Angiogenesis – introduction

lecture I

2nd March 2015
Angiogenesis

- **Angiogenesis** (an-jee-o-JEN-uh-sis):
  - angei-, angeio-, vessel or blood vessel, 
  - genesis-, origin or birth

**Angiogenesis:**
formation of new blood vessels via 
extension or remodeling from existing capillaries
Capillaries with pericytes

- Described in 1873 by the French scientist Charles-Marie Benjamin Rouget and were originally called Rouget cells.
- Play diverse roles: stabilization, controlling the hemodynamic processes of blood vessels, + they can sense angiogenic stimuli, guide sprouting tube, elicit endothelial survival functions and they exhibit tissue specific functions: brain (BBB), liver (hepatic stellate cells), kidney (mesangial cells).
- Pericytes are sometimes referred to as vascular smooth muscle cells.
- Desmin, α-SMA, PDGFRβ
The vascular wall

- **Tunica**
  - **Intima** = endothelial cells
  - **Media** = VSMC
  - **Adventitia** = connective tissue

- **Externa**

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**Endothelium**

**Internal elastic lamina**

**External elastic lamina**
Endothelium

vascular tone

thrombosis

vascular wall remodeling

angiogenesis

fibrinolysis

inflammation

transport
Organ-specific capillaries

- Structure of endothelium
- Basement membrane
- Glycocalyx
- Intercellular junctions
Endothelial junctions are formed by transmembrane adhesion proteins and their intracellular partners.
Any molecule for exchange must
1) move through the blood,
2) pass through the glycocalyx,
3) cross the endothelial barrier,
4) diffuse through the interstitial space to the target tissue where it can
5) interact with the target tissue.
Types of Capillaries

1. Continuous – without “gaps” open in its walls

2. Fenestrated – numerous “pores” in the endothelium found in intestinal lining, kidneys and endocrine organs

3. Discontinous – modified “leaky” capillaries like sinusoids in liver or spleen
Continuous Capillaries

• Continuous capillaries are abundant in the skin and muscles
  – Endothelial cells provide an uninterrupted lining
  – Adjacent cells are connected with tight junctions

• Continuous capillaries of the brain:
  – Have tight junctions completely around the endothelium
  – Constitute the blood-brain barrier
Fenestrated Capillaries

• Found wherever active capillary absorption or filtrate formation occurs (e.g., small intestines, endocrine glands, and kidneys)

• Characterized by:
  - An endothelium riddled with pores (fenestrations)
  - Greater permeability than other capillaries
Sinusoids

• Highly modified, leaky, fenestrated capillaries with large lumens

• Found in the liver, lymphoid tissue, and in some endocrine organs

• Allow large molecules (proteins and blood cells) to pass between the blood and surrounding tissues
Sinusoids

- Pericyte
- Endothelial cell
- Red blood cell in lumen
- Large intercellular cleft
- Tight junction
- Incomplete basement membrane
- Nucleus of endothelial cell
Capillary Exchange of Respiratory Gases and Nutrients

- Oxygen, carbon dioxide, nutrients, and metabolic wastes diffuse between the blood and interstitial fluid along concentration gradients
  - Oxygen and nutrients pass from the blood to tissues
  - Carbon dioxide and metabolic wastes pass from tissues to the blood
  - Water-soluble solutes pass through clefts and fenestrations
  - Lipid-soluble molecules diffuse directly through endothelial membranes
SHORT HISTORY

angiogenesis research
William Harvey - 1628

(1578-1657)

the first systematic description of circulatory system

- He accurately calculated the amount of blood in the body
The word "angiogenesis" was used for the first time in 1785 by British anatomist, Dr. John Hunter, to describe the growth of blood vessels in the reindeer antler.

The term "endothelium" was introduced by Wilhelm His in 1865 to differentiate the inner lining of body cavities from "epithelium".

A new classification of tissues based on histogenesis. In the present work, His put forth the basic concepts of tissue embryology. Using serial sections and three-dimensional models to illustrate his theories, he showed that the serous spaces in the embryo are mesodermal in origin and that they are lined by the special layer which he was the first to term "endothelial".
Blood vessel formation - short history of studies

- End of XIX/beginning XX century **Henryk Hoyer** – (Jagiellonian University) – description of lymphatic system

- Detailed study on vasculogenesis in chick embryo and lymphangiogenesis by Florence Sabin
• 1984 - The first angiogenic factor (basic fibroblast growth factor, bFGF) was purified by Yuen Shing and Michael Klagsbrun at Harvard Medical School.

