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Pivotal Advance: High-mobility group box 1 protein—a cytokine with a role in cardiac repair

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Abstract: The nuclear protein high-mobility group box 1 (HMGB1) has been largely characterized for its role in inflammation. However, HMGB1 released by inflammatory cells, as well as by necrotic cells, may also act as a signal of tissue damage and participate in tissue repair by recruiting stem cells to the injury site. The emergence of this function has focused the interest on HMGB1 as a molecule with an active role in tissue regeneration. We recently demonstrated that HMGB1 administration in a mouse model of myocardial infarction activates cardiac stem cells and promotes their differentiation into cardiomyocytes. The regenerative effect results in the improvement of cardiac function. In this review, we highlight the beneficial role of HMGB1 and discuss growth factor-based therapeutic approaches for the treatment of myocardial infarction. *J. Leukoc. Biol.* 81: 000–000; 2007.

Key Words: *stem cells · infarction*

INTRODUCTION

High-mobility group box-1 protein (HMGB1) is an ubiquitous highly conserved protein with a well-established role as a chromatin-binding factor [1]. Surprisingly, HMGB1 is also a cytokine that, when released by inflammatory and necrotic cells, mediates inflammation [2]. Although high levels of HMGB1 have been associated to pathologic conditions such as sepsis, arthritis, and cancer, its release during the physiological process of inflammation and regeneration may have a role in the local repair mechanism. Currently, there are several experimental findings that suggest the involvement of HMGB1 in this process.

In skeletal and, to a lesser extent, in cardiac tissue, regeneration requires the activation of stem cells, satellite cells, and cardiac stem cells (CSCs), respectively [3, 4]. Further, bone marrow-derived stem cells (BMSCs) are mobilized after injury and reach the damaged tissue through the circulation; these cells as well as resident stem cells play an important role in tissue regeneration [5]. Signals that modulate stem cell functions in injured tissue are only partially characterized, and HMGB1 may be one of these molecules. Interestingly, HMGB1 promotes vessel-associated stem cell (mesoangioblasts) proliferation in vitro and also has a chemotactic effect on mesoangioblasts, on bone marrow-derived c-kit$^+$ cells [6] and on endothelial precursor cells (EPC) [7]. It is noteworthy that HMGB1 induces mesoangioblast migration through an endothelial monolayer in vitro, and it may act in vivo as a signal to attract both mesoangioblasts and BMSCs [6]. The migratory response induced by HMGB1 results from its ability to modulate both endothelial and stem cell function. HMGB1 promotes cytoskeletal reorganization of smooth muscle cells and disrupts endothelial barrier function by disassembly of adherens junctions [8]. In addition, HMGB1 induces, in endothelial cells, a proinflammatory phenotype characterized by the upregulation of adhesion molecules VCAM-1 and ICAM, which are required for integrin-mediated adhesion of inflammatory cells and their subsequent transmigration into injured tissue. Stem cell homing and engraftment occur with a similar mechanism. Both EPC and BMSC require integrin β1 and β2 for their transmigration through the endothelial cell monolayer [9]. Interestingly, HMGB1-mediated migration of EPC involves integrin β1 and β2 [7].

HMGB1 activates the migratory response binding to the receptor for advanced glycation end products (RAGE) [10, 11]. Although HMGB1 also interacts with other receptors, including the Toll-like receptors (TLR)-2 and -4 [12], their role in HMGB1-induced cell migration has not been investigated so far. RAGE is a transmembrane protein belonging to the immunoglobulin superfamily, which is expressed on a variety of cells, including mesoangioblasts, BMSCs [6], EPCs [7], and CSCs [13]. The signaling pathways activated in the migratory response are not well characterized, and although it is known that HMGB1-induced smooth muscle cell migration involves mitogen-activated protein kinase (MAPK) signaling activation via RAGE, it has not been investigated whether a similar mechanism is activated in stem cells.

