CANCER STEM CELL BIOLOGY and CANCER VACCINES
(4)

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Cancer Stem Cell Niche
Angiogenesis
and
Metastasis

Cancer Stem Cell Niche

- Cell microenvironment is fundamental for cell growth, fate, and interaction with other cells in response to a specific stimulus.

- Recent studies have confirmed that the microenvironment can support generation and growth of solid tumors, and it is possible that alterations in paracrine signals from niche cells could initiate or enhance tumor formation from SSCs.

- These signals function to induce activation, differentiation, proliferation, and/or cell death.

- Moreover, these environmental stimuli are a part of a greater structure called “stem cell niche”.

Cancer Stem Cell Niche

- Niches are specialized microenvironments located within each tissue. Stem cells reside in the niche.
- The growth factors, cytokines, and small RNAs in the cellular microenvironment are essential for cell nutrition, intercellular communication, signal transduction, and cell fate.
- The mechanism by which the niche regulates CSC self-renewal, differentiation, tumorigenesis and metastasis is of fundamental importance.
- This niche refers to a specific microenvironment inside a discrete anatomic location where SSCs are found in an undifferentiated and selfrenewable state. These niches have been observed in different mammalian epithelial tissues, in the gastro intestinal tract, and in the neural and hematopoietic system where they regulate stem cell fate by directing cell-cell interaction and secretion factors.

- The TME is a complex and dynamic ecosystem made up of a heterogeneous population of cancer cells and resident or infiltrating non-cancer cells [mainly leukocytes, including lymphocytes and tumor-associated macrophages (TAMs), cancer-associated fibroblasts, endothelial cells, and pericytes].

- The niche consists of heterologous cell types that harbor stem cells and influence their fate through direct contact, thereby functioning to balance the quiescence and activation of stem cells.

- This balance is the key to homeostatic regulation of stem cells and ongoing tissue regeneration.

- The niche cells are surrounded by the ECM and a mixture of secreted molecules including lymphokines, cytokines, growth factors, and metabolites. Signaling molecules control stem cell number, proliferation, and fate determination.

- Soluble factors secreted from primary tumors can stimulate the recruitment of cells to the niche. Growth factors such as VEGF, TGF-β, and TNF-α have been identified as the major factors secreted from primary tumors which promote angiogenesis.

- In addition to the molecular and soluble factors, various cellular subsets of paranchyma tissue also influenced by the CSCs remodeling microenvironment.

- miRNA play a key role in the cellular microenvironment:

  -miR-17-92 cluster inhibits breast cancer cell proliferation and suppresses breast tumor cell invasion and migration through altering the cancer cellular niche.

  -miR-17-92 conditioned medium inhibited human breast cancer cell migration and invasion, which was mediated by the reduced abundance of plasminogen activators, cytokeratin 8/18, and IL-8.

Cancer Dormancy

- CSCs were known to be a very small and quiescent subpopulation.
- Indeed, recent evidence shows that CSCs can be relatively abundant (at least in some tumors), able to alternate between dormant and proliferating states, characterized by a high degree of heterogeneity and plasticity over space (i.e., in distinct tumor regions) and time (i.e., at distinct tumor progression stages).
- Moreover, subsets of CSCs were reported to differentiate into heterogeneous lineages of cancer cells including nonstem cells, and vice versa differentiated cells to undergo cell dedifferentiation and even adopt CSC features.
- CSCs reside in niches, preserving CSC survival and metastatic potential and regulating dormancy-reawakening switches.

Cancer Dormancy

- Enter and exit from dormancy is the ability of CSCs in the majority of cancer types, is essential condition for surviving therapy and initiating metastases, which are the two lethal features of CSCs.

- CSCs and dormant cells are two sides of the same coin; between dormant DCCs (dissemineted cancer cells) and CSCs there are a lot of similarities at the molecular level.

- For example, the activation of the p38 mitogen-activated protein kinase 1 (MAPK1) can induce dormancy in differentiated cancer cells as well as in CSCs . Similarly, the induction of the mammalian target of the rapamycin (mTOR) signaling pathway could preserve both the survival of dormant DCCs and the quiescence of CSCs.

