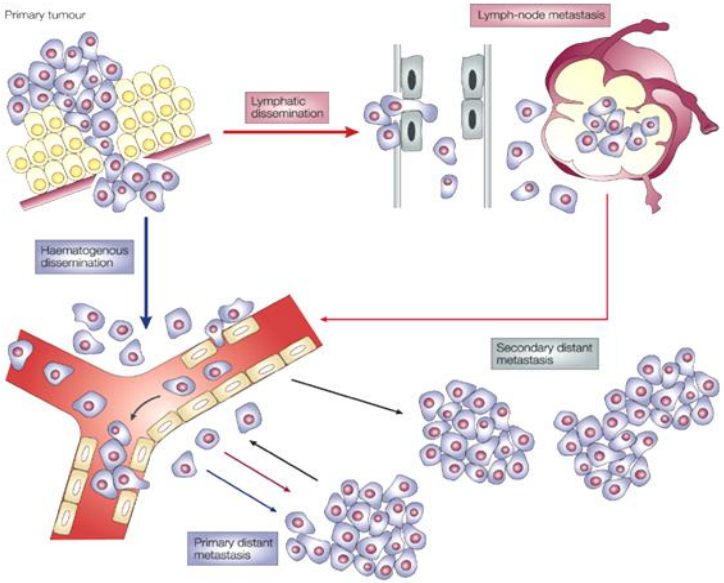
Teaching unit 10



Invasiveness and tumor metastasis

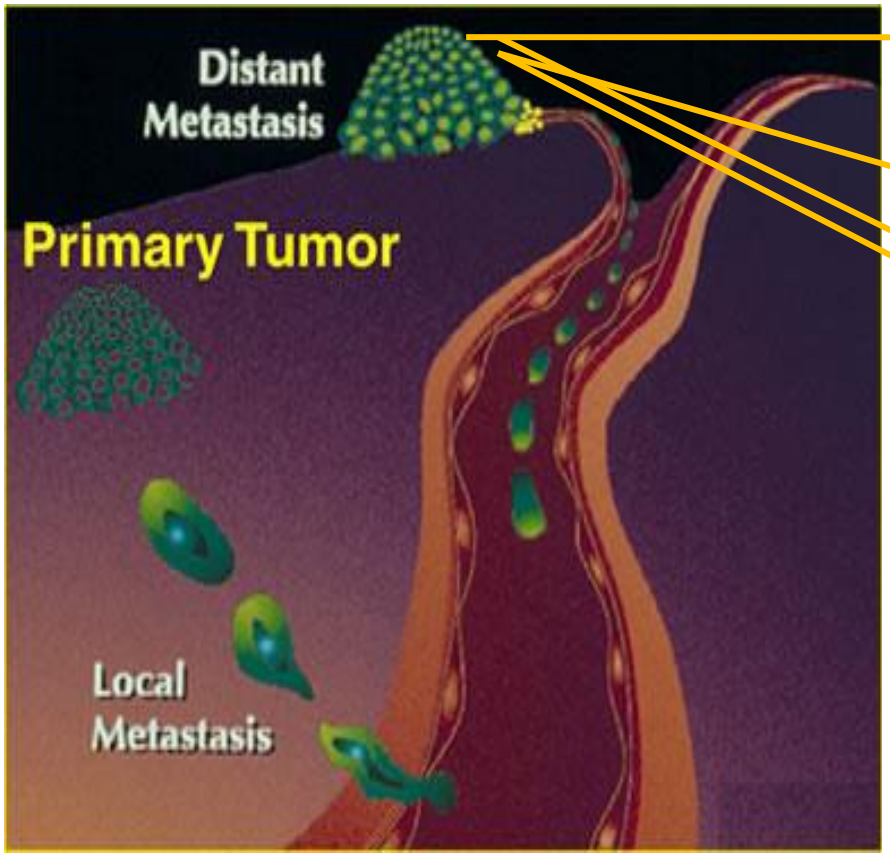
Basic principles of invasive tumor growth

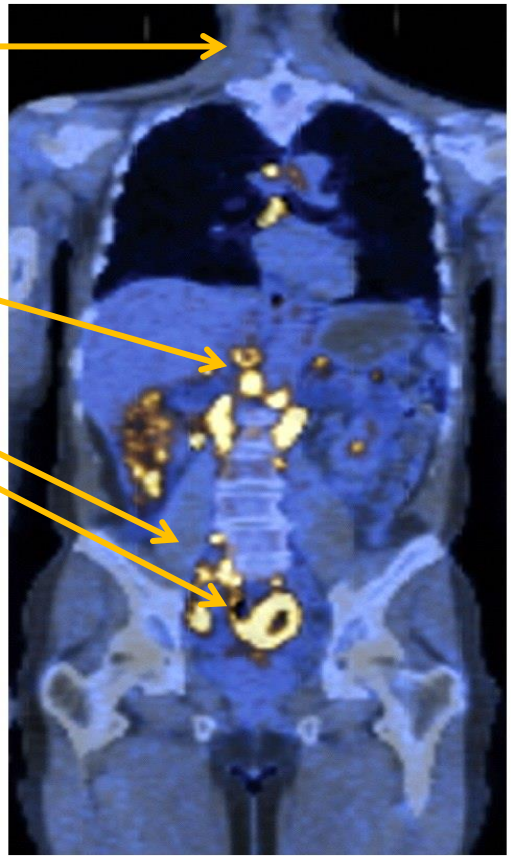
Genetic basis and mechanisms of metastasis

Tumor metastasis is the process of spread of malignant cells from the primary tumor to distant sites.

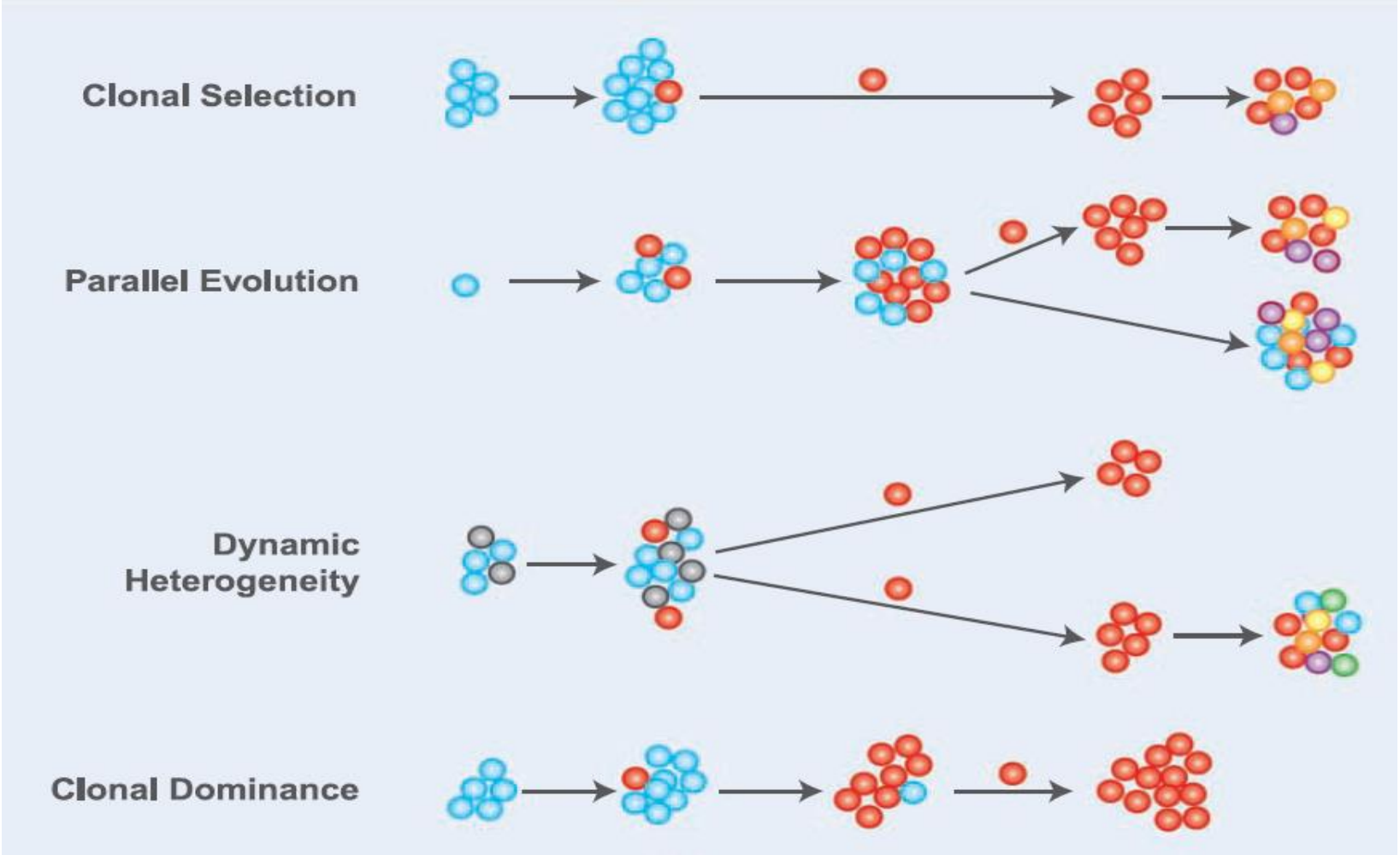
The establishment of metastases is the last qualitative step in the progression of malignant tumors.

The dynamics of progression depends on genetic mutations, cellular selection and tissue organization.





