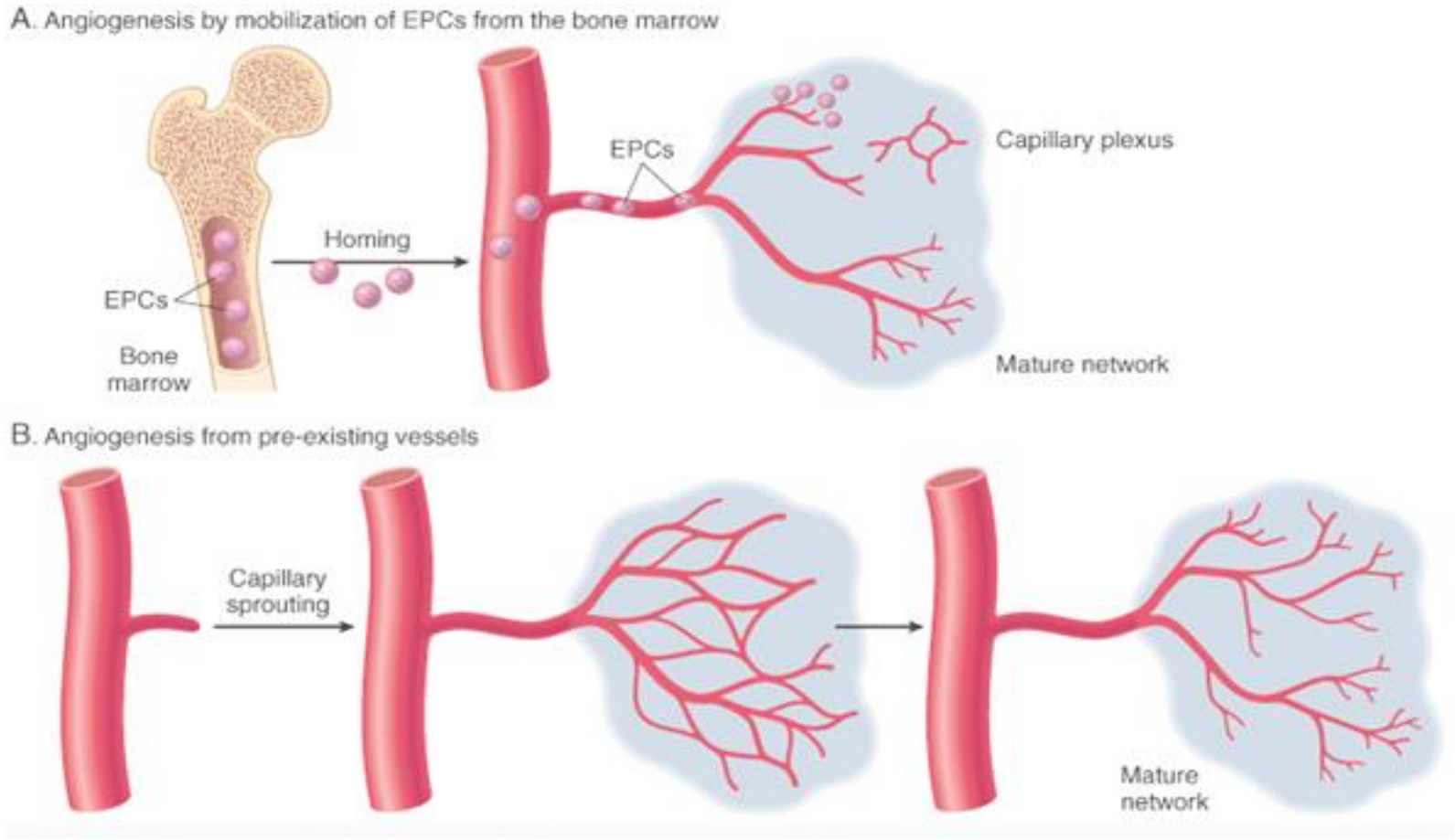
Pericytes are important components of blood vessels that modulate endothelial cell function and are critical for the **development of the mature vascular network**.

Pericytes regulate vascular function including **blood vessel diameter** (and thus blood flow) and **vascular permeability**.

They provide **mechanical support** and stability of the blood vessel wall and **enable the survival of endothelial cells** through direct intercellular contact and paracrine action.



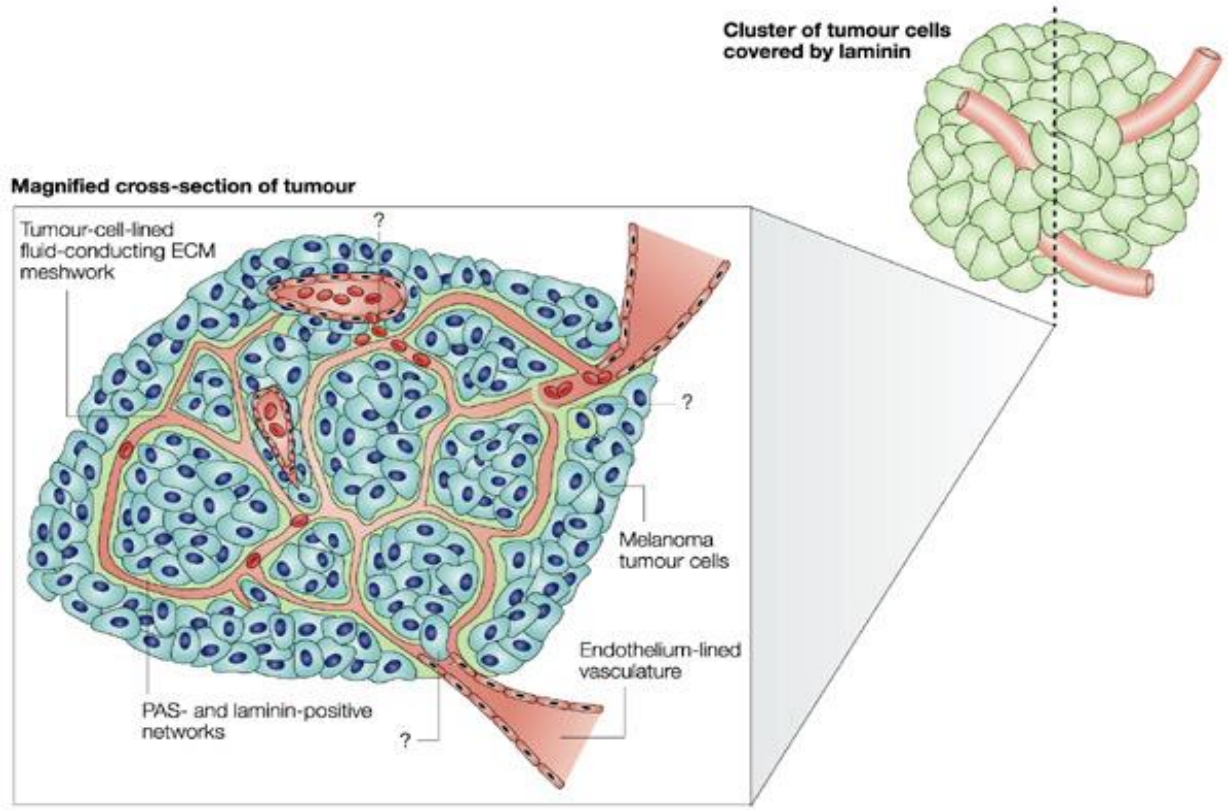
Tumors achieve their own blood supply in several ways...



A) The tumor recruit circulating endothelial cell precursors from the bone marrow that than participate in the formation of tumor blood vessels (a process known as **neovasculogenesis**).
B) In **tumor-induced angiogenesis**, which is similar to physiological angiogenesis, tumor cells release growth factors that stimulate the formation of new blood vessels from pre-existing capillaries.

C) Malignant cells and macrophages show marked plasticity; they show the ability to dedifferentiate into cells endothelial-like cells and thus enable the formation of their own vascular network. This phenomenon is known as **vascular mimicry**.

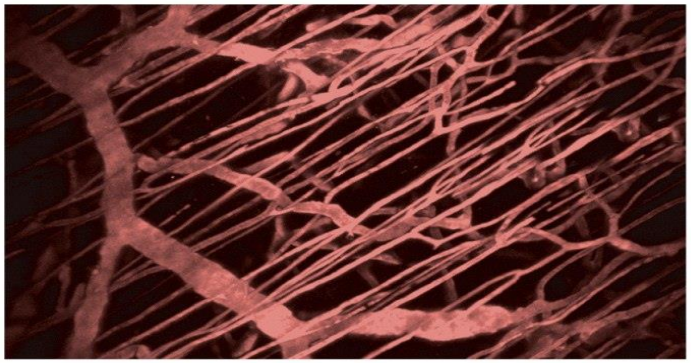
C)



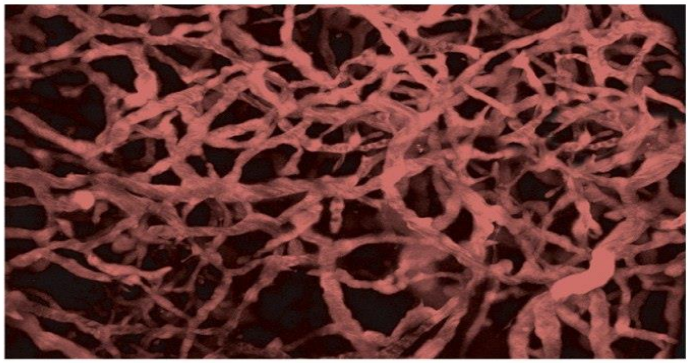
Tumor blood vessels show various structural abnormalities

Healthy vessel	Tumor vessel
	
Well organized	Disorganized
Defined arterioles and venules	Undefined arterioles and venules
Regularly distributed	Unevenly distributed
Non-dilated	Dilated
Non-permeable	Highly permeable
Mature and coated with mural cells	Premature and lack of mural cells
Low interstitial pressure	High interstitial pressure
Complete basement membrane	Lack basement membrane
Endothelial cell and mural cell	Mosaic cells
Appropriate expression of markers	High or low expression of markers
Normal rate of blood flow	Sluggish blood flow

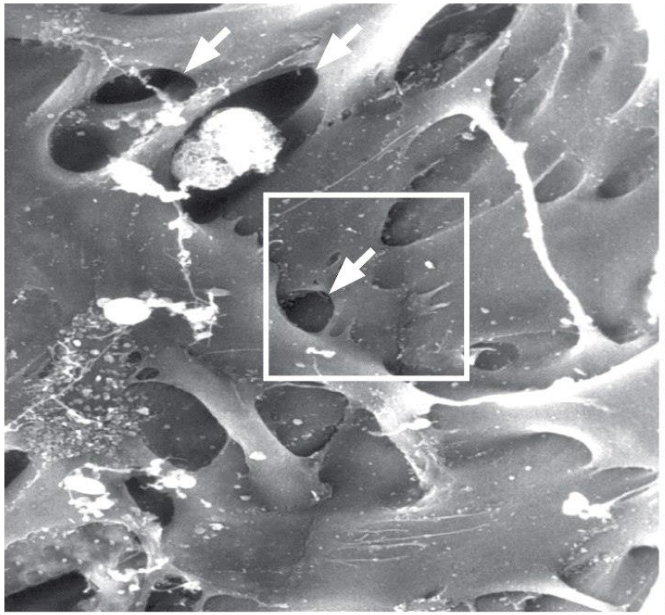
Due to the **incomplete basement membrane** and the **presence of fenestra (transcellular holes** or **large intracellular gaps between endothelial cells**), **tumor blood vessels are extremely permeable.**

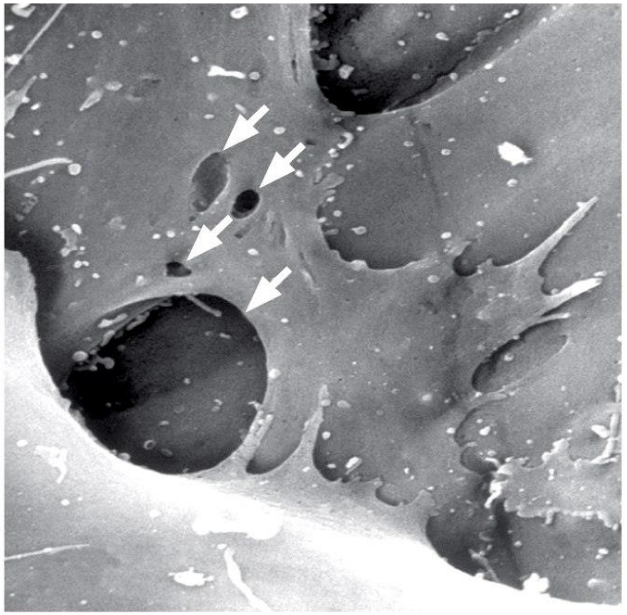


normal tissue



tumor





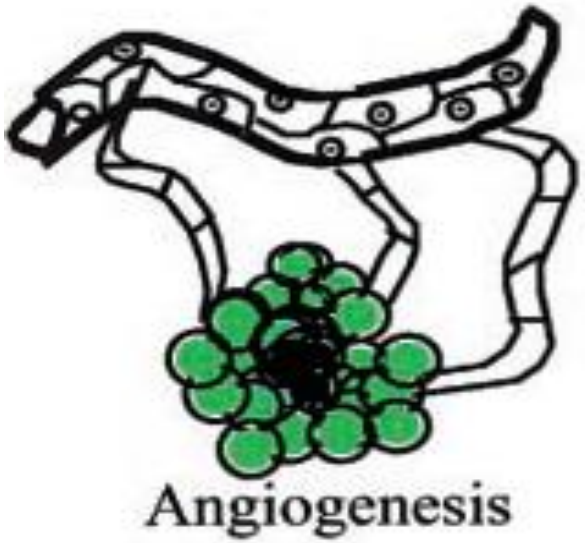
The mechanism of angiogenesis can be **organ**- and/or **tumor-specific**.