Cancer Dormancy

- Not all CSCs are dormant; and not all dormant cells are CSCs. Dormant cancer cells likely comprise both CSC and non-CSC subpopulations.
- Based on their tendency to enter dormancy, cancer (stem) cells can be broadly grouped into:

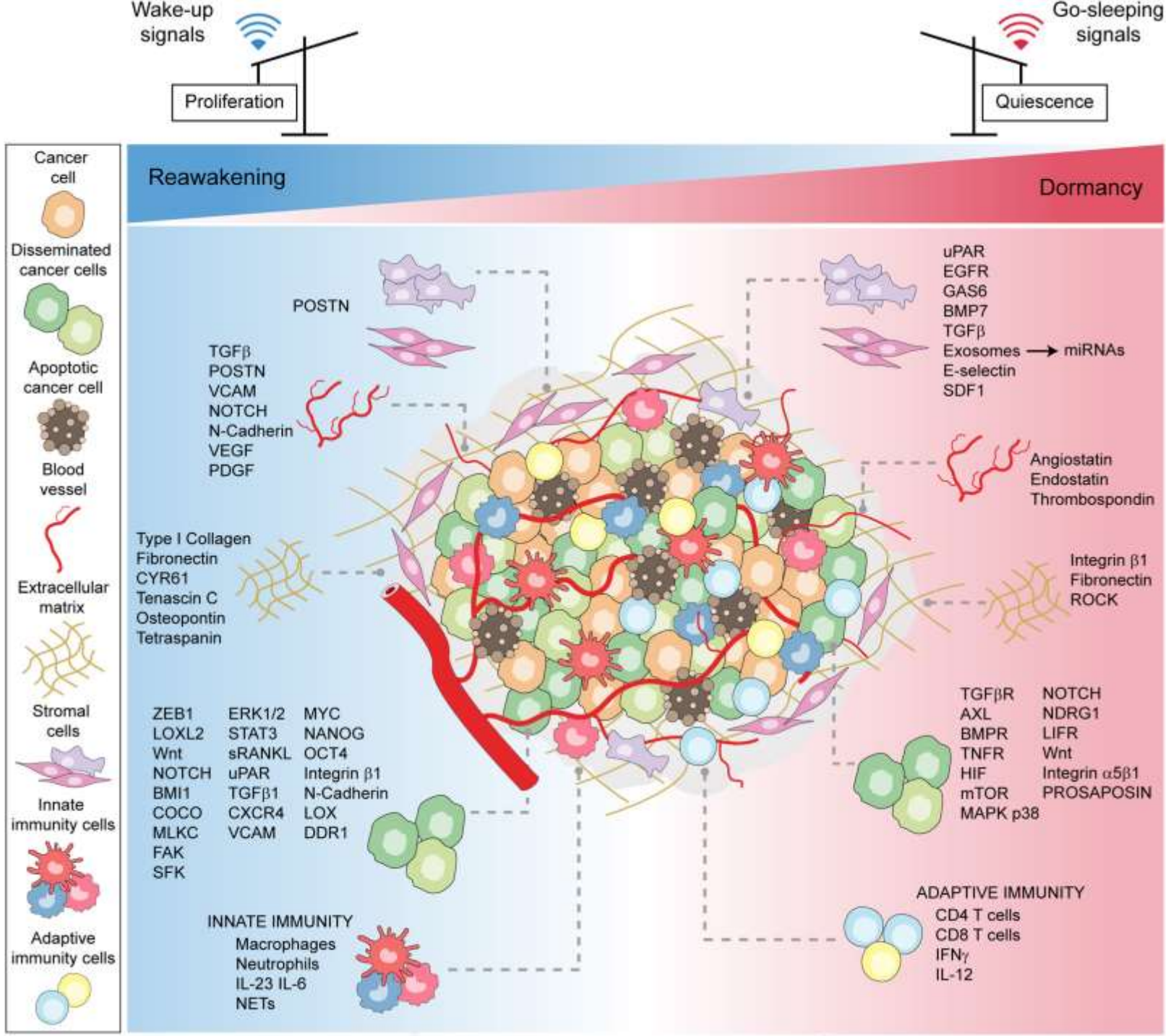
(i) dormancy-competent CSCs,

(ii) dormancy incompetent CSCs,

(iii) cancer repopulating cells, and

(iv) DCCs.

- Dormancy-competent CSCs are endowed with the ability to switch between dormancy and reawaking states, a plasticity that feed their metastatic potential and resistance to therapy.
- Conversely, dormancy-incompetent CSCs are usually enriched in advanced diseases and are characterized by a loss in the ability to enter dormancy, possibly due to the somatic mutations in the mechanisms of dormancy entry.



Sistigu et al., 2020

Metastatic Niches

- Metastasis is a complex process by which primary solid tumor cells invade adjacent and distant tissues and grow into secondary tumors.

- Metatstatic niche is a fertile environment of secondary organs (i.e., BM, lymph nodes, lungs, liver, and brain) that provide favorable conditions for the seeding of DCCs with stem-like and non-stem-like features.

