Neoplasia 2023/24 lecture 9

Dr Heyam Awad

MD, FRCPath

ILOS

- 1. understand the angiogenic switch in tumors and factors that stimulate and inhibit angiogenesis.
- 2. list the steps important for tumor metastasis and the mediators and genes responsible for them.
- 3. understand the concept of tumor dormancy and its clinical implications.

Hallmarks of cancer, a reminder:

- *1. self sufficiency in growth signals*
- *2. insensitivity to growth inhibitory signals*
- *3. evasion of cell death*
- *4. limitless replicative potential*
- *5. reprogramming of metabolism*
- 6. sustained angiogenesis
- 7. ability to invade and metastasize
- 8. evasion of the immune system

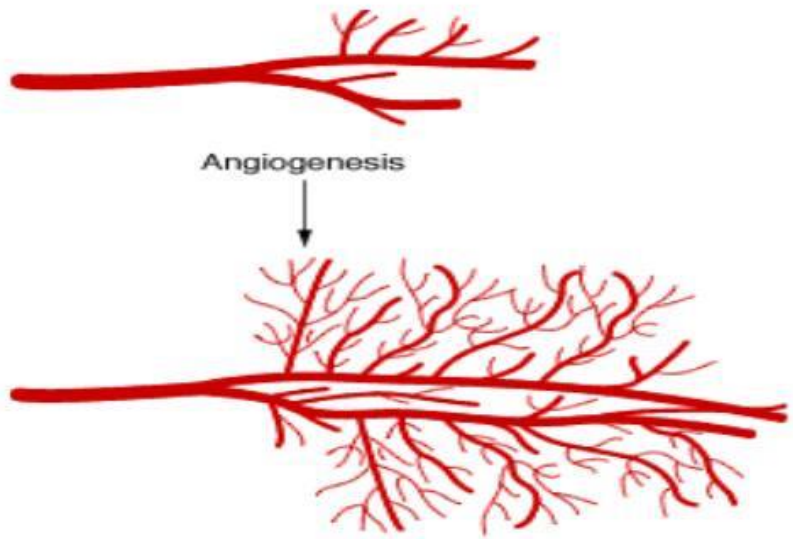
- Till now we covered the first five hallmarks.
- In this lectures we will cover hallmarks 6 and 7.

sixth hallmark: sustained angiogenesis

- Tumors cannot grow for more than 1-2mm without blood supply
- This 1-2 mm zone is the maximum direct diffusion distance.

- Angiogenesis important for tumors to:
- 1. supply oxygen and nutrients
- 2.Get rid of waste products
- 3. gain access to host blood vessels which is important for invasion and metastasis.
- 4.the endothelial cells in these vessels secrete growth factors that can help tumor growth

Angiogenesis



note

- Tumor blood vessels are abnormal : they are leaky, dilated and have haphazard pattern of connections

angiogenesis

- Angiogenesis is accomplished by factors secreted from the parenchymal tumor cells as well as the stroma. Also inflammatory cells surrounding the tumor can produce angiogenic factors.

- The balance between pro-angiogenic and anti-angiogenic factors controls formation of new blood vessels
- **Main pro-angiogenic: VEGF= vascular endothelial growth factor**
- **Main anti-angiogenic: TSP1= thrombospondin 1**

- Tumors usually stay in situ or small for several years… at this stage there is no angiogenesis

- Angiogenesis switch happens when VEGF (and other proangiogenic factors) increases and TSP 1 (or other antiangiogenic factors) decreases.

Angiogenic switch

- VEGF produced from tumor cells or macrophages
- Protease (secreted from tumor cells or stromal cells) can release FGF (an angiogenic agnt) from ECM

- TSP1 is produced from fibroblasts in response to tumor cells…. TSP is anti angiogenic
- Normal P53 induces synthesis of TSP1.. So if p53 is deleted.. Decreased TSP1

What causes the angiogenic switch

- Hypoxia is an important factor that favors angiogenesis
- Hypoxia.. **Stimulates production of hypoxia –inducible factor 1alpha (HIF 1 alpha)**
- HIF is a transcription factor which will stimulate production of VEGF

- HIF is destructed by VHL (von Hipple- Lindau)protein
- Hypoxia prevents VHL from recognizing HIF … no destruction ..more angiogenesis

Von Hippel- Lindau syndrome

- VHL gene is a tumor suppressor gene (because it decreases angiogenesis)
- Rarely some people inherit defective VHL gene… they develop tumors like renal cell carcinoma, pheochromocytoma..