CARDIAC STEM CELLS

Although until recently the heart has been regarded as a terminally differentiated organ, the recent identification of CSCs has changed this dogma and has opened new opportu-

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nities for myocardial repair. CSCs are committed to the cardiogenic lineage and can differentiate not only into cardiomyocytes but also into smooth muscle cells and endothelial cells [14].

Although CSCs are involved in the lifelong process of maintaining cardiac homeostasis and replacing, at least in part, dead cardiac cells, they are unable to completely regenerate cardiac tissue after acute and chronic injury. Nonetheless, CSCs represent a novel and promising tool to enhance the regenerative process in the heart [4]. It may be possible to induce CSCs to proliferate and differentiate ex vivo, before transplanting them into the damaged heart. Further, it may be possible to induce CSC proliferation and differentiation in vivo, without the need for ex vivo CSC expansion and subsequent in vivo transplantation.

HMGB1 AND CARDIAC STEM CELL ACTIVATION

CSCs have been identified as cells positive for stem cell antigens c-kit, Sca1 and MDR1 [14]. In mice hearts, c-kit-expressing cells are 0.2% of the total heart cells after separation from cardiomyocytes and increase up to 1% in acutely infarcted hearts (**Fig. 1**). CSCs express receptors for a variety of growth factors, cytokines, and chemokines; these include hepatocyte growth factor receptor (HGFR) c-Met, insulin growth factor receptor-1 (IGF-1R), vascular endothelial growth factor receptors (VEGFR), stem cell factor (SCF) receptor, epidermal growth factor receptor (EGFR), serum-derived factor (SDF)-1 receptor CXCR4 [15], and the HMGB1 receptor RAGE [13]. Therefore, growth factor treatment may modulate CSC function. Recently, this possibility has been addressed by Anversa's group: the combined administration of insulin growth factor-1 (IGF-1) and hepatocyte growth factor (HGF) in ischemic hearts [15, 16] increased CSC proliferation, migration, and survival. Moreover, we showed that HMGB1 delivery in the infarcted mouse heart enhanced CSC proliferation (**Fig. 2**): Twenty-four hours after infarction and HMGB1 treatment c-kit$^+$ cells increased ~2.5-fold compared with infarcted heart treated with a control protein. It is noteworthy that the HMGB1-mediated proliferative effect was also detected, albeit

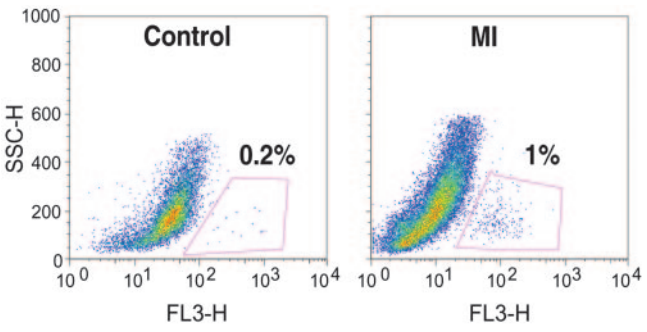

Fig. 1. c-kit$^+$ CSCs increase in the mouse-infarcted heart. Flow cytometric analysis shows c-kit$^+$ cells isolated from noninfarcted (control) and 24-h postmyocardial infarction (MI) mouse total heart. c-kit$^+$ cells increase from 0.2 to 1%.

