Vascular stem and progenitors cells

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Overview

- Development of blood vessel system – vasculogenesis, angiogenesis
- Embryonic stem cells
- Alternatives to embryonic stem cells
- Adult stem and progenitor cells and their prospective therapeutic applications
Overview

- Development of blood vessel system – vasculogenesis, angiogenesis
- Embryonic stem cells
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Vascular development – the beginning

Germ layers give rise to development of defined cell types

http://www.hhmi.org/biointeractive/stemcells/animations.html
Molecular mechanisms of stem-cell identity and fate
Fiona M. Watt and Kevin Eggan
EKTODERM

Molecular mechanisms of stem-cell identity and fate
Fiona M. Watt and Kevin Eggan
MESODERM – VASCULAR SYSTEM

Molecular mechanisms of stem-cell identity and fate
Fiona M. Watt and Kevin Eggan
Blood vessel formation

- Two distinct processes:
  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, tumour-angiogenesis…
Vasculogenesis

- The vascular system is one of the earliest organ system that develops during embryogenesis.
- 3 weeks after conception building of hemangioblastic cords.
- One of the first markers of vascular precursors: KDR (VEGF-R2).
- Further important early markers are: Brachyury and c-Kit.
Vasculogenesis

1. First phase
   - Initiated by the generation of hemangioblasts; differentiate in hematopoietic precursors and angioblasts

2. Second phase
   - Angioblasts proliferate and differentiate into endothelial cells

3. Third phase
   - Endothelial cells form primary capillary plexus
Vasculogenesis

- Extraembryonic Vasculogenesis
- Intraembryonic Vasculogenesis
Extraembryonic Vasculogenesis

- First apparent as blood islands in yolk sac
- Blood islands are foci of hemangioblasts
- Differentiate in situ:
  a.) loose inner mass of embryonic hematopoietic precursors
  b.) outer layer of angioblasts
- by the merge of individual blood islands capillary networks are formed
- Yolk sac vasculogenesis communicate with fetal circulation via the vitelline vein
Extraembryonic Vasculogenesis

(A) Yolk sac endoderm

Mesenchyme cells

Blood islands

Primitive hematopoietic precursors

Primitive blood cell

Endothelial cells of blood vesicles angioblasts
Cellular composition of the yolk sac

Human yolk sac with blood island

- Endoderm
- Mesoderm
- Blood island
- Endothelial cell
- Blood
ε-globin-lacZ transgenic mice

**β-LCR**

**pro**

Human ε-globin

**lacZ**

7.5 days pc: blood islands

8.5 days pc: vascular channels

12.5 days pc: vitelline circulation
Intraembryonic vasculogenesis

- para-aortic mesoderm = AGM (aorta-gonad-mesonephros)
- First **dorsal aorta** and **cardinal veins** are built
- **vascular plexus** of the endocardium is generated
- Development of **bilateral embryonic aortae**
- Then allantoic vasculature occurs
Embryonic circulatory system

Diagram showing the embryonic circulatory system with labels for various structures such as chorionic villus, vitelline artery and vein, posterior cardinal vein, common cardinal vein, dorsal aorta, lung bud, pharyngeal pouch IV, aortic arch III, ventral aortic root, anterior cardinal vein, internal carotid artery, placenta, umbilical vein, and yolk sac.
Intraembryonic vasculogenesis

- Subsequent vascular development mainly via angiogenesis

- Some endoderm derived organs, however, are also capable for vasculogenesis
Developmental 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
  - degradation of matrix proteins
  - detachment of ECs
  - migration of ECs
  - proliferation of ECs

Guided by endothelial tip cells and influenced by various attractant and repulsive factors (Ephrin, Netrin, Plexin…)
Sprouting angiogenesis

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 a tube.