• 1989 - One of the most important angiogenic factors, vascular endothelial growth factor (VEGF), was discovered by Dr. Napoleone Ferrara and by Dr. Jean Plouet. It turns out to be identical to a molecule called Vascular Permeability Factor (VPF) discovered in 1983 by Dr. Harold Dvorak.
• Gimbrone MA Jr, Cotran RS, Folkman J.
Endothelial regeneration: studies with human endothelial cells in culture.

• Jaffe EA, Nachman RL, Becker CG, Minick CR
Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria.

Endothelial cells were isolated from freshly obtained human umbilical cords by collagenase digestion of the interior of the umbilical vein. The cells were grown in tissue culture as a homogeneous population for periods up to 5 mo and some lines were subcultured for 10 serial passages......

• Ades EW, Candal FJ, Swerlick RA, George VG, Summers S, Bosse DC, Lawley TJ.
Endoglin is an auxiliary receptor for the transforming growth factor-beta family of cytokines and is required for angiogenesis and heart development.

vWF - Von Willebrand factor
## Endothelial cell markers used to identify microvasculature in tissues

<table>
<thead>
<tr>
<th>Marker</th>
<th>Ligand</th>
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<tr>
<td>CD31 (PECAM-1)</td>
<td>CD31 on endothelial cells, leukocytes; glycosaminoglycans</td>
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<tr>
<td>CD34</td>
<td>L-selectin</td>
</tr>
<tr>
<td>CD54 (ICAM-1)</td>
<td>LFA-1 integrin</td>
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<tr>
<td>CD62E (E-selectin)</td>
<td>Sialyl-Lewis-X antigen and other carbohydrates</td>
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<tr>
<td>CD105 (endoglin)</td>
<td>TGF-β1 and –β3</td>
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<td>CD106 (VCAM-1)</td>
<td>VLA-4 integrin</td>
</tr>
<tr>
<td>CD141 (thrombomodulin)</td>
<td>Thrombin</td>
</tr>
<tr>
<td>CD144 (VE-cadherin)</td>
<td>CD144 homotypic interaction</td>
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<tr>
<td>Endothelin receptor B (ETBR)</td>
<td>ET-1</td>
</tr>
<tr>
<td>Ephrin B2</td>
<td>EphB4</td>
</tr>
<tr>
<td>EphB4</td>
<td>Ephrin B2</td>
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<tr>
<td>Tie-2</td>
<td>Angiopoietins</td>
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<tr>
<td>VEGFR-2</td>
<td>VEGF</td>
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<tr>
<td>von Willebrand Factor (vWF)</td>
<td>Factor VIII</td>
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<tr>
<td>Uptake of Ac-LDL</td>
<td>Scavenger receptor</td>
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Model organisms in vascular research
Model organisms in vascular research

THE CHICKEN EMBRYO
an experimental model for development and angiogenesis

- Accessibility at all developmental stages
- Chorioallantoid membrane (CAM)
- Shell-less culture
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<tr>
<th><strong>Advantages</strong></th>
<th><strong>Limitations</strong></th>
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<tr>
<td><strong>Zebrafish husbandry:</strong></td>
<td><strong>Experimental limits:</strong></td>
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<tr>
<td>• It is relatively inexpensive to obtain and maintain large number of adult and embryo zebrafish.</td>
<td>• Few antibodies against zebrafish proteins are available so far. For this reason it is difficult to perform immunofluorescence analyses in zebrafish samples.</td>
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<td><strong>Genetic manipulation:</strong></td>
<td><strong>Embryo manipulation:</strong></td>
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<td>• Generation of larvae with deletion or overexpression of specific genes can be easily accomplished using available tools (e.g. morpholinos, RNA, mimics)</td>
<td>• Due relative small size of zebrafish larvae, you need skillful and careful trainees to perform experiments.</td>
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<td>• Considerable availability of genetically manipulated zebrafish strains that are defective or have acquired functions for specific gene products (e.g. zinc-fingers, ENU, tilling). And also Transgenic zebrafish lines that express reporter genes in particular cell types are also available in the scientific community.</td>
<td>• As most mammalian tumors grow at 37 °C, it is difficult to study the process of xenograft tumor growth at the optimal temperature.</td>
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<td><strong>Chemical screening:</strong></td>
<td><strong>Technical skills:</strong></td>
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<td>• Addition of active chemical stimulators or inhibitors to the water enables analysis of intervention of these compounds on physiological and pathological processes.</td>
<td>• Microinjection of tumor cells into the perivitelline space of a large number of zebrafish embryos is a tedious procedure and requires highly skillful micro-operations.</td>
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<td><strong>Anatomy of the zebrafish:</strong></td>
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<td>• Optical clarity of zebrafish embryos allows visualization of vascular and hematopoietic cells as well as tumor cell dissemination in living animals</td>
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<td>• Zebrafish embryos allows implantation of mammalian tumor cells, including human and mouse tumor cells, due to the absence of a functional immune system at this stage</td>
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Transgenic zebrafish allows analysis of endothelial cells in living embryos.

