Endothelial Progenitor Cells for tissue regeneration

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Isolation of Putative Progenitor Endothelial Cells for Angiogenesis

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The demonstration that HSCs from peripheral blood can provide sustained hematopoietic recovery is inferential evidence for circulating stem cells (5). Here, we have investigated the hypothesis that peripheral blood contains cells that can differentiate into ECs (6). We exploited two antigens that are shared by angioblasts and HSCs to isolate putative angioblasts from the leukocyte fraction of peripheral blood. CD34 is expressed by all HSCs but is lost by hematopoietic cells as they differentiate (7). It is also expressed by many including most activated ECs in the adult (8). Flk-1, a receptor for vascular endothelial growth factor (VEGF) (9), is also expressed by both early HSCs and ECs but ceases to be expressed during hematopoietic differentiation (10, 11).

CD34-positive mononuclear blood cells (MBs) were isolated from human peripheral blood by means of magnetic beads
Stepwise development of vessels

E17-18 (human)

Endothelial progenitor cell: a blood cell by many other names may serve similar functions

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<thead>
<tr>
<th></th>
<th>CFU-Hill</th>
<th>CAC</th>
<th>ECFC</th>
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<tbody>
<tr>
<td>Clonal proliferative status</td>
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<td>+</td>
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<td>Replacing ability</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>In vitro tube formation</td>
<td>+/-</td>
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<td>In vivo de novo vessel formation</td>
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<td>Homing to ischemic sites in vivo</td>
<td>+</td>
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<tr>
<td>Paracrine augmentation of angiogenesis</td>
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<tr>
<td>Phenotypic appearance</td>
<td>CD34⁺⁻⁻</td>
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<td></td>
<td>CD133⁺</td>
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<td>VEGFR2⁺</td>
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<td>CD45⁺⁻⁻</td>
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Yoder MC. J mol med. 2013
EPC and Stem Cells

Pluripotent SC

Ectodermal SC

Tissue SC

Skin SC

Epithelial cell

Neurons

Astrocyte

Oligodendrocyte

Melanocyte

Hair follicle

Neural SC

Hemangioblast

Hematopoietic SC

Lymphoid progenitor cell

Myeloid progenitor cell

T-lymphocyte

B-lymphocyte

NK cell

Erythrocyte

Platelet

Dendritic cell

Hematopoietic SC

Granulocytes

Endothelial progenitor cell

Endothelial cell

Vascular SC

Pericyte

Epiblast-like pluripotent SC

Mesodermal SC

Mesenchymal SC

Mesenchymal SC

Liver SC

Pancreatic SC

Intestinal SC

Endodermal SC

Osteoblast

Chondroblast

Myoblast

Cardiomyoblast

Pre-adipocyte

Fibroblast

Osteocyte

Chondrocyte

Myocyte

Cardiomyocyte

Adipocyte

Fibroblast

Smooth muscle cell

Hepatocyte

Islet cell

Intestinal cells

Asahara Am J Physiol Cell Physiol 287: C572–C579, 2004
EPC for cell therapy

Kalka et al. PNAS. 2000
Engrafted EPC fade away within a week after transplantation.

EPC: modes of action

Mobilization

Engraftment

Re-endothelization

Paracrine

Secretion of Cytokines

Angiogenesis

Vasculogenesis
Novel therapeutic approach: Cell-free (EPC-CM)

Similar or superior regenerative properties as Cell transplantation

Di Santo et al., Plos ONE 2009
EPC-CM for brain research?
Vascular and Nervous: coupled systems
Neurodegenerative disease

Vascular and Nervous: coupled systems
Development

<table>
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<tr>
<th>Organism</th>
<th>Role of VEGF in the development of Nerves</th>
<th>Role of VEGF in the development of Vessels</th>
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<td>Caenorhabditis elegans</td>
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<td>Drosophila melanogaster</td>
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<td>No (?)</td>
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<td>Homo sapiens</td>
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Angioneurins discovered through their neuronal effects (NGF, BDNF, NT3, NT4)

Angiogenesis
Neuroprotection

Angiogenesis
Neuroprotection
Other pleiotropic activities

Other angiogenic factors (VEGF, PDGF, ANG)

BDNF
TrkB receptor
Survival factor during neurodevelopment
Less important for adult neurons and angiogenesis

VEGF
Receptor 2
Angiogenesis
Neuroprotection

TGFβ1
IGF1
TGFβ type II receptor
Angiogenesis
Neuroprotection
Other pleiotropic activities

