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JAK-STAT signaling in cardiomyogenesis of cardiac stem cells

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Abbreviations: gp130, glycoprotein 130; IL, interleukin; EPC, endothelial progenitor cell; ES, embryonic stem; LIF, leukemia inhibitory factor

Recently various kinds of cardiac stem/progenitor cells have been identified and suggested to be involved in cardiac repair and regeneration in injured myocardium. In this review, we focus on the roles of JAK-STAT signaling in cardiac stem/progenitor cells in cardiomyogenesis. JAK-STAT signaling plays important roles in the differentiation of stem cells into cardiac lineage cells. The activation of JAK-STAT signal elicits the mobilization of mesenchymal stem cells as well, contributing to the maintenance of cardiac function. Thus we propose that JAK-STAT could be a target signaling pathway in cardiac regenerative therapy.

Introduction

JAK-STAT signaling pathway plays important roles in maintenance of cardiac homeostasis. To date, much attention has been paid to the biological and/or pathophysiological roles of JAK-STAT signal in cardiac myocytes. Accumulating evidence has indicated that JAK-STAT signaling pathway is activated in cardiac myocytes by various cytokines, such as interleukin (IL)-6-type cytokines, granulocyte colony-stimulating factor (G-CSF),[1] leptin,[2] erythropoietin[3] and so on, and that the cardiac activation of JAK-STAT pathway promotes cardiomyocyte survival and myocardial angiogenesis, protecting myocardium from pathological stresses.[4,5] Thus, the regulation of JAK-STAT activities in cardiomyocytes could be one of promising strategies for cardioprotection against cardiovascular diseases,[6,7] though their overactivation might be detrimental to the cardiac functions.[8,9]

It has been a long-standing belief that the mammalian hearts have the limited capacity of regeneration since postnatal cardiomyocytes substantially fail to proliferate. Therefore, the cardiac homeostasis has been thought to depend mainly on cardioprotection, not on de novo synthesis of cardiac myocytes. In this context, one of the most surprising findings in this decade is the discovery of cardiac stem/progenitor cells. Cells expressing c-kit,[10] Sca-1[11,12] or Islet-1[13] have been identified as resident cardiac stem/progenitor cells in myocardium. These cells possess the ability to differentiate into cardiac lineage cells, including cardiomyocytes, vascular smooth muscle cells and endothelial cells. Importantly, the transplantation of these cells results in the improved cardiac repair and regeneration after myocardial injury. In addition, bone marrow- or blood-derived cells, such as hematopoietic stem cells,[14-16] mesenchymal stem cells[17,18] and endothelial progenitor cells (EPCs),[19-21] have been also proposed as the endogenous source of cardiac lineage cells. Though the mechanisms of the differentiation of embryonic stem (ES) cells into cardiac lineage have been precisely investigated,[22,23] the signals responsible for the differentiation of cardiac resident and bone marrow-derived stem/progenitor cells remain to be fully elucidated. Interestingly, recent studies have suggested that JAK-STAT pathway could be involved in determining the cell fates of the stem/progenitor cells. In this review, we focus on the JAK-STAT-mediated regulation of cardiomyogenesis in ES cells, bone marrow-derived cardiac progenitor cells, and cardiac resident stem/progenitor cells.

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JAK-STAT Signal in Cardiomyogenesis in ES Cells

Since ES cells are attractive sources of cardiomyocytes in cardiac regenerative medicine, intensive studies have been performed to control ES cell differentiation into cardiac lineage cells. While JAK-STAT signal is essential for maintenance of self-renewal and pluripotency of ES cells,[24,25] several lines of studies have reported that STAT3 is involved in cardiomyogenesis in ES cells. In murine ES cells, Foshay et al. demonstrated that the blockade of JAK2-STAT3 signaling by pharmacological inhibitors or the by expression of their dominant-negative forms diminished beating areas in embryonic bodies, whereas the transfection of constitutively-active JAK2 increased the beating areas.[26]

