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# EPO and Super-EPO: Erythropoietins Direct Neoangiogenesis by Cardiac Progenitor Cells

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**Erythropoietin, the red blood cell-making cytokine, is also a potential cytoprotective agent in heart disease. In this issue of *Cell Stem Cell*, Hoch et al. (2011) use two heart failure models, including chemotherapeutic cardiotoxicity, to reveal a mechanistic connection between reduced cardiomyocyte production of erythropoietin and neoangiogenesis by cardiac progenitors.**

Heart failure—defective performance of cardiac muscle as a biomechanical pump—is the final common pathway in diverse forms of heart disease, e.g., those resulting from acute ischemic injury, chronic workload, human mutations, or adverse effects of certain drugs. Organ-level performance in these settings is compromised to varying degrees by defects in myocytes' mechanical function, accumulation of extracellular matrix, and, typically, an imbalance between cardiac muscle cell death and the scant capacity of adult mammalian hearts to execute effective regenerative growth. Hence, cardiac muscle cell number is an especially well-posed target for studies in regenerative medicine, ranging from empirical trials of cell grafting, to decoding the genetic networks for cardiac muscle cell creation and survival, to investigating dormant progenitor cells in adult hearts as a novel route to self-repair (Mercola et al., 2011). In this issue of *Cell Stem Cell*, Hoch et al. (2011) demonstrate defects in the Sca-1$^+$ cardiac progenitor cell (CPC) population (Oh et al., 2003) in two heart failure-prone models: mice with a cardiomyocyte-specific deletion of the transcription factor STAT3, and mice given doxorubicin, an anti-cancer drug with pronounced cardiotoxicity. They show that under heart failure conditions, CPCs are impaired in generating the new blood vessels that are essential to assist oxygen delivery and ensure organ homeostasis. The authors relate this defect to a diminished activation of the CCL2/CCR2 chemokine pathway, which is reduced in response to defective production of erythropoietin (EPO) in failing hearts.

Freshly isolated Sca-1$^+$ cardiac cells lacked the features of hematopoietic stem cells, endothelial progenitor cells, or differentiated endothelium, but in cell culture acquired a vascular endothelial phenotype, demonstrated by marker proteins and transcripts, activation of a VE-Cadherin-Cre lineage marker, and formation of a branching vascular web. A similar pattern of phenotypic and functional results was obtained using cloned Sca-1$^+$ cells from cardiac tissue, obviating the concern that the apparent progenitor function of these cells might have been inferred from a selectively expanding subpopulation of contaminating endothelium, rather than a true lineage decision by the cells. Endothelial differentiation was enriched in Sca-1$^+$ cells expressing both the CCL2 receptor (CCR2) and EPO receptor (EPOR), was reduced by a CCR2 blocker, and was suppressed in CPCs from CCR2-deficient mice. Similar defects were identified in CPCs from mice with a cardiomyocyte-restricted deletion of STAT3, which affected the CPCs in three further ways: impaired expression of CCL2, upregulation of the metalloproteinase MMP-12, and generation of an inhibitory CCL2 cleavage fragment. Conversely, cardiac Sca-1$^+$ cells' differentiation into endothelium was rescued when CCL2 was provided along with an MMP-12 inhibitor.

What drives CCL2 expression in Sca-1$^+$ CPCs? Coexpression of EPOR and CCR2 demarcated the cells that yielded endothelium with the greatest efficiency, suggesting that cardiomyocytes' expression of EPO was responsible (Hoch et al., 2011). Best known as a hematopoietic cytokine produced by the kidney to drive red blood cell production in bone marrow—and used to treat diverse anemias—EPO also acts as a cytoprotective and angiogenic cytokine on other diverse cell targets (Leist et al., 2004). EPO levels in the heart were suppressed by deleting STAT3 in cardiomyocytes, with analogous effects produced in cultured cardiomyocytes by STAT3 shRNA (Hoch et al., 2011). Conversely, treating the conditional STAT3 knockout mice with a long-acting EPO derivative (CERA: Continuous Erythropoiesis Receptor Activator, methoxy polyethylene glycol-epoetin beta) suppressed MMP-12 in cardiac Sca-1+ cells, rescued CCL2, suppressed the progressive loss of capillary density, and preserved cardiac pump function. In cultured CPCs from STAT3 conditional-knockout mice, EPO enhanced CCL2 expression, endothelial differentiation, and sprouting. By contrast, CERA and EPO had no effect on the hearts or CPCs of wild-type mice. Most features of this pathway were also evoked by cardiotoxic levels of the anticancer drug doxorubicin: loss of STAT3 and EPO in cardiomyocytes, reduced CCL2 in CPCs, impaired endothelial differentiation and capillary density, lethal heart failure, and a high degree of protection by CERA (Hoch et al., 2011). This intriguing paracrine and pathobiological circuit is summarized in Figure 1.