- Indeed, metastatic niches guarantee the nutrient and oxygen supply required for cell proliferation, thus setting the point for cancer (stem) cell proliferation or quiescence.

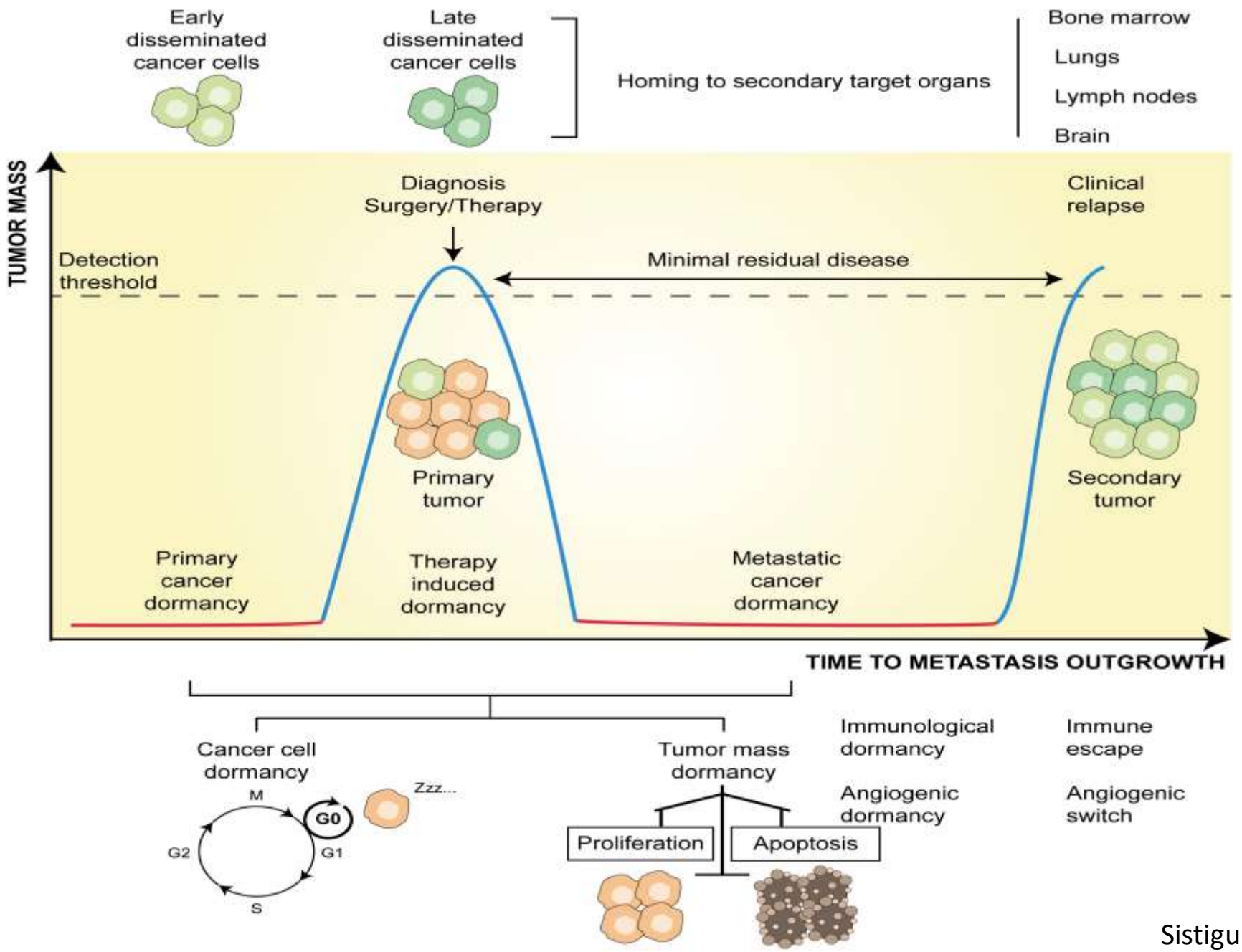
Metastatic Niches

- The molecules and underlying machinery used by normal stem cells for homing or mobilization and CSCs for invasion and metastasis are also realized to be similar.

- During HSC activation and mobilization, matrix metalloproteinase-9 (MMP-9) is required for proteolysis of the ECM components and converting stem cell factor from a membrane-bound form into a free form, which then promotes HSC proliferation and mobilization through a c-Kit receptor.

- The molecules of the MMP family are considered as key players in the process of cancer cell metastasis.

- In addition, cell surface receptors and the ligands required for their activation, such as SDF1 and CXCR4 are also expressed during normal stem cell homing and mobilization as well as cancer cell metastasis.



