Oncology for Scientists (RPN 530)

**Metastasis and Angiogenesis**

Chapter 13 and 14

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Department of Cancer Genetics
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About Exam!!

• First, think and understand the concepts. Don’t just memorize.
• Second, memorize scientific terms if they are necessary to understand.
• Third, explain and discuss cancer, angiogenesis and metastasis using the concepts and terms you have learned in this lecture with your friends and family.

• Check emphasized words (by bold, colored or high-lighted, etc) in handouts and notes because they are important and worth remembering.

Suggested resources:

If you have any questions...
Contact: masashi.muramatsu@roswellpark.org
Topics in this lecture

1. Angiogenesis
2. Metastasis
What are Blood vessels?

• Total length may be 100,000 km = they can encompass the earth by 2.5 times

• Blood vessels in the whole body are composed of macro (artery/vein) and microvessels

• Blood vessel: supply oxygen and nutrition from lung throughout the whole body

• Lymphatic vessel: drain the tissue fluid to blood circulation and protect from germs by immunity at lymph nodes

macrovessel  microvessel
What is (normal) Angiogenesis?

“Vasculogenesis” vs “Angiogenesis”

• Vasculogenesis is usually used for early developmental stage. First blood vessels are created from hemangioblast which is the multipotent precursor cells that differentiate to hematopoietic and endothelial cells. Vasculogenesis means the formation of new blood vessels when there are no pre-existing ones.
  • Create new blood vessels

• Angiogenesis is involved in formation, maturation and differentiation of blood vessels from pre-existing vessels. Angiogenesis can be observed in physiological and pathological conditions including growth, injury, inflammation and cancer. Occasionally, angiogenesis is called neovascularization.
  • Extend and expand blood vessels
Angiogenic factors

**Table 13.3 Important angiogenic factors**

<table>
<thead>
<tr>
<th>Name</th>
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<tbody>
<tr>
<td>Vascular endothelial GFs (VEGFs)</td>
</tr>
<tr>
<td>Basic fibroblast growth factor (bFGF)</td>
</tr>
<tr>
<td>Acidic fibroblast growth factor (aFGF)</td>
</tr>
<tr>
<td>Angiogenin</td>
</tr>
<tr>
<td>Transforming growth factor-α (TGF-α)</td>
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<tr>
<td>Transforming growth factor-β1 (TGF-β1)</td>
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<tr>
<td>Tumor necrosis factor-α (TNF-α)</td>
</tr>
<tr>
<td>Platelet-derived growth factor-B (PDGF-B)</td>
</tr>
<tr>
<td>Granulocyte colony-stimulating factor (G-CSF)</td>
</tr>
<tr>
<td>Placental growth factor (PIGF)</td>
</tr>
<tr>
<td>Interleukin-8 (IL-8)</td>
</tr>
<tr>
<td>Hepatocyte growth factor (HGF)</td>
</tr>
<tr>
<td>Proliferin</td>
</tr>
<tr>
<td>Angiopoietin</td>
</tr>
<tr>
<td>Leptin</td>
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</tbody>
</table>

*Paracrine:* interaction between different cells

*Autocrine:* interaction in the same cell

Diagram:

- **VEGF** interacts with **PDGFR-β**
- **PDGF** interacts with **VEGFR1/2**

(1) Paracrine: interaction between different cells

(2) Autocrine: Interaction in the same cell
Angiogenic signal (e.g. VEGF-VEGFR system)

Major angiogenic trigger
Hypoxia

HIF-1α: Hypoxia inducible factor-1α

Angiogenic signal (e.g. VEGF-VEGFR system)

Angiogenesis

Lymphangiogenesis
During the process of wound healing, the burst of angiogenesis must be shut down once the newly formed capillaries have reached a certain density.

**Derived form extracellular matrix**
- Endostatin
- Thrombospondin (TSP)-1 and -2, etc

**Non-matrix-derived**
- Angiostatin
- IFN-α
- IL-1β
- IL-18
- sFlt-1, etc
e.g. TSP-1 suppresses angiogenesis by induction of apoptosis in EC
What is tumor angiogenesis?

Angiogenic factors

I’m hungry!!

More! more!

OK, I’m stuffed. Time to move!