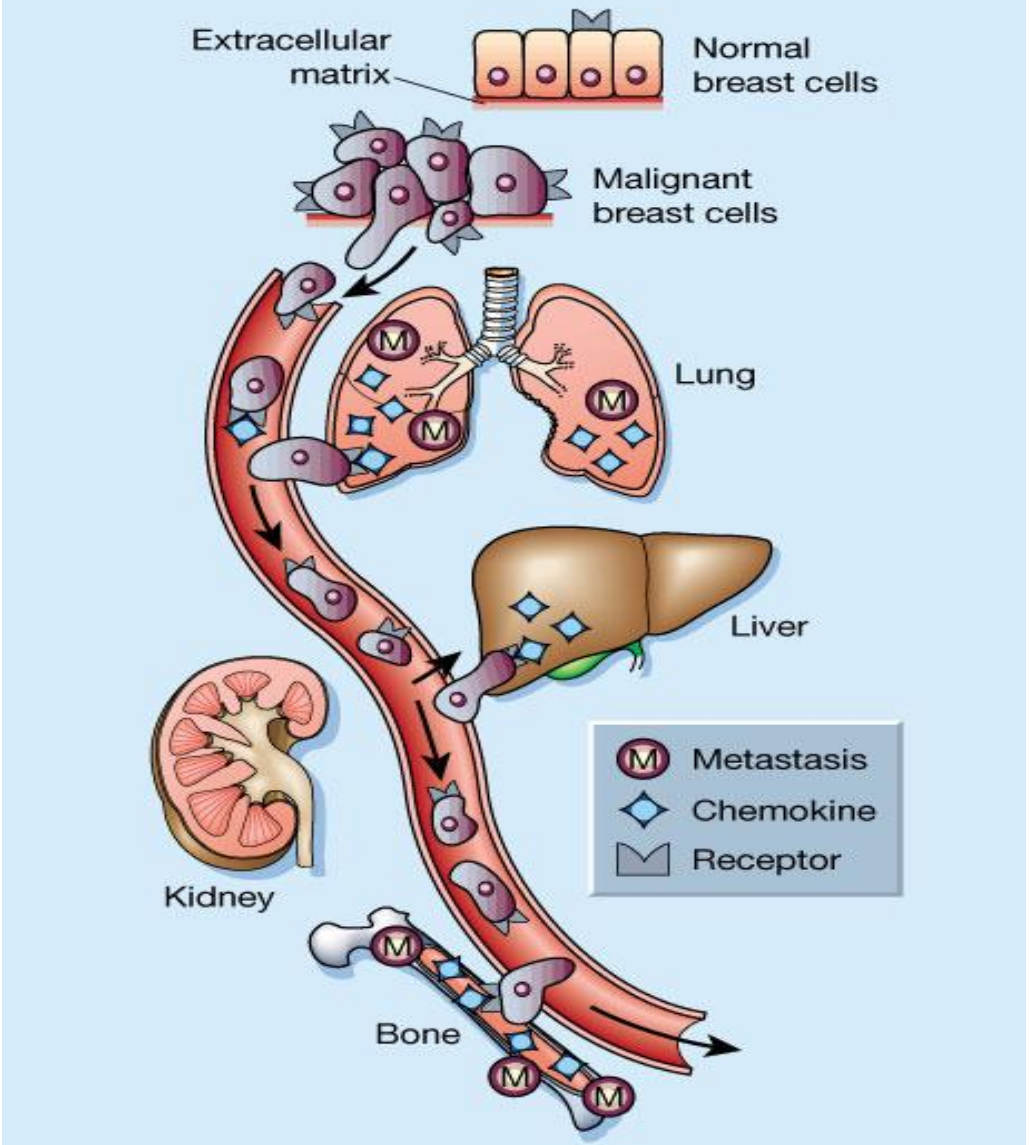
Any genetic change in a malignantly transformed cell is subject to clonal selection.



Stephen Paget (1855-1926)

In 1889, Stephen Paget put forward a theory of metastasis called "seed and soil".

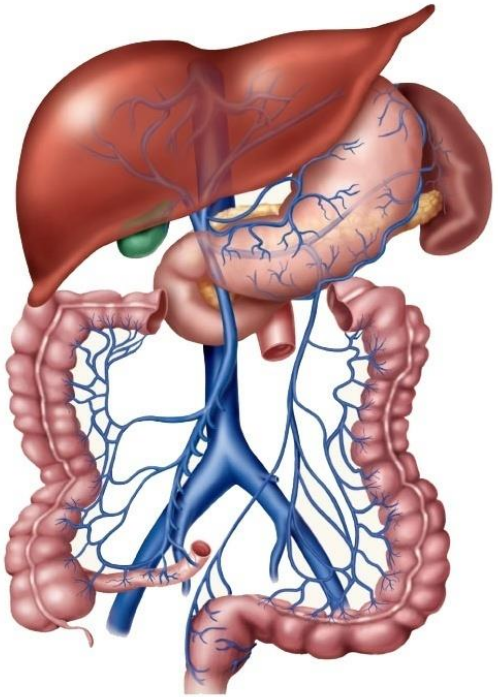
Organ-specific metastasis



James Ewing

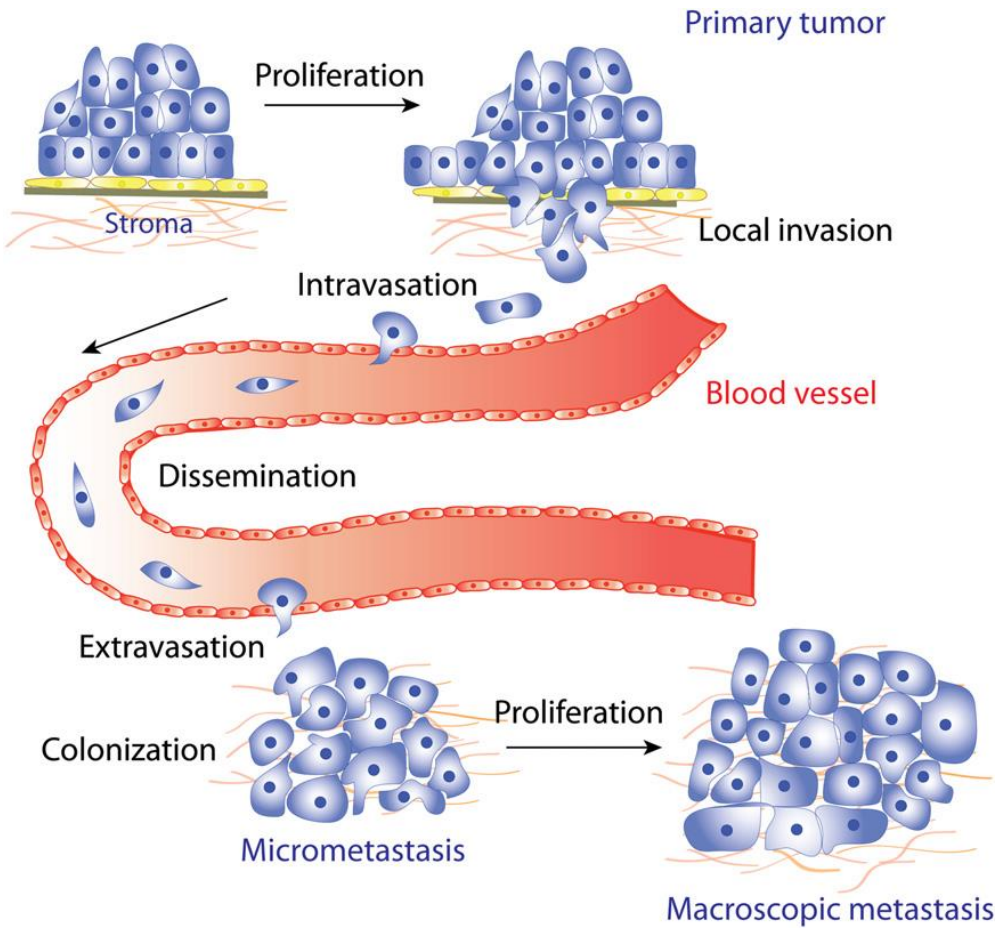
(1866- 1943)

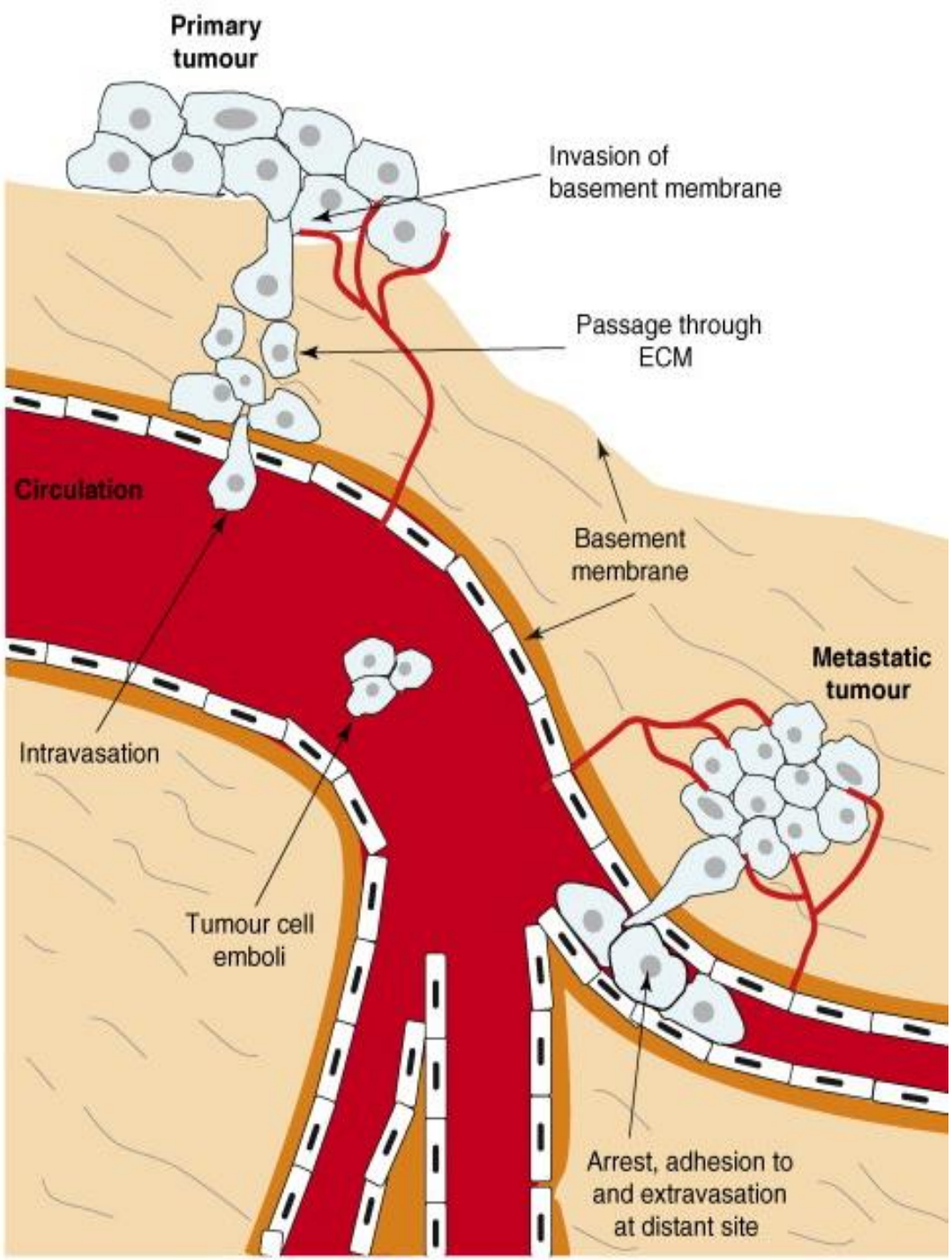
Tissue tropism of malignant cells results from mechanical factors and circulation patterns of the primary tumor.