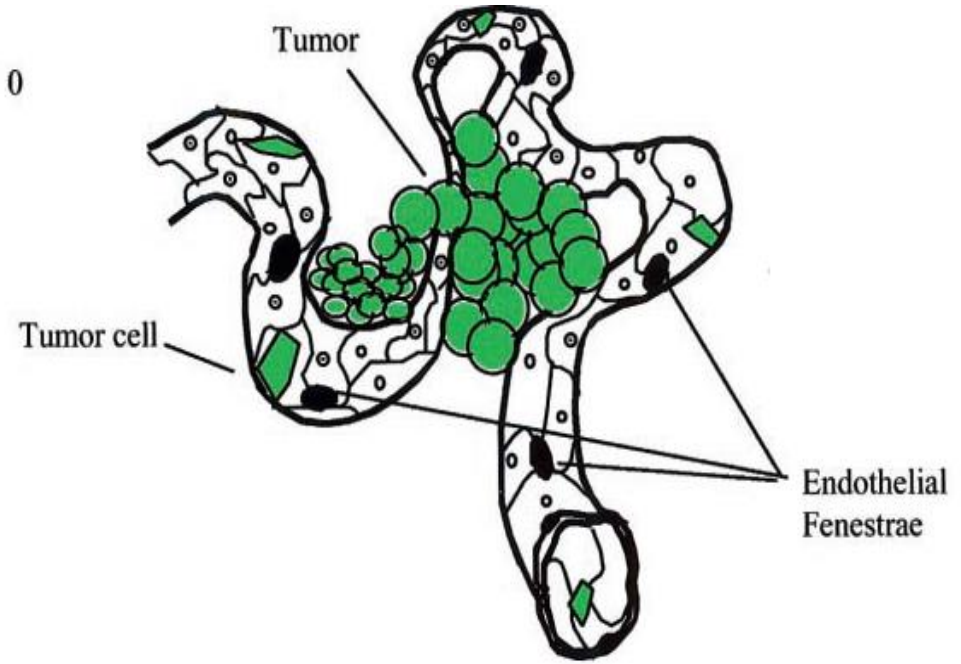
In tumors such as melanoma, parts of the blood vessel wall may contain partially (**mosaic blood vessels**) or completely (**vascular mimicry**) malignant melanocytes.

The walls of blood vessels are made up of tumor and endothelial cells. In peripheral blood vessels, functional pericytes are usually absent or rarely present, while the basement membrane is incomplete.

The tumor blood vessels are dilated and also tortuous or twisted.

Such aberrant blood vessels arise under the influence of dysregulated angiogenic signals in the tumor tissue, which is, among other things, the result of oncogene hyperactivation and loss of function of tumor-suppressor genes.



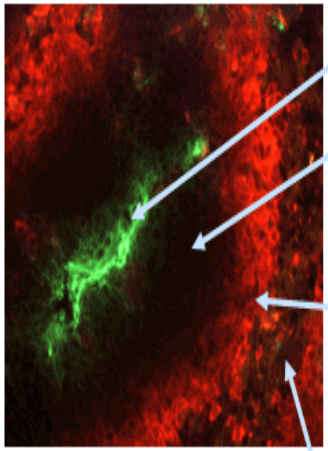


Dysfunction of tumor blood vessels

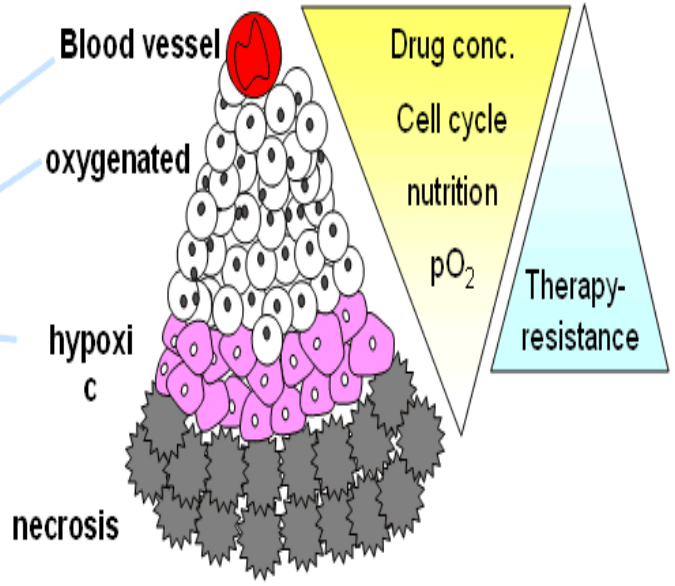
Structural abnormalities of blood vessels are variable within a solid tumor mass and heterogeneity is also reflected in the density of blood vessels.

Blood flow in the tumor environment is also heterogeneous. In some tumor areas, the blood flow is slow, which is why those areas are deprived of oxygen and nutrients, resulting in severe hypoxia.

Increased permeability of tumor blood vessels leads to extravasation of plasma proteins, as well as fluid in the extracellular microenvironment within the tumor, resulting in an **increase in interstitial pressure**.



Green: CD31
Red: anti-Pimonidazole



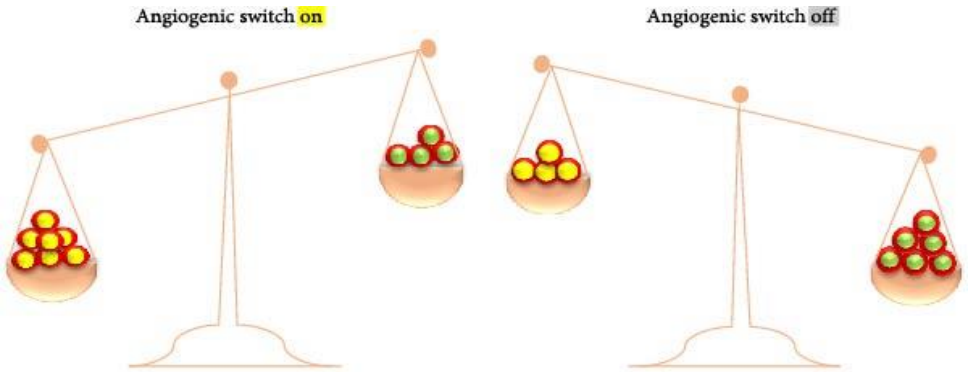
This nature of tumor blood vessels limits the effectiveness of therapy, especially chemotherapy, and due to impaired oxygen supply, the effectiveness of radiotherapy also decreases.

Mediators of angiogenesis

Angiogenic phenotype of tumor cells

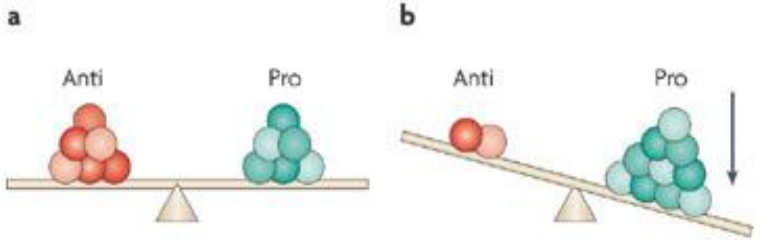
The malignant cells express an angiogenic phenotype resulting from an "turning on of angiogenic switch". The angiogenic balance between angiogenic activators and inhibitors tightly regulates angiogenic switch mechanism.

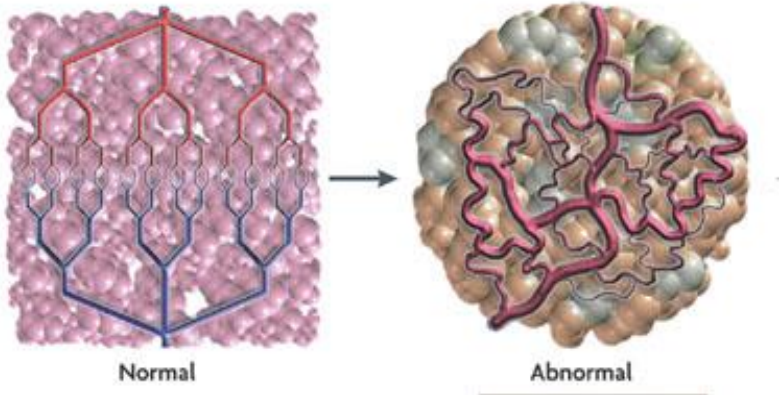
The beginning of the process of angiogenesis in a tumor is related to **changes in the local balance between proangiogenic and antiangiogenic molecules. This change with the dominance of proangiogenic factors leads to the "turning on" of the angiogenic switch.**



Activator
VEGF
FGF
PDGF
Angiopoietins
APLN
Chemokines
Angiogenin
Interleukin-8
Hepatocyte growth factor

Inhibitor
Angiostatin
Prolactin
Thrombospondin ½
Endostatin
Restin
Pigment epithelium-derived factor
Tumstatin
Canstatin
Arresten