Sistigu et al., 2020

Metastatic Niches

- Pre-metastatic niche formation may be an initial event of metastasis:

  - Bone marrow-derived hematopoietic progenitor cells home to tumor-specific pre-metastatic sites and form cellular clusters before the arrival of tumor cells.

- The BM frequently hosts DCCs derived from different primary organs, including breast, colon, prostate, head, and neck, although these DCCs rarely develop bone metastases .

- This observation suggests that BM metastatic niches could delay or even prevent tumor mass sprouting by inducing a state of dormancy, a situation observed in expanded hematopoietic stem cells (HSCs) undergoing differentiation.

- In line with this hypothesis, metastatic niches reportedly provide unique signals promoting quiescence and long-term survival.

Metastatic Niches

- **Notch2**, which is known to induce cancer cell proliferation in primary breast carcinomas, was recently shown to have an opposite effect in metastatic BM niches, favoring the quiescence and long-term survival of disseminated breast CSCs.

- The **Wnt** pathway, which in its canonical form acts as a regulator of processes like cell proliferation and cell stemness, is also inversely associated with cancer cell dormancy, was reported to induce dormancy of prostate cancer cells populating the BM niches, via a mechanism involving the non-canonical receptor tyrosine kinase-like orphan receptor 2 (ROR2)/Siah E3 Ubiquitin Protein Ligase 2 (SIAH2) signal, resulting in the inhibition of the canonical Wnt/b-catenin pathway.

- **TGF-b, bone morphogenetic proteins (BMPs)**, and **LIFR**. Firstly described as a potent inhibitor of HSC proliferation, TGF-b is now recognized as another major factor that, once released by osteoblasts (one main BM stromal cell type), keeps DCCs and CSCs in a state of protracted dormancy. Similarly, the production of BMPs by BM stromal cells was associated with DCC hibernation. Finally, in breast cancer patients, low LIFR levels were shown to correlate with poor prognosis and with the appearance of overtmetastasis along with the loss of CSC-associated genes.

Metastatic Niches

- Beyond reacting to soluble factors, DCCs also engage with other cell types of the metastatic niche, as well as with the ECM.
- Experimental studies show that breast cancer cells prime mesenchymal stem cells (MSCs) residing in BM niches to transfer microRNAs (miRNAs) via exosomes, which in turn promote cancer cell quiescence and drug resistance.
- Using a 3D co-culture model, it is demonstrated that DCCs from breast tumors cannibalize surrounding MSCs, resulting in an increased survival and tumor mass dormancy. Osteoblasts and osteoclasts, which are BM stromal cells with opposite physiological functions, also play opposite roles in the regulation of DCC dormancy.