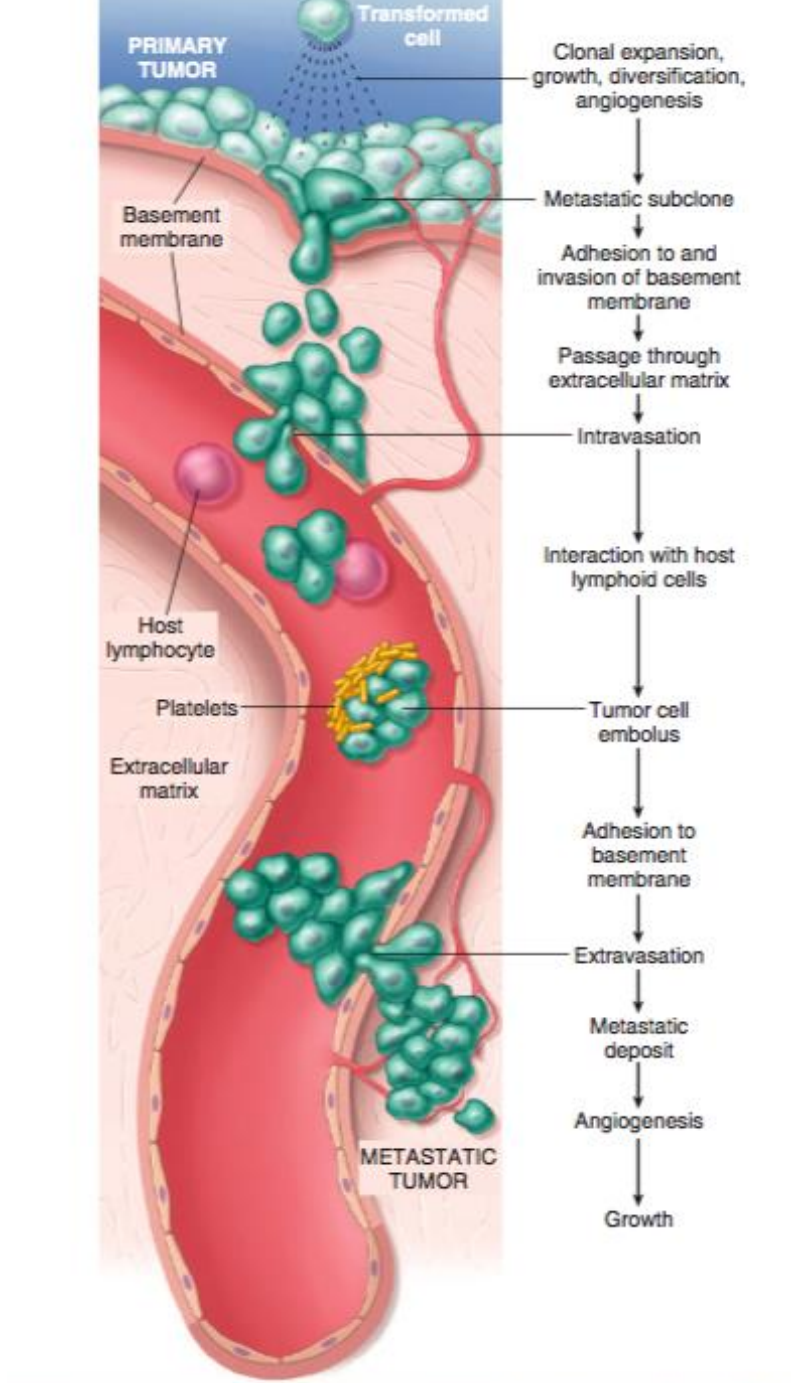
7th hallmark: ability to invade and metastasize

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

Invasion-metastatic cascade

Steps needed for metastatic spread are called: invasion -metastatic cascade

See next picture for the steps


Transformed cell
PRIMARY TUMOR
Clonal expansion, growth, diversification, angiogenesis
Basement membrane
Metastatic subclone
Adhesion to and invasion of basement membrane
Passage through extracellular matrix
Intravasation
Interaction with host lymphoid cells
Host lymphocyte
Platelets
Tumor cell embolus
Extracellular matrix
Adhesion to basement membrane
Extravasation
Metastatic deposit
Angiogenesis
METASTATIC TUMOR
Growth

- The two main steps are: invasion of ECM and vascular dissemination and homing

ECM invasion

In order to metastasize, cells need to enter the blood vessels .

