Biology of perivascular progenitor cells

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“Basic Mechanisms translated to the Clinic”
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Structure of presentation

• Introducing mesenchymal stem cells (MSCs).
• Revisiting the identity/equivalence of perivascular MSCs, adventitial progenitor cells and pericytes.
• Illustrating the biology and therapeutic prospect of pericytes and adventitial progenitor cells.
The family of Mesenchymal Stem Cells (MSCs)

(ii) possessing self-renewal capacity and ability to differentiate \textit{in vitro} into chondrogenic, osteogenic, adipogenic and myogenic lineages,

(iii) expressing CD73, CD90 and CD105 and being negative for CD34, CD11, CD19, CD45, CD79a, CD14, histocompatibility locus antigen HLA-DR.
The term MSC was coined by Caplan after Friedenstein’s discovery of multipotent stromal cells endowed of self renewal and plasticity.

Uccelli et al. Nature Rev Immunology, 2008
MSCs are part of the stromal cell pool that support the endosteal and vascular niches in bone marrow

Uccelli et al Nature Rev Immunology, 2008
Interaction between MSCs and cells of acquired and innate immunity

Uccelli et al Nature Rev Immunology, 2008
MSCs are abundantly present in adult tissues: the adipose tissue paradigm.
Pericytes: stabilizers of the vasculature

Hamilton et al. Frontiers in Neuroenergetics 2010

Pericytes: stabilizers of the vasculature

NG2+ pericytes in mouse heart

Hamilton et al. Frontiers in Neuroenergetics 2010

Mitchel and Madeddu, unpublished

Berry et al. Circ Cardiovasc Imaging 2010
Perivascular CD146+ pericytes are present in different organs

Crisan et al. Cell Stem Cell 2008
CD146+ Pericytes expanded in culture regenerate skeletal muscle in dystrophin-deficient mice

Crisan et al. Cell Stem Cell 2008
- The presence of early progenitor cells, named mesoangioblasts because of their ability to differentiate into endothelial cells and other mesodermal lineages, has been already demonstrated in the embryonic dorsal aorta.

- Moreover, hematopoietic stem cells are generated from *hemogenic endothelium* in the embryonic aortic wall.

Minasi MG, Riminucci M, De Angelis L, *et al.* The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues.

Hypothetical scheme of the `vasculogenic zone'

Zengin E et al. Development 2006;133:1543-1551
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<thead>
<tr>
<th></th>
<th>Pericytes</th>
<th>Adventitial cells</th>
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<tbody>
<tr>
<td>Perivascular location</td>
<td>Capillaries and microvessels</td>
<td>Large vessels</td>
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<tr>
<td>Human tissue origin</td>
<td>Adult, foetal and embryonic skeletal muscle and pancreas, adult WAT, foetal skin, small intestine, brain, foetal and embryonic BM, term and mid-term placenta</td>
<td>Adult WAT, foetal skeletal muscle, lung and BM</td>
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<tr>
<td>FACS selection</td>
<td>CD146+CD34-CD56-CD45-</td>
<td>CD34+CD31-CD146-CD45-</td>
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<td>Markers in vitro</td>
<td>CD146, NG2, PDGFRβ, αSMA, CD90, CD73, CD105, CD44, ALP, nestin, vimentin</td>
<td>CD34, CD90, CD73, CD105, CD44, vimentin</td>
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<td>Markers in vivo</td>
<td>CD146, NG2, PDGFRβ, α SMA, CD90, CD73, CD105, CD44, ALP</td>
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<td>Documented differentiation</td>
<td>Osteogenic, adipogenic, chondrogenic, myogenic</td>
<td>Osteogenic, adipogenic, chondrogenic, pericytic</td>
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<td>potential</td>
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<td>Cell Line Identifiable Markers</td>
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<td><strong>Mesangioblasts</strong></td>
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<td>Mesenchymal: CD13&lt;sup&gt;+&lt;/sup&gt;, CD73&lt;sup&gt;+&lt;/sup&gt;, CD44&lt;sup&gt;+&lt;/sup&gt;, CD49b&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Morosetti, 2011, Acta Myol.</td>
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<td>Pericyte: NG2&lt;sup&gt;+&lt;/sup&gt;, CD105&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>Endothelial: Tie2&lt;sup&gt;+&lt;/sup&gt;, KDR&lt;sup&gt;+&lt;/sup&gt;, CD31&lt;sup&gt;+&lt;/sup&gt;, CD34&lt;sup&gt;+&lt;/sup&gt;</td>
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<td><strong>Cordblood MSC</strong></td>
<td>Bosch, 2012, Stem Cell Dev</td>
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<td>Mesenchymal: CD71&lt;sup&gt;+&lt;/sup&gt;, CD73&lt;sup&gt;+&lt;/sup&gt;, CD80&lt;sup&gt;+&lt;/sup&gt;, CD105&lt;sup&gt;+&lt;/sup&gt;</td>
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<td><strong>Humbelical cord perivascular cells</strong></td>
<td>Bosch, 2012, Stem Cell Dev</td>
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<td>Mesenchymal: CD56&lt;sup&gt;+&lt;/sup&gt;, CD71&lt;sup&gt;+&lt;/sup&gt;, CD73&lt;sup&gt;+&lt;/sup&gt;, CD90&lt;sup&gt;+&lt;/sup&gt;,</td>
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<td>Pericyte: RGS5&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>Endothelial: CD34&lt;sup&gt;+&lt;/sup&gt;</td>
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<td><strong>Human foetal aorta VPCs</strong></td>
<td>Invernici, 2007, Am. J. Pathol.</td>
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<td>Mesenchymal: desmin&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Invernici, 2008, Cytotechnology</td>
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<td>Corselli, 2012, Stem Cells Dev</td>
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<td>aSMa&lt;sup&gt;+&lt;/sup&gt;, ALP</td>
<td>Crisan, 2008, Cell Stem Cell</td>
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<td>Psatis, 2011, J. Cardiovasc. Tranl. Res.</td>
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<td><strong>Vascular wall resident multipotent SCs</strong></td>
<td>Ergun, 2010, Antioxid Redox Signal</td>
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<td>Mesenchymal: CD73&lt;sup&gt;+&lt;/sup&gt;, CD44&lt;sup&gt;+&lt;/sup&gt;, CD90&lt;sup&gt;+&lt;/sup&gt;, desmin&lt;sup&gt;+&lt;/sup&gt;,</td>
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<td><strong>SVPs</strong></td>
<td>Campagnolo, 2010, Circulation</td>
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Adventitial Progenitor Cells Contribute to Arteriosclerosis