The main steps in the metastatic cascade are...

- ✓ Invasion and mobility
- ✓ Intravasation and survival in circulation
- ✓ Extravasation of malignant cells into parenchymatous organs
- ✓ Growth of metastatic colonies in distant organs

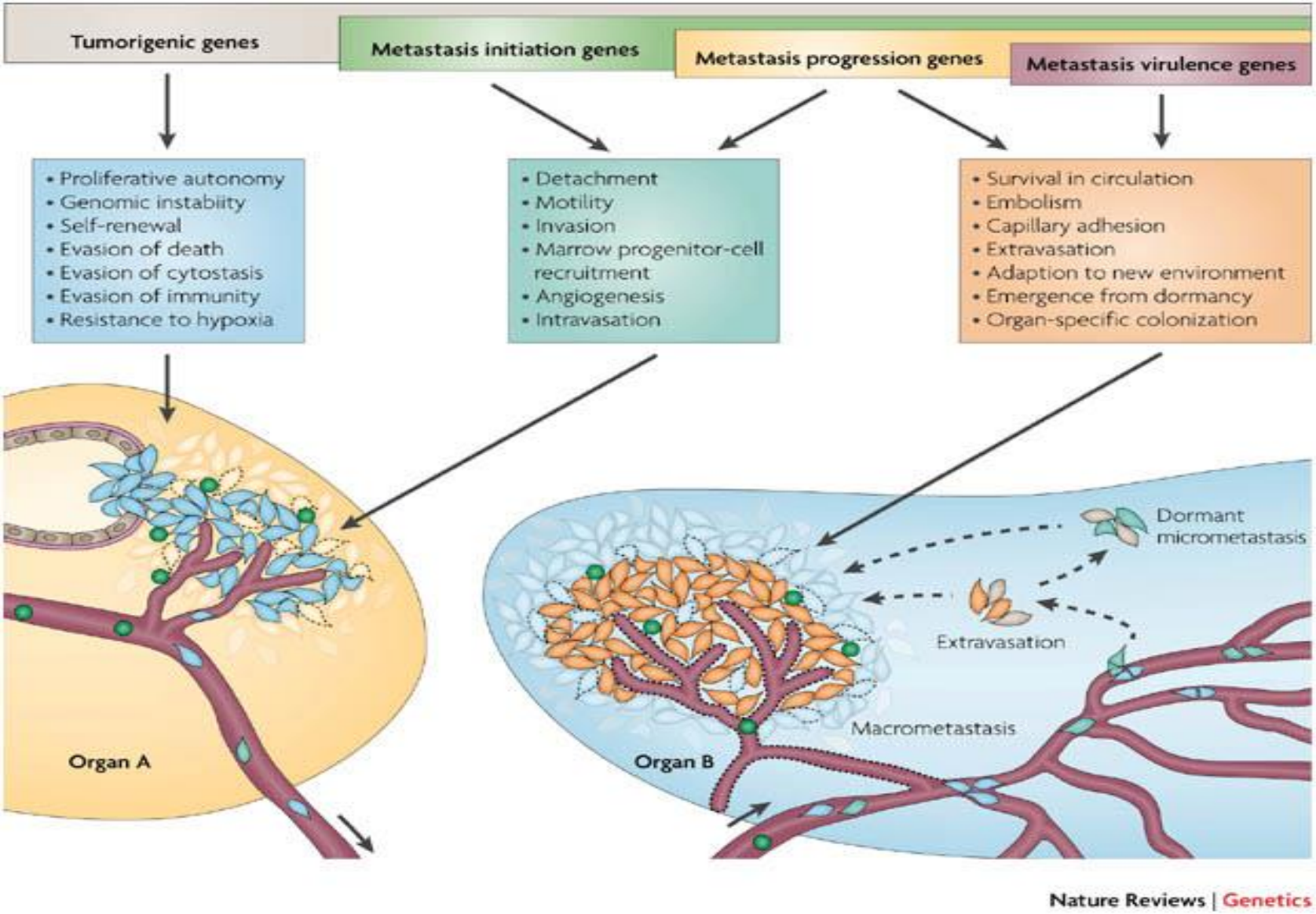




Metastatic cascade

All steps in the metastatic cascade are necessary for the successful formation of metastases. If only one of the steps is missing, tumor cell metastasis is unsuccessful.

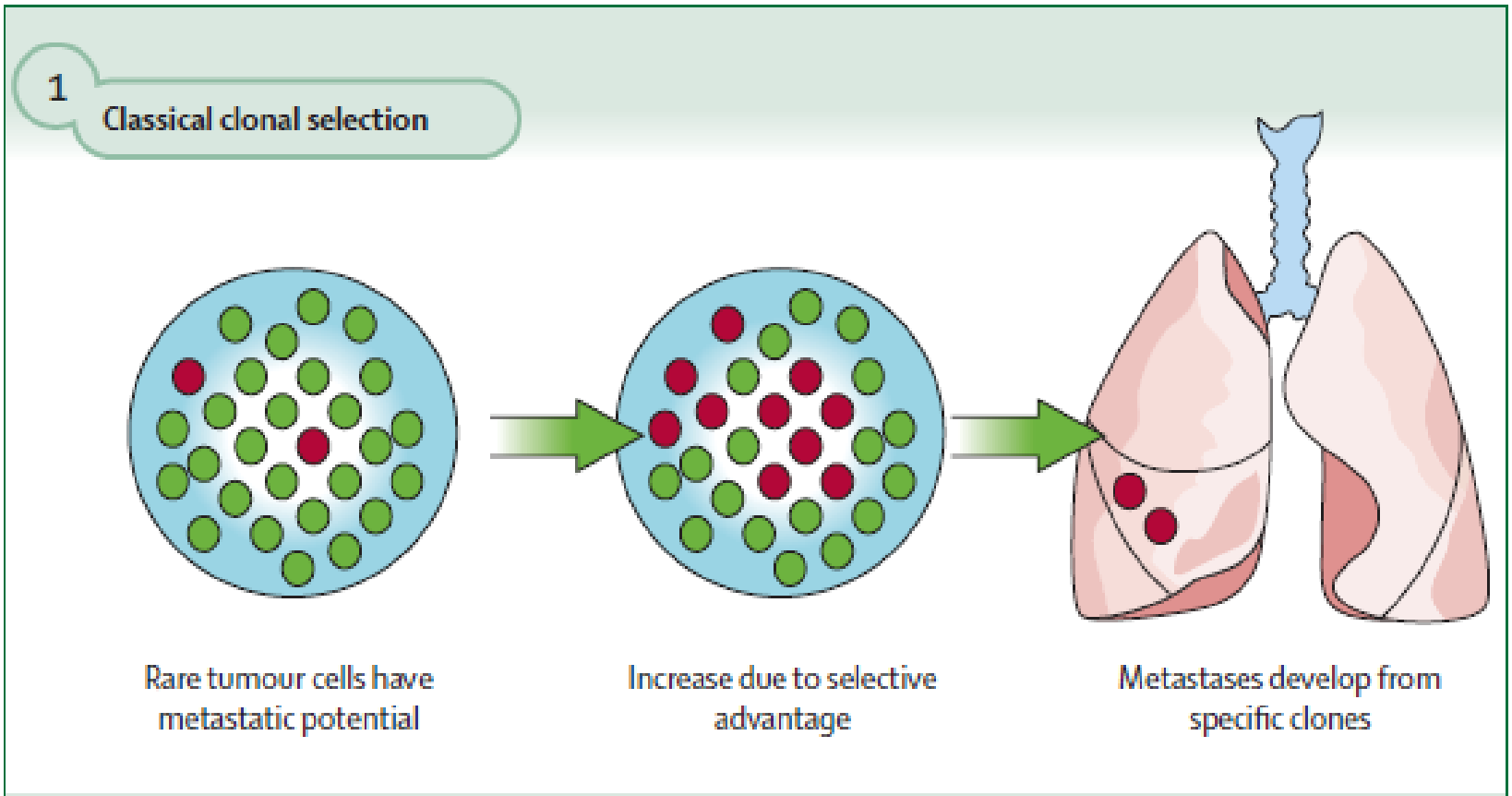
Only those tumor cells that have undergone all the necessary genetic changes can successfully form metastatic foci.



Numerous genes are responsible for each individual step in the metastatic cascade. These **genes** are classified into three groups - genes for the **initiation of metastases**, genes responsible for the **progression of metastases** and genes of **metastatic "virulence".**

In the tumor tissue, only a **small number of tumor cells** manage to accumulate all the genetic changes necessary for the cells' **full metastatic potential.**

A dominant cell clone in a primary tumor undergoes a series of genetic changes and thus acquires a "**selective advantage**" over other clones, as well as the ability to metastasize.