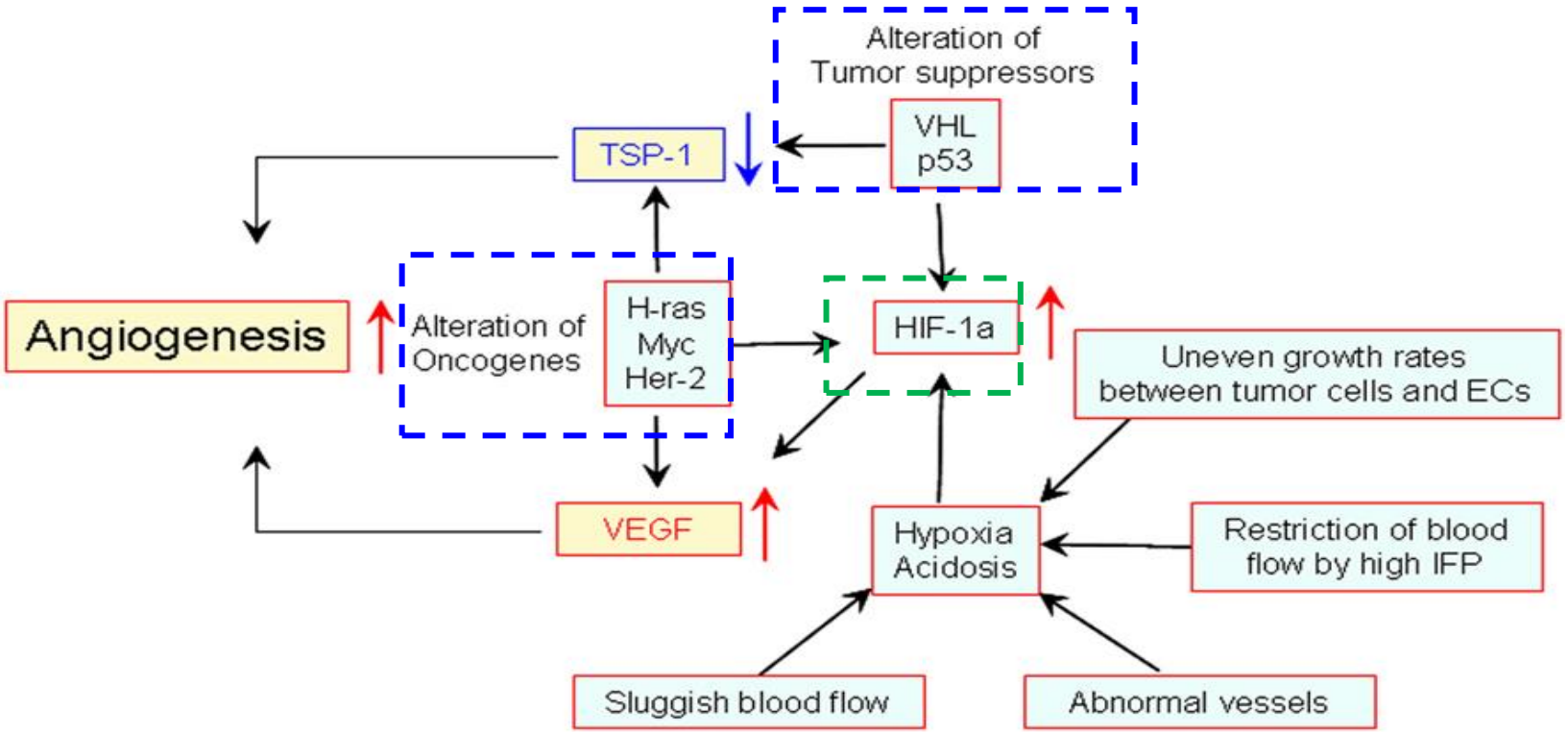




... Tumor angiogenesis takes place in two phases…

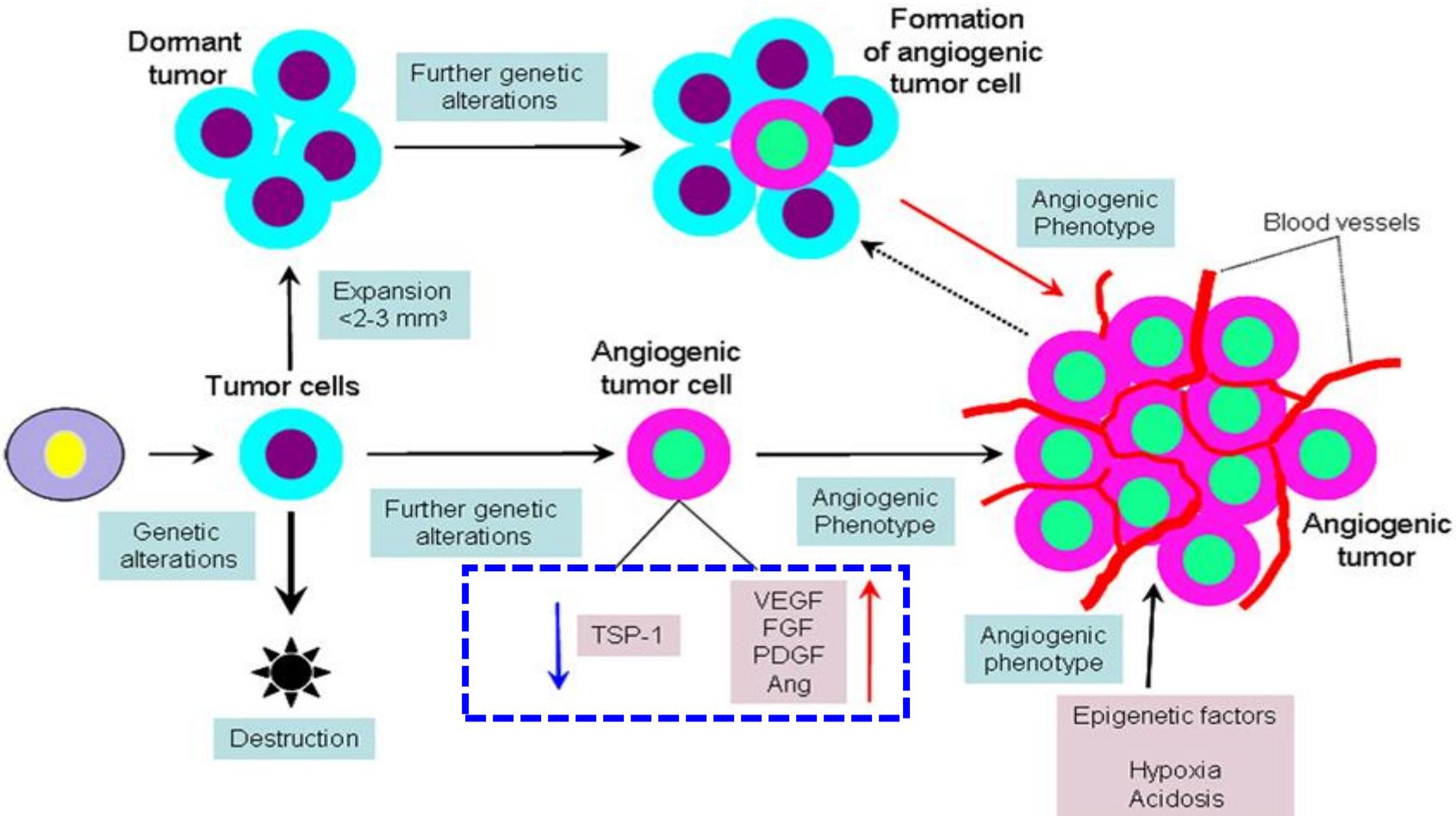
…In the first phase, **activation of an oncogenes (e.g. HER-2)** and/or **mutation and inactivation of an anti-oncogene (e.g., p53)** induce the expression of genes that encode proangiogenic factors.

In the second phase, malignant cells are additionally exposed to **various stress factors** in the tumor environment that stimulate angiogenesis.

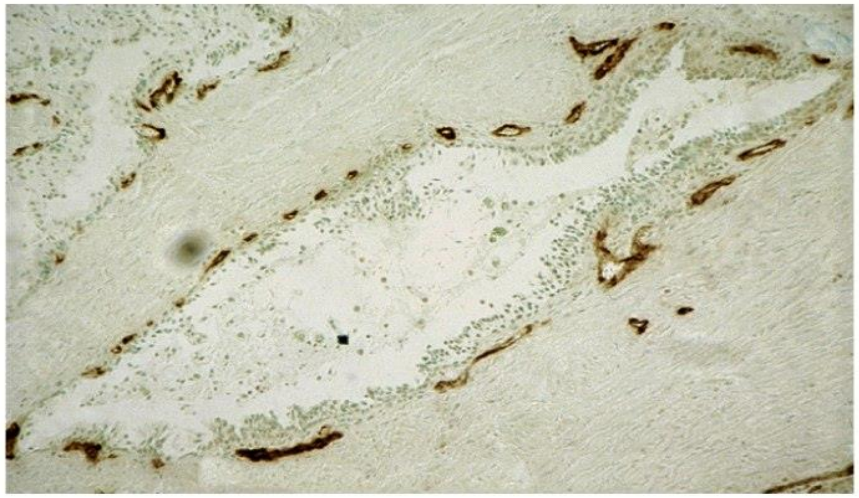


Induction of neoangiogenesis requires, on the one hand, increased expression of one or more proangiogenic factors (e.g.,VEGF), and on the other hand, reduced expression of one or more endogenous factors (e.g., thrombospondin-1).

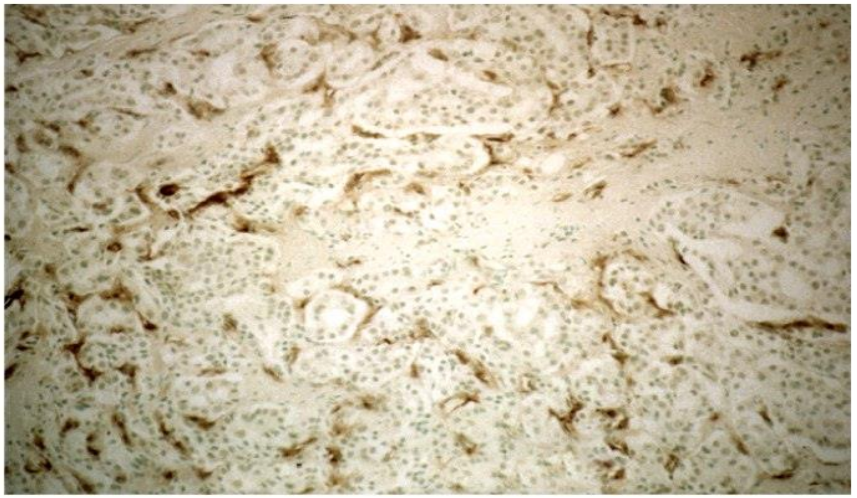
This dominance of proangiogenic factors also enhance tumor progression.



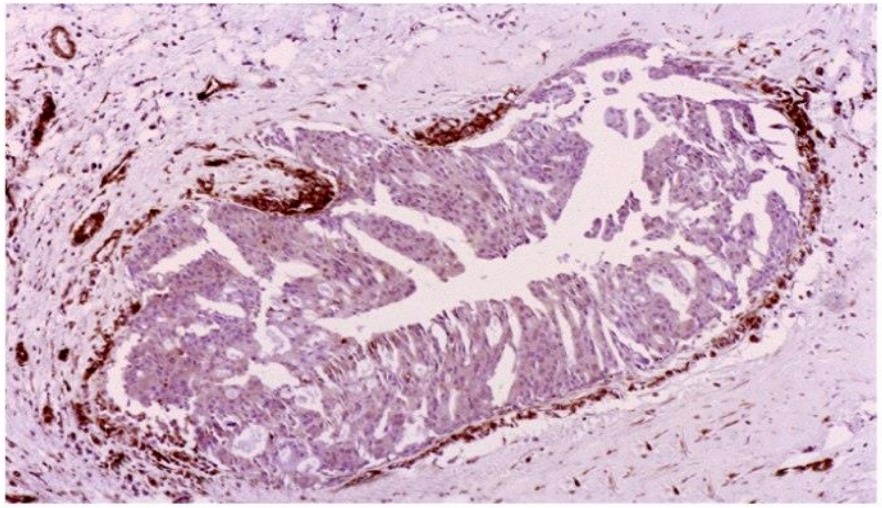
An angiogenic tumor phenotype is closely related to the increased proliferation of malignant cells, as well as the acquisition of invasive and metastatic potential.



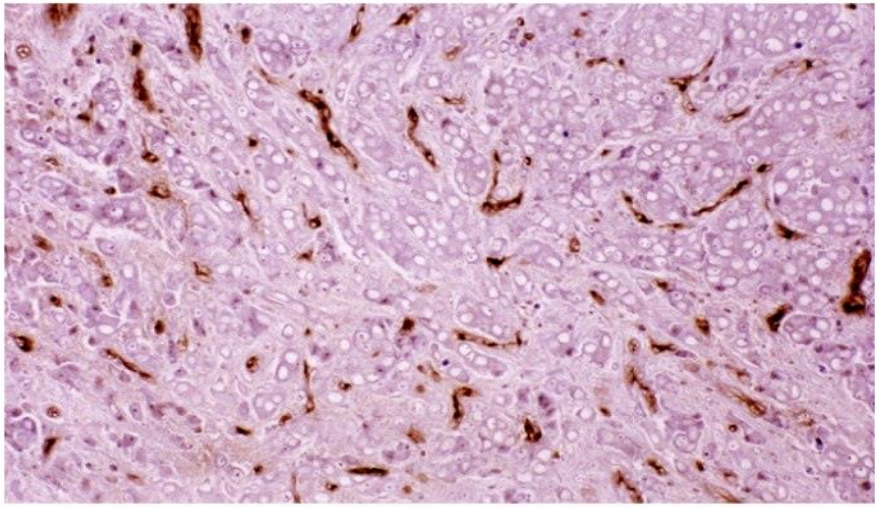
prostate cancer (PIN; *in situ*)



invasive prostate cancer



human breast cancer (*in situ*)



invasive human breast cancer

Angiogenesis regulation factors

The different stages of angiogenesis are specific regulated by:

.... adhesive molecules (e.g., integrins, selectins and cadherins) that enable cell adhesion to components of the extracellular matrix (ECM).