Tumor angiogenesis emerges circulating tumor cells (CTCs) and contributes to cancer progression and metastasis.
Features of tumor angiogenesis

Extreme and chaotic expression of angogenic factors

Disorganized vascular structure
Low adhesion and pericyte coverage

Hypoxic stress, metabolic changes, cancer cell intravasation and less effect of chemotherapy

Green: blood vessels
Red: hypoxic area

human melanoma
**Stroma contributes to tumor angiogenesis**

*Tumor microenvironment* (Tumor-associated stroma): induced by cytokines and chemokines secreted from tumor cells

- Macrophage: Tumor-Associated Macrophages (TAMs)
- Fibroblast: Carcinoma-Associated Fibroblasts (CAFs)
- Myeloid cell: Bone Marrow Derived Cells (BMDCs)
- Extracellular matrix (ECM)

**e.g.** The degree of tumor-associated stroma (TAMs) infiltration into tumor tissue correlate with tumor angiogenesis and cancer malignancy
Macrophages play a major role in releasing mitogenic factors for carcinoma cells as well as reorganizing the tumor stroma in order to facilitate angiogenesis and, in some tumors, carcinoma cell invasiveness.
We have proposed the term “anti-angiogenesis” to mean the prevention of new vessel sprouts from penetrating into an early tumor implant. If a tumor could be held indefinitely in the nonvascularized dormant state, there are a number of theoretical benefits. It is possible that metastasis will not arise from a nonvascularized tumor.

Dr. Judah Folkman described in NEJM. 1971 proposed anti-angiogenesis therapy.
Efficiency of anti-angiogenesis therapy

- Endostatin / Angiostatin
- Avastin (bevacizumab): monoclonal antibody for VEGF-A
- SU5416: tyrosine kinase inhibitor for VEGFR-2
- SU6668: tyrosine kinase inhibitor for PDGFR
- Sunitinib: multi-targeted receptor tyrosine kinase inhibitor

From Genentech USA Inc. website
**Paradox of anti-angiogenesis therapy**

The median survival benefit of 4.4 months does not persist, with an equivalent fraction of each treatment group (placebo plus IFL (irinotecan, 5-FU and leucovorin) versus bevacizumab plus IFL) showing progression-free survival at 20 months.

- Resistance: expression other angiogenic factors such as bFGF and PDGF
- HIF-1 can induce EMT and promote invasiveness via cytokine expression.
- Normalize disorganized tumor blood vessels
- Side effect (high blood pressure, bleeding and coronary artery disease, etc)
Summary of angiogenesis

- Angiogenesis is induced by hypoxic trigger (transcription factor), HIF-1α.
- Major angiogenic factors are VEGF-A, bFGF, TGF-β, PlGF and PDGF.
- Tumor angiogenesis is required to supply oxygen and nutrition for tumor growth.
- Tumor microenvironmental cells such as TAM, CAFs, BMDCs and ECM contribute to tumor angiogenesis.
- Tumor angiogenesis contributes to cancer progression and metastasis.
- Avastin is a monoclonal antibody for VEGF-A which is used as an anti-angiogenesis therapy.
- Sunitinib is a multi-targeted receptor tyrosine kinase inhibitor which inhibit activity of VEGFR-2 and PDGFR.
1. Angiogenesis

2. Metastasis
What is metastasis?

- Over 90% of cancer related mortality is caused by metastasis.

- Cancer cells that have left the primary tumor and traveled by the body’s highways-blood and lymphatic vessels-to seek out new sites throughout the body where they may form new colonies.

- **The dissemination of cancer cells throughout the body has already occurred by the time a primary tumor is first detected**: at the time of initial diagnosis or after dissection of primary tumor, these scattered cells will be undetectable because they only form minute tumor colonies, known as micrometastasis.
The invasion-metastasis cascade

- Formation of metastasis has multiple steps such as angiogenesis → EMT → invasion → intravasation → circulation → rolling and adhesion → extravasation → MET → micrometastasis → colonization → macrometastasis.

- Each step is very complicated and remains poorly understood.
The invasion-metastasis cascade

Figure 14.18b The Biology of Cancer (© Garland Science 2014)
EMT=Epithelial-Mesenchymal Transition

• The great majority (>80%) of life-threatening cancers occur in epithelial tissues.

• In order to acquire motility and invasiveness, carcinoma cells must shed many of their epithelial phenotypes, detach from epithelial sheets, and undergo a drastic alteration.

• Once carcinoma cells acquire access to the EMT program, they can exploit it to profoundly change their own morphology, motility and ability to invade nearby cell layers.

• In the absent of EMT signals, the carcinoma cells may then undergo a mesenchymal-epithelial transition (MET) and revert to the phenotype of the primary tumor.
Features of the EMT

**Epithelial cells**
- ✓ Cytokeratin expression
- ✓ Epithelial adherence junction protein (E-cadherin)
- ✓ Epithelial cell polarity

**Mesenchymal cells**
- ✓ Fibroblast-like shape
- ✓ Motility
- ✓ Invasiveness
- ✓ Mesenchymal adherence junction protein (N-cadherin)
- ✓ Protease secretion (MMPs)
- ✓ Vimentin expression
They look like....