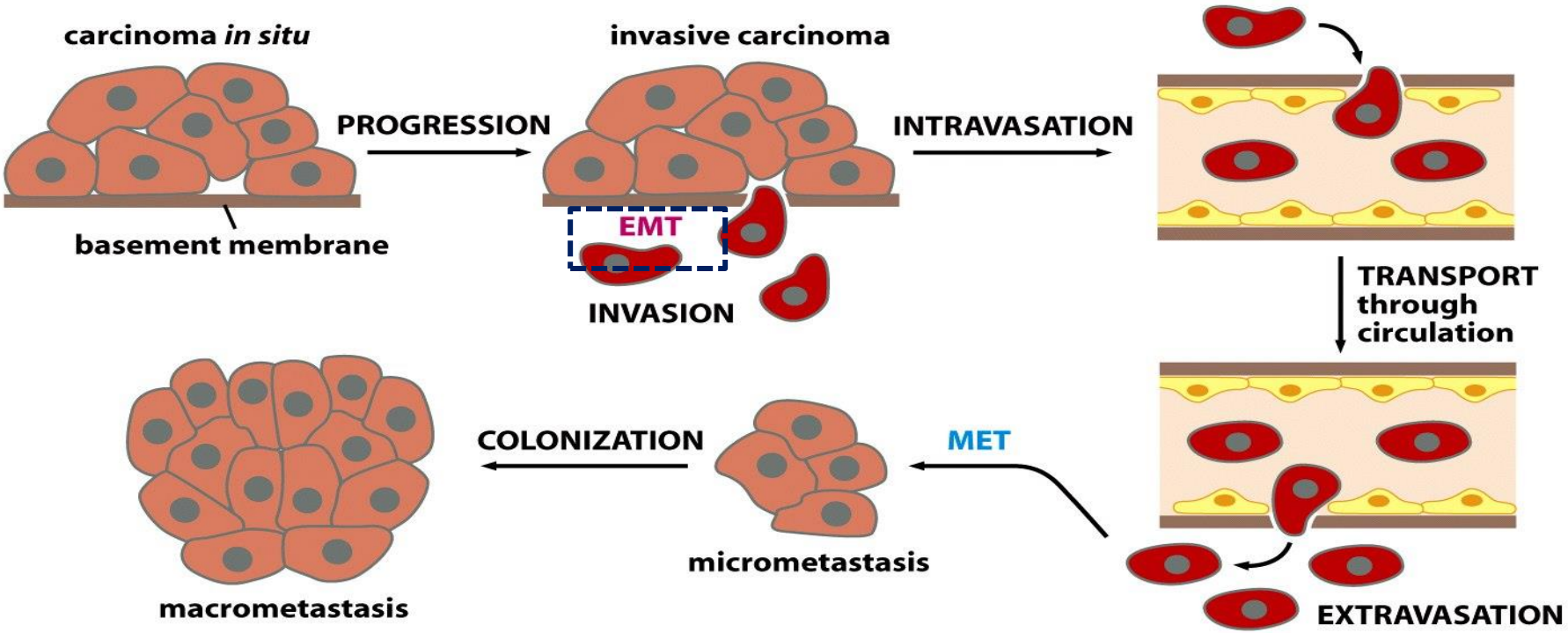


Selective pressure in the primary tumor affects the acquisition of metastatic potential

Hypoxia and **inflammation** play an important role in metastasis and influence the tumor to cooperate with numerous cells such as bone marrow-derived cells (**BMDCs**), among which myeloid suppressor cells (**MDSCs**) are important suppressor cells and Mesenchymal Stem Cells (**MSCs**).

The ability of malignant cells to initiate in the primary tumor **the epithelial-to-mesenchymal transition (EMT) program** is also a result of the selective pressure they face in the primary tumor.

Initiation of EMT in metastatic cells results in their **migration, invasion and intravasation.** It also represents the main mechanism by which the emergence of circulating malignant cells is promoted.



Hypoxia in tumor tissue

Under hypoxic conditions, **HIF1α** and **HIF2α** are stabilized resulting in the **transcription of more than 100 genes**.

These target genes are involved in **angiogenesis**, **glycolysis**, and **invasion**, which together help tumor cells adapt to hypoxic conditions.

By adapting to the **selective pressure of hypoxia**, tumor cells acquire the ability to withstand unfavorable microenvironmental conditions by **inducing glycolysis switching, resistance to apoptosis, angiogenesis and invasion** through the extracellular matrix.

All these acquired functions enable tumor cells to metastasize to distant places.

Various genes for glycolysis are expressed and the metabolic products released affect the acidification of the extracellular space.

Since these conditions are toxic to

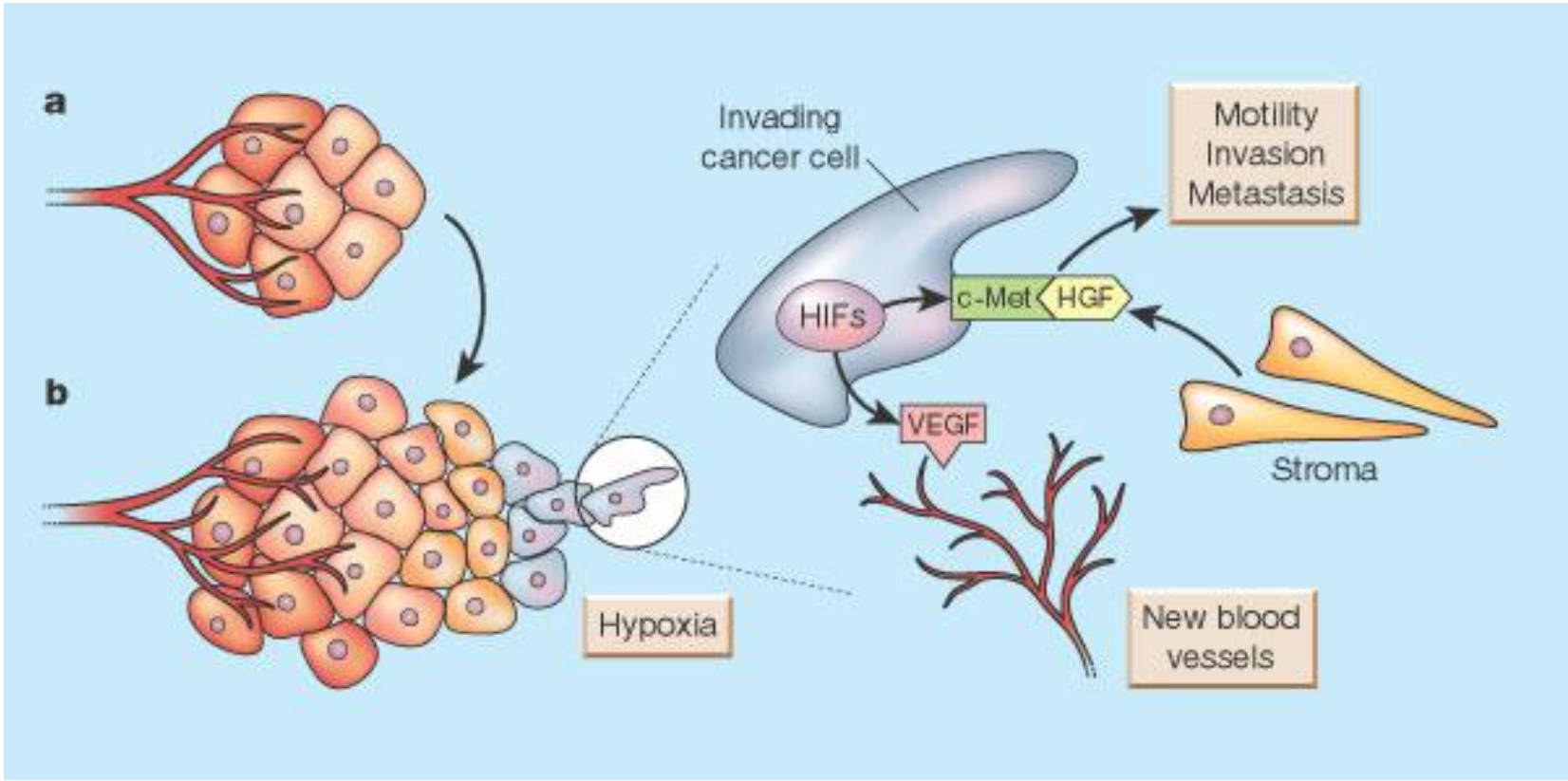
cells, further adaptation of tumor cells is necessary, which implies how

increased expression of the H+ transporter

as well as the acquisition of resistance to apoptosis.

Anaerobic metabolism and resistance to apoptosis are essential for the survival of metastatic cells both in the circulation and in distant organs.

Hypoxia promotes angiogenesis and tumor invasion

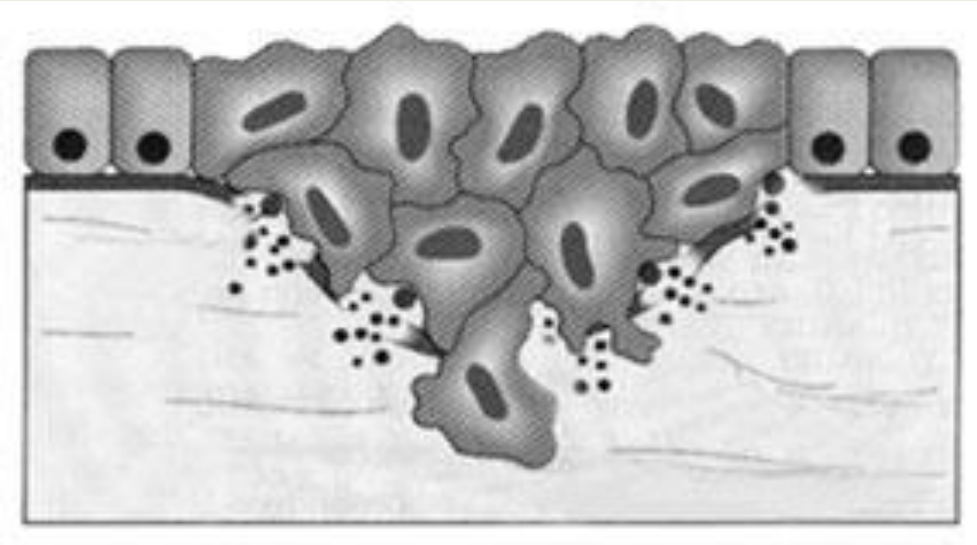


... **The expression of genes for angiogenesis increases**, such as **VEGF**. The increased permeability of blood vessels results in the extravasation of proteins that participate in the remodeling of blood vessels and the extracellular matrix in the immediate vicinity. These proteins also participate in the activation of endothelial cells involved **in the creation of a new vascularized region in the tumor tissue**.

Angiogenesis also contributes to the **successful adaptation** of **metastatic cells** in the **parenchyma of distant organs**.