First tumor cells need to invade the underlying basement membrane then through interstitial connective tissue and then penetrate vascular basement membrane

This process is repeated when tumor cells exit the blood vessel to the metastatic site

- Invasion of ECM (both basement membrane or interstitial matrix) is a dynamic process that needs several steps.
- 1.loosening of tumor cells
- 2. degradation of ECM
- 3.Changes in attachment of tumor cells to ECM proteins
- 4.locomotion

First step: loosening of tumor cells

- E cadherin works as a glue that keeps cells together
- For cells to become loose, they need to decrease E cadherin.

Second step

Degradation of ECM

- Proteases degrade ECM components…
- These proteases are produced from tumor cells, OR the tumor cells send signals to stromal cells or inflammatory cells to secret them

Third step: change in attachment

- Normal epithelial cells have integrin receptors that attach to collagen and laminin in ECM
- These receptors help maintain cells in the resting differentiated state
- If this normal adhesion is lost cells die by apoptosis
- Cancer cells lose this adhesion, but they evade apoptosis.
- Also, the ECM is modified by collagenase and other proteases actions that create new adhesion sites.

Fourth step: locomotion

- = migration of the tumor cells through the ECM.
- Complex process that uses receptors and signaling proteins that affect actin cytoskeleton

Factors used for locomotion include:

- Tumor derived cytokines(autocrine motility factor
- Cleavage products of matrix components have chemotactic activity
- Some growth factors(insulin like growth factor) have chemotactic activity that facilitates locomotion
- Stromal cells secrete hepatocyte GF / scatter factor (HGF/SCF)

Vascular dissemination and homing of tumor cells

- After the steps mentioned previously the tumor cells can enter the blood vessel
- Once in the blood vessels, they can be destroyed by the immune cells… so they need to evade this (next lecture)
- Some tumor cells circulate in the blood individually, others form emboli (small aggregates) that bind leukocytes and platelets to protect themselves from being recognized by the immune system

- These tumor cells circulate in the blood, but at a certain point they must exit the vessel to tissues
- The site of extravasation (site of metastatic deposit) generally can be predicted by the location of the primary tumor and its vascular and lymphatic drainage
- Many tumors metastasize to the organ that presents the first capillary bed they encounter.
- However, in many cases the natural pathway of drainage doesn’t explain the distribution of metastasis

- Why tumors choose certain sites for their metastatic spread and not others???
- This is related to :
- A. expression of adhesion molecules in the tumor cells, whose ligands are present in the endothelium of target organs
- B. expression of chemokines and their receptors
- C. once they reach the target site, tumor cells must colonize the site . Their growth in the metastatic site depends on the host stroma.. If the host stroma at a specific site doesn’t allow the tumor cells to live there, they cannot survive.

- Although tumor cells can escape their site of origin it is more difficult for them to colonize new sites
- Tumor cells are continually shed from tumors, some of which can be detected in the blood even in people who will never have metastases. Because theses cells fail to live in the new environment
- Some though might live for long periods and be dormant and form metastases later when there are suitable conditions
- Tumor dormancy is described mainly in melanoma, breast and prostate cancer. This means these tumors can recur a long time after initial treatment.