.... ECM components (such as collagen, fibronectin, laminin and proteoglycans) that are responsible for cell- ECM interactions.

... enzymes of the ECM (e.g., matrix metalloproteinases) which cause proteolysis of ECM components.

... cytokines (such as IL-1β, IL-8 and TNF).

... proangiogenic growth factors (e.g., VEGF, bFGF, TGF-β, EGF and angiopoietin-1).

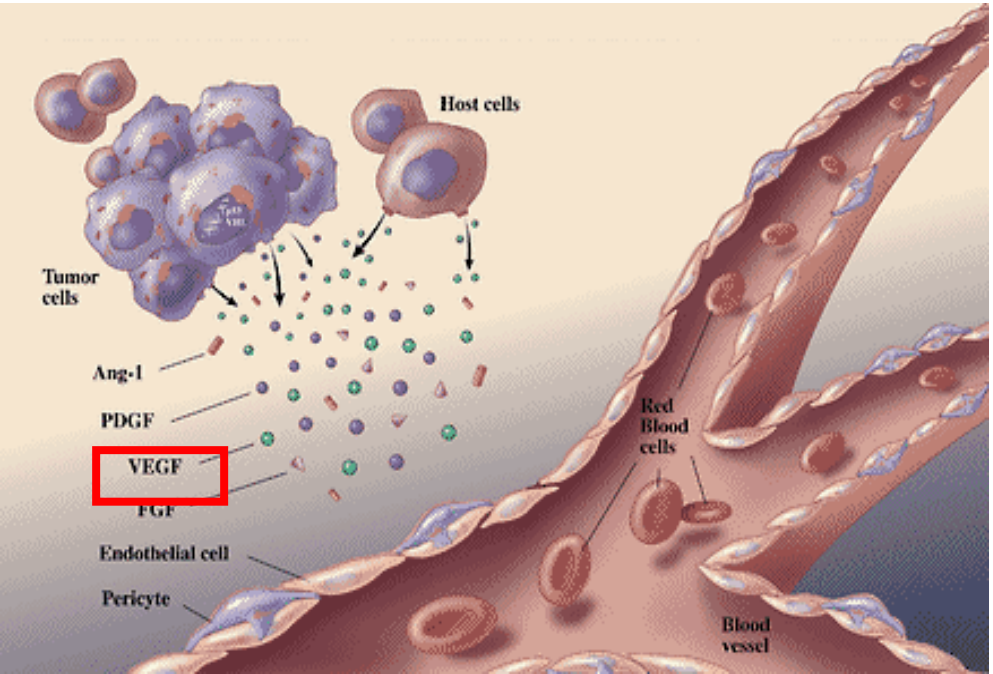
Several different families of growth factors (proangiogenic factors) are known.

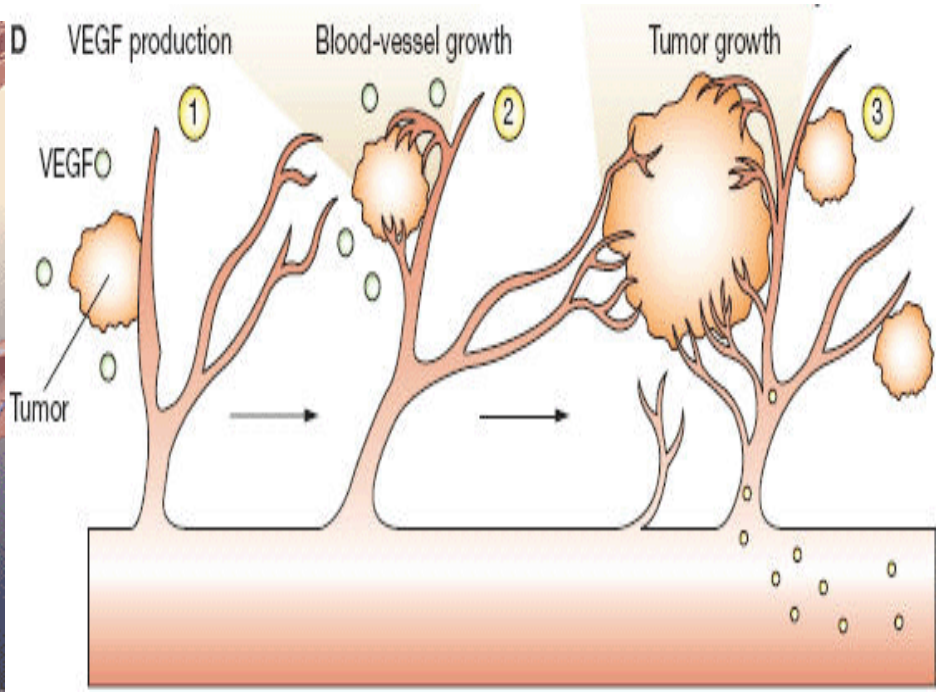
Some factors, such as vascular endothelial growth factor (VEGF), exert their effect directly. They show a high (but not absolute) degree of specificity for endothelial cells, and especially for activated endothelial cells.

There are other factors that act indirectly. These factors (e.g., TGF-β, TNF-α, IL-8, IL-6, SDF-1) act either by stimulating the expression of the main factors of angiogenesis, or by activating cells at the site of angiogenesis that then enhance angiogenic processes.

Proangiogenic factors

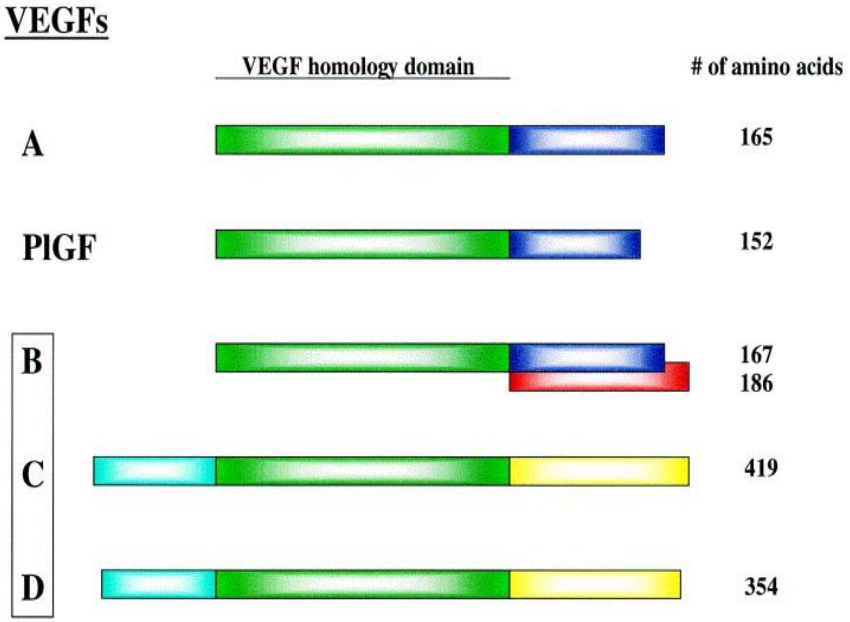
One of the most important and strongest stimulators of tumor angiogenesis are **vascular endothelial growth factor** (**VEGF**). It is a key mediator of tumor angiogenesis.





VEGF (VEGF-A)

The VEGF family includes: VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF).



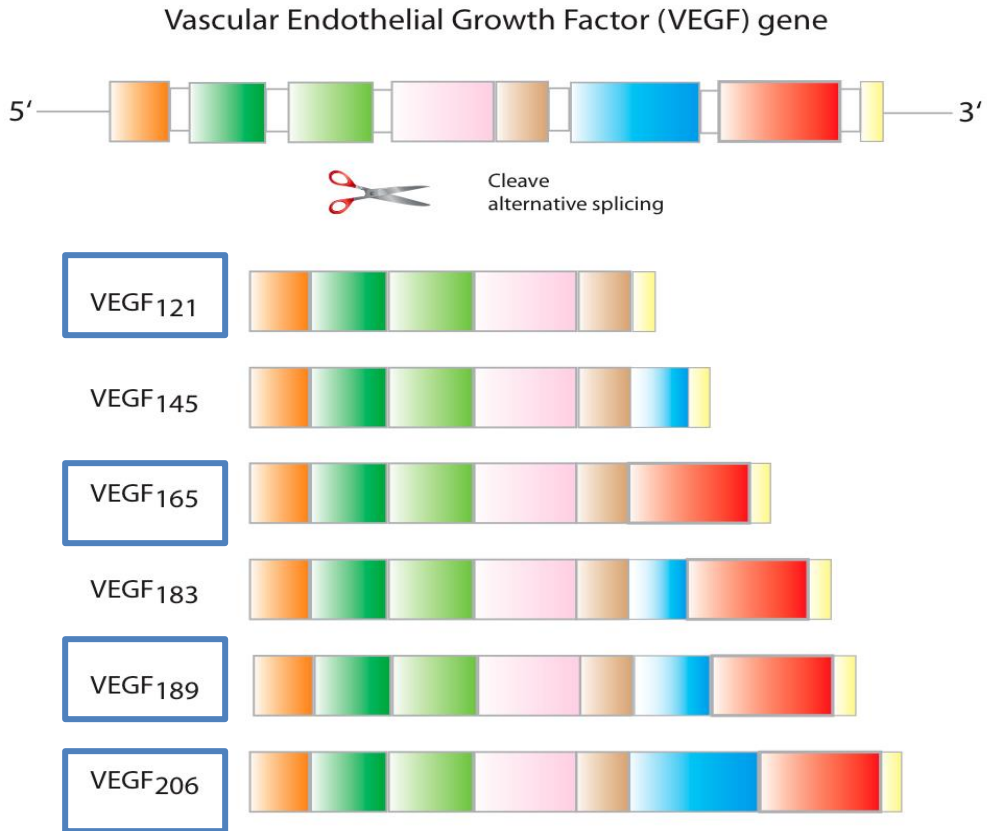
VEGF was first discovered as a vascular permeability factor.

Increased vascular permeability is thought to be due to the presence of intracellular fenestrae, reduced pericyte support, and/or the presence of specialized endothelial organelles called vesiculovacuolar organelles.

VEGF is a highly specific and potent endothelial cell mitogen.

Alternative splicing of the VEGF gene results in several isoforms: $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ и $VEGF_{206}$. **$VEGF_{121}$** is the shortest isoform that is present in circulating form, while **$VEGF_{189}$** and **$VEGF_{206}$** are bound to the cell surface or are sequestered in the ECM in an inactive form and are activated by proteases. **$VEGF_{165}$** is also bound to the cell surface, but is also present in a circulating form.

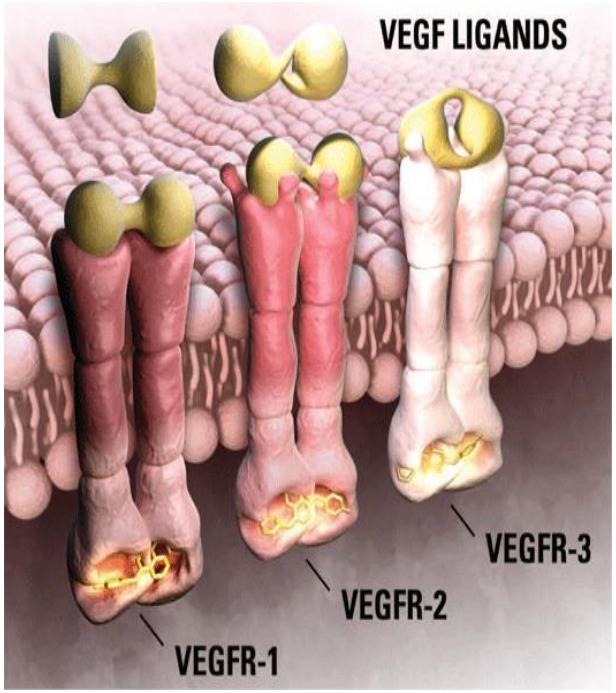
$VEGF_{121}$ and $VEGF_{165}$ isoforms are thought to be the main mediators of angiogenesis.