HIFα increases expression **MMP1** и **MMP2**, **LOX** (*Lysyl Oxidase*) and chemokine receptor **CXCR4** and thereby supports the **invasion of tumor cells** into the newly vascularized region.

Degradation of the basement membrane under the influence of MMP2 and changes in the extracellular matrix under the influence of MMP1 and LOX enable the physical barrier to be removed, which results in the migration of malignant cells. By expressing CXCR4 on their surface, malignant cells migrate to regions of angiogenesis.



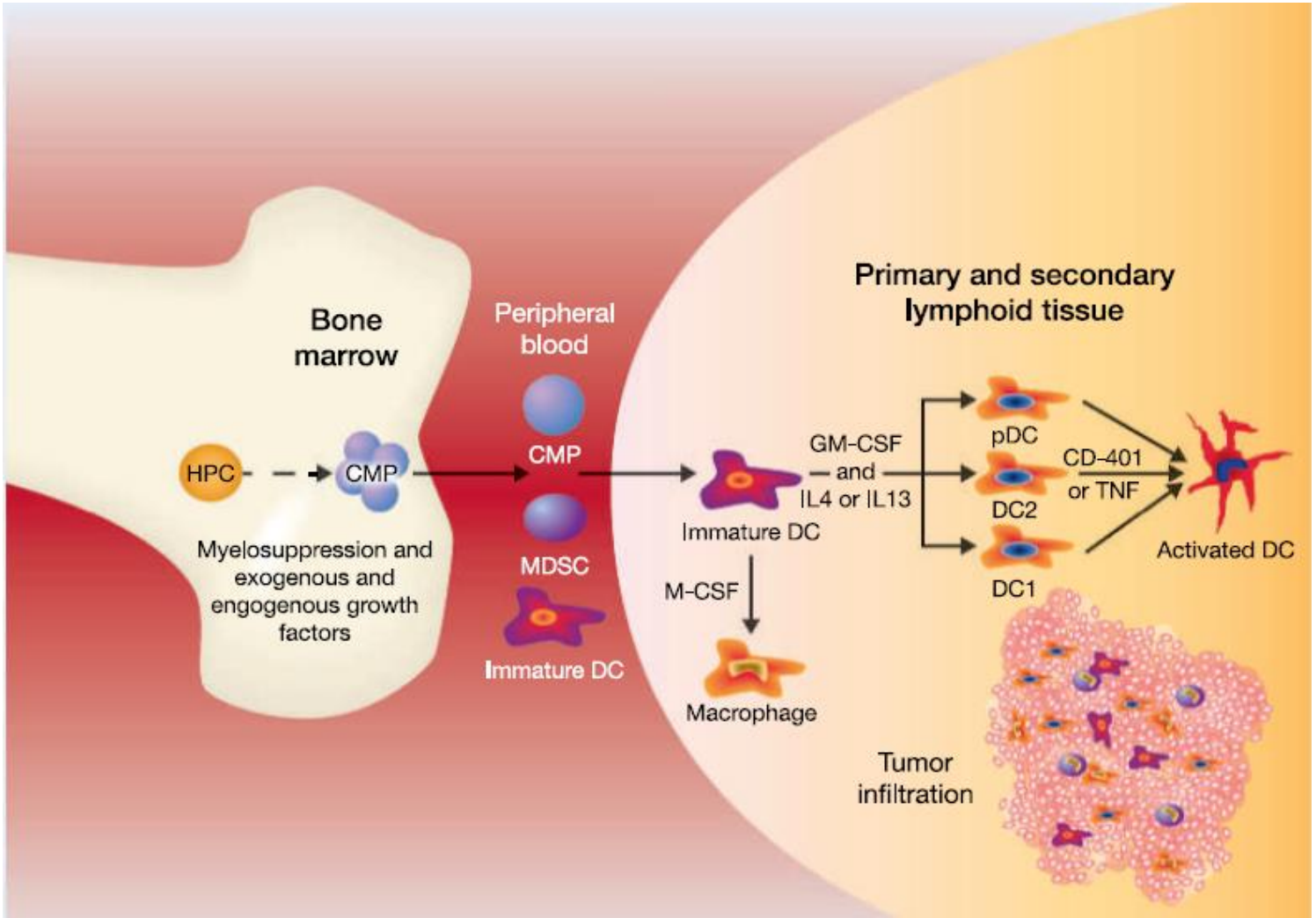
The ability of malignant cells to invade and migrate through the extracellular matrix simultaneously **allows them to enter the circulation as well as leave the blood vessel**.

Inflammation in the tumor tissue

Because of the similarity between the processes that occur during carcinogenesis and the processes during wound healing, cancer resembles a "**wound**" **that never heals...**

... **The inflammatory response** in the tumor tissue plays a significant role in the selective pressure on tumor cells.

The tumor selects an immunosuppressive environment, and at the same time uses the cells of the innate immunity for its own progression.



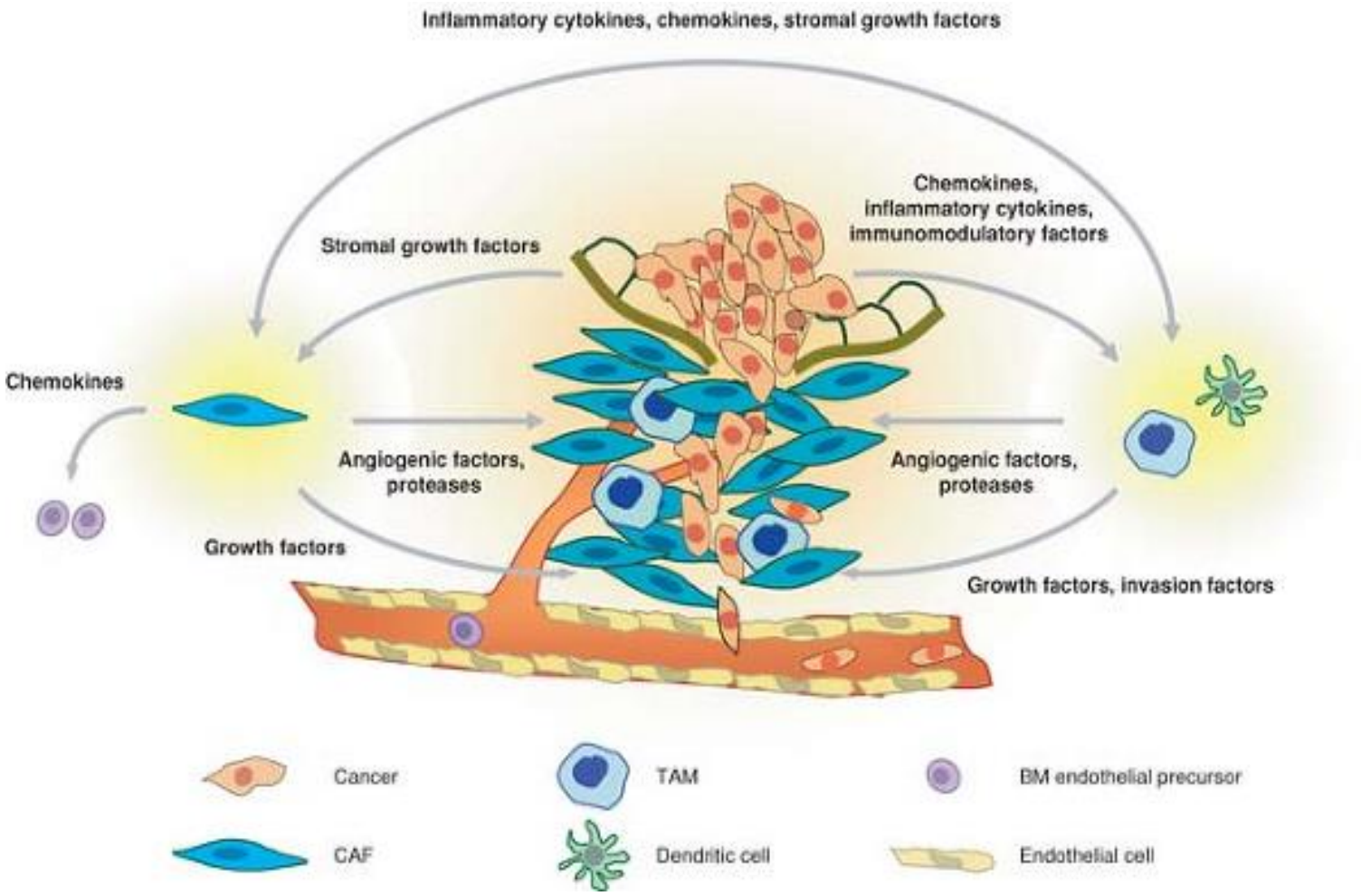
The tumor microenvironment **selects those cells** that stimulate production **immunomodulatory factors**

(**TGF-β, COX2**, macrophage growth factor **CSF-1** (colony-stimulating factor-1), **IL-10** and**IL-6**)

All the mentioned factors **inhibit the maturation of dendritic cells** and **affect the phenotype of tumor-associated macrophages (TAM).**

The tumor **recruits MDSCs**. These cells increase the local production of immunosuppressive **TGF-β**, thus blocking the functions of T lymphocytes and inhibiting the activation of NK cells.

Chronic activation of the tumor microenvironment, primarily cells of the innate immunity, polarizes chronic inflammation, which promotes tumor progression in various ways.

