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Hematopoietic stem cell transplantation for cardiac and peripheral vascular disease

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Summary:

Recent studies have suggested that marrow and blood hematopoietic stem cells may contribute to nonhematopoietic tissue repair in multiple organ systems. In animal models and more recently in limited human trials, unpurified marrow mononuclear cells and/or subsets of adult hematopoietic stem cells have been reported to contribute to neoangiogenesis. Since the subset of hematopoietic stem cells (HSCs) that are both CD34$^+$ and AC133$^+$ are endothelial cell precursors, clinical trials using autologous AC133$^+$ HSCs isolated with the Miltenyi CLIMACS cell separator and transplanted into patients with ischemic and refractory peripheral vascular or coronary artery disease are being implemented at Northwestern University.
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The field of adult stem cell plasticity is developing so rapidly that terminology is lagging. Most tissues or organ systems have a stem cell compartment to repair damage or replace aged cells. Previously it had been assumed that adult stem cells are faithful to a tissue-specific lineage. Recently, the term plasticity has been coined and refers to the ability of adult stem cells and in particular hematopoietic stem cells (HSCs) to repair tissues other than their original tissue compartment. Metamoirosis, a Greek term for 'change in fate' may be more appropriate than the term plasticity (verbal communication Diane Krause, Yale University). The metamoirosis or plasticity debate will probably continue for sometime and revolves around questions like: How purified or conversely heterogeneous was the hematopoietic 'stem' cell population used in the experiment? What markers are used to demonstrate hematopoietic stem cell origin? What markers are used to determine that the hematopoietic stem cell has undergone metamoirosis to a new tissue lineage? What assays are used to demonstrate normal functionality of the tissue-specific cell derived from a hematopoietic stem cell?

Terminology for vessel formation in adults from hematopoietic stem cells is similarly lagging. In the field of vessel formation, angiogenesis refers to the development of new blood vessels from already existing vasculature. In contrast, vasculogenesis is the embryonic genesis of new vessels. Therefore, in this paper, the term neoangiogenesis will refer to metamoirosis of blood stem cells into new blood vessels, a postembryonic vasculogenesis occurring in the adult.

Despite these caveats that require ongoing critical assessment, experimental data support metamoirosis of adult hematopoietic stem cells into the liver,[1–4] brain,[5] skeletal muscle,[6] heart,[7] lung,[8,9] kidney,[10] gut,[11] and skin.[11] Again for this paper, discussion will be restricted to evidence for neoangiogenesis, identification of the hematopoietic progenitor cell subset involved in neoangiogenesis, and the potential of autologous hematopoietic stem cell transplantation to treat ischemic vascular diseases.

Evidence supporting neoangiogenesis

A stem cell is characterized by both self-renewal and differentiation into functionally distinct lineages. Hematopoietic stem cells may be CD34$^+$ AC133$^+$ or CD34$^-$ AC133$^+$ or CD34$^+$ AC133$^-$. Vascular development is regulated by growth factors and their receptors such as vascular endothelial growth factor (VEGF) and VEGF tyrosine kinase receptors such as VEGFR-1 (flt-1) or VEGFR-2 (KDR or flk-1).[12] Other growth factors such as angiopoietin-1 that bind a tyrosine kinase receptor Tie-2 (pronounced Tek) may be involved in completing the vascular architecture by assembling pericytes and smooth muscle cells around endothelial cells.[12] Marrow or peripheral blood CD34$^+$ hematopoietic stem cells express VEGFR and Tie.[13] When cultured *ex vivo* in fibronectin-coated flasks with VEGF, CD34$^+$ AC133$^+$ cells differentiate into endothelial cells characterized by morphology, acetylated low-density lipoprotein (LDL) incorporation, nitric oxide (NO) release, Von Willebrand factor expression, and lectin binding.[14]

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In animal models, marrow mononuclear cells injected into ischemic extremities or hibernating myocardium improve regional blood flow.[15,16] The unfractionated mixture of hematopoietic mononuclear cells includes more differentiated cells that are thought to provide angiogenic cytokines as well as stem cells that become incorporated into collateral vessels by a process of neoangiogenesis. In clinical trials, Tateishi-Yuyama *et al* injected autologous bone marrow mononuclear cells into patients with ischemic peripheral vascular disease (PVD).[17] Patients were selected for chronic ischemic extremity pain or nonhealing ischemic ulcers or both and a resting blood pressure ankle-brachial index (ABI) less than 0.6 (normal >1.0). Bone marrow cells were collected under general anesthesia from the posterior superior iliac crest and with a 26-gauge needle injected into the gastrocnemius muscle of the ischemic leg in multiple sites divided by a 3×3 cm grid. Significant improvement in the ABI, transcutaneous oxygen pressure, and pain-free walking occurred following treatment.

Hung-Fat Tse *et al* injected autologous bone marrow mononuclear cells into ischemic myocardium using a catheter guided by electromechanical mapping.[18] Patients were selected for stable angina refractory to medical therapy. The ischemic area was injected intramyocardially with a mixture of $CD34^+$, $CD3^+$ T cells, and granulocytes. Following treatment, the number of anginal episodes and nitroglycerin tablet usage decreased. Postinjection cardiac MRI demonstrated improved wall motion and thickness.