Inflammatory Niche

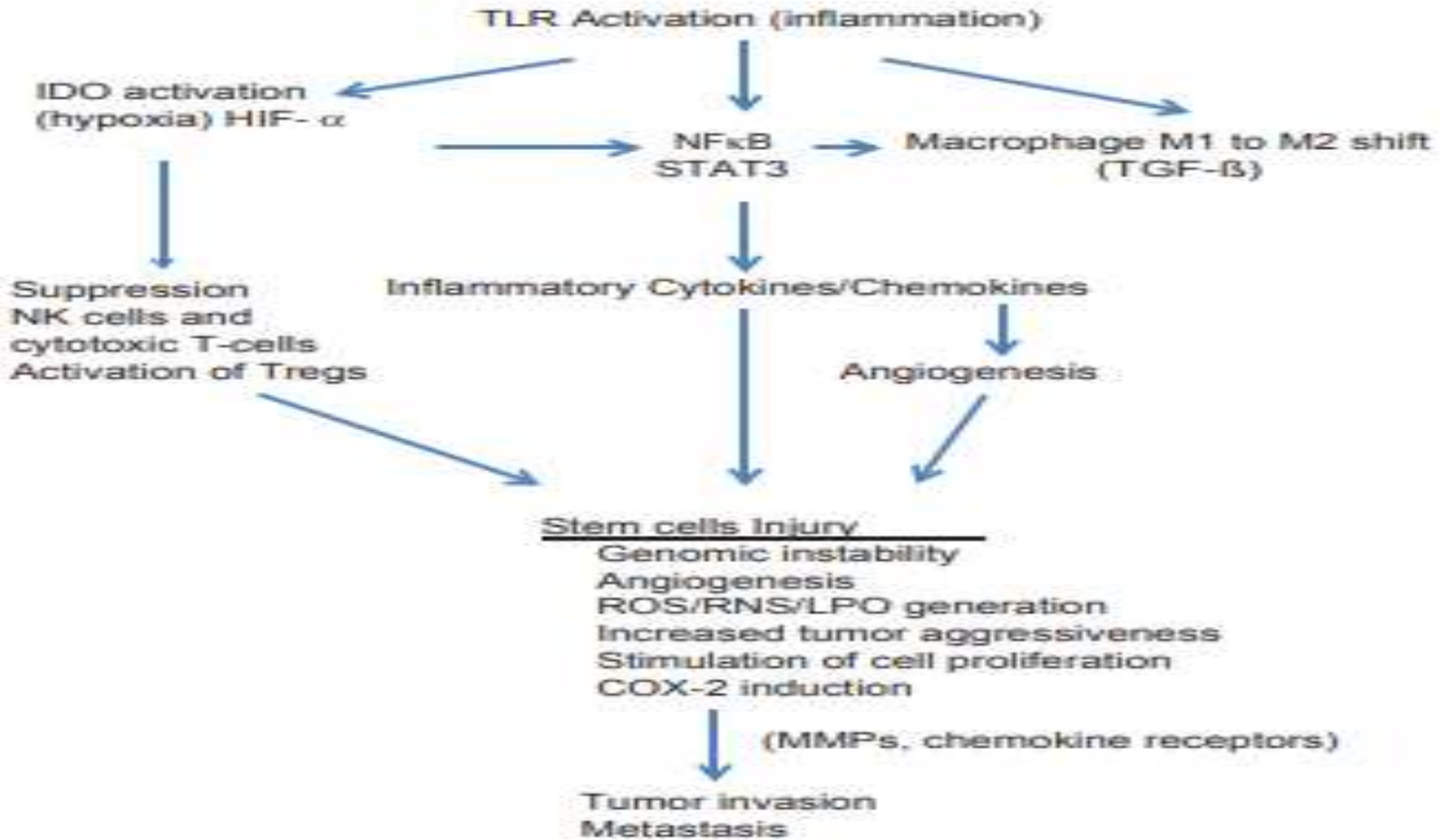
- Chronic inflammation is another important environmental factor driving tumor progression.
- This condition may be one of the principal factors in CSCs expansion and tumor dissemination.
- This inflammatory response can be initiated by the activation of toll-like receptors (TLRs), stimulated by pathogen-associated-molecular patterns (PAMPs) of carcinogenic microbes or by products released from cancer cells.
- Consequently, nuclear factor kappa B (NF-κB) is activated inducing an inflammatory response that could increase self-renewal activity in cancer cells.

- Prolonged inflammation, infection, exposure to toxins, or autoimmune diseases may cause changes in the environmental milieu and can lead to reprogramming of SSCs turning them into cancer stem cells after.
- Tumor environment may cause transformation of the SSCs by secreting TGF-β.
- This cytokine will enhance the transition from SSCs to CSCs by inducing zinc finger E-box-binding homeobox1 (ZEB1) transcription factor expression.
- ZEB1 contributes to cancer dissemination and the activation of epithelial-mesenchymal transition (EMT), a process that has been linked to cancer metastasis. Additional evidence suggests that ZEB1 is responsible for the maintenance of CSC-like phenotypes.

- Malignant tumors often develop at sites of chronic inflammation and tissue injury; this may support the role of inflammation in cancer progression.
- Helicobacter pylori causing chronic viral hepatitis, general gastric inflammation, gastritis, inflammatory bowel disease, and several other chronic inflammation conditions are also shown to increase the risk of cancer development, and the induction of CSCs.
- Tumor stroma also contains activated fibroblasts, inflammatory cells, and nascent blood capillaries. The formation of such microenvironments facilitates induction of an inflammatory response that causes cell migration and epithelial cell proliferation.
- This results in tissue repair that can occasionally turn into uncontrolled cell proliferation and dissemination

Hypoxia and Metastasis

- A critically important aspect of tumor microenvironment is hypoxia. It has been shown that hypoxia can predict the likelihood of tumor aggressiveness, invasion, metastasis, tumor recurrence, resistance to chemotherapy and radiotherapy, and patient survival.
- Hypoxia, by increasing the release of HIF-1α in the microenvironment, induces the expression of the chemokine receptor CXCR4 on the membrane surface of stem cells, which is responsible for migration and metastasis of cancer stem cells.
- One way hypoxia increases stem cell aggressiveness is by activating NADPH oxidase within tumor cells, which has been demonstrated in glioblastoma tumor cells.
- This leads to the production of high levels of ROS – principally the superoxide radical, which rapidly reacts with NO to form the powerful radical peroxynitrite. This radical powerfully inhibits mitochondrial function leading to the production of a whole array of ROS.