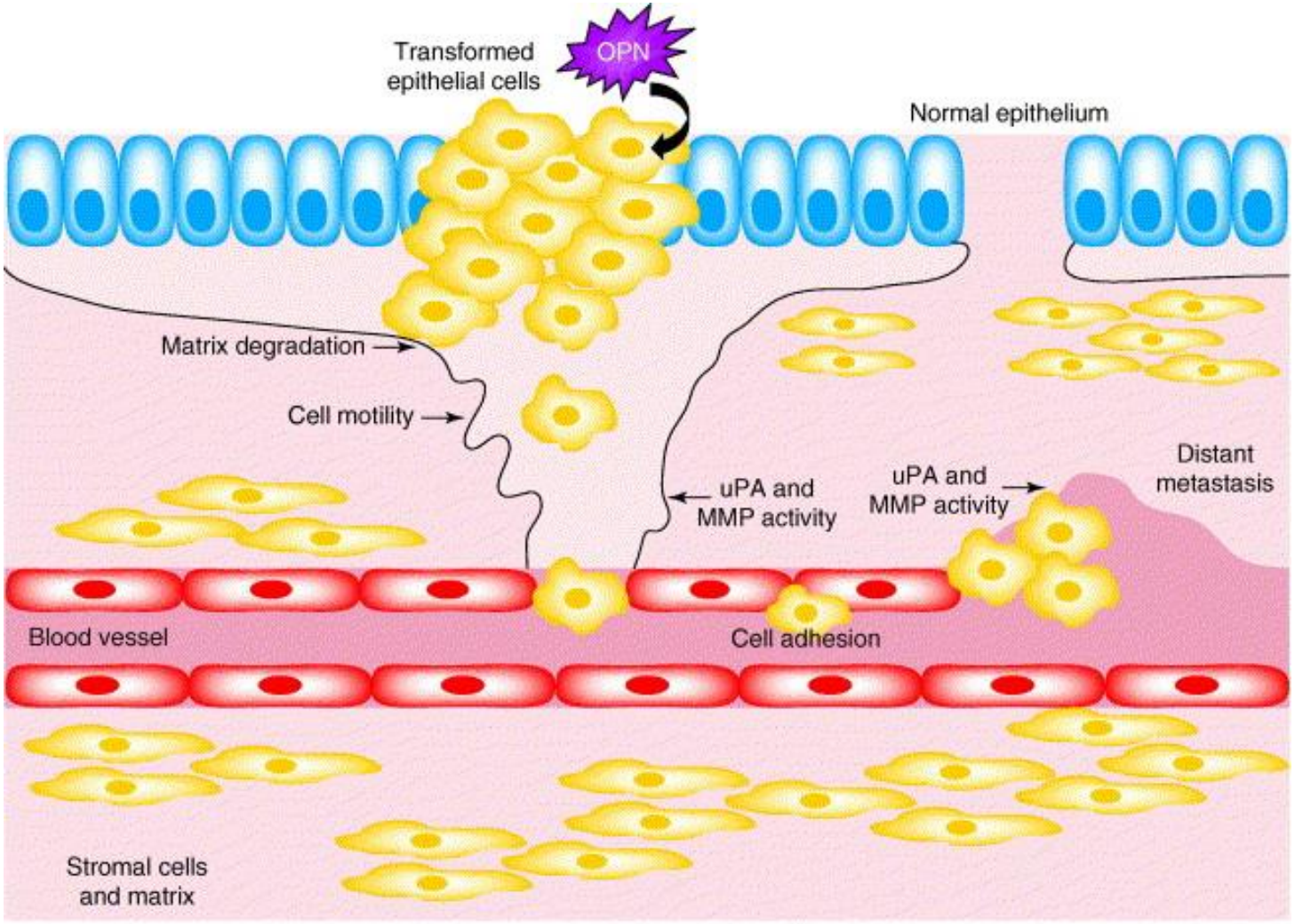


Interactions of tumor cells with the stroma stimulate tumor invasion and metastasis

... Tumor cells **stimulate inflammatory mechanisms** that they then use for their own purposes.

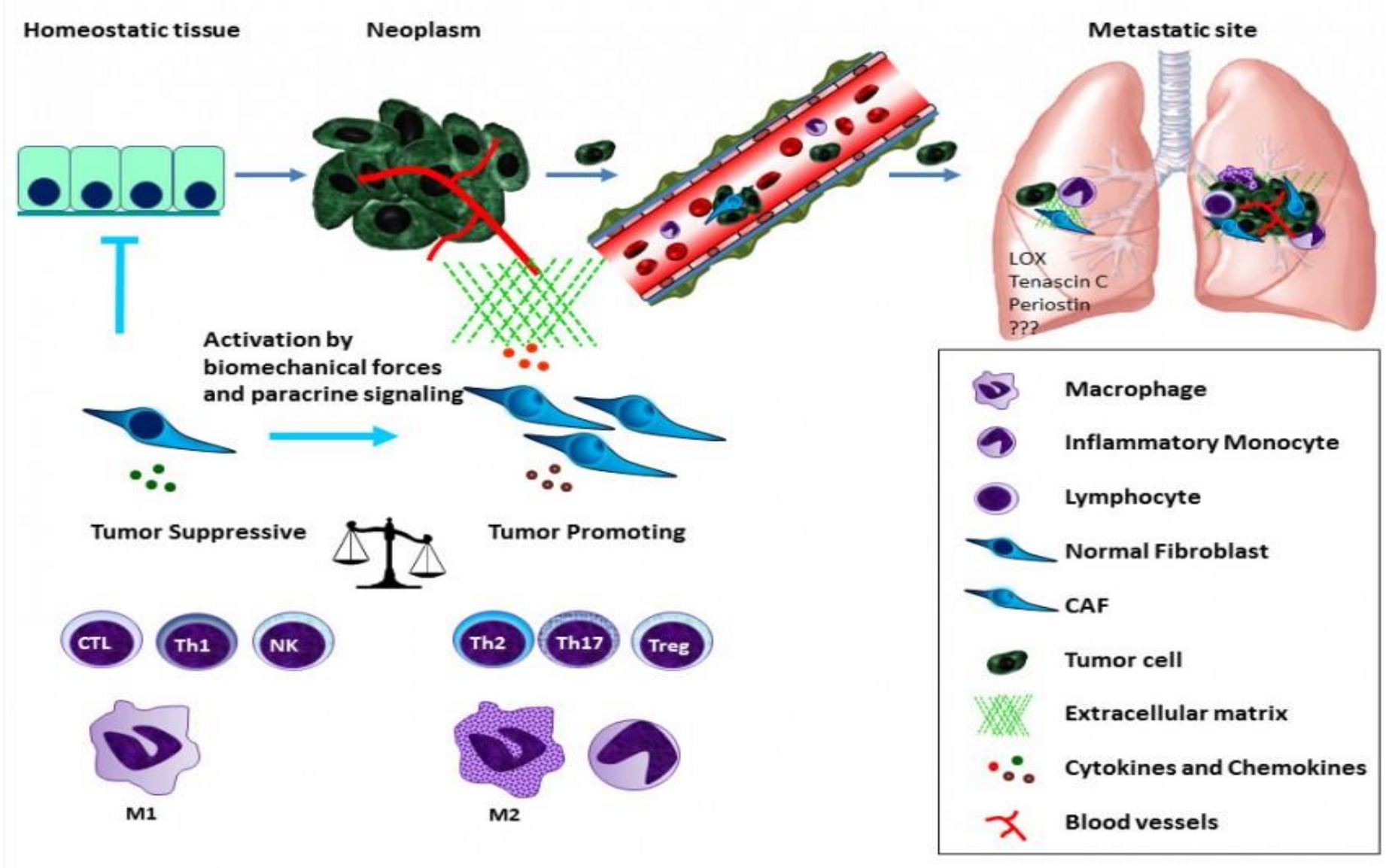
MDSCs and **TAMs** participate in the breakdown of the basement membrane, by producing **uPA and MMPs** that help tumor cells to break down the components of the extracellular matrix, which results in the migration and invasion of malignant cells.

Carcinoma-associated Fibroblasts (CAFs) promote tumor growth by secreting **CXCL12** which stimulates the expression of CXCR4 on tumor cells. CAFs also stimulate angiogenesis by means of CXCL12, which induces the recruitment of precursor endothelial cells. Also, these cells act on TAMs that are recruited to regions of hypoxia where they produce VEGF.



TRENDS in Cell Biology

Tumor cells redirect the immune system as an "enemy" into their "accomplice" in different ways.



Avoiding apoptosis and aging

The most **common internal cellular triggers of apoptosis** are excessive activation or loss of function of some proteins.

External triggers of apoptosis are hypoxia, low pH, reactive oxygen radicals, loss of cellular contact and killing mediated by immune mechanisms...

... Tumor cells ignore the above signals and this resistance to apoptosis is one of the prerequisites for their successful metastasis.

... Ectopic expression of antiapoptotic genes in malignant cells, such as **BCL2** and **BCL-XL**, not only makes these cells resistant to a wide range of insults (hypoxia, low pH and reactive oxygen radicals), but also increases their metastatic capacity.

... **Cellular senescence** represents another important barrier in carcinogenesis.

Pressure on tumor cells to avoid cellular senescence results in either inactivation or mutation of the p53 gene in malignant cells.

The ability to self-renew

While many tumor cells may have limited proliferative potential, some cells acquire the ability to self-renew.

Tumor stem cells are a small subpopulation of cells that have the ability to self-renew.

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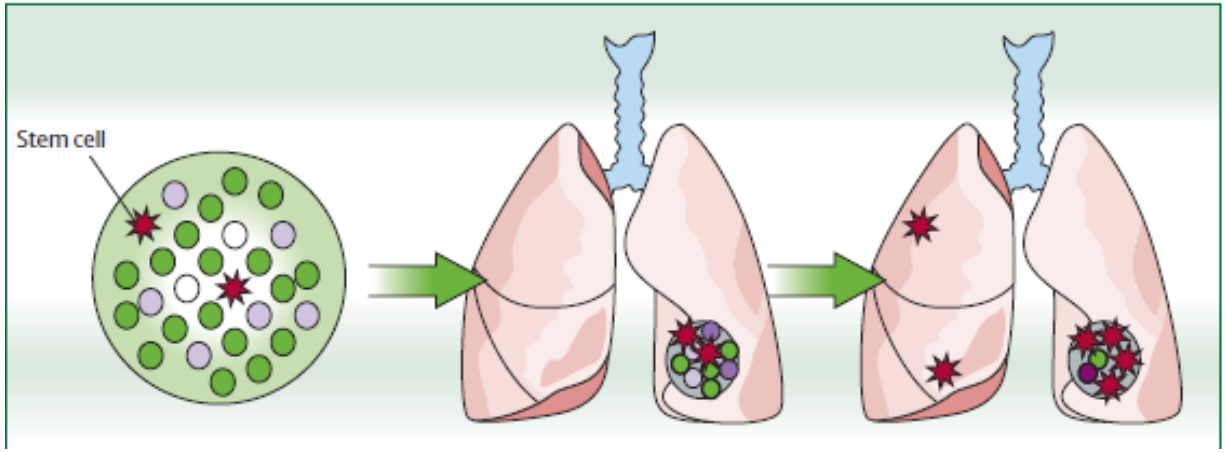
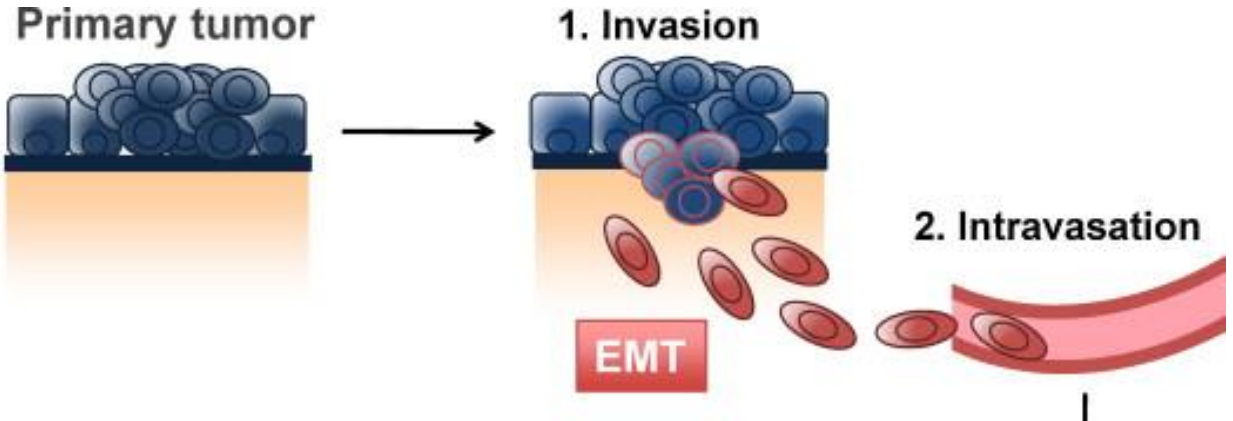