Figure 22-25. Molecular Biology of the Cell, 4th Edition.

sprouting.mp4
Intussusceptive angiogenesis

- remodelling of existing vessels
- vessel enlarges
- pinches inward
- splits into two vessels
Intussusceptive angiogenesis

Figure 1. (a–d) Three-dimensional schematic illustrating the steps in the generation of new vascular segments by intussusceptive growth. The process begins with the protrusion of portions of the walls from opposite sides into the vessel lumen (a, b). After contact has been established and fortified (c), the endothelial bilayer becomes perforated centrally and a transmural pillar is formed (d); (a′–d′). Two-dimensional representation of the events depicted in a–d. Endothelial cells (EC) situated on opposite sides of a capillary protrude into its lumen until they contact each other (a′–c′). Once established, this contact is fortified by the formation of interendothelial junctions and then reorganized in such a manner that the endothelial bilayer is perforated centrally. The endothelial cells then retract, and the newly formed pillar increases in girth after being invaded by fibroblasts (FB) and pericytes (Pr), which lay down collagen fibrils (Co in d′). After [30]. (For colored picture see color plate 2.)

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Building of blood vessels in adulthood

- Endothelial precursors
- Intussusceptive growth
- Angiogenic sprouting
- Lymphangiogenesis
Important factors guiding angiogenesis - VEGF
Important factors guiding angiogenesis

- **bFGF**: proliferation, differentiation, maturation
- **TGFβ**: stabilize the mature capillary network by strengthen the ECM structures
- **PDGF**: recruits the pericytes to provide the mechanical flexibility to the capillary
- **MMP inhibitors**: suppresses angiogenesis
- **Endostatin**: binds to VEGF to interfere the binding to VEGFR
Summary part 1

- Vascular system develops from mesodermal germ layer
- Two categories of blood vessel building:
  a.) vasculogenesis: vascular progenitors
  b.) angiogenesis: sprouting, intussusceptive; from preexisting vessels
- Extraembryonic vasculogenesis: yolk sac, blood islands, vascular plexus
- Intraembryonic vasculogenesis: AGM region – dorsal aorta and cardinal veins
- Majority of blood vessels built by angiogenesis (embryo and adult)
- Proangiogenic factors: VEGF, bFGF, angiopoietins
- Maturation and stabilization of blood vessels: TGFβ and PDGF
- Anti-angiogenic: MMP inhibitors, Endostatin
Overview

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- Embryonic stem cells
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Embryonic stem cells as a tool to study vascular development

- Generation of stem cells
- Differentiation of stem cells into various cell types
Stem cells – What kind of cells are they?

Characteristic of stem cells

a.) self renewal: indefinite life span

b.) potency to differentiate into all cell types
Stem cells - differences

- **Origin**
  - embryonic
  - adult

- **Potency**
  - *pluripotent*:
    - can differentiate to most of the cell types
  - *multipotent*:
    - can differentiate into some different cell types
    - (cell type of one organ)
Embryonic stem cells

- Can differentiate into nearly all different cell types of the body

- They are an artificial product: normally they do not occur during embryonic development nor in adult organism

- They can be generated by treating the inner cell mass of blastocysts with a special cytokine cocktail
Where do the blastocytes come from?

- They are generated during \textit{in vitro} fertilization
- Superovulation induced in women (hormonal therapy)
- Egg cells fertilized with sperms in petri dishes
- These fertilized eggs are cultivated \textit{in vitro} until they reach the stage of blastocytes
- Blastocysts are implanted into uterus to induce pregnancy
What happens with blastocytes generated during *in vitro* fertilization?

- During the process of *in vitro* fertilization a lot of blastocytes are generated.
- Only a few of them are used for implantation into the uterus.
- What happens with the rest?
  - a.) stored for second trial to induce pregnancy
  - b.) eternal storage
  - c.) destruction
  - d.) released for adoption
  - e.) offered for scientific research
Generation of stem cell lines

- derived from blastocyst: 3-5 day-old embryo
- Grown on mouse feeder cell layer
- Some clones grow out
- Expanded
- Used for differentiation into various cell types

Creating lines-sm.mov
How to test if cell line is really an embryonic cell line?

A. Subculturing for many months

B. Specific surface markers: Oct-4, Sox2, NANOG

C. Testing if cells are pluripotent: differentiation in various cell types *in vitro*;

D.injecting *in vivo* - teratoma should be built
Stem cell lines

- There are 21 cell lines available, which were generated before August 2001

- USA, Singapore, Korea, Sweden, China,…

- In most of the countries it is allowed to work with these cell lines
Why should new lines be generated?