Hu et al. J Clin Invest. 2004

Evelyn Torsney, Yanhua Hu, Qingbo Xu
Trends in Cardiovascular Medicine Volume 15, Issue 2 2005 64 - 68
Human fetal aorta contains vascular progenitor cells capable of inducing vasculogenesis, angiogenesis, and myogenesis.


Spheroids

Clonogenic assay

Immunocytochemistry

Tie-2
Desmin

Human fetal aorta vascular progenitor cells transplantation in a murine model of peripheral ischemia

A convenient source of autologous progenitor cells
Localisation of SVPs in human saphenous vein adventitia

Campagnolo et al. Circulation 2010
Expansion and characterization of SVPs from polyclonal preparations

Campagnolo et al. Circulation 2010
Current standard operating protocol

- Current SOP allowed successful expansion in 63% of 35 tested lines, which reached the therapeutic target of 30-50 million viable SVPs at passage 8 (P8) in ~10 weeks.
- Functional tests in 15 SV pericyte (SVP) lines, of which 10 derived from leftovers of coronary artery bypass grafts (CABG-SVP) and 5 from wastes of varicose SV from subjects with no evidence of coronary disease (NC-SVP)
The roadmap to first-in-man clinical trial

- Expansion SOP
- Limb ischemia
- Myocardial Infarction
- Mechanisms
- Combination cardiac stem cells
- GMP upgrading
- Safety
- Swine MI model
- First-in-man Study

SOP: Standard Operating Procedure
GMP: Good Manufacturing Practice
TSCR: Translational Scientific Collaboration Research
MRC: Medical Research Council
NIHR: National Institute for Health Research
BHF: British Heart Foundation
NIHR: National Institute for Health Research
GMP upgrade and quality controls
NHS-BT, NHS-BLG and Cambridge Cancer UK
SVPs transplantation in a limb ischaemia model

Protocol:
Male 8-week-old CD1 Foxn1
nu/nu mice underwent operative limb ischaemia and 1 day later received 8× 10^4 DiI-labeled SVPs (passage 6), so-called early-culture endothelial progenitor cells, or vehicle (DMEM, 30 μL) in 3 different points of the ischaemic adductor muscle (n=7 mice per group).

Endpoints:
Blood flow recovery and reparative angiogenesis 14d post-ischaemia
SVPs transplantation in a acute MI model

Protocol:
MI was induced in 8-week-old male immunocompetent CD1 mice (n= 13 per group) by occlusion of the left anterior descending coronary artery, followed by injection of Dil-stained SVPs (1×10^6 cells per heart), human bone marrow mesenchymal stem cells (MSCs; 1×10^6 per heart) or PBS at 3 different sites along the infarct border zone.

Endpoints: Late functional recovery and angiogenesis.
SVPs transplantation Improves neovascularization

Katare et al. Circ Res 2011
Tracking cell engraftment in the ischaemic limb

Campagnolo et al. Circulation 2010
Tracking cell engraftment in the infarcted heart

Katare et al. Circ Res 2011
Mechanisms of therapeutic action
Acknowledgments

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