1. Processes important in the initiation of metastasis

Epithelial-to-Mesenchymal Transition(EMT) program is one of the most significant characteristics of those cancers that tend to metastasize.

Hypoxia increases the activity of β-catenin which then promotes the expression of the transcription factor Snail and consequently the initiation of EMT.

TNF-α, which is secreted by TAMs, participates in the stabilization of β-catenin and Snail, and thus this **inflammatory mediator** increases the motility of malignant cells.



Туморске ћелије које користе програм EMT карактеришу ...

.... **increased resistance to apoptosis**
which is probably related to the transcription factor **Snail.**

... **the ability to escape selective pressure**
of apotosis and cellular aging.

... acquisition of characteristics
tumor stem cells.

Tumor invasion begins due to the **loss of cell adhesion**. In most cases, the **decreased expression of E-cadherin** is responsible for the loss of cell adhesion.

Loss of E-cadherin expression disrupts intercellular adhesion and promotes detachment of tumor cells from the epithelium. Tumor cells altered in this way gain the ability to invade and metastasize.

After separating from neighboring cells, malignant cells :

... they induce **degradation** and **remodeling of the extracellular matrix** and thus invade this structure.

... tend to **alter integrin expression**.

During migration, malignant cells they show filapoda (protrusions on the periphery of the cell). Integrins support the movement of malignant cells through the locally degraded extracellular matrix.

2. Processes important in metastatic progression

Among the genes that are selected to support the growth of the primary tumor, some genes are also necessary in the later processes of tumor progression, that is, for the dissemination of malignant cells. These genes provide functions that are specific for metastasis and have been designated as genes responsible for metastatic progression.

The premetastatic niche

Premetastatic niches are often localized **around terminal veins** in distant organs and contain **recruited hematopoietic precursor cells of the myeloid lineage as well as stromal cells**. These niches provide cytokines, growth factors and adhesive molecules that support the arrival of metastatic cells.

Malignant cells, in the primary tumor, secrete:

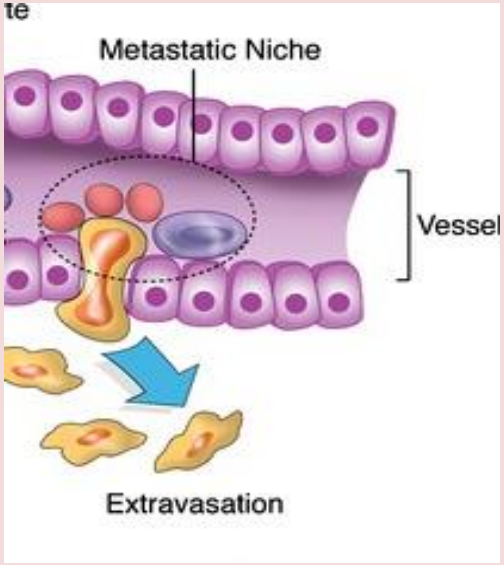
VEGF and PlGF can mobilize **VEGFR1+ myeloid cells** from bone marrow into specific target tissues.

VEGF, TGF-β andTNF-α, can induce synthesis **S100A8 и S100A9** specifically in the lung parenchyma. This results in infiltration myeloid cells in the lungs and consequently formation of a premetastatic niche.

LOX (*Lysyl OXidase*), can also direct formation of a premetastatic niche.

...

Cytokines and growth factors that accompany inflammation and hypoxia in the primary tumor not only stimulate the growth of the primary tumor, but also participate in the creation of the microenvironment in distant organs after the dissemination of malignant cells.

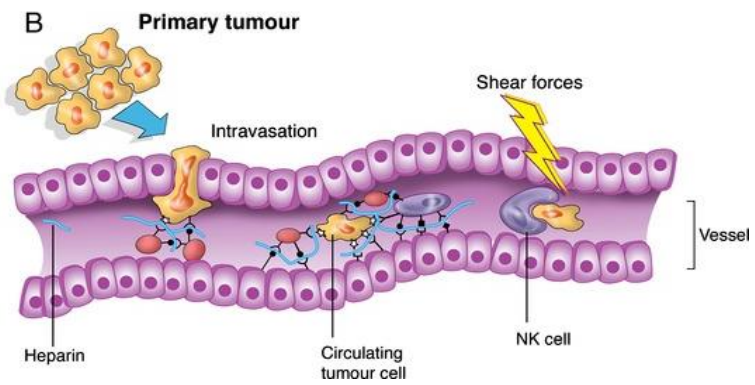


Local environment in distant organs is changed and **it begins to resemble a primary tumor lesion**. Once malignant cells detach from the primary tumor and enter the circulation**, target organs with established premetastatic niches**

they become "better soil" which facilitates anchoring, retention, survival and growth of metastatic cells.

3. Survival of metastatic cells in the circulation

The growth of the primary tumor is the result of **selection** those tumor cells that are **more resistant to apoptosis**, and these cells have an advantage over other tumor cells and proliferate intensively. **Increased expression of antiapoptotic genes BCL2 and BCLXL or decreased expression of proapoptotic genes as well as genes for TNF-family receptors results in increased metastatic capacity of tumor cells**.



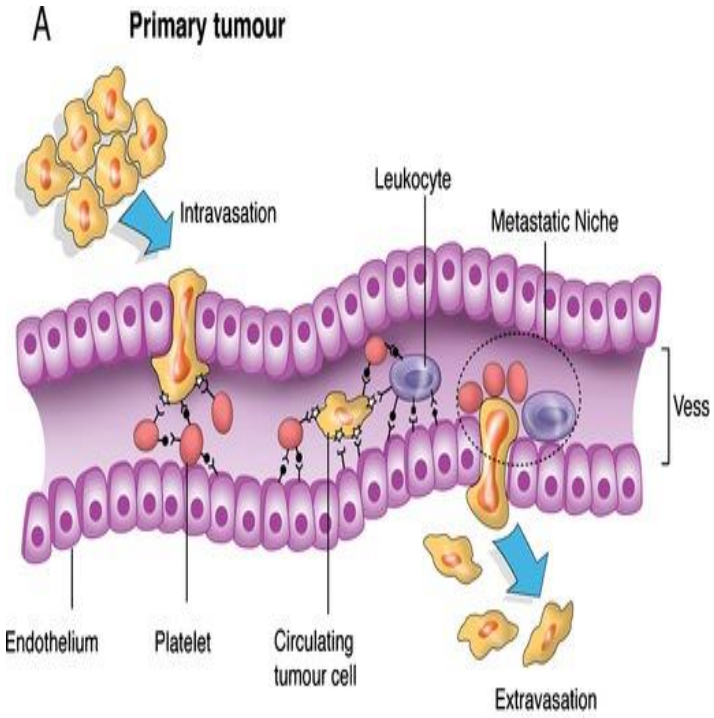
Expression of αVβ3 integrin on circulating tumor cells and platelets promotes their aggregation, which thus forms **tumor emboli**. These tumor emboli not only facilitate the retention of tumor cells, but also protect them from the action of NK cells.

4. Екстравазација и колонизација метастатских ћелија

During extravasation, tumor cells mimic leukocytes because they use the same adhesive molecules as leukocytes.

Tumor cells bind to **E- and P-selectin** on endothelium in target organs. **VEGF** released from tumor cells disrupts intercellular connections between endothelial cells and increases vascular permeability, which together facilitate extravasation of tumor cells.

