Neoplasia
Lecture 7

Development of Sustained Angiogenesis

- **Tumors cannot enlarge beyond 1-2 mm in diameter unless they are vascularized.**
- **Cancer cells can stimulate neo-angiogenesis during which new vessels sprout from previously existing capillaries or by vasculogenesis in which endothelial cells are recruited from the bone marrow.**

- **Growing cancers stimulate neoangiogenesis during which vessels sprout from previously existing capillaries.**
- **Neovascularization has a dual effect on tumor growth:**

1. **Perfusion supplies needed nutrients and oxygen**
2. **Newly formed endothelial cells stimulate the growth of adjacent tumor cells by secreting growth factors, such as insulin-like growth factors (IGFs) and PDGF.**

- **Tumor vasculature is abnormal leaky dilated, and have a haphazard pattern of connection.**

- **Angiogenesis is required not only for continued tumor growth but also for access to the vasculature and hence for metastasis.**
- **Angiogenesis *is thus a necessary biologic correlate of malignancy.***

- **Angiogenesis is controlled by a balance between angiogenesis promoters and inhibitors**
- **In angiogenic tumors this balance is in favor of promoters.**

- **The angiogenic switch involves increased production of angiogenic factors and/or loss of angiogenesis inhibitors.**
- **Angiogenic factors (promotors) may be produced:**

1- Directly by the tumor cells themselves

2- By inflammatory cells (e.g., macrophages)

3- By stromal cells associated with the tumors

- **The angiogenic inhibitors are Proteases that either elaborated by the tumor cells or by stromal cells in response to the tumor angiogenic and anti-angiogenic factors.**
- **The angiogenesis inhibitors as angiostatin and endostatin are produced by proteolytic cleavage of plasminogen and collagen respectively.**

- **The angiogenic switch is controlled by several physiologic stimuli, such as hypoxia.**
- **Relative lack of oxygen → activation of hypoxia-induced factor-1α (HIF1α), an oxygen-sensitive transcription factor → stimulates production of pro-angiogenic cytokines as VEGF.**

- **HIF1α is continuously produced but in normal conditions the von Hippel-Lindau protein (VHL) binds to HIF1α, leading to ubiquitination and destruction of HIF1α.**

- **In hypoxic conditions, such as a tumor that has reached a critical size→ the lack of oxygen → prevents HIF1α recognition by VHL protein →no destruction of HIF1α → HIF1α translocates to the nucleus and activates transcription of its target genes such as VEGF.**

- **VHL acts as a tumor suppressor gene, and germ-line mutations of the *VHL* gene are associated with hereditary *VHL syndrome :***

1- Renal cell cancers

2- Pheochromocytomas

3- Hemangiomas of the CNS

4- Retinal angiomas

5- Renal cysts

- **Mutations involving tumor suppressors and oncogenes in cancers also tilt the balance in favor of angiogenesis.**
- **For example**

- **p53 normally stimulates expression of antiangiogenic molecules, such as thrombospondin-1, and represses expression of proangiogenic molecules such as VEGF.**

  Common loss of p53 in tumor cells provides a permissive environment for angiogenesis.