Receptors for VEGF

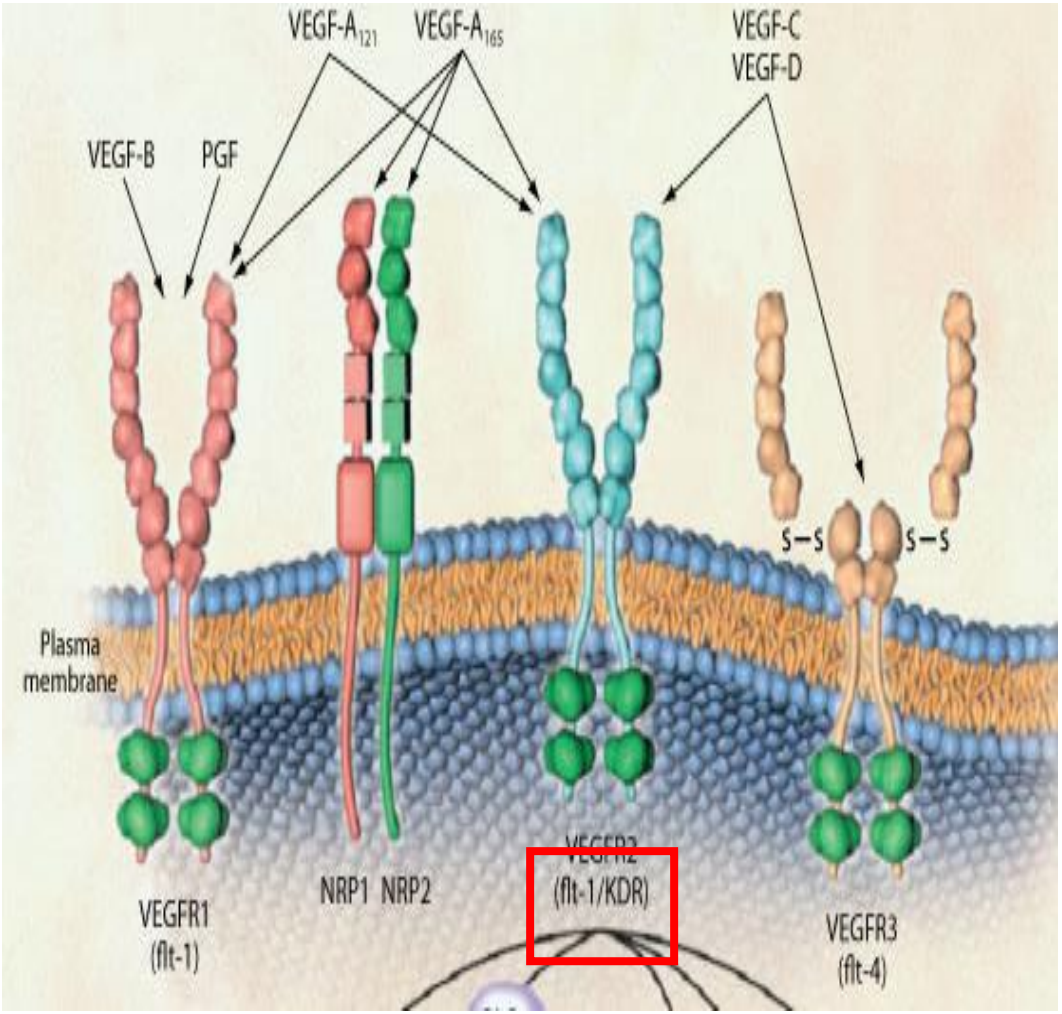
There are three types of receptors with tyrosine kinase activity **VEGFR-1**, **VEGFR-2** and **VEGFR-3**.

VEGFR-1 and VEGFR-2 are expressed on endothelial cells of blood vessels, while VEGFR-3 is expressed on endothelial cells of lymphatic vessels.

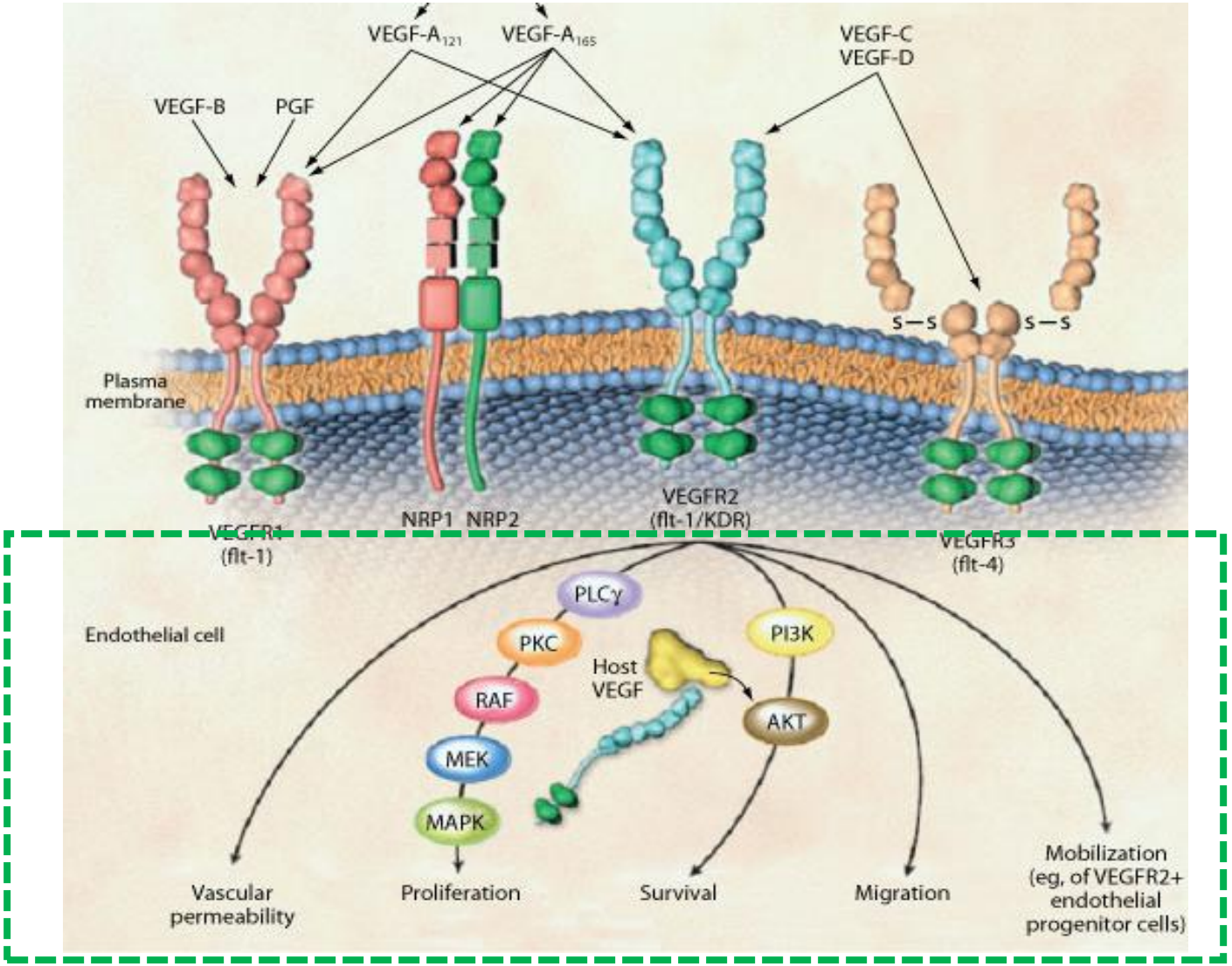


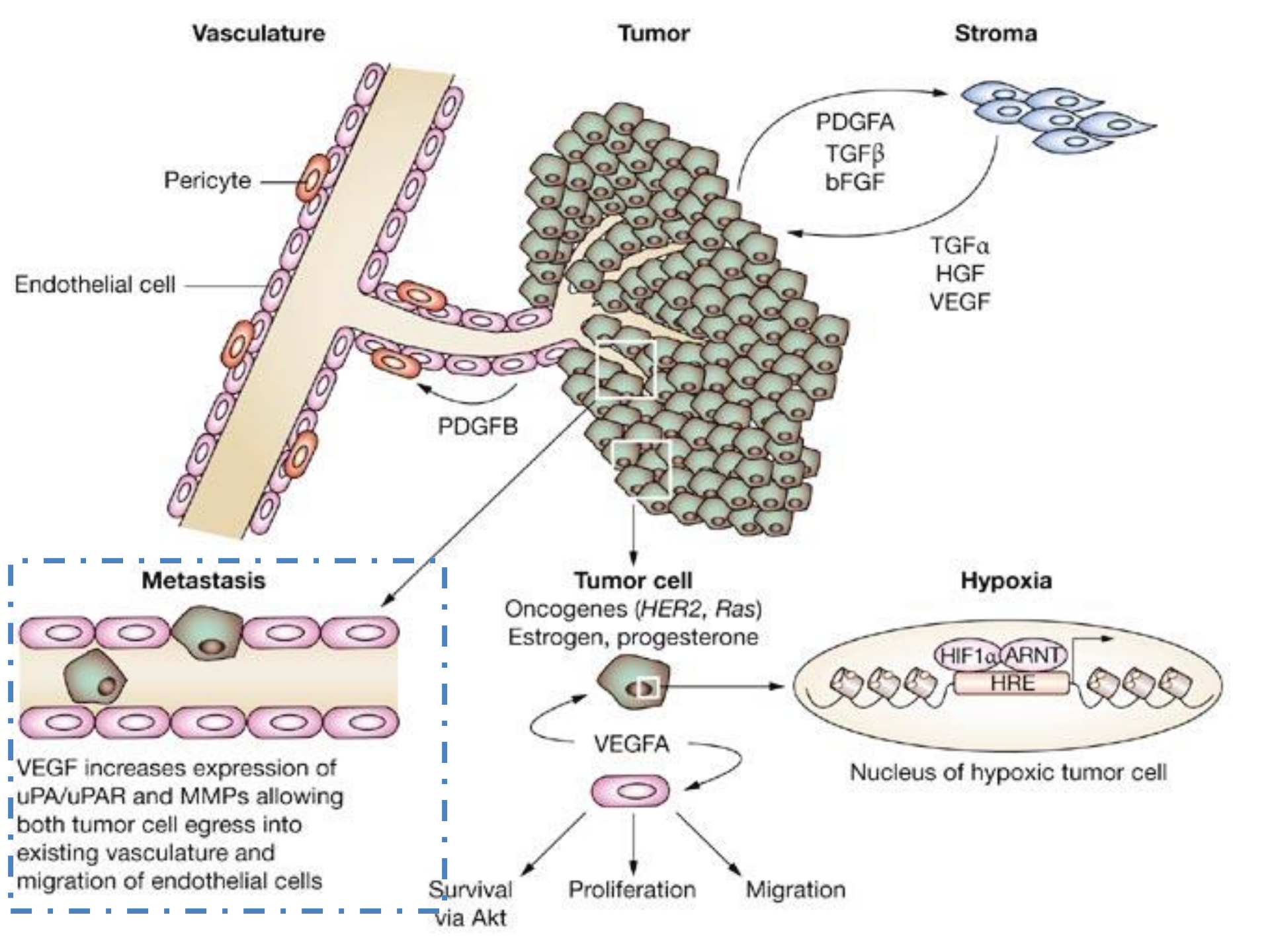
The main signaling receptor in angiogenesis is VEGFR-2.

It is assumed that the soluble form of VEGFR-1 serves as a negative regulator in physiological angiogenesis.