Rajasingh et al. reported that leukemia inhibitory factor (LIF) and bone morphogenetic protein (BMP)-2 synergistically differentiated murine ES cells into cardiomyocytes. Importantly, inhibition of STAT3 or mitogen-activated protein kinase (MAPK) repressed synergistic effect of LIF and BMP-2 on cardiomyocyte differentiation in murine ES cells.[27] Interestingly, the intracardiac injection of ES cells, pretreated with LIF and BMP-2, improved postinfarct left ventricular functions in a

murine acute myocardial infarction model and decreased area of fibrosis. Furthermore, the intracardially injected murine ES cells exhibited the expression of cardiomyocyte and endothelial cell markers in vivo and increased capillary density. These results suggested that activation of glycoprotein 130 (gp130)-JAK2-STAT3 signaling pathway leads to the differentiation of ES cells into cardiac lineage, though the JAK-STAT-independent differentiation is also reported.[28,29]

The Role of JAK-STAT Signal in Bone Marrow-Derived Cardiac Progenitor Cells

Previously, it has been proposed that bone marrow-derived cells, such as hematopoietic stem cells, bone marrow stromal cells and EPCs, transdifferentiate into cardiac lineage cells, including cardiomyocytes, vascular smooth muscle cells and endothelial cells.[14,15,17,30] On the other hand, several groups demonstrated that transplanted hematopoietic stem cells transdifferentiated into cardiomyocytes at negligible frequency in infarct myocardium.[31,32] Thus, the ability of bone marrow-derived cells to differentiate into cardiomyocytes is controversial; however, it is widely accepted that the transplantation of these cells improves cardiac function in injured hearts through paracrine factors from transplanted cells, accompanied by neovascularization.[33] Interestingly, several studies have revealed that these paracrine circuits are mediated by JAK-STAT signaling.

Recently, it has been demonstrated that the injection of bone marrow mesenchymal stem cells into the hind limb muscles significantly improved ventricular function in the cardiomyopathic hamsters, although the existence of intramuscularly injected mesenchymal stem cells were limited in the injected muscles.[34,35] Those results indicate that the mesenchymal stem cell injection resulted in the activation of JAK-STAT3 signaling pathway and that upregulation of growth factors not only in skeletal muscles and but also in the diseased hearts. In addition, the administration of JAK-STAT inhibitors abrogated mesenchymal stem cell-mediated growth factor expression and functional improvement in the diseased hearts, suggesting that the tissue trophic paracrine network is activated by mesenchymal stem cell-mediated JAK-STAT3 signaling. The similar findings were also observed in EPCs. IL-10 augments EPC-mediated functional improvement and neovascularization in the ischemic myocardium possibly through enhancement of EPC mobilization and survival.[36] Since IL-10-mediated vascular endothelial growth factor (VEGF) expression in EPCs was abrogated by a STAT3 inhibitor, it is suggested that IL-10 might enhance EPC survival and function through activation of STAT3.

The IL-6-type cytokine is also a candidate that regulates the dynamics of bone marrow-derived cells in cardiac repair. The intramuscular injection of LIF cDNA after myocardial infarction attenuated infarct size and improved cardiac function, accompanied by the increased myocardial neovascularization.[37] Interestingly, increased level of serum LIF led to the differentiation of bone marrow cells into cardiomyocytes and endothelial cells, possibly by enhancing the cell migration. Moreover, it is proposed that LIF-mediated recruitment of bone marrow-derived cells is essential for the cardiac repair as an endogenous regenerative mechanism. The postnatal bone marrow contains a population of nonhematopoietic Sca-1+/Lin-/CD45- mononuclear cells that express early cardiac lineage markers such as Nkx2.5/Csx and GATA-4 and these cells migrate in response to several cytokines after myocardial infarction.[38] Interestingly, supernatants from infracted myocardial tissue increased the motility of the bone marrow-derived mononuclear cells, and inhibition of LIF signaling by anti-LIF receptor neutralizing antibodies suppressed the cell motility. Since LIF utilizes JAK-STAT signaling pathway as is the case with other IL-6-type cytokines, it is likely that JAK-STAT pathway plays important roles in the LIF-mediated differentiation and migration of bone marrow-derived cells after myocardial injury.