- Epithelial cell polarity
- Benign

Now, they seem to have a.....

- Motility
- Invasiveness
- Malignancy
The EMT-inducing transcription factors

- Twist
- Snail (SNAI1)
- Slug (SNAI2)
- Goosecoid
- FOXC2
- ZEB1 (δEF1)
- ZEB2 (SIP1)
- E12/E47 (Tcf3)

<table>
<thead>
<tr>
<th>Name</th>
<th>Where first identified</th>
<th>Type of transcription factor</th>
<th>Cancer association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snail (SNAI1)</td>
<td>mesoderm induction in Drosophila; neural crest migration in vertebrates</td>
<td>C2H2-type zinc finger</td>
<td>invasive ductal carcinoma</td>
</tr>
<tr>
<td>Slug (SNAI2)</td>
<td>delamination of the neural crest and early mesoderm in chicken</td>
<td>C2H2-type zinc finger</td>
<td>breast cancer cell lines, melanoma</td>
</tr>
<tr>
<td>Twist</td>
<td>mesoderm induction in Drosophila; emigration from neural crest</td>
<td>bHLH</td>
<td>various carcinomas, high-grade melanoma, neuroblastoma</td>
</tr>
<tr>
<td>Goosecoid</td>
<td>gastrulation in frog</td>
<td>paired homeodomain</td>
<td>various carcinomas</td>
</tr>
<tr>
<td>FOXC2</td>
<td>mesenchyme formation</td>
<td>winged helix/forkhead</td>
<td>basal-like breast cancer</td>
</tr>
<tr>
<td>ZEB1 (δEF1)</td>
<td>postgastrulation mesodermal tissue formation</td>
<td>2-handed zinc finger/homeodomain</td>
<td>wide variety of cancers</td>
</tr>
<tr>
<td>ZEB2 (SIP1)</td>
<td>neurogenesis</td>
<td>2-handed zinc finger/homeodomain</td>
<td>ovarian, breast, liver carcinomas</td>
</tr>
<tr>
<td>E12/E47 (Tcf3)</td>
<td>associated with E-cadherin promoter</td>
<td>bHLH</td>
<td>gastric cancer</td>
</tr>
</tbody>
</table>

*It remains unclear whether E12/E47 can function on its own to induce an EMT, or whether this bHLH functions as a subunit of a heterodimeric TF complex formed with other well-validated EMT-TF proteins such as Twist.*

They can induce EMT...

- ✓ Loss of epithelial markers like E-cadherin, β-catenin
- ✓ Gain of fibronectin and vimentin and fibroblast-like morphology
Alteration of the gene profiles by the EMT

(A) Control vector vs. Twist vector

(B) Western blot analysis

Figure 14.15 The Biology of Cancer (© Garland Science 2014)
Signals trigger the EMT from stroma

Figure 14.24 The Biology of Cancer (© Garland Science 2014)
Human mammary epithelial cell were implanted in an immunocompromised mouse host.

These antibodies can stain human cell but not mouse cell

- Cytokeratin: epithelial marker
- Vimentin: mesenchymal marker
Clinical evidences: the EMT progresses human cancer

Increasing evidence correlates the expression of EMT-inducing TFs with the induction of malignant behavior in cancer cells in patients.
The invasion-metastasis cascade

- carcinoma in situ
- invasive carcinoma
- EMT
- INVASION
- PROGRESSION
- INTRAVASATION
- TRANSPORT through circulation
- MET
- COLONIZATION
- micrometastasis
- macrometastasis
- extravasation

Figure 14.18b The Biology of Cancer (© Garland Science 2014)
Signaling pathway of cell motility-related protein production

Lamellipodia

Filopodia
Small GTPase family play a key role of cancer cell motility

The detailed management of cell shape and motility is under the control of members of a group of Ras-related proteins belonging to the Rho family.

- Cytokines
- Chemokines
- Extracellular matrix

Rho family
- Rho
- Rac
- Cdc42

Stress fiber
Focal adhesion
Lamellipodia
Filopodia
The invasion-metastasis cascade
The mechanism of cancer cell adhesion during circulation

Adhesion molecule related to rolling, adherence and extravasation

- Integrons (integrin α4β1, VCAM-1, ICAM-1),
- Selectins (P-selectin, L-selectin and E-selectin)
- CD44, etc

These molecules are expressed on not only normal cell such as endothelial cells and leukocytes but also cancer cell. Some cancer cell exploit leukocytes and platelets function to adherent endothelial wall.
Platelets support tumor cell extravasation

Formation of microthrombus (attachment of platelets) and proliferation in the lumen of the capillary
Lodging and extravasation of circulating tumor cells

Once lodged in the blood vessels of various tissues, cancer cells must escape from the lumina of these vessels and penetrate into the surrounding tissue—the step termed **extravasation**.