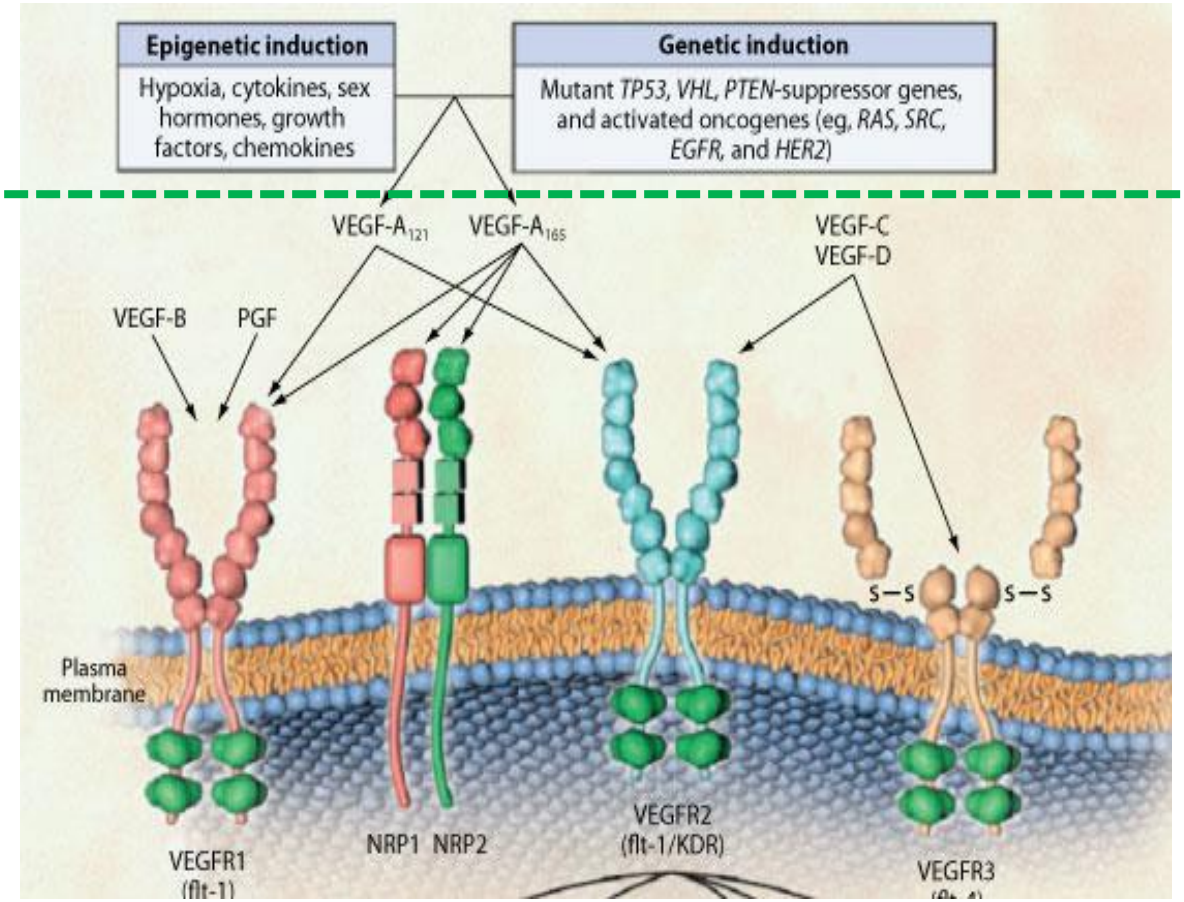
The role of VEGF in tumor angiogenesis

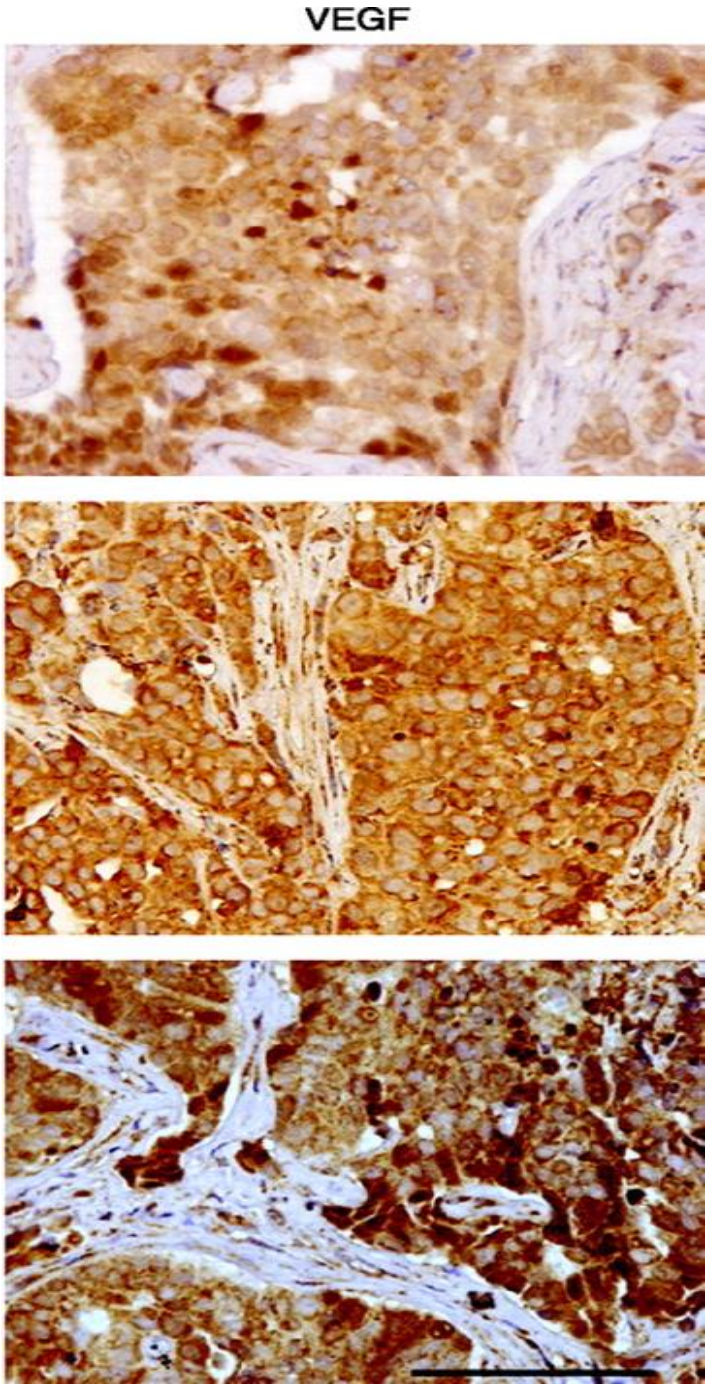



Vasculature
Tumor
Stroma
Pericyte
Endothelial cell
PDGFA
TGFβ
bFGF
TGFα
HGF
VEGF
PDGFB
Metastasis
VEGF increases expression of uPA/uPAR and MMPs allowing both tumor cell egress into existing vasculature and migration of endothelial cells
Tumor cell
Oncogenes (HER2, Ras)
Estrogen, progesterone
VEGFA
Survival via Akt
Proliferation
Migration
Hypoxia
HIF1α
ARNT
HRE
Nucleus of hypoxic tumor cell

... VEGF is produced by many types of cells, such as tumor cells, macrophages, vascular smooth muscle cells, and fibroblasts.

VEGF is overexpressed in many human tumors compared with normal tissue.

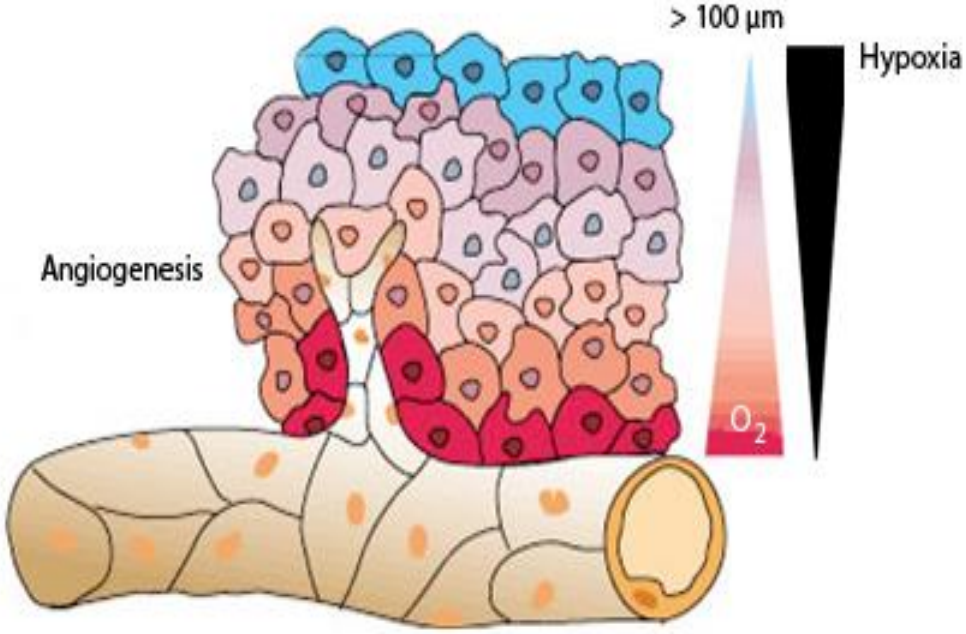


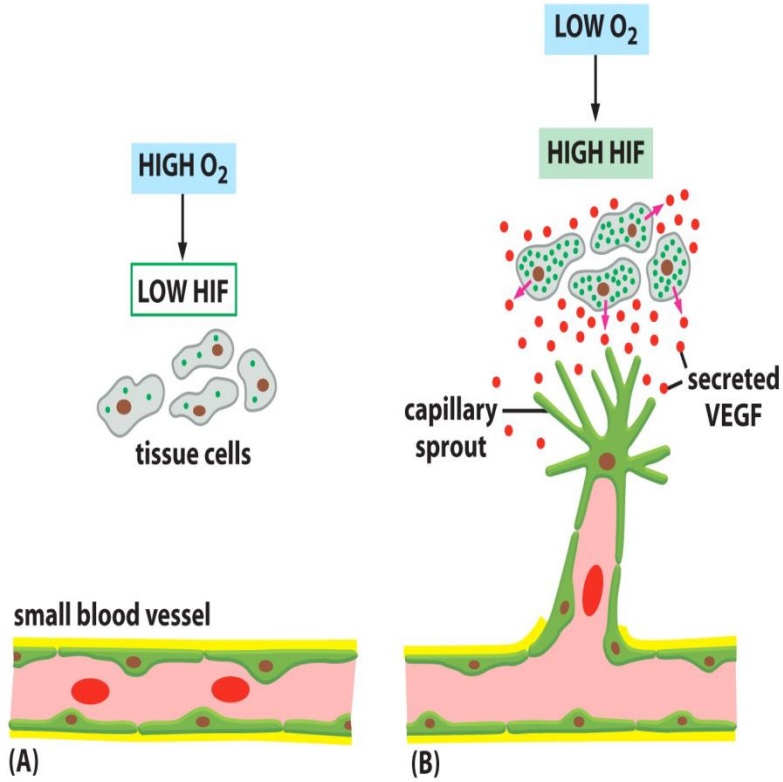


Expression of VEGF in tumor tissue

The role of hypoxia in tumor angiogenesis

Tissue hypoxia is an important stimulus for VEGF expression. Lack of oxygen induces an increased intracellular level of the HIF-1α active form. HIF-1α stimulates the VEGF transcription, which is secreted, diffuses through the tissue, reaches endothelial cells and binds to specific receptors on their surface.

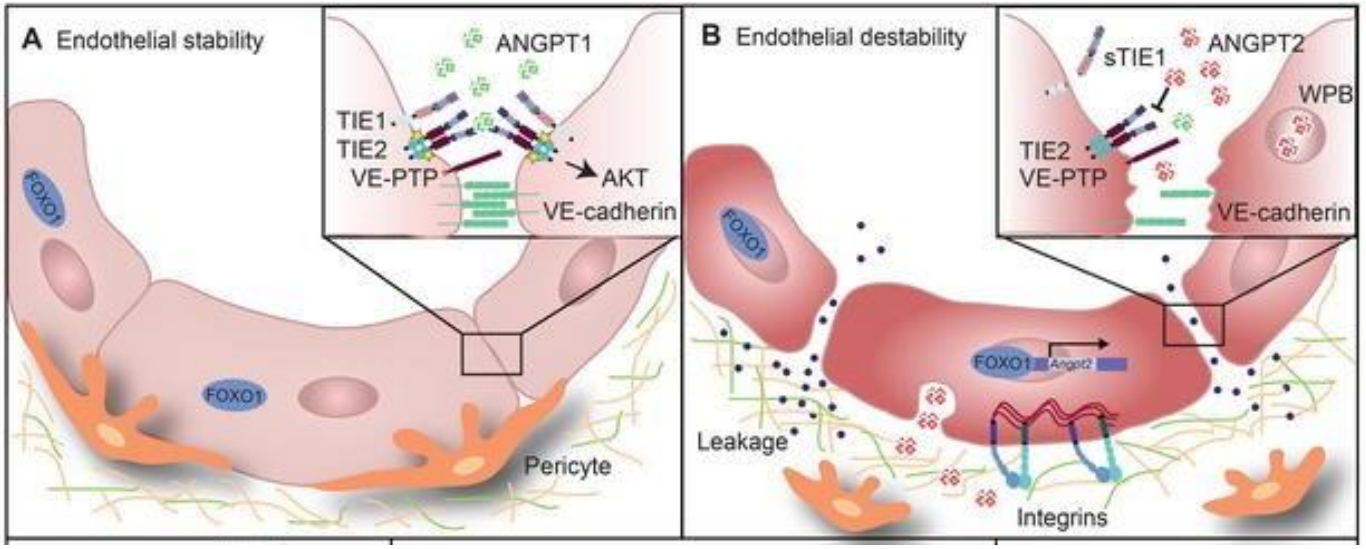




HIF (Hypoxia- inducible factor)
-the main transcription factor of VEGF
- stable in conditions of hypoxia

↑ O_2 causes degradation of HIF1-α
↓O_2 stabilizes HIF1- α

Angiopoietin/tie-2 signaling pathway



The next important factors that regulate angiogenesis are **angiopoietin-1** and **angiopoietin-2.**

They bind to the **receptor tie-2.**

Angiopoietin-1 promotes the maturation and stabilization of newly formed blood vessels by triggering the activation of the Akt/survivin signaling pathway.

