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PRE-CLINICAL RESEARCH

Plasminogen Regulates Cardiac Repair After Myocardial Infarction Through its Noncanonical Function in Stem Cell Homing to the Infarcted Heart

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Objectives The purpose of this study was to investigate the role of plasminogen (Plg) in stem cell–mediated cardiac repair and regeneration after myocardial infarction (MI).

Background An MI induces irreversible tissue damage, eventually leading to heart failure. Bone marrow (BM)–derived stem cells promote tissue repair and regeneration after MI. Thrombolytic treatment with Plg activators significantly improves the clinical outcome in MI by restoring cardiac perfusion. However, the role of Plg in stem cell–mediated cardiac repair remains unclear.

Methods An MI was induced in Plg-deficient ($Plg^{-/-}$) and wild-type ($Plg^{+/+}$) mice by ligation of the left anterior descending coronary artery. Stem cells were visualized by in vivo tracking of green fluorescent protein (GFP)-expressing BM cells after BM transplantation. Cardiac function, stem cell homing, and signaling pathways downstream of Plg were examined.

Results Granulocyte colony-stimulating factor, a stem cell mobilizer, significantly promoted BM-derived stem cell ($GFP^{+}c\text{-}kit^{+}$ cell) recruitment into the infarcted heart and stem cell–mediated cardiac repair in $Plg^{+/+}$ mice. However, Plg deficiency markedly inhibited stem cell homing and cardiac repair, suggesting that Plg is critical for stem cell–mediated cardiac repair. Moreover, Plg regulated C-X-C chemokine receptor type 4 (CXCR4) expression in stem cells in vivo and in vitro through matrix metalloproteinase-9. Lentiviral reconstitution of CXCR4 expression in BM cells successfully rescued stem cell homing to the infarcted heart in Plg-deficient mice, indicating that CXCR4 has a critical role in Plg-mediated stem cell homing after MI.

Conclusions These findings have identified a novel role for Plg in stem cell–mediated cardiac repair after MI. Thus, targeting Plg may offer a new therapeutic strategy for stem cell–mediated cardiac repair after MI. (J Am Coll Cardiol 2014;63:2862–72) © 2014 by the American College of Cardiology Foundation

Ischemic heart disease, including myocardial infarction (MI), is a major cause of death and disability worldwide. Obstruction of coronary arteries leads to MI, with the associated death of cardiomyocytes. Treatments aimed at restoring blood supply rapidly in the infarct heart, thrombolytic therapy and primary angioplasty, have significantly decreased early mortality of patients with MI. However, continuous overloads in the surviving myocardium eventually leads to heart failure. Epidemiological data have shown that about 60% of heart failure results from ischemic heart disease (1). Standard therapy for heart failure that addresses the fundamental problem of cardiomyocytes loss is cardiac transplantation, but this treatment has limited application because of insufficient donor hearts and the need for long-term immunosuppressive therapy. New discoveries on the regenerative potential of stem cells for preventing heart

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failure have transformed experimental research and led to an explosion in clinical investigation (2–6).

Stem cell homing to the heart to promote cardiac repair and regeneration after MI is a naturally occurring process. However, it occurs too slowly, and recruited stem cells are not sufficient to be meaningful for the recovery of heart function (7). To reach the efficient regeneration of damaged myocardium, therapeutic treatments have been developed by transplantation of a large number of stem cells into the bloodstream or infarcted heart or enhanced mobilization of stem cells from bone marrow by treatments with cytokines, such as granulocyte colony-stimulating factor (G-CSF) (8,9). However, insufficient stem cell engraftment and survival during these treatments have limited the application of stem cell therapy for treating MI (10,11). Therefore, a better understanding of the underlying mechanisms regulating stem cell function during cardiac repair may lead to the development of novel approaches for stem cell therapies.

Plasminogen (Plg) is the main enzyme responsible for fibrinolysis. The Plg system contains a proenzyme, Plg, which is converted to the active enzyme plasmin by tissue Plg activator or urokinase Plg activator (12). As a thrombolytic agent, tissue Plg activator (tPA) has been utilized as the first line of treatment of acute MI for almost 2 decades. In addition to its canonical function, Plg is critical for cardiac repair after MI, wound healing, and liver injury (13–15). However, the mechanism for Plg-regulated cardiac repair remains largely unknown.