Blaylock, 2015

Chemokines

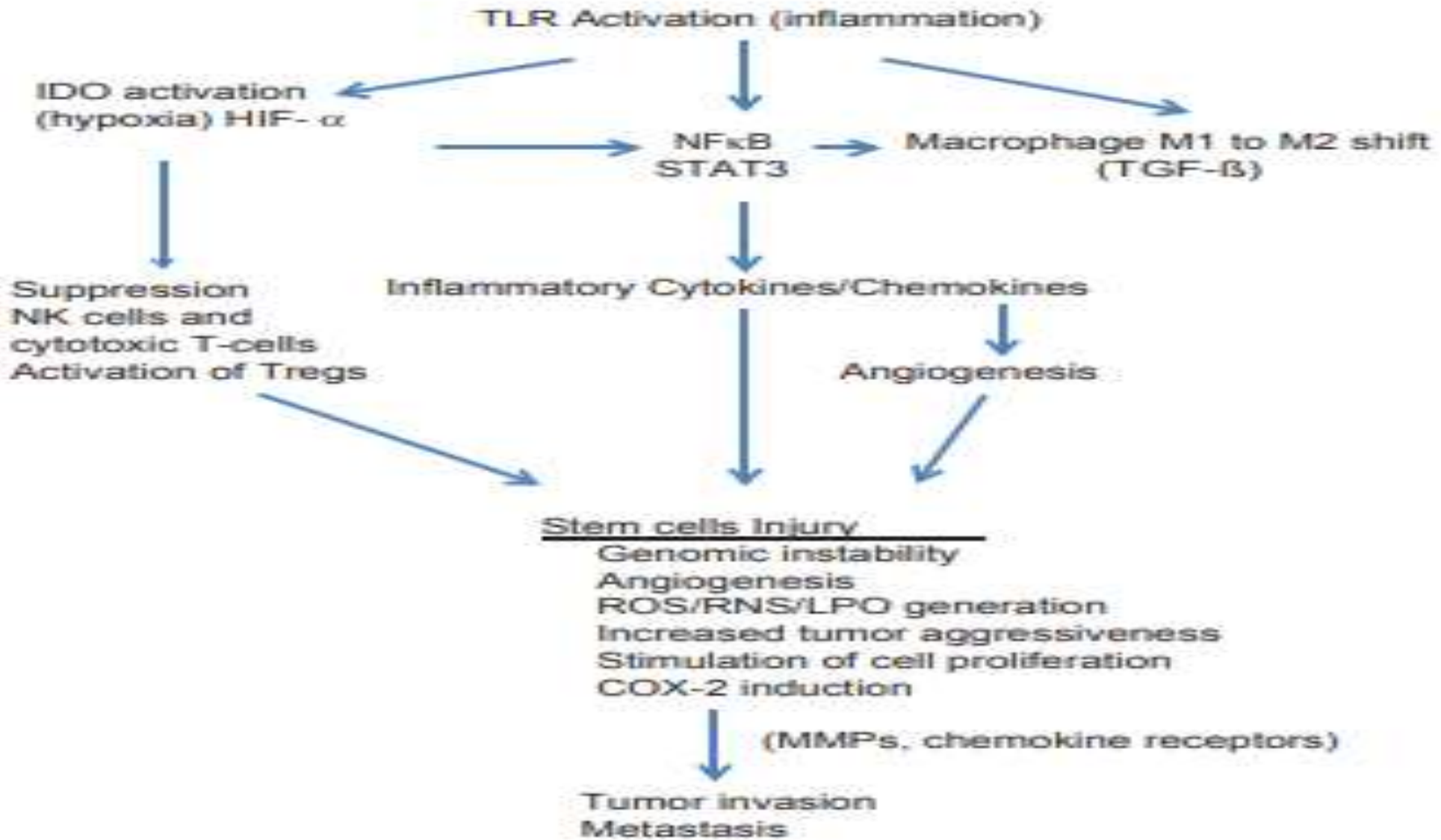
- There is a strong link between inflammation, invasion and metastasis of cancers. In some animal studies, inflammation was necessary for a cancer to metastasize.
- A strong relationship also exists between the presence of chemokine receptors and metastasis.
- These receptors, in conjunction with their ligands, not only attract increasing numbers of immune cells to the microenvironment, but also when appearing on the cancer cells themselves stimulate mobility of these malignant cells.
- This has been shown to drive metastasis to distant sites and that the specific sites of metastasis are also chosen based on the presence of these chemokine receptors. One of the better-studied chemokine receptors includes CXCR4 and its ligand CXCL12, which is frequently expressed by malignant cells.
- Other chemokine receptors expressed by malignant cells include CX3CR1, CCR1, CCR7, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR5, and CXCR7.
- Malignant melanomas express a number of chemokine receptors and may explain its high propensity to metastasize to a number of sites.