Angiopoietin-2 induces destabilization of blood vessels, detachment of pericytes and budding of existing blood vessels.

basic Fibroblast Growth Factor (bFGF)

Together with VEGF, bFGF stimulates the formation of new blood vessels, and one of the functions of this growth factor is to induce the secretion of enzymes, MMPs, plasminogen activators, as well as collagenases, all of which are responsible for the ECM breakdown.

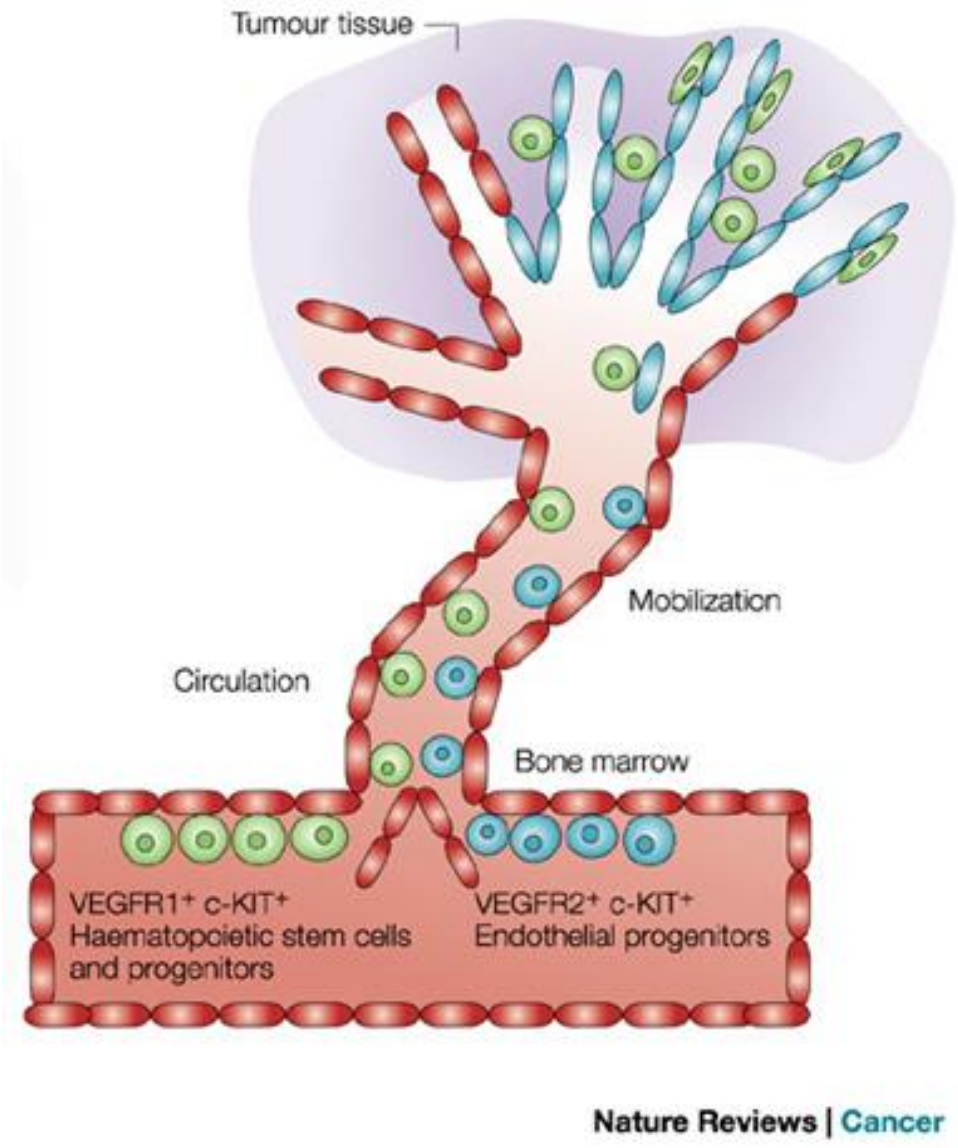
It also works by increasing the production of VEGF in smooth muscle cells of blood vessels, as well as the VEGFR expression on ECs.

Platelet-derived growth factor-B (PDGF-B)

Increased PDGF-B expression promotes pericyte recruitment and blood vessel stabilization.

Inhibition of the PDGF-B signaling pathway reduces pericyte recruitment and consequently increases endothelial cell apoptosis.

Angiogenesis and circulating bone marrow-derived cells

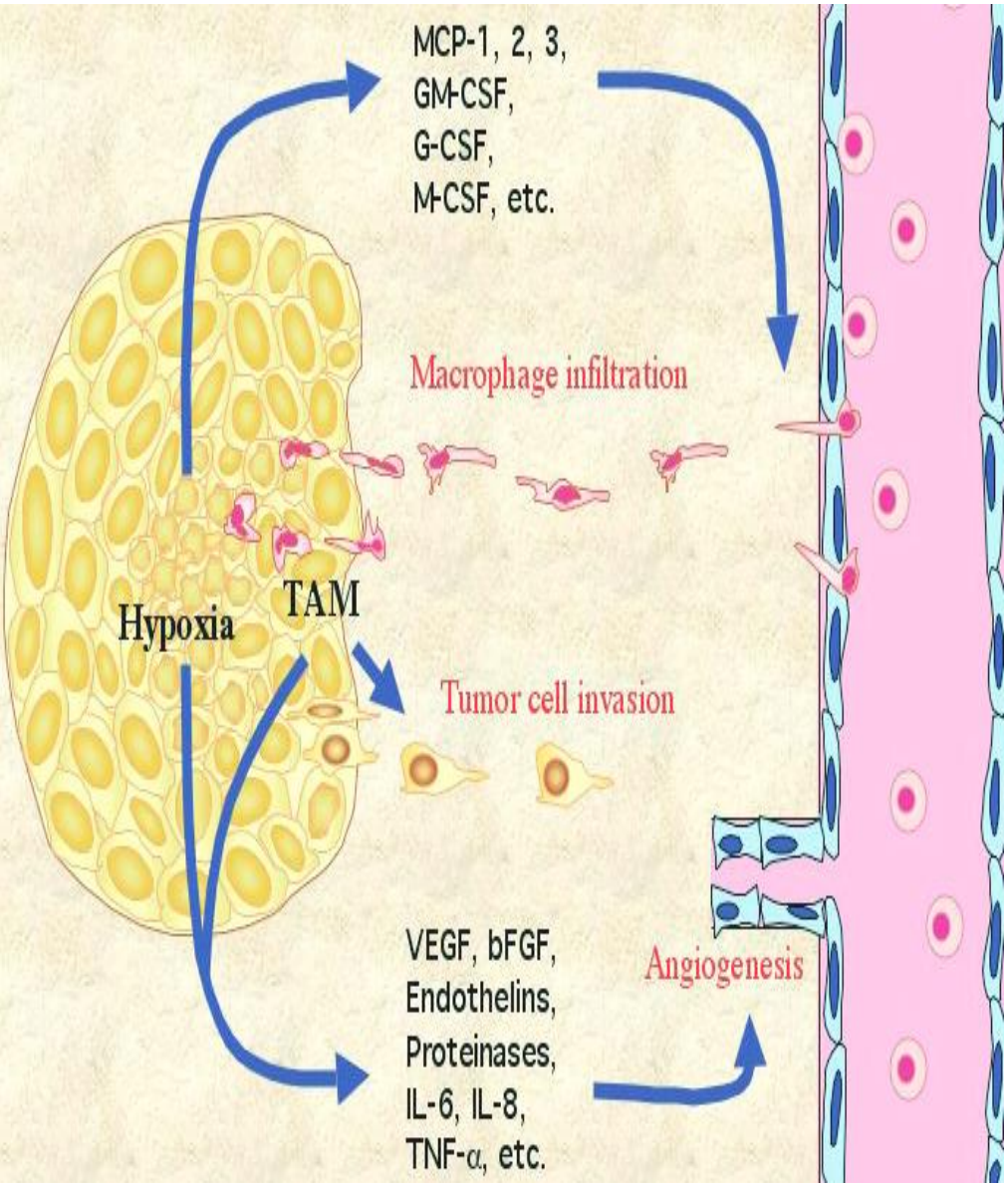


Many types of cells derived from the bone marrow can be mobilized to the site of the formation of new blood vessels, where they amplify the angiogenesis processes.

Hematopoietic (CD45+) cells, such as monocytes and other myeloid cells, may be involved in angiogenesis processes.

Non-hematopoietic CD45- cells are also involved in tumor angiogenesis. Circulating endothelial cell precursors are thought to be incorporated into the wall of growing blood vessels, where they then differentiate into endothelial cells.

The role of TAM-2 in tumor angiogenesis

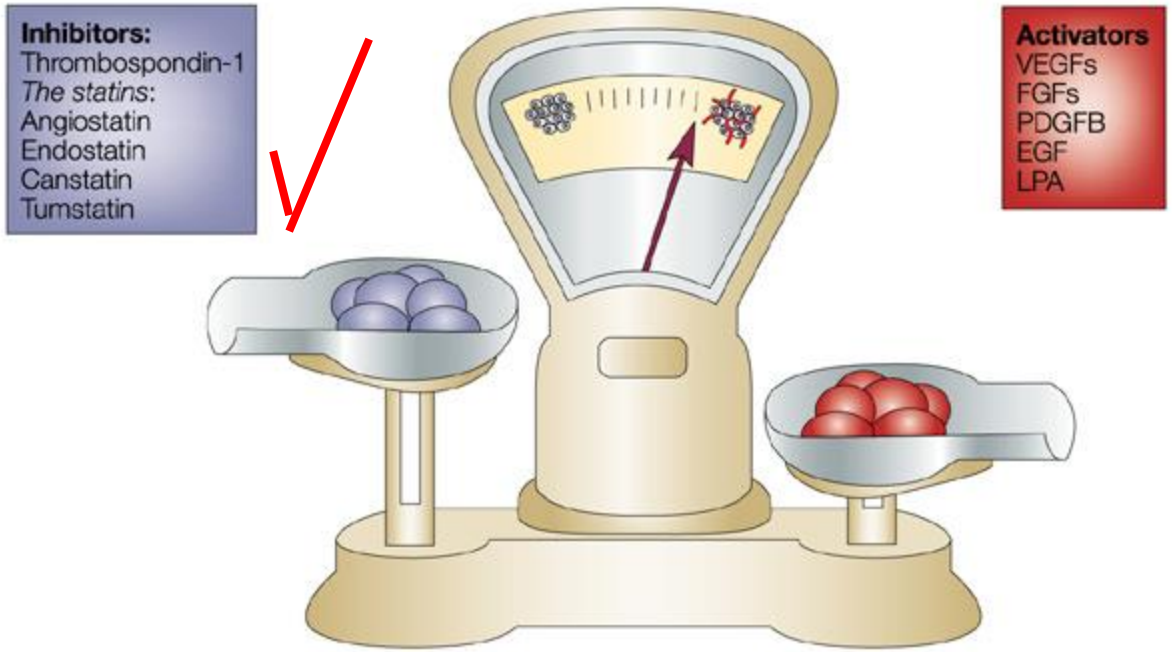


In 1863. Virchow was the first to discover leukocytes in tumor tissue as well as tumor enviroments. The tumor-associated macrophages (TAMs) comprise large portion of the leukocyte infiltrate of many tumors (including primary tumors and metastases).