In this study, using a knockout mouse model, stem cell tracking and genetic lentiviral approaches, and an experimental MI model, we have elucidated a novel mechanism by which Plg induces recruitment of bone marrow (BM)–derived stem cells to the infarcted heart, promoting cardiac repair after MI. Moreover, our data reveal that Plg induces C-X-C chemokine receptor type 4 (CXCR4) expression in migrating BM stem cells and may contribute to stem cell recruitment to the infarcted heart.

Methods

All animal procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee. The BM transplantation and induction of MI, flow cytometric analysis, histochemistry and immunohistology of heart sections, Western blot of CXCR4 expression, zymography of matrix metalloproteinase-9 (MMP-9) activity, in vitro chemotaxis assay, and data analysis are described in the Online Appendix.

Results

Plg is critical for G-CSF–stimulated cardiac function recovery and tissue repair after infarction. G-CSF promotes the recruitment of BM-derived stem cell to injured tissue, leading to improvement of tissue repair and heart function recovery in MI models (9,16–18). To investigate the role of Plg in stem cell–mediated tissue repair and heart function recovery, MI was induced in $Plg^{+/+}$ and $Plg^{-/-}$ mice by ligation of the left anterior descending coronary artery. Mice were treated with G-CSF to stimulate stem cell mobilization from BM and recruitment to the infarct heart, and cardiac function was analyzed by echocardiography. Plg deficiency did not alter normal heart function, as indicated by the similar ejection fraction of the left ventricle (LV) observed in $Plg^{+/+}$ (86.7 ± 2.4%, n = 6) and $Plg^{-/-}$ (87.6 ± 0.8%, n = 8) mice (Fig. 1A). The ligation surgery induced inadequate cardiac function, reducing LV ejection fraction by about 50% in $Plg^{+/+}$ and $Plg^{-/-}$ mice (Fig. 1A). No spontaneous recovery of heart function was observed in the 28 days after MI surgery, as shown by decreased ejection faction detected in $Plg^{+/+}$ and $Plg^{-/-}$ mice. Importantly, G-CSF significantly increased ejection fraction by one-half in $Plg^{+/+}$, but not in $Plg^{-/-}$ mice (Fig. 1A). Consistently, G-CSF reduced LV internal diameter by approximately 30% to 40% in $Plg^{+/+}$, but not in $Plg^{-/-}$ mice (Fig. 1B). These data indicate that Plg is critical for G-CSF–stimulated heart function recovery after MI.

Abbreviations and Acronyms

BM = bone marrow
BMNC = bone marrow mononuclear cell
CXCR4 = C-X-C chemokine receptor type 4
EC = endothelial cells
FACS = fluorescence activated cell sorting
G-CSF = granulocyte colony-stimulating factor
GFP = green fluorescent protein
LV = left ventricle/ventricular
MMP = matrix metalloproteinase
Plg = plasminogen
SDF = stromal cell-derived factor
SMC = vascular smooth muscle cells

We investigated the role of Plg in G-CSF–mediated tissue repair after MI. G-CSF treatment significantly improved post-MI tissue repair in $Plg^{+/+}$ mice 4 weeks after MI surgery, as evidenced by decreased infarct size by 30% (Figs. 1C and 1D). In contrast, $Plg^{-/-}$ mice had even slightly larger infarct size, indicating that Plg is required for G-CSF–stimulated tissue repair. Formation of new blood vessels (i.e., neovascularization) is a fundamental process during cardiac repair after MI. G-CSF induced a significant, 2-fold increase in microvascular density in the infarcted area in $Plg^{+/+}$ mice (Figs. 1E and 1F). Plg deficiency slightly decreased basal neovascularization (without G-CSF treatment), and completely abolished G-CSF–induced neovascularization. Together, these data establish a critical role of Plg in G-CSF–stimulated tissue repair and cardiac function recovery after MI, implicating that Plg may promote stem cell recruitment to improve cardiac repair and regeneration.

Plg is required for BM-derived stem cell recruitment in the infarcted heart. G-CSF treatment is widely used to enhance stem cell mobilization and recruitment to improve cardiac repair after MI (9,18,19). Our previous work has shown that Plg is required for G-CSF–induced stem cell mobilization from BM to the circulation (20). To evaluate