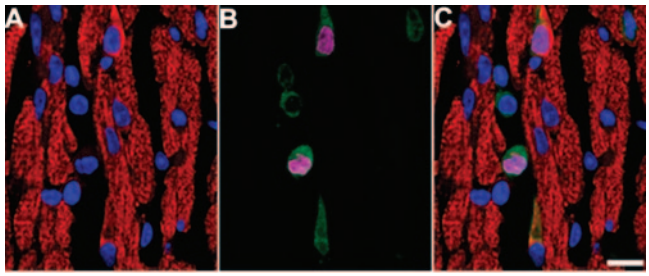

Fig. 2. HMGB1 induces cardiac c-kit+ cell proliferation. Immunohistochemistry shows cycling c-kit cells in infarcted-HMGB1-treated hearts. Myocardial infarction and HMGB1 treatment were carried out in kit/GFP transgenic mice, in which green fluorescente protein (GFP) was driven by the c-kit promoter; therefore, GFP$^+$ cells were c-kit$^+$ cells. To identify GFP cells that entered the cell cycle, bromodeoxyuridin(BrdU) was administered intraperitoneum in mice immediately after infarction, and immunohistochemical analysis was performed with anti-BrdU antibody. (A) α-sarcomeric actin immunostaining (red fluorescence). (B) GFP$^+$ cells in the same section as in (A) incorporated BrdU (magenta fluorescence). (C) Merge of A and B. Blue false color, Propidium iodide in nuclei. Scale bar = 10 μm.

to a lesser extent, in the absence of infarction, suggesting that exogenously delivered HMGB1 did not require tissue injury to activate CSCs [13].

These data suggest that a growth factor-based therapy may represent a useful approach to modulate endogenous CSC functions.

HMGB1 AND CARDIAC REPAIR

Maturation of CSCs to a functional cardiac phenotype represents an important step required for the functional recovery of the heart. HGF and IGF-1 delivery in mouse and dog infarcted hearts induced CSC migration in the injured region differentiation into cardiomyocytes and recovery of cardiac function [15, 16]. Similarly to HGF and IGF-1, HMGB1 administration into the infarcted mouse heart, enhanced CSC proliferation 24 h after myocardial infarction and induced their differentiation at later time points. Three days after infarction, CSCs started expressing the cardiac transcription factor MEF2C, and after 1 week, MEF2C-expressing cells occupied the infarcted area. The newly formed cells were also positive for α-sarcomeric actin, another marker of the cardiogenic lineage (**Fig. 3**) [13]. One important issue concerns the dimension and the functional competence of CSC-derived cardiomyocytes. In mouse cardiac tissue, cardiomyocytes are integrated in a syncitium through gap junctions, which ensure their synchronous contraction during the action potential propagation. One week after myocardial infarction and HMGB1 treatment, CSC-derived cardiomyocytes had a volume between 300 and 600 μm^3, and they expressed connexin 43, a gap junction protein required for electromechanical coupling between myocytes, suggesting that newly formed cells were functionally competent. However, adult murine myocytes have an average volume of 20,000 μm^3, suggesting that myocardial regeneration was due to the formation of immature cardiac cells that resemble a fetal phenotype.

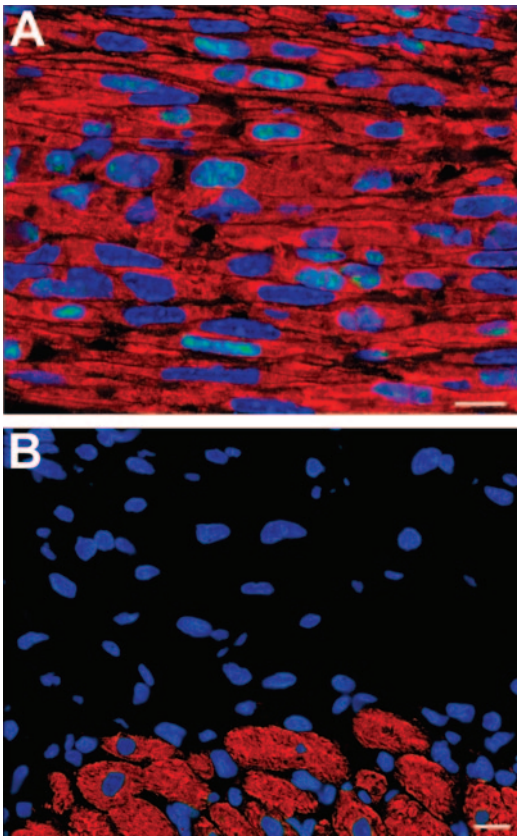

Fig. 3. HMGB1 promotes cardiac tissue regeneration. Newly formed myocytes are detected in infarcted HMGB1-treated heart (A) but not in infracted heart treated with a control protein (B). Newly formed cells express α-sarcomeric actin (red fluorescence) together with the cardiac transcription factor MEF2C (arrowheads and light blue fluorescence). Blue false color, Propidium iodide in nuclei. Scale bar = 10 μm. Modified from [13].