The Role of JAK-STAT Signal in Cardiac Resident Stem/Progenitor Cells

Recently various kinds of cardiac resident stem/progenitor cells expressing c-kit,[10] Sca-1[11,12] or Islet-1[13] have been identified in the myocardium. These cells were reported to differentiate into cardiac lineage cells including cardiomyocytes, vascular endothelial and smooth muscle cells; however, little has been known about signaling pathways that determine their cell fates.

Since JAK-STAT pathway plays important roles in stem cell differentiation, as described above, we examined the biological significances of IL-6-type cytokines in the cardiac stem/progenitor cells.[39,40] IL-6-type cytokines, such as LIF, cardiotrophin-1 and IL-11, induced the expression of the endothelial cell marker genes and proteins, but not cardiomyocyte or smooth muscle cell markers, in cultured cardiac Sca-1$^+$ cells, accompanied by activation of STAT3 and ERK1/2 but not Akt. Similarly, cardiac c-kit+ cells are also differentiated into endothelial cells by LIF-mediated STAT3 activation.[40] In contrast, IL-6 failed to induce the endothelial differentiation because of the lacking of its receptor in Sca-1$^+$ cells; however, IL-6 exhibited the activity to induce the endothelial differentiation and STAT3 phosphorylation in the presence of soluble IL-6 receptor, an agonistic receptor. Previously, it was reported that IL-6 deficiency reduced capillary density in the heart.[41] Thus, IL-6 might contribute to vascular formation by activating cardiac stem cell in the presence of soluble IL-6 receptor. Importantly, the inhibition of STAT3 pathway by the transduction with its dominant negative form or with siRNA abrogated the IL-6-type cytokines-induced differentiation of Sca-1$^+$ cells into endothelial cells and the inhibition of ERK1/2 with the MEK1/2 inhibitor U0126 also prevented the endothelial differentiation, suggesting that IL-6-type cytokines could elicit the endothelial differentiation of cardiac Sca-1$^+$ cells through gp130-STAT3 in coordination with gp130/ERK pathway.

Although cardiac Sca-1$^+$ cells were differentiated into beating cardiomyocytes by oxytocin,[12] JAK-STAT signaling did not induced the differentiation of cardiac Sca-1$^+$ cells into cardiomyocytes. In contrast, JAK-STAT3 signaling pathway evokes the differentiation of murine ES cells into beating cardiomyocytes[26,27] as described above, suggesting that the signaling pathways