Large numbers of cancer cells arrested in the microvessels (of the liver).
The invasion-metastasis cascade

- **invasion**:
  - Carcinoma in situ
  - Normal stroma
  - Basement membrane

- **metastasis**:
  - Invasive carcinoma
  - Reactive stroma
  - EMT
  - Intravasation
  - Transport through circulation
  - Extravasation

- **colonization**:
  - Micrometastasis
  - Normal stroma
  - Basement membrane

**Figure 14.18b The Biology of Cancer (© Garland Science 2014)**
Blood flow pattern strongly involved in metastasis behavior

- 66%: vascular pattern
- 20%: tropism rather than blood flow
- 14%: negative interaction
He analogized the seeding of cancer cells to the dispersal of the seed of plants. After studying the clinical course of 735 breast cancer patients, Paget concluded that the patterns of metastasis formation in the body or by the patterns of dispersal from the breast through the general circulation. He wrote, “a plant goes to seed, its seed are carried in all directions; but they can only live and grow if they fall on congenial soil”
Pre-metastatic niche formation

A comfortable condition (pre-metastatic niche) for cancer cells has already made up at the metastatic site in distal organ by something secreted from primary tumor before cancer cells colonize at the site.

Adapted from Bethan Psaila & David Lyden, Nature Reviews Cancer 9, 285-293 (April 2009)
Current perspective on cancer cell metastasis

Das S et al. (2008) Cancer stem cells and glioma
Nat Clin Pract Neurol 10.1038

Chaffer CL, Weinberg RA. Science. 2011 Mar 25;331(6024):1559-64
Current perspective on cancer cell metastasis 2

Current perspective on cancer cell metastasis 3

Quail DF, Joyce JA.
Metastasis suppressor genes contribute to regulating the metastatic phenotype

<table>
<thead>
<tr>
<th>Name</th>
<th>Cellular location</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRMS1</td>
<td>nuclear protein</td>
<td>involved in chromatin remodeling</td>
</tr>
<tr>
<td>CRSP3</td>
<td>nuclear protein</td>
<td>transcription factor</td>
</tr>
<tr>
<td>KAI1/CD82</td>
<td>transmembrane protein</td>
<td>cell–cell associations</td>
</tr>
<tr>
<td>KISS1</td>
<td>secreted protein</td>
<td>ligand of G-protein–coupled receptor</td>
</tr>
<tr>
<td>NM23</td>
<td>cytoplasmic kinase</td>
<td>regulator of MAPK cascade (?)</td>
</tr>
<tr>
<td>p63</td>
<td>nuclear transcription factor</td>
<td>multiple targets</td>
</tr>
<tr>
<td>RhoGDI-2</td>
<td>cytoplasmic protein</td>
<td>negative regulator of Rho action</td>
</tr>
<tr>
<td>SseCKs</td>
<td>cytoplasm</td>
<td>cytoskeleton-associated protein</td>
</tr>
<tr>
<td>VDUP1</td>
<td>cytoplasm</td>
<td>regulator of MAPK cascade (?)</td>
</tr>
<tr>
<td>CDH1 (= E-cadherin)</td>
<td>cell surface adhesion</td>
<td>favors formation of epithelial cell sheets</td>
</tr>
<tr>
<td>TIMPs</td>
<td>secreted protein</td>
<td>inhibitor of metalloproteinases</td>
</tr>
<tr>
<td>MKK4</td>
<td>cytoplasm</td>
<td>protein kinase component of MAPK cascade</td>
</tr>
<tr>
<td>DICER</td>
<td>cytoplasm</td>
<td>miRNA processing</td>
</tr>
</tbody>
</table>

SSeCKS gene loss in metastasis
Summary of Metastasis cascade

- Carcinoma in situ
- Invasive carcinoma
- Reactive stroma
- Micrometastasis
- Normal stroma
- Basement membrane
- Epithelial
- Mesenchymal

Figure 14.18b: The Biology of Cancer (© Garland Science 2014)
About Exam!!

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- Check emphasized words (by **bold**, colored or high-lighted, etc) in handouts and notes because they are important and worth remembering.

- Please, please and please! Use legible and reader-friendly hand writing. If I cannot understand and read, you won’t get points even though your answer is awesome. This is so sad.
Do you have any questions?

The volcano known as Eyrjafjallajökull, is closest to what Icelandic city?

A: Reykjavic
B: Kopavogur
C: Hafnarfjöurvei
D: Fjarðabyggð

From Google.com