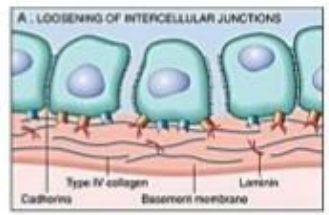
Tumor dormancy

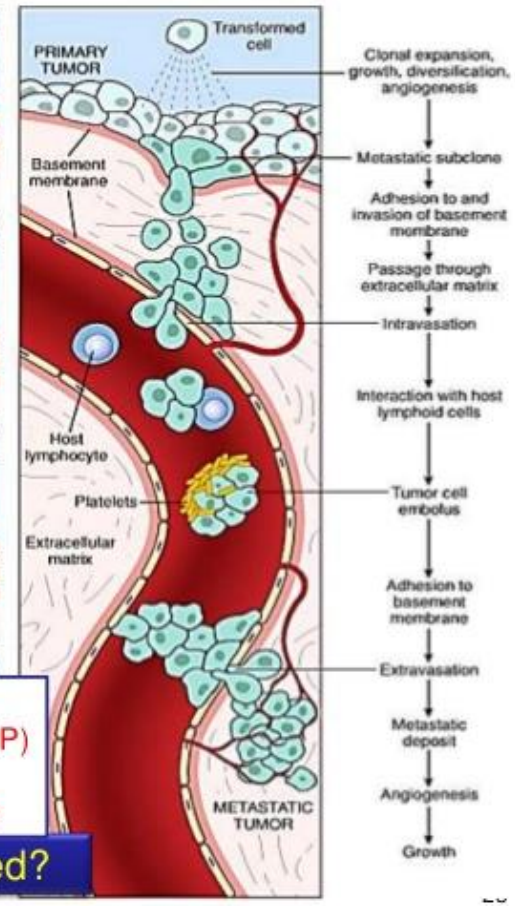
- Prolonged survival of micro-metastases without progression

Metastasis:

Pathogenesis:

1. Cell loosening
2. BM degradation
3. Invasion
4. Locomotion
5. BV adhesion
6. Intra-vasation
7. Tumour embolus
8. Adhesion
9. Extra-vasation
10. Angiogenesis
11. Growth.





- E-Cadherin, β-catenin
- Matrix Metalloproteinases (MMP)
- Collagenase (not in benign)
- Actin Cytoskeleton, chemokine

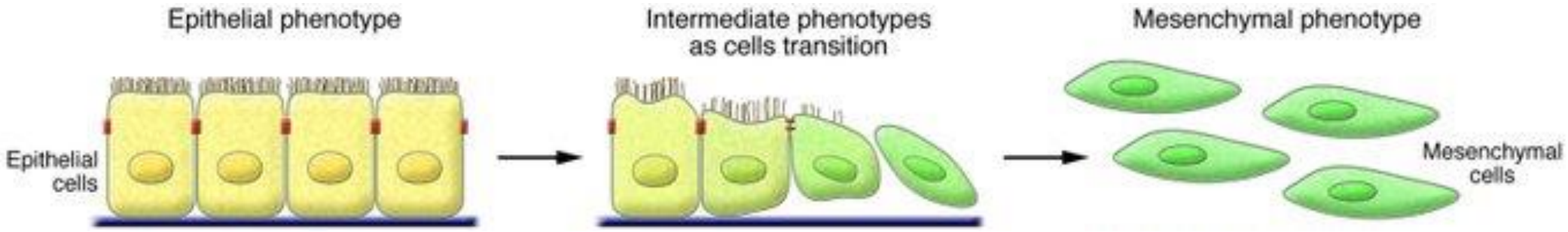
What chemical mediators are involved?

Molecular genetics of metastases

- Are there any genes that control the metastatic phenotype?
- Possibly TWIST and SNAIL/ SLUG .. They promote epithelial to mesenchymal transition (EMT)

EMT

- = tumor cells downregulate some epithelial markers like E cadherin and upregulate some mesenchymal markers like vimentin and sma (smooth muscle actin)
- These molecular changes are associated with phenotypic changes, so the cells become spindly, and functional change (they are more capable to invade and metastasize)



Clinical aspects related to metastasis

- Metastasis is the single most important factor dictating outcome of cancer.
- localized cancer that is confined to the organ it originated from has the best prognosis.
- We measure prognosis by the five year survival rate.
- 5 year survival means the percentage of people who live at least **5** years after being diagnosed with cancer.
- For example, **5-year survival** rate of 65% means that 65 out of 100 people who are diagnosed with cancer will be still alive **5** years after the initial diagnosis.
- 5 year survival of Localised colorectal cancer is around 95% whereas it is 6% for metastatic colorectal cancer… so metastasis dramatically affects survival.

Tumor stage

- Tumor stage measures the extent of tumor spread in the body.
- For each tumor, there is stage called TNM stage.
- T measures the tumor size/ or extent of local invasion (depending on the tumor type)
- N measures lymph node involvement.
- M measures the presence of metastasis.
- The higher T, N or M, the higher the stage, which means the worse the prognosis.