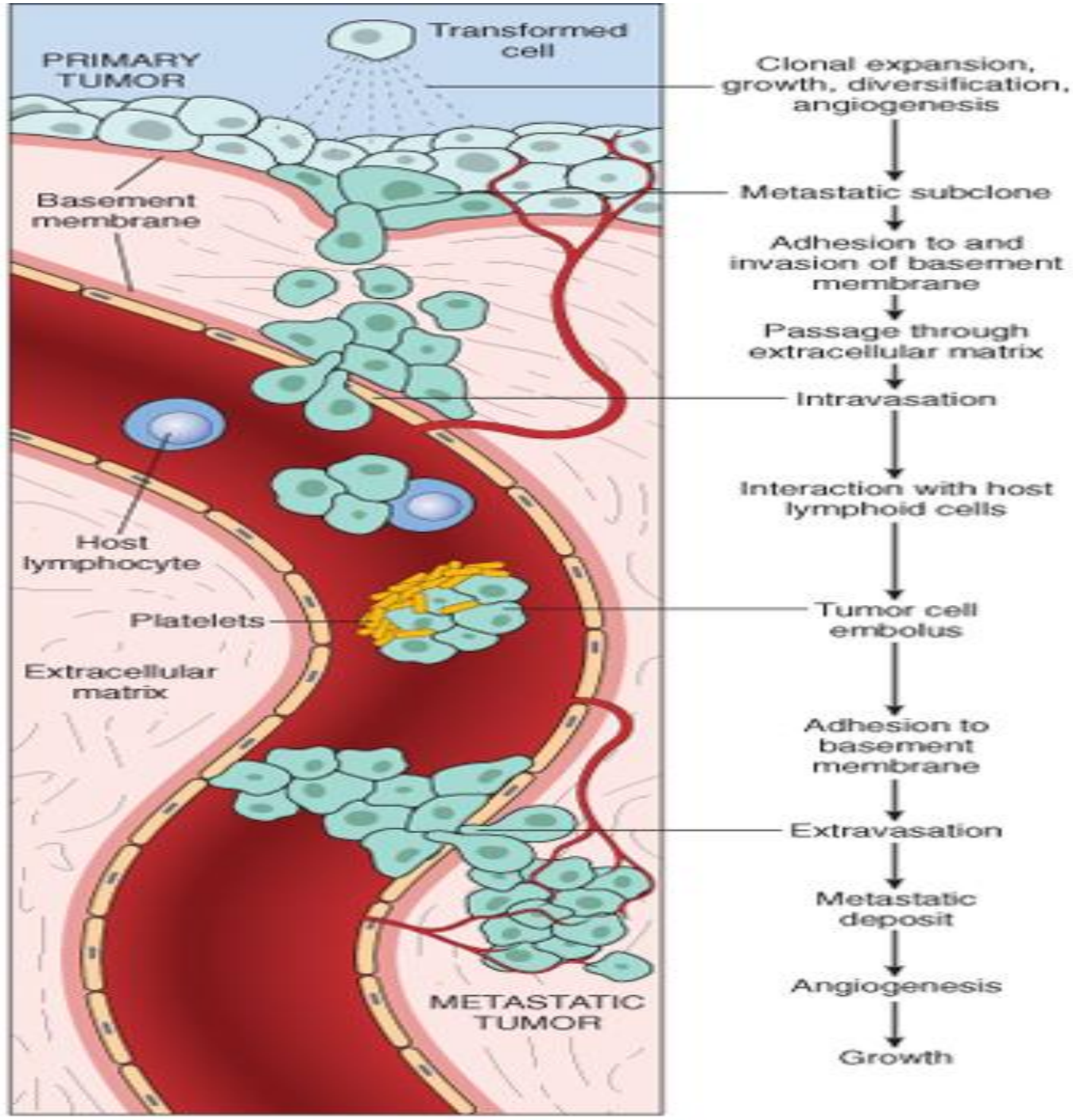
- **The transcription of VEGF is also influenced by signals from the RAS-MAP kinase pathway, and mutations of *RAS* or *M*YC up-regulate the production of VEGF.**
- **anti-VEGF antibody is now approved for the treatment of several types of cancers.**

Invasion and Metastasis

- **Invasion and metastasis are the major causes of cancer related morbidity and mortality result from complex interactions involving cancer cells, stromal cells, and the extracellular matrix (ECM).**

Steps of metastasis

1. **Local invasion**
2. **Intravasation into blood and lymph vessels**
3. **Transit through the vasculature**
4. **Extravasation from the vessels**
5. **Formation of micrometastases**
6. **Growth of micrometastases into macroscopic tumors**



The metastatic cascade can be subdivided into two phases:

(1)Invasion of ECM

(2)Vascular dissemination and homing of tumor cells

Invasion of Extracellular Matrix (ECM)

- **Human tissues are organized into a series of compartments separated from each other by two types of ECM:**
- **1-Basement membranes .**
- **2-Interstitial connective tissue.**

- **ECM is composed of :**

1-Collagens

2-Glycoproteins

3-Proteoglycans

- **Invasion of the ECM is an active process that requires four steps :**
- **1-Detachment of tumor cells from each other.**
- **2-Degradation of ECM .**
- **3-Attachment to novel ECM components .**
- **4-Migration of tumor cells .**

- ***loosening*** **of tumor cells needs to loss of E-cadherins that act as intercellular glues that keep the cells together.**
- **Their cytoplasmic portions bind to β-catenin .**
- **E-cadherin can transmit antigrowth signals by sequestering β-catenin.**

- ***<u>E-cadherin function is lost in almost all epithelial cancers by :</u>***
- ***1- mutational inactivation of E-cadherin***

  genes.
- ***2- by activation of β-catenin genes.***
- ***3-by inappropriate expression of the SNAIL and TWIST transcription factors, which suppress E-cadherin expression .***

- **oncogenes are SNAIL and TWIST, which encode transcription factors whose primary function is to promote a process called epithelial-to-mesenchymal transition (EMT).**
- **In EMT, carcinoma cells down-regulate certain epithelial markers (e.g., E-cadherin) and up-regulate certain mesenchymal markers (e.g., vimentin and smooth muscle actin).**
- **These changes are believed to favor the development of a promigratory phenotype that is essential for metastasis.**
- **Loss of E-cadherin expression seems to be a key event in EMT, and SNAIL and TWIST are transcriptional repressors that promote EMT by down-regulating E-cadherin expression.**
- **EMT has been documented mainly in breast cancers.**