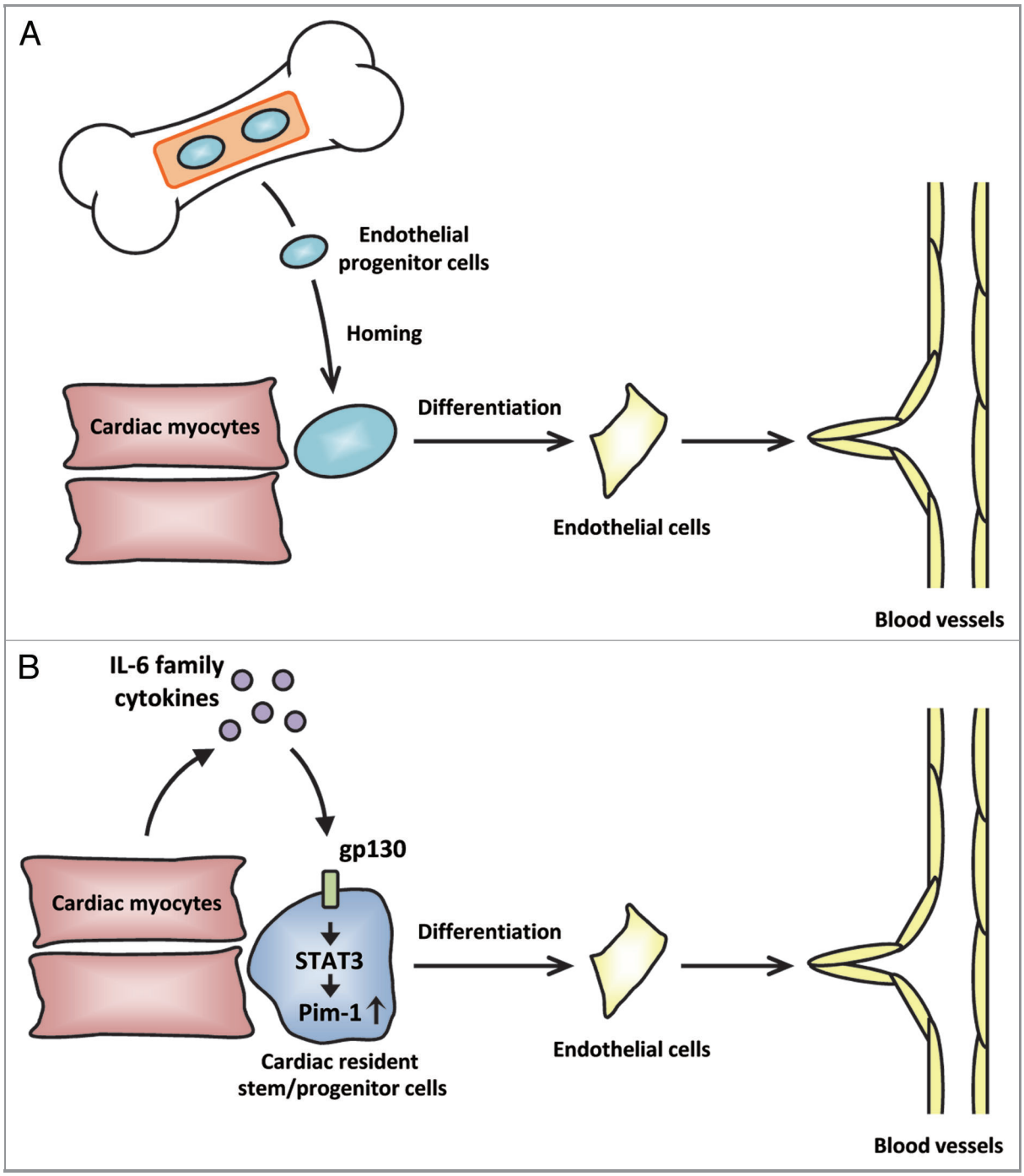

Figure 1. The proposed mechanism of STAT3-mediated vasculogenesis by cardiac stem/progenitor cells. (A) In the vasculogenesis by bone marrow-derived cells, endothelial progenitor cells migrate from the bone marrow, home to peripheral organs, and participate in the postnatal neovascularization. (B) JAK-STAT pathway in cardiac resident stem/progenitor cells may contribute to neovascularlization by inducing the endothelial differentiation. IL-6-type cytokines, secreted from myocardium, stimulate gp130-JAK-STAT-Pim-1 signaling pathway in cardiac resident stem/progenitor cells, and induce the endothelial differentiation. The newly differentiated endothelial cells may participate in vessel formation without homing process, designated as "in situ vasculogenesis."

responsible for the regulation of differentiation into cardiomyocytes might be different between ES cells and cardiac Sca-1$^+$ cells.

We have addressed molecular mechanisms for JAK-STAT-mediated endothelial cell differentiation of Sca-1$^+$ cells, focusing on Pim-1 kinase.[42] Pim-1 has been originally identified as an oncogenic serine/threonine kinase involved in the regulation of cell survival, proliferation and differentiation.[43] In cardiac Sca-1$^+$ cells, Pim-1 is upregulated in response to LIF through STAT3 signaling pathway. The blockade of STAT3 pathway abrogated the upregulation of Pim-1 expression by LIF and the

gene transduction of constitutively-active form of STAT3 cDNA induced expression of Pim-1, indicating that STAT3 activation is necessary and sufficient for Pim-1 induction. Importantly, the overexpression of dominant negative form of Pim-1 abrogated the endothelial differentiation, indicating that Pim-1 kinase activity is essential for STAT3-mediated endothelial cell differentiation of cardiac Sca-1$^+$ cells. Furthermore, the functional roles of Pim-1 in cardiac Sca-1$^+$ cells were evaluated in vivo. The transplantation of Sca-1$^+$ cells increased the capillary density in myocardium after myocardial infarction, associated with the improvement of cardiac function, as reported previously.[11] Interestingly, these beneficial effects were abrogated by the overexpression of dominant-negative form of Pim-1, suggesting that JAK-STAT-Pim-1 pathway could contribute to neovascularization, promoting cardiac repair/regeneration in vivo.