Macrophages

- In a breast cancer model, it is found that macrophage-deficient mice developed the tumor normally, but it would not metastasize to the lung. TAMs appear to be major players in controlling tumor biology, including angiogenesis, invasion, and metastasis. TAMs are attracted to the tumor, beginning at the earliest stages of carcinogenesis, by chemokines.
- Switching from the antitumor M1 phenotype macrophage to the M2 protumor mode is accomplished by activation of NFκB and this promotes proliferations, invasion, and metastasis of the tumor.
- TAMs promote angiogenesis and lymphangiogenesis as well as promoting immune escape and therefore increase the likelihood of metastasis.
- A recent study found the presence of M2 macrophages in the tumor stroma, but not tumor nest, was a strong marker for tumor size, invasion risk, and as an independent prognostic factor for reducing breast cancer survival.

ECM

- The ECM, commonly defined as the non-cellular component of a tissue, is a highly dynamic and physiologically active structure, that provides biochemical and biophysical support for surrounding cellular components.
- ECM also represents a biological barrier, an anchorage site, and a movement track, playing major roles in regulating cellular interactions and communications.
- The ECM is tightly organized during embryogenesis and tissue homeostasis, but becomes extremely deregulated and deranged in cancer.
- Emerging evidence suggests that the ECM may serve as a niche for DCCs and CSCs, influencing cell survival and proliferation, and thus dormancy.



Blaylock, 2015

- Another function of the tumor niche is the active recruitment of new endothelial and stromal cells into tumors that is essential for developing a pro-angiogenic environment that enhances tumor survival under adverse conditions.

Angiogenic/Perivascular Niches

- A tumor promoting milieu made up of a multitude of microvessels, regulates dormancy of cancer cells disseminated into BM, lungs, and brain from different primary tumors.

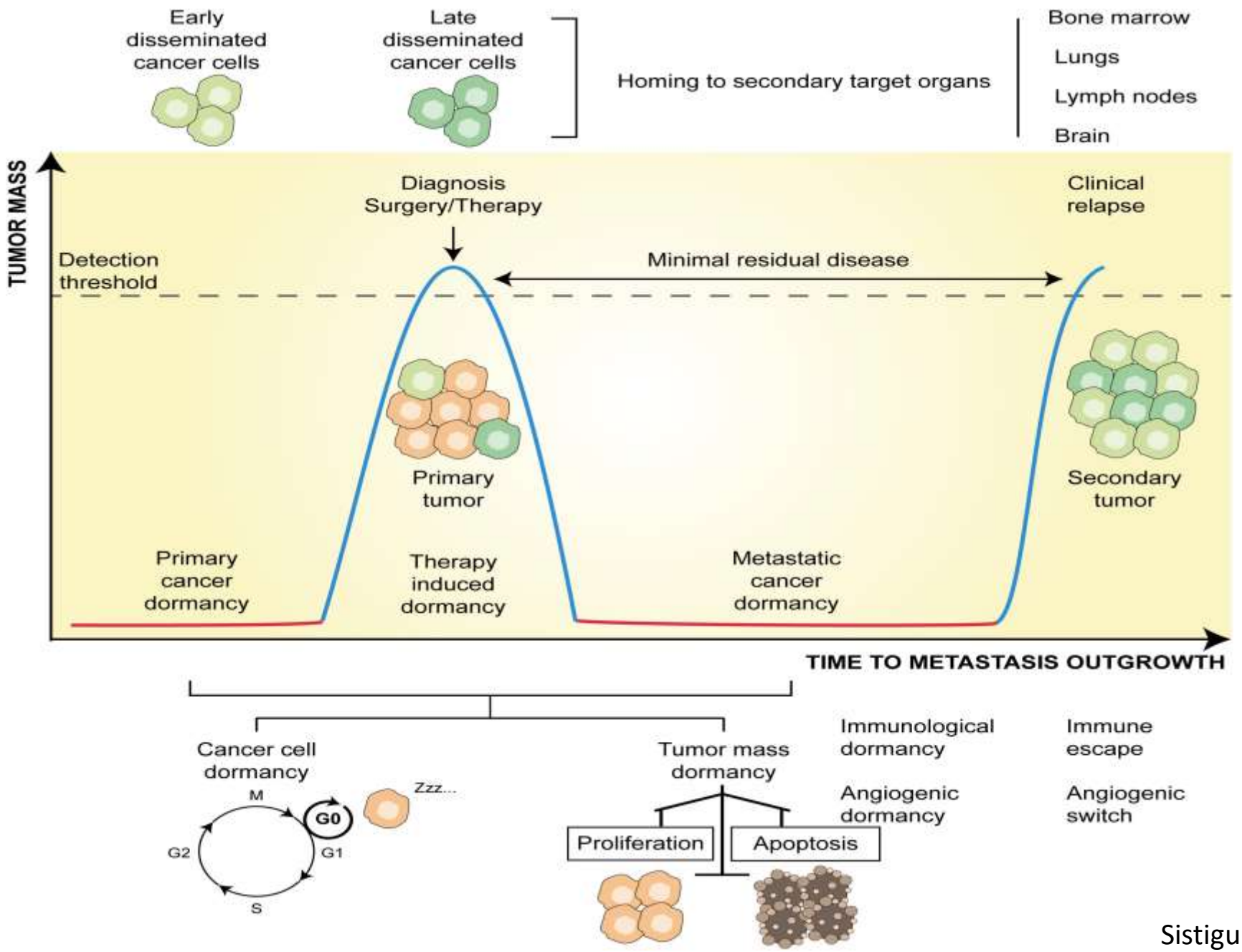
- Perivascular niches are characterized by the high availability of oxygen, nutrients, and paracrine factors, which renders them a permissive environment for the proliferation of DCCs and CSCs.