- Dorsal Aorta (DA)
- Posterior Cardinal Vein (PCV)
- Dorsal Longitudinal Anastomotic Vessel (DLAV)
- Intersegmental Vessels (Se)
- Caudal Vein
- Capillary Plexus
VEGF is required for vasculogenesis in zebrafish

Microangiography allows high resolution mapping of mature vessels
Performing zebrafish tumor xenograft experiments. Tumor cells (red) are microinjected in the yolk sac of 2 or 3 dpf zebrafish embryo. 24h after injection new vessels (green) sprouted from the subintestinal plexus (SIV) and vascularize the tumor mass. DA, dorsal aorta; PCV: posterior cardinal vein; Se: intersegmental vessel; SIV: subintestinal veins.
Whereas embryos and early stage larvae from zebrafish and other fish species are highly exploited due to their transparent nature, these fish start losing transparency already at 2-3 days of age unless pigmentation is inhibited with chemicals, which themselves may affect the development of the fish. On the other hand, the glass catfish remain transparent throughout their lifespan, in a non-genetically manipulated setting, which is a unique quality of this species.
How the blood vessels are formed?

Blood vessels in the small intestine

Susuma Nishinaga/Science Photo Library
Blood vessel formation

a) vasculogenesis: 
*de novo* blood vessel generation from vascular progenitor cells

b) angiogenesis: 
formation of new blood vessels via extension or remodeling of existing blood vessels
Blood vessel formation

- **Vasculogenesis:**
  a) during embryonic development;
  b) during adulthood associated with circulating progenitor cells

- **Angiogenesis:**
  a) embryonic development
  b) adulthood: wound healing, menstrual cycle, tumor-angiogenesis...
Physiological angiogenesis in adults is restricted to the placenta, uterus, hair growth, and wound healing.
New capillary formation in response to wounding
Angiogenesis may be impaired in many diseases.
Three ways of formation of blood vessels

**Vasculogenesis**
capillaries are formed from vascular progenitor cells

**Angiogenesis**
formation of new blood vessels from pre-existing vessels

**Arteriogenesis**
formation of mature blood vessels; differentiation into veins and arteries

angioblast → bFGF → VEGF

VEGF

Ang1, bFGF

MCP-1, PDGF

capillary

angioblast → VEGF → Ang-2

capillary

angioblast → Ang1, bFGF → MCP-1, PDGF

MCP-1, PDGF

angioblast → VEGF → Ang-2

angioblast → Ang1, bFGF → MCP-1, PDGF

angioblast → VEGF → Ang-2

angioblast → Ang1, bFGF → MCP-1, PDGF

angioblast → VEGF → Ang-2

angioblast → Ang1, bFGF → MCP-1, PDGF

angioblast → VEGF → Ang-2
Major growth factors and receptors involved in blood vessels formation

VEGF – vascular endothelial growth factors
VEGF-A – *crucial mediator of angiogenesis*

VEGF-R – receptors for vascular endothelial growth factors

Angiopoietins (Ang-1, 2)
Tie-2 – receptor for Ang-1, -2

FGFs – fibroblast growth factors

PDGF – platelet-derived growth factor
Angiogenesis

- Majority of vascular development occurs via angiogenesis
- Growth of new blood vessels from existing vessels
- Two distinct mechanisms available
  a) sprouting angiogenesis
  b) intussusceptive angiogenesis
Sprouting angiogenesis

- **Sprouting**: invasion of new capillaries into unvascularized tissue from existing mature vasculature.

![Illustration of sprouting angiogenesis](image)

- This endothelial cell will generate a new capillary branch.
- Pseudopodial process guides the development of the capillary sprout as it grows into the surrounding connective tissue.
- Capillary sprout hollows out to form tube.