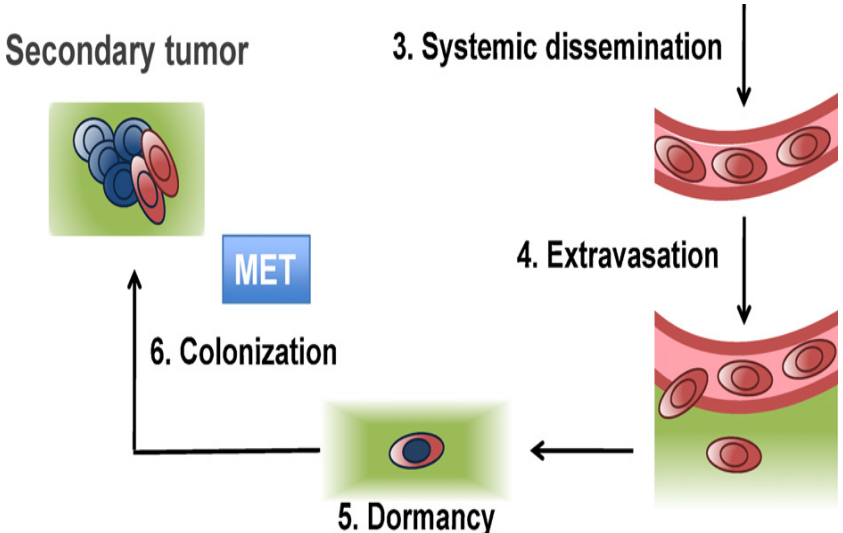
The expression of **CXCR4** on circulating tumor cells enables selective extravasation into certain organs. This selective extravasation is due to the production of **CXCL12 in certain organs**

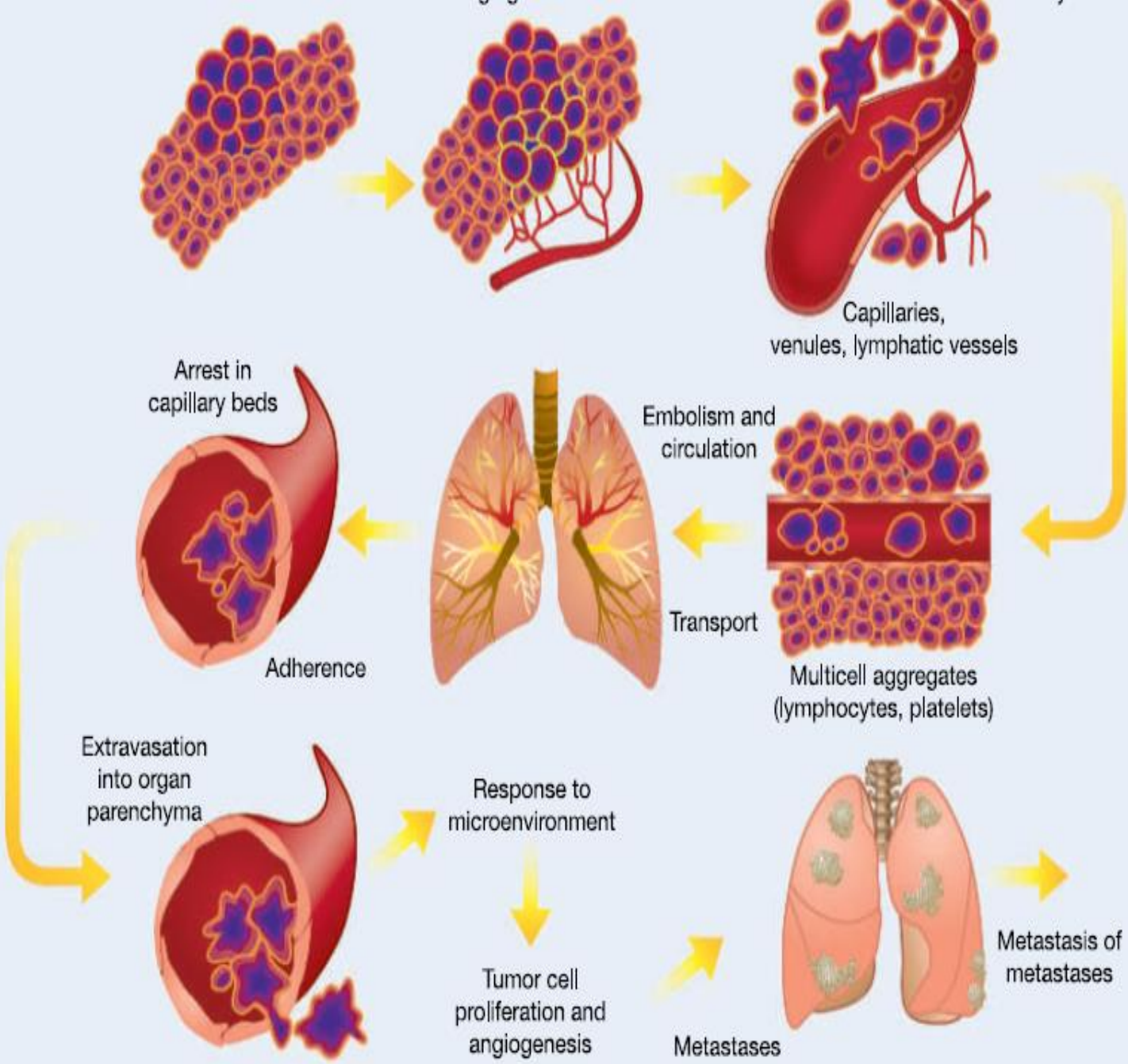


5. Growth of metastatic colonies in distant organs

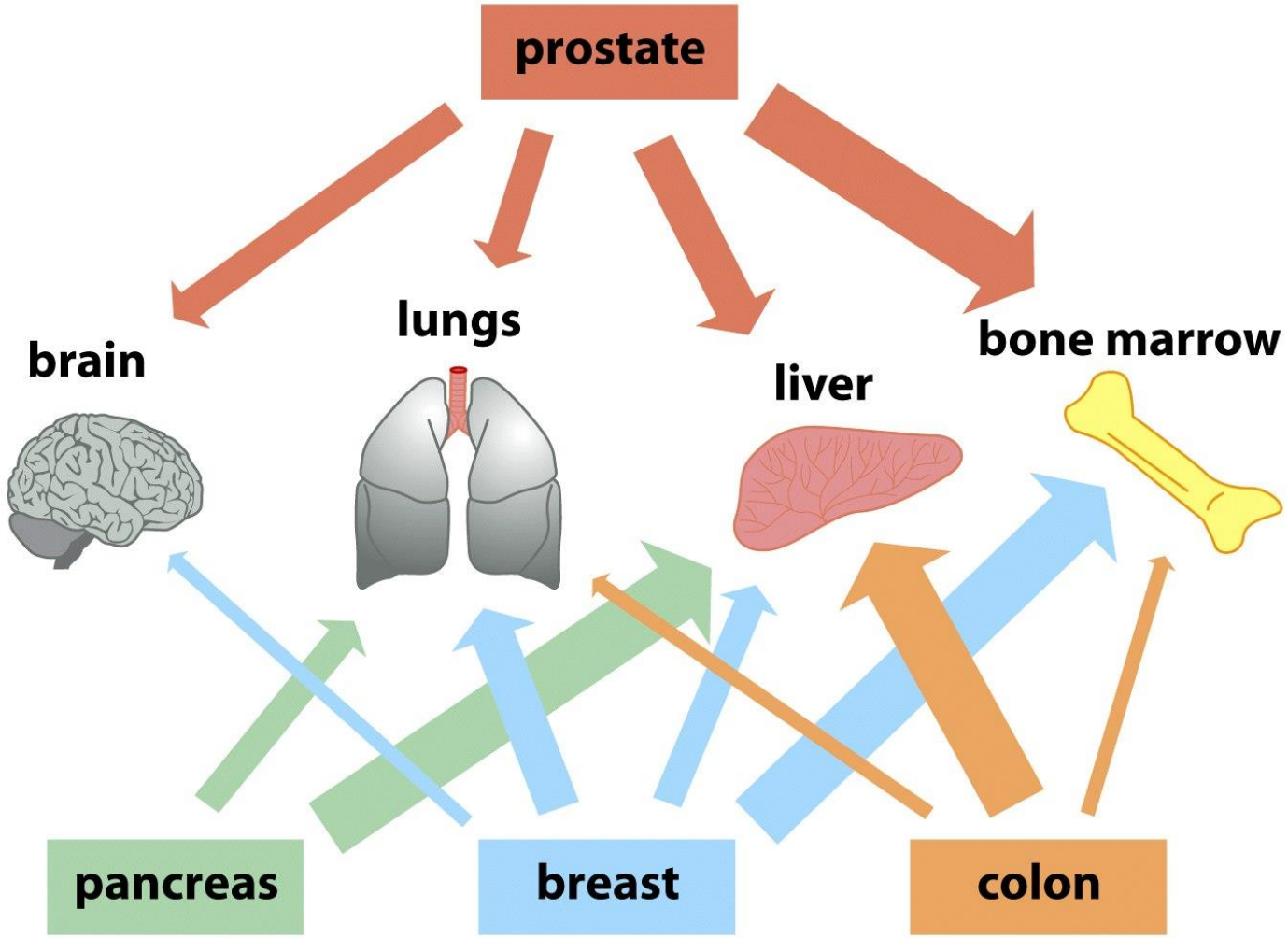
One of the major limiting steps in the metastatic cascade is the **ability** of metastatic cells to **continue to grow in distant organs after extravasation**..

Successful adaptation to the new microenvironment promotes the establishment of metastatic colonies in distant organs and implies the **existence of growth factors** as well as the **preservation of the sensitivity of metastatic cells** to those factors.




Capillaries,
venules, lymphatic vessels
Embolism and
circulation
Transport
Multicell aggregates
(lymphocytes, platelets)
Arrest in
capillary beds
Adherence
Extravasation
into organ
parenchyma
Response to
microenvironment
Tumor cell
proliferation and
angiogenesis
Metastases
Metastasis of
metastases

Metastatic tropism



Bones - site of lung, kidney and breast cancer metastases.

Lungs - the site of metastases of melanoma, breast cancer, colon cancer, bladder cancer... **MMP-9** production in the lungs during the pre-metastatic phase is critical for the invasion of disseminated tumor cells into the lung tissue. Integrins participate in the "guiding" of malignant melanocytes into the lungs.

Colorectal cancer is characterized by the formation of metastases in the liver, where malignant cells reach this place via the **portal circulation**. Tumor cells from melanoma and lung and breast cancer reach the liver through the **systemic circulation**.

Brain - the site of metastasis of lung, breast, kidney, colorectal cancer, melanoma... Malignant melanocytes that metastasize to the brain have increased **STAT3** activity. This transcription factor increases the synthesis of FGF, VEGF and MMP2 responsible for angiogenesis and invasion.

Lymphangiogenesis (the process of creating new lymphatic vessels) is also characteristic of advanced tumors.

VEGF-C and **VEGF-D** are involved in lymphangiogenesis. They bind to **VEGFR-3.**

The expression of VEGF-C and VEGF-D is induced by inflammation, but not by hypoxia.

... Tumor metastasis to **regional lymph nodes** is one of the early signs of metastatic potential and/or spread to distant organs..

Lymph nodes secrete **CXCL12** which interacts with CXCR4 expressed on malignant cells. **CXCR3** plays a significant role in tumor metastasis to lymph nodes.