During progression, numerous regions of hypoxia are formed. Different factors are secreted in these regions, which act **chemotactically and attract macrophages to the tumor microenvironment**.

TAM2, can be an important source of VEGF and matrix metalloproteinases involved in the process of tumor angiogenesis.

Endogenous inhibitors of tumor angiogenesis



Thrombospondin-1

... extracellular matrix glycoprotein that binds to the CD36 receptor, and functions as a potent endogenous inhibitor of angiogenesis. Tumor suppressor gene p53 has been shown to increase the expression of thrombospondin-1 in various malignancies.

It inhibits proliferation and migration of endothelial cells and at the same time induces apoptosis of these cells.

...

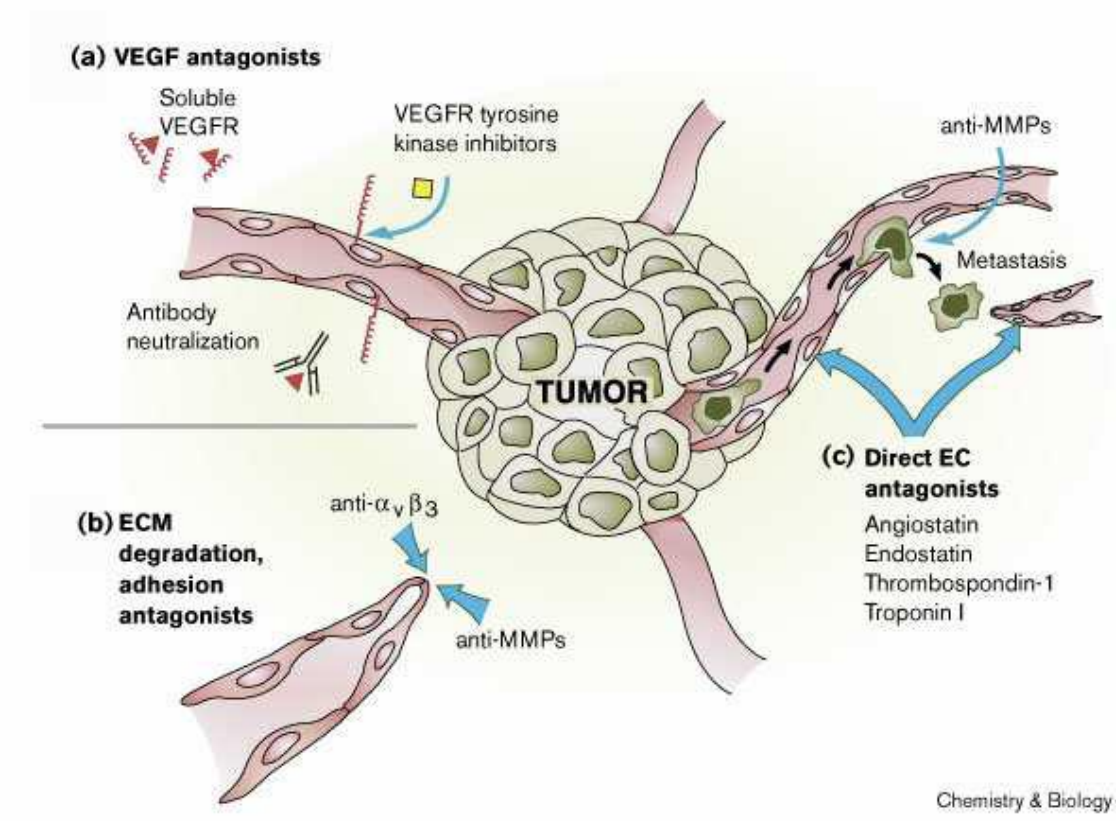
Angiostatin, a fragment of plasminogen, inhibits cell proliferation and induces endothelial cell apoptosis.

Angiogenesis inhibitors are also **type IV collagen fragments** such as endostatin.

Endogenous inhibitor is a fragment of calreticulin also known as **vasostatin**.

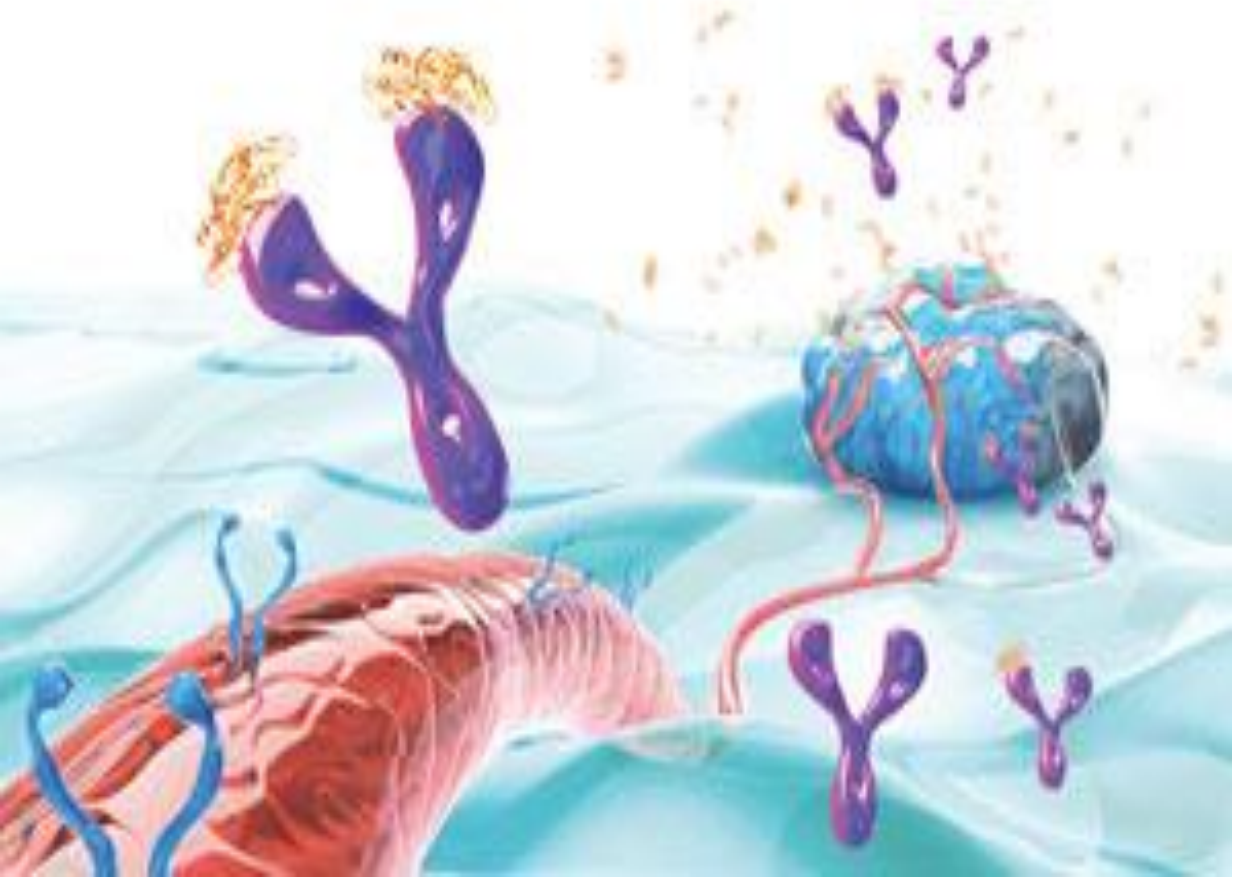
Inhibition of tumor angiogenesis in different stages

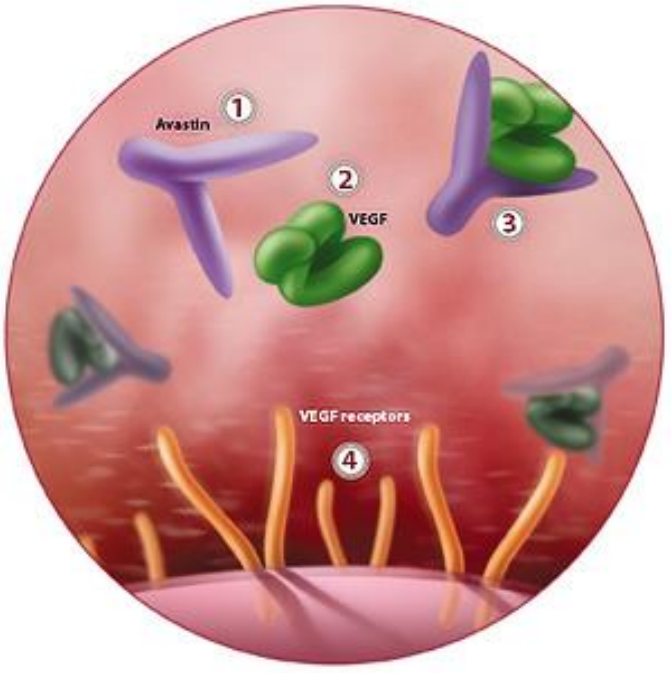
One of the major strategies to kill the cancerous cells is hindering the blood supply to these cells.

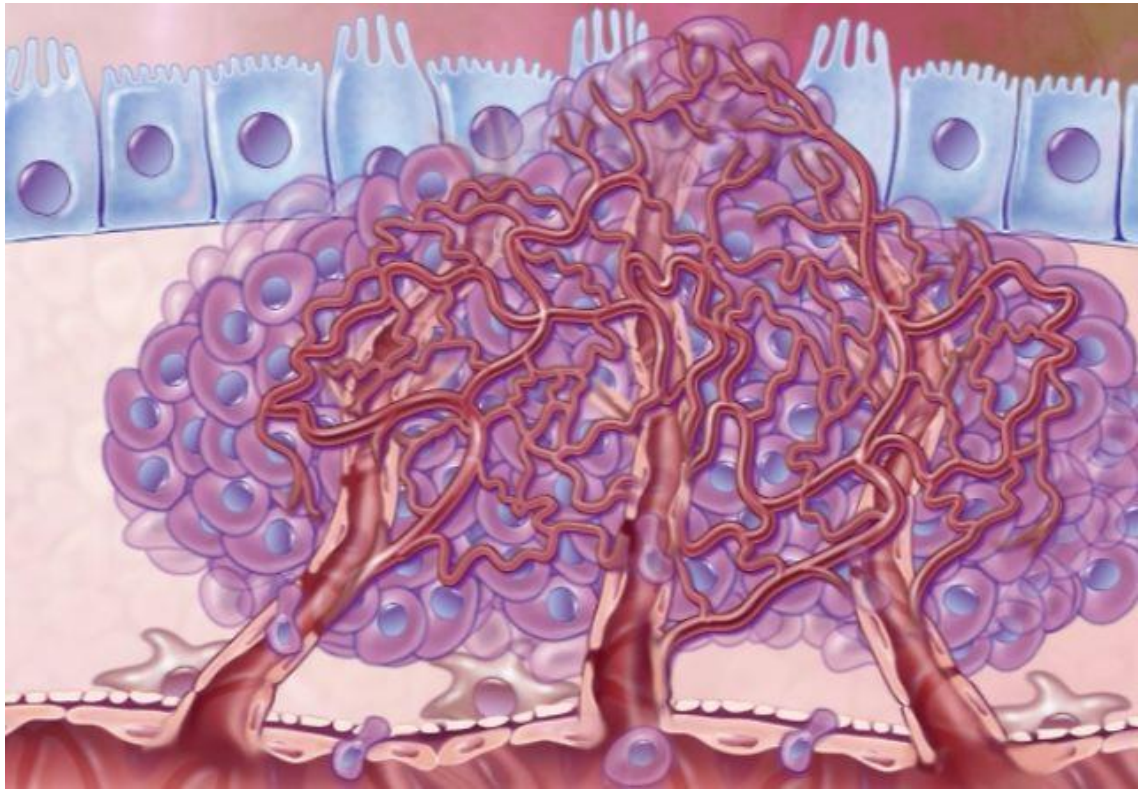


Bevacizumab

Humanized anti-VEGF monoclonal antibody







http://vimeo.com/28439795