A growing body of work emphasizes the cardioprotective properties of EPO, via mechanisms unrelated to this cytokine's eponymous effect on the oxygen-carrying capacity of blood. For example, in rats, EPO blocks cardiac apoptosis after coronary artery obstruction (Moon et al., 2003), ascribed to an inhibition of the mitochondrial membrane permeability transition pore (Juhaszova et al., 2004). Notably, protection is evoked in cultured cardiomyocytes and experimental animals by an 11 amino acid peptide from the

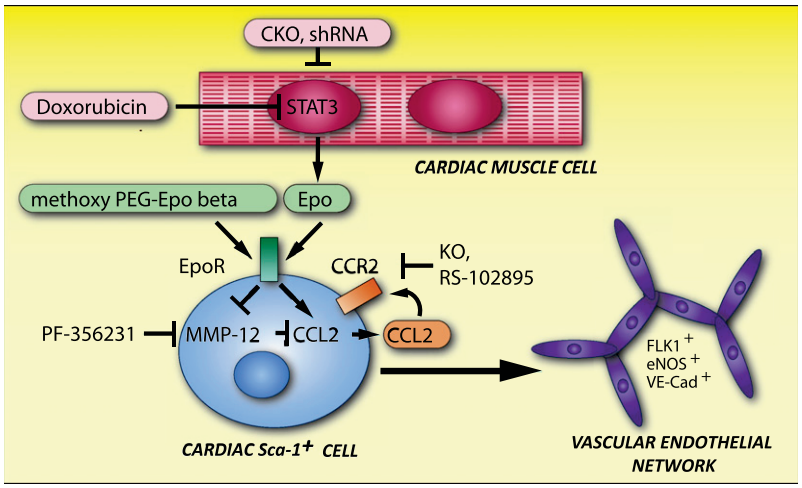


**Figure 1. The STAT3-EPO-CCL2 Pathway for Cardiac Blood Vessel Formation and Its Dysregulation in Heart Failure**

Cardiomyocytes' expression of STAT3 is essential for local EPO production, which directs the differentiation of Sca-1$^+$ CPCs into vascular endothelium. Methoxy polyethylene glycol-epoetin beta, a long-lasting EPO derivative, can bypass the requirement for endogenous EPO production, activate blood vessel formation by Sca-1$^+$ CPCs, improve cardiac pump function in the conditional knockout of STAT3, and overcome doxorubicin-induced cardiotoxicity.

aqueous face of EPO helix B (Ueba et al., 2010). This peptide serves as one of several means used to dissociate EPO's capacity for cytoprotection from erythrocyte production, and therefore may overcome the hazard of thrombosis that can occur in response to EPO therapy (Leist et al., 2004). Acute protection from cardiac injury is likely mediated by the expression of EPOR in cardiomyocytes and is contingent on its forming a heterodimer with CD131, a subunit for many cytokines (Brines et al., 2008). In contrast to this ostensibly direct block of cardiomyocyte death, Hoch and colleagues have unmasked a distinct, indirect protective mechanism for EPO, by virtue of its ability to rescue impaired CPC differentiation into a vascular network.

While the present investigations have been conducted in mouse models, the observed downregulation of STAT3 and EPO protein levels in failing human hearts suggests that this pathway might operate in a clinically relevant context as well. Conceivably, this circuit is also disrupted by other important anticancer drugs that exhibit cardiotoxicity, like tyrosine kinase inhibitors (Force and Kolaja, 2011). As a cautionary note, the authors have not yet proven that resident Sca-1$^+$ cells are the critical target for EPO in vivo (rather than circulating progenitor cells, preformed endothelium, cardiomyocytes, or even epicardium) (Brade et al., 2011). Impaired cardiac muscle creation by the CPCs (Oh et al., 2003) would also be consistent with the authors' findings and is a tantalizing alternative yet to be addressed. Despite these opportunities for future refinement, it is always welcome to see that an FDA-approved agent exhibits additional candidate therapeutic activity, especially, such as here, when novel mechanistic insights are revealed.

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