The biological significances of Pim-1 in the regulation of stem cell differentiation have been previously described. Similar to the findings described above, it was reported that Pim-1 is required for VEGF-induced endothelial cell differentiation of Flk-1$^+$ ES cells.[44] Therefore, it is possible that cardiac Sca-1$^+$ cells utilize the common signaling pathway with Flk-1$^+$ ES cells, in the process of the endothelial differentiation. In cardiac c-kit$^+$ stem/progenitor cells, other aspects of Pim-1 have been reported. Transplantation of cardiac c-kit$^+$ stem/progenitor cells overexpressing Pim-1 into infract myocardium significantly enhanced myocardial regeneration accompanied with reduction of infarct size, upregulation of c-kit$^+$ cells, and increased vasculature in the damaged region.[45] Furthermore, Pim-1 stimulates cell cycling and promotes asymmetric division in cardiac c-kit$^+$ progenitor cells, leading to a preservation of the progenitor cell pool as well as cardiogenic daughter cells.[46] Taken together, Pim-1 may exhibit the differential functions, depending on the kinetics of its activity.

The neovascularization is classified into two categories; angiogenesis and vasculogenesis. In angiogenic process, the preexisting endothelium grows and contributes to the formation of vascular network, while vasculogenesis is defined as a de novo vessel formation from vascular endothelial precursor cells. In postnatal neovascularization, it is certain that angiogenesis plays important roles in vessel formation; however, recent studies have proposed that the bone marrow-derived cells, such as EPCs,[47] participate in the vascular formation, by homing to the target organs and by differentiating into endothelial cells. Considering that IL-6-type cytokines, which activate JAK-STAT pathway in cardiac stem cells, are induced in injured myocardium,[40] it is suggested that cardiac resident stem cells contribute to postnatal vasculogenesis as novel endothelial precursor cells. It should be noted that the cardiac resident stem cells could mediate vasculogenesis without homing process, designated as "in situ vasculogenesis," unlike the bone marrow-derived cells (**Fig. 1**). Interestingly, recent studies have described the existence of tissue resident EPCs in non-cardiac tissues.[48] Further studies would be required to elucidate whether the activation of JAK-STAT pathway in these non-cardiac resident stem cells also induces their differentiation.

Perspectives

In order to develop novel therapeutic strategies that target cardiac stem cells, it is essential to elucidate the signaling pathways that determine their cell fates. In this review, we discussed the roles of JAK-STAT pathway in the differentiation of stem/progenitor cells into cardiac lineage cells. And we have described that JAK-STAT-Pim-1 pathway plays critical roles in the endothelial differentiation of cardiac Sca-1$^+$ stem/progenitor cells.[39,40,42] Toward the clinical application of these findings, we have to solve at least two problems. First, we should identify the human cells that correspond to murine Sca-1$^+$ cells and to make clear whether these cells differentiate into endothelial cells in response to activation of JAK-STAT signaling pathway. Especially in clinical situations in cardiovascular medicine, stem cell therapies would be performed in elderly patients whose stem/progenitor cells, such as EPCs, are likely to be senescent.[49] In this context, it is important to maintain the ability of cardiac stem cells to differentiate into endothelial cells. Interestingly, it has recently been demonstrated that erythropoietin secreted by cardiomyocytes restored the endothelial cell differentiation of cardiac Sca-1$^+$ progenitor cells, and preserved the cardiac microvasculature and cardiac function in murine heart failure model.[50] Second, it is required to develop the interventional methods that safely activate JAK-STAT-Pim-1 and their downstream pathways. To address this problem, further effort should be made to identify Pim-1 substrates responsible for the endothelial differentiation. In spite of these difficulties, targeting JAK-STAT signaling in cardiac resident stem/progenitor cells might be a promising therapeutic strategy against cardiovascular diseases since the neovascularization is critical to preserve myocardium in failing hearts.

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