Grading and staging of cancer

- Grading is determined by cytologic and histologic appearance of the tumor
- In general well differentiated tumors are less aggressive than poorly differentiated ones
- However.. **Staging is more important than grading in determining outcome and prognosis**

Example of staging: colon cancer

- T : describes the extent of involvement of the wall.
- T1: tumor invades the mucosa and submucosa
- T2: tumor invades muscularis propria (muscularis externa)
- T3: subserosa (serosal fat) involved
- T4: direct invasion to adjacent structures.

- N: describes the number of LN involved.. See next slide.
- M: describes if there is metastasis or not.

Example: TNM of colon cancer

TNM Staging of cancer colon

Tis	Carcinoma in situ
T1	Tumor invades submucosa
T2	Extending into the muscularis propria
T3	Penetrating through the muscularis propria into subserosa
T4	Tumor directly invades other organs or structures
N0	No regional lymph node metastasis
N1	Metastasis in 1 to 3 lymph nodes
N2	Metastasis in 4 or more lymph nodes
M0	No distant metastasis
M1	Distant metastasis

Question

- Refer to the previous slide to determine the T, N, M stage of the following patient:
- A 65 year old male had a 5 cm mass in the caecum. The tumor was composed of well defined glands that invade the muscularis propria but not reaching the subserosa. 20 lymph nodes were examined histologically, six of which showed metastatic deposits. The CT scan showed a metastatic deposit in the liver.

answer

- Refer to the previous slide to determine the T, N, M stage of the following patient:
- A 65 year old male had a 5 cm mass(the size of colon cancer is irrelevant, T stage of the colon depends on extent of local invasion of the wall of the colon) in the caecum. The tumor was composed of well defined glands (This is also irrelevant, because the architecture describes the grade not the stage) that invade the muscularis mucosa but not reaching the subserosa (this is T2. the muscle is involved but the the subserosal fat isn't). 20 lymph nodes were examined histologically, six of which showed metastatic deposits (this means six nodes involved by the tumor, the other 14 are negative, not involved, and this is N2). The CT scan showed a metastatic deposit in the liver.(metastasis is M1)
- So this patient has T2 N2 M1.

Question

- A 48-year-old woman goes to her physician for a routine physical examination. A 4 cm diameter non-tender mass is palpated in her right breast. The mass appears fixed to the chest wall. Another 2 cm non-tender mass is palpable in the left axilla. A chest radiograph reveals multiple 0.5 to 2 cm nodules in both lungs. Which of the following classifications best indicates the stage of her disease?
- A T1 N1 M0
- B T1 N0 M1
- C T2 N1 M0
- D T3 N0 M0
- E T4 N1 M1

answer

- A 48-year-old woman goes to her physician for a routine physical examination. A 4 cm diameter(size is important in breast cancer, however you don't have to memorize the sizes corresponding to each T to answer this question) non-tender mass is palpated in her right breast. The mass appears fixed to the chest wall. Another 2 cm non-tender mass is palpable in the left axilla(this means there is a lymph node involvement, this patient definitely isn't N0, she's N1). A chest radiograph reveals multiple 0.5 to 2 cm nodules in both lungs (this means there is metastasis, so we are talking about M1). Which of the following classifications best indicates the stage of her disease? Having realized that the tumor is N1 M1 you can answer this question although you don't know the T , this is the philosophy of such questions, we want you to use your knowledge of the concept of what T N or M mean)
- A T1 N1 M0
- B T1 N0 M1
- C T2 N1 M0
- D T3 N0 M0
- E T4 N1 M1

Summary

- Angiogenesis provides tumors with blood, oxygen, nutrients and growth factors.
- The balance between pro-angiogenic (VEGF) and anti-angiogenic factors (TSP1) controls formation of new blood vessels. The balance is tipped towards more neoangiogenesis under the influence of HIF.
- Metastatic spread of cancer occurs via the invasion-metastatic cascade, the most important steps of which include degradation of ECM, vascular dissemination and homing.
- Tumor metastasis is controlled by genes including TWIST and SNAIL/ SLUG which promote epithelial to mesenchymal transition (EMT)
- Metastasis is the most important factor that determines the outcome which is measured by the 5 year survival.
- TNM stage describes the extent of tumor spread. Each tumor has a specific and different TNM stage.
- T refers to the size in some tumors or to extent of local invasion in others. N refers to lymph node involvement. M to distant metastasis.

Thank you