![Images of sprouting angiogenesis](image)
Sprouting of developing blood vessels is mediated by specialized endothelial cells localized at the tips of growing capillaries – **TIPS CELLS** --> **MIGRATION**

behind the tip cells, endothelial **STALK CELLS** form the capillary lumen --> **PROLIFERATION**

- **TIP**
  - Induced by VEGF
  - High VEGFR signalling
  - Low Notch signalling
  - Extension of filopodia
  - Highly motile
  - Lead new sprouts
  - Guide migration

- **STALK**
  - High Notch signalling
  - Low VEGFR signalling
  - Non-motile
  - Trail tip cells
  - Lumen morphogenesis
  - Maintain junctions
  - Connect to parent vessel
Intussusceptive or non sprouting angiogenesis

- remodelling of existing vessels
- interendothelial contact is needed
- splits into two vessels

**INTRALUMINAL PILLARS ARE FORMED**

- The endothelial walls of the opposite sides of a vessel migrate to each other, forming an intraluminal pillar
- Subsequently, the pillar is invaded by pericytes and myofibroblasts that deposit extracellular matrix into the pillar
- Finally, several pillars increase in size and fuse with each other, splitting up the initial capillary into two new capillaries
Stages of angiogenesis
Angiogenesis is a dynamic and context determined process.
Main proangiogenic factor

Vascular Permeability Factor = Vascular Endothelial Growth Factor

1983, Dr H. Dvorak

V
P
F

vascular permeability factor
endothelial cell survival factor
endothelial cell proliferation
endothelial cell migration

1989, Dr N. Ferrara
Dr J. Plouet

V
E
G
F
Stages of angiogenesis

1. increase in vessel permeability
2. loosening of pericyte contact
3. proteinase release from endothelial cells
4. digestion of basement membrane and extracellular matrix
5. migration and proliferation of endothelial cells
6. formation of vascular structures
7. fusion of new vessels
8. initiation of blood flow
   - inhibition of endothelial cell proliferation
   - inhibition of the migration of endothelial cells
9. formation of basement membrane
MMPs are pro and anti-angiogenic

PRO-ANGIOGENIC

- Degradation of basement membrane and ECM to allow cell detachment and migration
- Cleavage of VE-cadherin cell-cell adhesion
- Release of active VEGF from ECM stores
- Cleavage of basement membrane to release bFGF and to release and activate TGFβ
MMPs are pro and anti-angiogenic

ANTI-ANGIOGENIC

- Generation of antiangiogenic factors
  - angiostatin from plasminogen
  - endostatin, tumostatin, arrestin, and canstatin from type XVIII and IV collagen
Balance between MMPs and TIMPs

Matrix metalloproteinases (MMPs)
  zinc-dependent endopeptidases

  Tissue inhibitors of metalloproteinases (TIMPs)
Summary of the mechanisms of angiogenesis

(a) Angiogenic factors (FGF, VEGF) bind to EC receptors
(b) MMPs are activated and degrade matrix; EC migrate
(c) $\alpha_v\beta_3$ facilitates EC adhesion, migration
(d) Mesenchymal cells release angiopoietin I which binds to Tie-2
   - Sprouting?
   - Pericyte recruitment?
   - Vessel stabilization?
(e) EC release of PDGF-BB recruits pericytes to EC

arterio/venous differentiation
(ephrrins/Eph)
Angiogenesis is dependent on the balance between pro- and anti-angiogenic mediators.
Angiogenesis may be impaired in many diseases
Tumor angiogenesis

Small avascular tumour

Angiogenic switch
tumour secretion of angiogenic factors is triggered by hypoxia

VEGF  bFGF  EGF

Metastasis formation

Pro-angiogenic factors stimulate
the migration and proliferation of
endothelial cells
rapid tumour growth

Loboda et al., 2012
Different mechanisms of blood formation in tumors by Loboda et al., 2012.
The mechanisms involved in blood vessel formation are evolutionary very conservative.
Dissolved T. rex bone yielded flexible, branching vessels some of which contain cell-like structures.
Presence of VEGF-like proteins in different animals

• In the nematode *Caenorhabditis elegans* four possible homologs of PDGF/VEGF receptors (VER-1 to VER-4) and one ligand (PVF-1) are known.

• PVF-1 has the ability to bind to human receptors VEGFR-1 and VEGFR-2 and to induce angiogenesis in two model systems derived from vertebrates.

Drosophila melanogaster respiratory (tracheal) system

- Branching tubular system of trachea delivers oxygen to the tissues of insects.

- Its development shows parallels to the angiogenesis

- Branchless (a homolog of mammalian FGF), PVF1, PVF2, PVF3 (homologs of mammalian VEGF/PDGF) and PVR receptor regulate the migration of early hemocytes and are necessary for formation of tracheal system.

Tracheal tree of Drosophila embryo

DB – dorsal branch; DT – dorsal trunk;
GB – ganglionic branch; VB – visceral branch
Take home messages

1. Endothelial cells form the inner part of blood vessels

2. Three main mechanisms of formation of blood vessels are known

3. Numerous mediators are involved in blood vessels formation

4. Physiological angiogenesis in adults is restricted, but it is a significant component of numerous diseases, such as cancer or atherosclerosis

5. Angiogenesis is a multi-step process including activation of endothelial cells, their proliferation and migration