- Because the existing lines are not completely the same
- Lines contaminated with mouse feeder cells
- Chromosomal defects
- What else???
- Not known
Laws regulating stem cell research in Austria

☐ Art. 17: Freiheit der Wissenschaft gehört zu Grundrecht

☐ It is NOT FORBIDDEN to work with existing stem cell lines (no laws which prohibit this)

☐ Generation of new lines is FORBIDDEN
What can stem cells be used for?

- Reservoir to generate various tissue types
- Can be genetically modified in order to repair gene defects
- A tool to study human embryonic development
What kind of cell types can be generated so far?

- Nerve cells
- Cardiomyocytes
- Pancreatic cells
- Liver cells
- **Endothelial and smooth muscle cells**

List will be highly extended in next years
Differentiation of stem cells to vascular cells
Differentiation of ES cells to vascular cells

- Stem cells cultivated with a defined cocktail mix (BMP-4, VEGF, SCF, Tpo, Flt3-ligand) in serum free medium to generate embryoid bodies

- EBs dissociated and cultivated in specific medium or EBs seeded for outgrowth
$KDR^{\text{low}}/C{-}Kit^{\text{neg}}$ population gives rise to cardiomyocytes, SMCs and Ecs – common progenitor

Lei Yang et al., Nature Letters, 2008
Embryonic stem cells are generated from the inner cell mass of blastocystes (3 – 5 dpc)

Characteristics: indefinite life span, pluripotent – can give rise to nearly every cell type

Primarily cultivated on feeder cells for expansion of undifferentiated cells

Generation of ECs through stimulation with various cytokine cocktail via Embryoid bodies
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Alternatives to human embryonic stem cells

- Stem cells derived from single blastomeres
- Stem cells through nuclear reprogramming – overview
- Induced pluripotent stem cells (iPS) through expression of stem cell specific proteins in differentiated cells
Human embryonic cell lines derived from single blastomeres

Figure 1. Derivation and Characterization of hESC Lines from Single Blastomeres without Embryo Destruction

(A) Stages of derivation of hES cells from single blastomere. (a) Blastomere biopsy, (b) biopsied blastomere (arrow) and parent embryo are developing next to each other, (c) initial outgrowth of single blastomere on MEFs, 6 days, and (d) colony of single blastomere-derived hES cells.
Stem cells through nuclear reprogramming - overview

- Adult and stem cells are **genetically equivalent**
- Differential gene expression is a result of **epigenetic changes** during development
- **Nuclear reprogramming:** reversal of the differentiation state of a mature cell to one that is characteristic of the undifferentiated embryonic state
  - A. Nuclear transfer
  - B. Cell extracts
  - C. Culture induced reprogramming
  - D. Transdifferentiation
Stem cells through nuclear reprogramming – Nuclear transfer
Nuclear exchange to generate stem cells

http://www.hhmi.org/biointeractive/stemcells/animations.html
Transfer of somatic nucleus

- Generate your own stem cells
- Advantage: no immunoreaction
- Disadvantage: extremely inefficient
  not really accepted ethically
Transfer of somatic nucleus

- 2001: J. B. Cibelli et al., MA (USA); 6-cell stadium generated; not reproducible
- 2005: Hwang Woo Suk; cheating
- 2008: Stemagen, CA (USA); claim that they generated 5 embryos; not really accepted
Cybrid embryos - human chromosomes with animal eggs

**Figure 1** The generation of embryonic stem cells through *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI) and somatic cell nuclear transfer (SCNT). Following maturation, the oocyte is fertilized with sperm by either sperm fusion (IVF) or sperm injection (ICSI). Usually, the resultant embryos will be homoplasmic for oocyte mtDNA. For SCNT, the nucleus from a matured oocyte is removed (enucleated) and replaced with a donor somatic cell consisting of a nucleus plus other cytoplasmic components such as mitochondria. Consequently, the reconstructed oocytes will possess either two populations of mtDNA (heteroplasm) or recipient oocyte mtDNA only (homoplasm). The population of mtDNA present in the ESCs will be representative of the mtDNA populations present at fertilization or oocyte reconstruction but, for heteroplasm, one population may be preferentially selected for, which could in turn result in homoplasm.
Stem cells through nuclear reprogramming - overview