The mixture of marrow mononuclear cells used in the above experiments makes it impossible to interpret as to which cells were therapeutic and whether repair was from cytokine-induced local repair and/or neoangiogenesis from the injected marrow stem cells or both. These human trials did demonstrate that cells could be injected safely without arrhythmias, ectopic calcification or vessel thrombosis and provided sufficient evidence of efficacy to merit further studies. Stamm *et al* purified $AC133^+$ stem cells from the bone marrow and injected the purified HSC into the border zones of myocardial infarcts at the time of coronary artery bypass grafting (CABG).[19] The procedure was safe without complications and neoangiogenesis was suggested by improved myocardial perfusion scintigraphy (SPECT). However, reasons for beneficial myocardial performance are confounded by the CABG.

Autologous hematopoietic stem cell transplantation for peripheral vascular disease or ischemic cardiac disease

Patients with PVD or coronary artery disease (CAD) who are not surgical candidates are being considered at Northwestern University for trials of neoangiogenesis from autologous $AC133^+$ HSC. Mobilizing or harvesting stem cells in patients with vascular atherosclerosis has potential risks. Cytokines such as G-CSF that are used for peripheral blood stem cell mobilization have in rare reports been associated with thrombosis and angina pectoris.[20] Alternatively, general anesthesia to harvest marrow from the ileum has increased risks in patients with either CAD or PVD (who probably also have CAD). After collection, $AC133^+$ stem cells may be purified with the Miltenyi CLINIMACS device. Injection of HSC into a peripheral extremity may be performed cutaneously under local anesthesia using a 26-gauge needle into grid-marked sections of the ischemic gastrocnemius muscle. Cardiac injection is a higher risk procedure that may be complicated by arrhythmias or angina. Routes of administration include a mini-thoracotomy transthoracic approach or an intramyocardial approach with an injection needle catheter advanced from the femoral artery.

Regeneration of cardiac myocytes

$AC133^+$ HSC have a potential role for therapeutic trials of neoangiogenesis in ischemia vascular disease. Besides endothelial and vascular smooth muscle cells, HSC may also undergo metamoirosis into cardiac myocytes. Following ligation of murine coronary arteries and injection of green fluorescent protein (GFP^+) marrow stem cells (Lin^- $ckit^+$), an area of regenerating GFP^+ cardiomyocytes may be visualized 9 days after injection.[7] Orlic *et al*[21] have also demonstrated myocardial regeneration in murine models using G-CSF and stem cell factor (SCF) to mobilize HSCs into the peripheral blood around the time of coronary artery ligation and myocardial infarction. These results are being confirmed at Northwestern University in a coronary artery ligation/reperfusion model. GFP^+ transgenic mice are used as donors for marrow transplantation of normal Balb/c mice. The resulting chimeric mice whose blood and marrow are GFP^+ undergo coronary artery ligation and 1 hour later reperfusion followed by either injection with GFP^+ marrow into the infarct site or treatment with G-CSF for 7 days to mobilize HSCs into the blood. When evaluated 1 month later, virtually all cells within an infarcted area were GFP^+ (Figure 1) and therefore arose from the

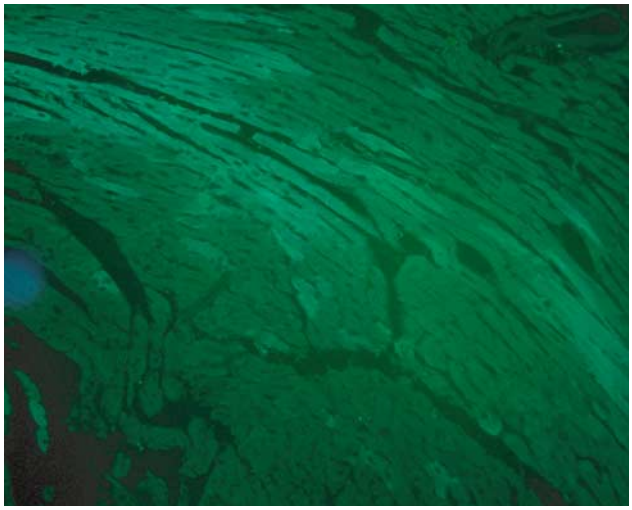

Figure 1 A normal mouse underwent total body irradiation and bone marrow transplantation from a transgenic GFP mouse. One month later GFP^+ hematopoietic chimerism was confirmed by visualizing peripheral blood in the recipient mouse. The GFP^+ chimeric mouse underwent coronary ligation and reperfusion followed by 7 days of G-CSF to mobilize stem cell into the blood. One month later, the area of infarcted myocardium was repopulated with GFP^+ nonhematopoietic cells

HSC compartment. The phenotype and functionality of the myocardial GFP^+ cells are now being investigated.

Conclusion

The reactivation of dormant genes has been documented in nuclear transfer experiments in which the nucleus of a single differentiated adult cell can clone an entire organism. It is, therefore, a reasonable possibility that adult stem cells may reactivate dormant and distinct genetic repertoires resulting in lineage switch. Several questions remain unanswered about adult stem cell metamoirosis. Does it occur in human beings? Can it occur with enough frequency to be clinically practical? Are the differentiated cells functional? Is the process of metamoirosis stable, that is, can the differentiated cells revert to their former phenotype? Hematopoietic stem cells are a unique source of adult stem cells that can be easily obtained in preparative quantities. It is possible that hematopoietic stem cell fate is not set at birth. Its destiny may be what we make of it.

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