PREVENTION OF HEART FAILURE BY HMGB1 TREATMENT

The newly formed myocytes in HMGB1-treated hearts, enhanced cardiac performance. Contraction of the infarcted free wall of the left ventricle reappeared 1 wk after myocardial infarction and HMGB1 treatment. Ejection fraction as well as other functional parameters improved at 2 and 4 wk after HMGB1 treatment. The administration of HMGB1 had also an influence on cardiac anatomy. Compared with untreated infarcted mice hearts, infarcted hearts that received HMGB1 were characterized by an attenuated left ventricular dilation and an increased infarcted wall thickness. Thus, diastolic wall stress was lower, up to 1 mo postinfarction, in treated animals [13]. It is noteworthy that the improvement of myocardial function after HMGB1 treatment occurred in the presence of a comparable infarct size. Moreover, for a given infarct size, the degree of impairment was always higher in the untreated group, as shown by the values of diastolic wall stress at all time points (**Fig. 4**).

These results show that HMGB1, through the induction of regeneration, can prevent remodeling of the postinfarcted heart and can improve cardiac performance.

HMGB1 AND BONE MARROW STEM CELLS

The origin of CSCs has not been determined, and it is an open question whether CSCs derive from the bone marrow, which, as a major reservoir of stem cells in the organism, may replenish the heart with undifferentiated cells. Current knowledge supports the notion that cardiac injury enhances the number of circulating stem cells and their recruitment in to the heart [17–19]. Although such an effect is too low to be of clinical relevance, some factors, including granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) [20], further increase the number of circulating stem cells and enhance their recruitment into the damaged tissue. HMGB1 injection into the infarcted heart neither mobilized BMSCs into the systemic circulation nor enhanced their cardiac homing [13]. Bromodeoxyuridin (BrdU)-labeled c-kit$^+$ BMSCs were introduced into the mouse systemic circulation through the tail