Experimental Approach:
Nuclear reprogramming of somatic genome in hybrids generated with pluripotent cells; in most hybrids less differentiated partner is predominant

Mechanistic insights:
Allows study of genetics of reprogramming
Question: chromosomes of somatic cells reprogrammed or silenced; nucleus or cytoblast required for molecular reprogramming

Limitations:
Fusion rate is very low
Tetraploid cells are generated
Stem cells through nuclear reprogramming - overview

**Experimental Approach:**
Exposure of somatic nuclei or permeabilized cells to extracts from oocytes or pluripotent cells;

**Mechanistic insights:**
Allows biochemical and kinetic analysis of reprogramming

**Limitations:**
No functional reprogramming done
Stem cells through nuclear reprogramming - overview

- Pluripotency maintained by a combination of extra- and intracellular signals

- **Extracellular signals**: STAT3, BMP, WNT, …

- **Intracellular signals**: factors at transcriptional level (Oct-4, Nanog, Sox2…)
Induced pluripotent stem cells (iPS)

- Break through!!
- Shinya Yamanaka; Cell; 2006
- Inducing pluripotency in human skin cells by transfer of defined gene combination
- Reproduced in various other labs!!
Induced pluripotent stem cells (iPS)

- Initially 24 genes selected (retroviral vectors)
- Transduced into mouse embryonic fibroblasts
- Resistance gene for G418 under control of Fbx15 promoter, which is only active in pluripotent cells
- Drug resistant colonies appeared, which resembled ES cells
- Expressed transcripts and proteins considered to be part of ES cell signature
- Termed: induced pluripotent stem cells (iPS)
- Formed all three germ layers in vitro and in vivo
- Best combination: Oct-4, Sox2, c-Myc, Klf4
Induced pluripotent stem cells (iPS) – What can be done better?

1. **Better protocols** for more efficient generation of iPS (2007)

2. **Less factors** for inducing iPS:
   - 2007: 3 factors
   - 2008: 2 factors + 2 chemical inhibitors
   - 2 factors only

3. **Reprogramming without modification of DNA**
Induced pluripotent stem cells (iPS) – what can be done better

3. Reprogramming without retroviral vectors
2008: Adenoviral vectors (transient), not very efficient
2008: Plasmids
2009: Transposons for gene transfer: very efficient (DNA segments with ability to move in the genome; vectors for gene manipulation)

4. Using only chemicals for reprogramming next 5-10 years
Induction and Isolation of Vascular Cells From Human-Induced Pluripotent Stem Cells

- Daisuke Taura, Shinya Yamanaka, Kazuwa Nakao et al; Arterioscler, Thromb and Vasc Biol
- Investigation of 3 hES cell lines and 4 iPS lines
- 2D culture system to induce EC differentiation
- 1-5% VE-cadherin+ after 10 days
- Sorted for positive cells
- Similar results from hES and iPS
Transdifferentiation

- Turn one cell type into another in a controlled manner

- Doug Melton, Harvard, succeeded to generate pancreatic β-cells by transducing pancreatic exocrine cells with viral vectors encoding for 3 different factors (Ngn3, Pdx1, Mafa); Nature, 2008
Stem cell therapy in the clinic

- 2009: first prae-clinical trial with human embryonic stem cells
- 1. proposal 4 years ago
- Study has begun in January 2009 in Menlow park California
- Phase I clinical trial (only security not effectivness tested)
- 8-10 paraplegic people
- Neuronal stem cells should be tested
- Controversial: not up to date any more; danger of generation of tumours
Frontiers in stem cell research

- iPS without gene transfer
- Directed, complete differentiation of embryonic stem cells
- Transdifferentiation
- Tumour stem cells – are they involved in tumour development?
Summary part 3