- **The second step in invasion is local *degradation of the basement membrane and interstitial connective tissue*.**
- **Tumor cells may either secrete proteolytic enzymes themselves or induce stromal cells (e.g., fibroblasts and inflammatory cells) to elaborate proteases.**

- **Multiple different families of proteases are present :**
- **1-matrix metalloproteinases (MMPs).**
- **2- cathepsin D.**
- **3-urokinase plasminogen activator.**

- **MMPs regulate tumor invasion not only by remodeling insoluble components of the basement membrane and interstitial matrix but also by releasing ECM-sequestered growth factors.**
- **cleavage products of collagen and proteoglycans also have chemotactic, angiogenic, and growth-promoting effects.**

- **MMP-9 is a gelatinase that cleaves type IV collagen of the epithelial and vascular basement membrane and also stimulates release of VEGF from ECM-sequestered pools.**

- **Benign tumors of the breast, colon, and stomach show little type IV collagenase activity**
- **whereas their malignant counterparts overexpress this enzyme.**
- **the levels of metalloproteinase inhibitors are reduced so that the balance is tilted greatly toward tissue degradation.**

- **overexpression of MMPs and other proteases have been reported for many tumors. Because of these observations, attempts are being made to use protease inhibitors as therapeutic agents.**

- **The third step in invasion involves *changes in attachment of tumor cells to ECM proteins*.**
- **Normal epithelial cells have receptors, such as integrins, for basement membrane laminin and collagens that are polarized at their basal surface.**
- **these receptors help to maintain the cells in a resting, differentiated state.**
- **Loss of adhesion in normal cells leads to induction of apoptosis.**

- **cleavage of the basement membrane proteins collagen IV and laminin by MMP-2 or MMP-9 generates novel sites that bind to receptors on tumor cells and stimulate migration.**

- ***Locomotion*** **is the final step of invasion.**
- **Migration is a complex, multistep process that involves many families of receptors and signaling proteins that eventually impinge on the actin cytoskeleton.**
- **Such movement seems to be potentiated and directed by tumor cell-derived cytokines, such as autocrine motility factors.**

- **Cleavage products of matrix components (e.g., collagen, laminin) and some growth factors (e.g., insulin-like growth factors I and II) have chemotactic activity for tumor cells.**
- **Stromal cells also produce paracrine effectors of cell motility, such as hepatocyte growth factor/scatter factor (HGF/SCF), which bind to receptors on tumor cells.**
- **Concentrations of HGF/SCF are elevated at the advancing edges of the highly invasive brain tumor glioblastoma multiforme, supporting their role in motility.**

Vascular Dissemination and Homing of Tumor Cells

- **In the bloodstream, some tumor cells form emboli by aggregating and adhering to circulating leukocytes, particularly platelets.**
- **Aggregated tumor cells are thus afforded some protection from the antitumor host effector cells.**
- **Most tumor cells, however, circulate as single cells.**

- **Extravasation of free tumor cells or tumor emboli involves adhesion to the vascular endothelium, followed by egress through the basement membrane into the organ parenchyma by mechanisms similar to those involved in invasion.**

- **<u>The site at which metastases is related to two factors:</u>**

1. **The anatomic location and vascular drainage of the primary tumor.**
2. **The tropism of particular tumors for specific tissues.**

- **The site of extravasation and the organ distribution of metastases generally can be predicted by the location of the primary tumor and its vascular or lymphatic drainage.**
- **Many tumors metastasize to the organ that represents the first capillary bed they encounter after entering the circulation.**
- **In many cases the natural pathways of drainage do not readily explain the distribution of metastases.**

- **e.g.lung cancers tend to involve the adrenals with some regularity but almost never spread to skeletal muscle.**
- **<u>The mechanisms of site-specific homing involves :</u>**
- **1-the expression of adhesion molecules by tumor cells whose ligands are expressed preferentially on the endothelium of target organs.**
- **2-chemokines and their receptors.**
- **chemokines participate in directed movement (chemotaxis) of leukocytes.**

- **Human breast cancer cells express high levels of the chemokine receptors *CXCR4* and *CCR7.***
- **The ligands for these receptors (i.e., chemokines CXCL12 and CCL21) are highly expressed only in those organs where breast cancer cells metastasize.**
- **it is speculated that blockade of chemokine receptors may limit metastases.**

- **After extravasation tumor cells are dependent on a receptive stroma for growth.**
- **Tumors may fail to metastasize to certain target tissues because they present a nonpermissive growth environment.**
- **The precise localization of metastases cannot be predicted with any form of cancer**