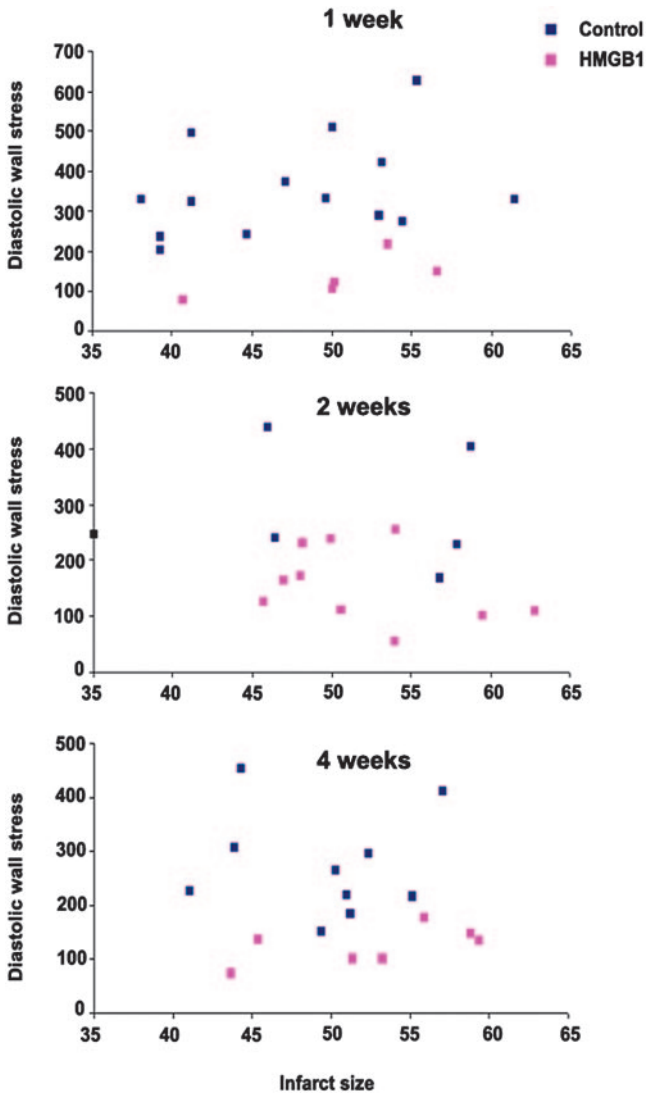

Fig. 4. Correlation between infarct size and diastolic wall stress in infarcted HMGB1-treated hearts at the indicated time points. Infarct size ranged between 40 and 65% of the left ventricle and was comparable between control and HMGB1-treated hearts. For any given infarct size, diastolic wall stress reduction in HMGB1-treated hearts vs. control was consistently observed.

vein, immediately after infarction and intramyocardial HMGB1 delivery. These cells were identified in the heart in a very low number, whereas the infarcted region was repopulated with cells of cardiac origin, unequivocally demonstrating the intracardiac origin of cardiomyocytes.

FUTURE PERSPECTIVES

In recent years, basic scientists and clinicians have looked at stem cells as the promising approach to promote cardiac regeneration. The recent identification of CSCs has provided a new potential approach to cardiac repair: differently from stem cells of other adult tissues, CSCs are committed to the cardiogenic lineage and therefore may represent the best tool to form new cardiomyocytes in the infarcted heart. Unfortunately, in basal conditions CSCs are unable to regenerate cardiac tissue after acute/chronic injury, possibly also because of their low number. Therefore, the possibility of applying a growth factor-based therapy to enhance CSC migration, proliferation, and differentiation, provides an important alternative to CSC isolation from a cardiac biopsy, their expansion in vitro, and reimplantation into the damaged heart. HMGB1 seems to have all the requisites to accomplish the indicated functions when used at low concentration. An important point to consider is the detrimental effect of high concentrations of HMGB1; indeed, HMGB1 has been involved in several pathologic conditions such as sepsis, hemorrhagic shock, arthritis, and cancer [21]. It is noteworthy that in our study, we used 200 ng of HMGB1, whereas 500 μg are required to induce septic shock in mice [22].

There are important questions that need to be addressed in order to elucidate HMGB1's effects on CSCs. How does HMGB1 induce CSC proliferation and differentiation? Which signaling mechanisms are involved in CSC's response to HMGB1? Does HMGB1, in addition to enhancing CSC migration, proliferation, and differentiation also inhibit cell death? Recent studies and our preliminary results show that HMGB1 also modulates endothelial cell function and induces angiogenesis [23, 24]. Thus, endothelial cell response is likely to contribute to cardiac tissue repair.

Finally, the effect of HMGB1 in the postischemic myocardium after the occurrence of cardiomyopathy, remains to be established. It is noteworthy that chronic infarcts, as well as the age-related decline of cardiac function, are characterized by an increasing growth of CSCs, but apoptosis prevails on proliferation, and this imbalance reduces the number of functionally competent CSCs [25–27]. Moreover, in patients with cardiac aging and heart failure, the number of apoptotic CSCs is high and is associated with the expression of p16, an established marker of cellular senescence. Therefore, additional studies will be required to elucidate HMGB1's mechanism of action and establish whether it induces repair also in the absence of acute ischemia when chronic heart failure has developed. The possibility to activate endogenous CSCs has been recently demonstrated: the combined administration of IGF-1 and HGF in the infarcted heart enhanced CSC migration, proliferation, and survival [15, 16]. It is noteworthy that VEGF, FGF-1, FGF-2, erythropoietin, and HGF have been delivered either as recombinant protein or by gene therapy approaches to protect cardiac cells and to increase blood supply in animal models of myocardial ischemia, infarction, and heart failure [28]. With the identification of CSCs, the results reported in these studies should be reevaluated in order to determine whether there was any effect on CSCs as well.

In conclusion, activation of endogenous CSC with a growth factor delivery system represents a potential therapeutic approach for the treatment of heart diseases. Future studies will help to understand the feasibility of such an approach.

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