Nature Reviews | Neuroscience
EPC-CM: set-up

Peripheral blood

„Endothelial Priming“ of MNC

7 days

EPC

In vitro

Collect medium

Centrifugation

In vivo

Filtration

Freezing

Hypoxia

2 days
Experimental Approach

Effects of EPC-CM on neurons / brain Endothelial Cells

Effects of EPC-CM on brain resident Neuronal Stem/Progenitor Cells
Results
Effects of EPC-CM on brain Endothelial Cells

rBCEC4 cells
Experimental Methods

2. Migration
Endothelium

3. Proliferation

4. Vascular Tube Formation
(Matrigel)

Wound Healing Assay
(Scratch Test)

Presto Blue Assay

Tube Formation Assay

Experimental Methods

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<tr>
<th>a</th>
<th>b</th>
<th>c</th>
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<tbody>
<tr>
<td>Confuent monolayer</td>
<td>MDCK/CAM/</td>
<td>MDCK-dual/</td>
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<tr>
<td>Scratch the monolayer surface carefully and wash (Time 0 h)</td>
<td>Epi-thelial behavior (adhesive unidirectional migration)</td>
<td>Mesenchymal behavior (random migration)</td>
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<tr>
<td>Take pictures at timely intervals of the wounded area until its closure</td>
<td></td>
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<tr>
<td>0 h</td>
<td>6 h</td>
<td>12 h</td>
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Viable Cell
Reduction Reactions

Resazurin

Resorufin

Emit's fluorescence at 590nm
EPC-CM is angiogenic for brain endothelial cells

**Migration**

- Ctr
- CM

**Tube formation**

- Ctr
- CM

**Proliferation**

- Ctr
- CM

**Vessel sprouting**

- Ctr
- CM

* indicates statistical significance.
Cytoprotective properties of EPC-CM

OGD
6 hrs
95% N2/5% CO2
Glucose free medium

Reperfusion
24 hrs
20% O2/5% CO2
5 mM Glucose

OGD= Oxygen and Glucose Deprivation

OGD/CM
OGD/Ctrl
Control

In progress: IL-8 neutraliz. and prot. K
Effects of EPC-CM on neurons rat brain embryonic primary cultures
Experimental Set-up
Fetal rat brain cell cultures

Cerebral cortex (CTX)
Striatum (GE)
Midbrain (VM)
EPC-CM increases the number of neurons (β-III-tubulin) in CTX cultures
EPC-CM increases TH-positive cell densities in VM cultures
EPC-CM is neuroprotective against MPP+ induced toxicity in VM cultures
EPC-CM increases striatal GABA-positive cell densities but fails to protect against 3-NP induced toxicity
In vivo effects of EPC-CM on NSC
In vivo effects of EPC-CM on NSC: Doublecortin (DCX)

Kemperman G. et al., Trends in Neurosciences, 2004
Subventricular Zone (SVZ)

Neuronal lineage:

- B → C → A → Immature and mature neurons

Cell identity:
- SVZ astrocyte
- Putative stem cell
- Transit amplifying progenitor
- Migrating neuroblast

Identified markers:
- GFAP
- Vimentin
- Nestin
- Dlx2
- PSA-NCAM
- Dlx2
- Tuj1
- Hu
- DCX, calretinin
- NeuN
- TH
- Calbindin
- GAD65

Abrous D.N: Physiological Reviews 2005
Subgranular Zone-Hippocampus (SGZ)

Abrous D.N: Physiological Reviews 2005
Experimental Set-up
EPC-CM injection
Experimental Set-up
Areas analyzed
EPC-CM promotes DCX cells in the SVZ

![Image of brain sections with DCX staining under Ctr and CM conditions with corresponding quantification graphs showing increased cell numbers in the SVZ regions V1, V2, and V3 under CM treatment compared to Ctr, marked with asterisks indicating statistical significance.](image-url)
EPC-CM promotes DCX cells in the SGZ
Summary (I)

• EPC-CM promotes brain endothelial cells functions

• EPC-CM protects brain endothelial cells against in vitro ischemia

• EPC-CM promotes neuronal viability
  ➢ Dopaminergic cells (relevance for Parkinson’s Disease?)
Summary (II)

• EPC-CM has specific neuroprotective capacity

• EPC-CM increases the number of neuronal progenitors in the neurogenic niches of naïve rat brain.
Angiogenic factors in EPC-CM

A

B

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