- Generation of ESCs from single blastomere

- Reprogramming of differentiated cells via:
  - nuclear transfer: molecular cloning
  - transduction with stem cell genes – iPS
  - transdifferentiation
Overview

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Adult progenitor and stem cells

- Cells of the body are constantly renewed
- Undifferentiated cells found among differentiated ones
- Can renew themselves (20 to 30 PD, divide rarely)
- Mainly generate cell types of the tissue in which they reside (multipotent)
- Identified in various tissues (bone marrow, brain, blood vessels, muscles, skin, pancreas, stomach, intestine, liver)
# Adult progenitor and stem cells – sources and transdifferentiation

<table>
<thead>
<tr>
<th>Bone Marrow</th>
<th>Adult Stem Cells</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marrow</td>
<td>Peripheral Blood</td>
<td>Brain</td>
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<tr>
<td>Bone</td>
<td>Bone Marrow</td>
<td>Nerves</td>
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<tr>
<td>Cartilage</td>
<td>Blood cells</td>
<td>Blood cells</td>
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<tr>
<td>Tendon</td>
<td>Nerves</td>
<td>Muscle</td>
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<tr>
<td>Muscle</td>
<td>Skin</td>
<td>All Tissues</td>
</tr>
<tr>
<td>Fat</td>
<td>Brain</td>
<td>Cornea</td>
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<tr>
<td>Liver</td>
<td>Smooth Muscle</td>
<td>Retina</td>
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<tr>
<td>Brain/Nerve</td>
<td>Fat</td>
<td>Pancreas</td>
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<tr>
<td>Blood cells</td>
<td>Gastrointestinal</td>
<td>Liver</td>
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<tr>
<td>Heart</td>
<td>Esophagus</td>
<td>Heart</td>
</tr>
<tr>
<td>All Tissues</td>
<td>Stomach</td>
<td>Lung</td>
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<tr>
<td>Stem Cells from Fat</td>
<td>Small Intestine</td>
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<tr>
<td>Bone</td>
<td>Large Intestine/Colon</td>
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<td>Cartilage</td>
<td>Placenta</td>
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<td>Nerves</td>
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<td>Bone</td>
<td>Umbilical Cord Matrix</td>
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<tr>
<td>Cartilage</td>
<td>CORD BLOOD</td>
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<td>Muscle</td>
<td>Various Tissues</td>
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<td>Tendon</td>
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<td>Bone Marrow</td>
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<tr>
<td>Blood vessel</td>
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</tbody>
</table>
Adult stem cells

☐ Task: Maintenance and repair of tissue

☐ Most promising adult stem cells are: MESENCHYMAL stem cells (umbilical cord blood, bone marrow are the sources for these cells)