- Accordingly, distinct types of CSCs and DCCs localize in the perivascular niches, growing in the proximity of capillaries.

- It recently emerged that bidirectional interactions between these cells and components of the perivascular niche, including endothelial cells, are relevant for tumor evolution.

Angiogenic/Perivascular Niches

- A hallmark of progressive cancer growth, in both primary and secondary tumors, is the induction of tumor vasculature, a process termed the **“angiogenic switch”**.
- Indeed, like healthy tissues, tumors need both an appropriate supply of oxygen/nutrients and a way to remove waste products.
- However, unlike physiological angiogenesis, in which new vessel sprouting is a highly regulated and self-limited process, tumor angiogenesis lacks growth controls resulting in continuous and deregulated vessel production. This leads to a structurally and functionally abnormal tumor vascular network characterized by new vessels with dead ends, which results in low oxygen tension (hypoxia), the paucity of metabolites, and imbalanced expression of angiogenic factors.
- This latter eventually stimulates further abnormal angiogenesis. As neovascular supply is crucial for tumor growth, cancer cells, including those integrated into the vessel walls, undergo **«angiogenic dormancy»**. During angiogenic dormancy, cancer cell proliferation rate is balanced by enhanced apoptosis induction. This equilibrium maintains tumors that are microscopic and undetectable, for extended times.



Sistigu et al., 2020

Angiogenic/Perivascular Niches

- The balance between the angiogenic switch and angiogenic dormancy is a finely-tuned process regulated by integrated microenvironmental factors, including the pro-angiogenic vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), anti-angiogenic thrombospondin-1, angiostatin, and endostatin.
- Prosaposin has been described as another regulator of metastatic growth arrest. Once produced by cancer cells, prosaposin acts in a paracrine and endocrine fashion inducing the expression of thrombospondin-1 in stromal cells at primary and distant tumor sites, which blocks neoangiogenesis and delays tumor growth.
- Many niche components also play a role in regulating angiogenesis. Indeed, CSCs, seem able to transdifferentiate and directly contribute to the formation of abnormal vessels, thus supplying for the absence of true angiogenesis.
- Moreover, CSCs often promote a considerable enhancement of VEGF levels, both by a direct production and by stimulating a pro-angiogenic activity in stromal cells localized in the proximity of the niche.

Angiogenic/Perivascular Niches

- Notch also act as angiogenesis promoters, while anti-angiogenic factors (i.e., thrombospondin-1) are associated with inactivation of the stem-related transcriptional factors (i.e., MYC), which in turn promote dormancy.

- In a seminal work, a transcriptional rewiring of cancer cells undergoing an angiogenic switch is characterized. This switch was associated with downregulation of the angiogenesis inhibitor thrombospondin and upregulation of genes not hitherto linked to tumor dormancy;

  - such as endothelial cell specific molecule 1 (ESM1), 5'-ectonucleotidase, tissue inhibitör of metalloproteinase 3 (TIMP3), epidermal growth factor receptor (EGFR), insulin-like growth factor receptor (IGF1R), phosphatidylinositol 3-kinase (PI3K) signaling, Eph receptor A5 (EphA5), and histone H2BK.

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