☐ Problem: only few cells, difficult to isolate
Adult stem cells – vascular progenitors
## Adult progenitor cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Origin</th>
<th>Specific phenotypic markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPCs</td>
<td>Bone marrow, vascular parenchyma, organ-specific EPCs, cord blood</td>
<td>Human: CD133 (AC133)<em>, VEGFR2</em>, VE-cadherin*, CD34*</td>
</tr>
<tr>
<td>CEPs</td>
<td>Peripheral blood, cytokine-mobilized peripheral blood</td>
<td>Mouse: VEGFR2*, Lin&lt;sup&gt;−&lt;/sup&gt;−c-Kit&lt;sup&gt;+&lt;/sup&gt;Sca1*, VE-cadherin*</td>
</tr>
<tr>
<td>Lymphatic endothelial progenitors</td>
<td>Bone marrow</td>
<td>Human: CD133*, VEGFR3*, VEGFR2*</td>
</tr>
<tr>
<td>CECs</td>
<td>Vessel wall mature endothelium</td>
<td>Human: CD133&lt;sup&gt;Neg&lt;/sup&gt;, VEGFR2*, CD146*, VE-cadherin*, CD34*</td>
</tr>
<tr>
<td>CECs</td>
<td></td>
<td>Mouse: VEGFR2*, VE-cadherin*, Tie-2*</td>
</tr>
<tr>
<td>HSCs</td>
<td>Bone marrow</td>
<td>Human: CD34&lt;sup&gt;+&lt;/sup&gt;CD38&lt;sup&gt;Neg&lt;/sup&gt;, CD133&lt;sup&gt;+&lt;/sup&gt;CD34&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>HSCs</td>
<td></td>
<td>Mouse: Lin&lt;sup&gt;−&lt;/sup&gt;−c-Kit&lt;sup&gt;+&lt;/sup&gt;Sca1&lt;sup&gt;+&lt;/sup&gt;Thy&lt;sup&gt;low&lt;/sup&gt;</td>
</tr>
<tr>
<td>HPCs</td>
<td>Bone marrow</td>
<td>Lineage-specific markers (common marker: CD45+, CD133&lt;sup&gt;Neg&lt;/sup&gt;)</td>
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<tr>
<td></td>
<td></td>
<td>Myeloid: CD11b&lt;sup&gt;+&lt;/sup&gt;, CD14&lt;sup&gt;+&lt;/sup&gt;, CD15&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>Erythroid: Glycophorin-A&lt;sup&gt;+&lt;/sup&gt;, Ter-119&lt;sup&gt;+&lt;/sup&gt;</td>
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<td></td>
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<td>Megakaryocytic: CD41a&lt;sup&gt;+&lt;/sup&gt;, CD42b&lt;sup&gt;+&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>Lymphoid: CD3&lt;sup&gt;+&lt;/sup&gt;, CD4&lt;sup&gt;+&lt;/sup&gt;, CD8&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Common to EPCs, CEPs, HSCs and HPCs</td>
<td></td>
<td>CD31 (PECAM)&lt;sup&gt;+&lt;/sup&gt;, vWF&lt;sup&gt;+&lt;/sup&gt;, BS-1 or <em>Ulex europeaus</em> binding,</td>
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<tr>
<td></td>
<td></td>
<td>Ac-LDL uptake, VEGFR1&lt;sup&gt;+&lt;/sup&gt;, CD146 (S-endo-1, P1H12)&lt;sup&gt;+&lt;/sup&gt;, CD34&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Mobilization of vascular progenitors

- Steady state 0.01% MNCs in blood are CEPs
- Amount of **circulating progenitors is increased** after trauma, infectious injuries, tumour growth, chemotherapy, vascular disruptive agents
- 24 h after injury: 12%
- Tissue ischemia results in recruitment progenitor cells
Mobilization of vascular progenitors

Mobilization mediated in response to various cytokines:

- **SDF-1α** (stroma cell derived factor 1α)
- Metalloproteinases (**MMP9**)
- **VEGF**
- **PLGF** (placental growth factor)
- **G-CSF** (granulocyte-colony stimulating factor)
- **CCL2, CCL5** (chemokine ligands)
Isolation of adult vascular progenitors

A. Mononuclear cells from peripheral blood seeded on fibronectin (or kanguru collagen) coated tissue culture plates; some cells adhere and build colonies – EPC

B. FACS sorting of cells: CD34+, KDR+, CD133+

C. In vitro colony forming cell assay: ECFC (endothelial colony forming cell assay)
Prospective therapeutic applications

- Tumour homing – Trojan horse principle
- Organ revascularization and regeneration
- Wound healing
- Heart diseases
- Blood diseases
Tumour homing – Trojan horse principle

EPCs home to places of active neoangiogenesis

Vascular progenitors transduced with a therapeutic gene

Vehicle for targeting therapeutic gene expression to tumour

Bystander effect of advantage

Gene directed enzyme prodrug therapy (CYT-P450-Ifosfamide; HSV-TK/Ganciclovir)
Organ vascularization and regeneration

- After pathological ischemic events in the body, exogenous introduction of vascular progenitors may facilitate restoration.
- Bone marrow, rich reservoir of tissue-specific stem and progenitor cells.
- Possible applications: ischemic limbs, postmyocardial infarction, endothelialization of vascular grafts, atherosclerosis, retinal and lymphoid organ neovascularization.
Potential use of adult stem and progenitor cells
Peripheral Artery Disease

Figure 5. Angiographic analysis of collateral vessel formation in patients in group A. Collateral branches were strikingly increased at (A) knee and upper-tibia and (B) lower-tibia, ankle, and foot before and 24 weeks after marrow implantation. Contrast densities in suprafemoral, posterior-tibial, and dorsal pedal arteries (arrows) are similar before and after implantation.

Tateishi-Yuyuama et al., The Lancet, Aug, 2002
Impaired wound healing

Figure 4. Limb salvage after marrow implantation in two patients in group ANon-healing ulcer on heel (A) and ischaemic necrosis on big toe (B) showed improvement 8 weeks after implantation.

Tateishi-Yuyuama et al., The Lancet, Aug, 2002
Summary part 4

- Adult progenitors are undifferentiated cells with capacity to proliferate
- Can be found in many different tissues
- Differentiate to resident cell types (cell type of one organ)
- Can be isolated by sorting for progenitor markers, differentiated and used for clinical applications
- Various potential applications
- Tissue vascularization and regeneration, heart diseases, …
- Ongoing preclinical and clinical trials
Literature I

Endothelial Biomedicine
William C. AIRD
Cambridge University Press, 2007

Yolk Sac with Blood islands
New England Journal of medicine
Volume 340:617, February 1999

Developmental Biology, 8th Edition
Sinauer Associates, 2006

Hematopoietic induction and respecification of A-P identity by visceral endoderm signaling in the mouse embryo
Maria Belaoussoff et al.
Development, November 1998

Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population
Lei Yang et al.

Generation of functional hemangioblasts from human embryonic stem cells
Shi-Jiang Lu et al.
Nature Methods, May 2007

Human embryonic stem cell lines derived from single blastomeres
Irina Klimanskaya et al.
Nature Letters, November 2006
Literature II

Nuclear Reprogramming and pluripotency
Konrad Hochedlinger et al.
Nature, June 2006

Human-animal cytoplasmic hybrid embryos, mitochondria, and an energetic debate
Justin St Jon et al.
Nature Cell Biology, September 2007

Induced pluripotency and cellular alchemy
Anthony C F Perry
Nature Biotechnology, November 2006

Endothelial progenitor cells for cancer gene therapy
K-M Debatin et al.
Gene Therapy, 2008

Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration
Shahin Rafii et al.
Nature Medicine, June 2003

Translating preclinical finding of (endothelial) progenitor cell mobilization into the clinic; from bedside to bench and back
J.M.L. Roodhart et al.
Biochimica et Biophysica Acta, April 2009
Emerging potential of transposons for gene therapy and generation of induced pluripotent stem cells
T. VandenDriessche et al.
Blood, May 2009

Induction and Isolation of Vascular Cells Fromm Human-Induced Pluripotent Stem Cells
D. Taura et al.
Arterioscler Thromb Vasc Biol, April 2009

Transposon-mediated genome manipulation in vertebrates
Z. Ivics et al.
Nature methods, June 2009

Multipotent Progenitor Cells Are Present in Human Peripheral Blood
D. Cesselli et al.
Circulation Research, 2009

The definition of EPCs and other bone marrow cells contributing to neoangiogenesis and tumour growth: Is there common ground for understanding the roles of numerous marrow-derived cells in the neoangiogenic process?
M.C. Yoder et al.
Biochimica et Biophysica Acta, April 2009

Somatic Cell Nuclear Transfer in Humans; Pronuclear and Early Embryonic Development
J.B. Cibelli et al.
The Journal of Regenerative Medicine, November 2001
Literature IV

Induction of Pluripotent Stem Cells form Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors
K. Takahashi, Shinya Yamanaka
Cell, August 2006

Induced pluripotent Stem Cell Lines Derived from Human Somatic Cells
J. Yu et al,
Science, December 2007

Induced Pluripotent Stem Cells Generated Without Viral Integration (Adenoviral vectors)
M. Stadtfeld et al;
Science, September 2008

Generation of Mouse Induced Pluripotent Stem Cells Without Viral Vectors
K. Okita, S. Yamanaka et al.
Science, November 2008

In vivo reprogramming of adult pancreatic exocrine cells to β-cells
Q. Zhou et al.
Nature, October 2008