Human progenitor cells from bone marrow or adipose tissue produce VEGF, HGF, and IGF-I in response to TNF by a p38 MAPK-dependent mechanism

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Methods

Progenitor cell therapy is a promising treatment modality for a variety of degenerative diseases (9, 10). Although it has been reported that adult stem cells, delivered into ischemic myocardium, improved myocardial function and survival through transdifferentiation into cardiomyocytes (1, 18, 25), additional studies have demonstrated a very low level of transdifferentiation of stem cells into cardiomyocytes in ischemic myocardial tissue (2, 23). Thus there has been a growing recognition that stem cells may mediate organ-protective effects through enhanced neovascularization at sites of injured myocardium or through production of local factors. Dernbach and colleagues (5) reported antioxidant properties in circulating progenitor cells. Rehman et al. (27) demonstrated that human adipose stromal cells secrete angiogenic and antiapoptotic growth factors, such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), in response to hypoxia and that conditioned media containing these growth factors increased cell growth and suppressed cell apoptosis. Indeed, our previous study showed that adult progenitor cell differentiation is not required for myocardial functional protection; pretreatment of isolated heart with human mesenchymal stem cells (hMSCs) or human adipose progenitor cells (hAPCs) improved posts ischemic myocardial function, decreased myocardial inflammation, and reduced activation of apoptotic caspases (41). Progenitor cells exert cardioprotection, but not by transdifferentiation, soon after (40 min) an infarct. However, the mechanism whereby they may positively affect wound healing and the components of the wound milieu that stimulate progenitor cells to produce growth factors remain unclear.

Tumor necrosis factor-α (TNF-α) is induced in response to various injuries (21) and plays a central role in postinjury organ dysfunction and local tissue cell apoptosis, as well as induction of proinflammatory signaling (7, 17, 20). TNF, working as an upstream cytokine, may induce other cytokines via p38 mitogen-activated protein kinase (MAPK). It has been reported that TNF-induced production of IL-1β and IL-6 is regulated by p38 MAPK activation. Secretion of VEGF, HGF, and IGF-I in hMSCs and hAPCs was significantly increased by stimulation with TNF and was associated with increased activation of p38 MAPK. The p38 MAPK inhibitor decreased production of TNF-stimulated VEGF, HGF, and IGF-I in hMSCs and hAPCs. However, p38 MAPK inhibitor alone had no effect on production of growth factors. These data demonstrate that progenitor cells are potent sources of VEGF, HGF, and IGF-I. TNF, a prominent tissue cytokine, strongly stimulated production of growth factors by hMSCs and hAPCs via a p38 MAPK-dependent mechanism.

tumor necrosis factor; growth factors; human mesenchymal stem cells

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cultured from normal human bone marrow. Primary human preadipocytes were isolated from subcutaneous adipose tissue. The cells were thawed, and the culture process was initiated according to the manufacturer’s instructions. hMSCs and hAPCs were plated in T-225 tissue culture flasks (Corning, Corning, NY) and cultured with mesenchymal stem cell growth medium and preadipocyte growth medium, respectively (Cambrex Bio Science) at 37°C in 5% CO2 and 90% humidity. The medium was changed every 3 days.

**Experimental groups.** After they attained 70% confluence, hMSCs and hAPCs were plated in 12-well plates (Corning) at 0.1 × 10⁶ cells-well⁻¹-ml⁻¹. The cells were divided into groups as follows: control, p38 MAPK inhibitor [10 μM SB-203580 (p38MKI)], TNF (50 ng/ml), and TNF + p38MKI. After 24 h of incubation, supernatants were harvested for assay of VEGF, HGF, and IGF-I.

**ELISA of VEGF, IGF-I, and HGF.** Production of VEGF, IGF-I, and HGF from hMSCs and hAPCs was determined by ELISA using a commercially available kit (R & D Systems, Minneapolis, MN; BD Biosciences, San Diego, CA) according to the manufacturer’s instructions. All samples and standards were measured in duplicate.

**Protein isolation and Western blot analysis.** The p38 MAPK pathway was studied by Western blot analysis. The cells were collected in cold buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO₄, 1 μg/ml leupeptin, and 1 mM PMSF and centrifuged at 12,000 rpm for 5 min. The protein extracts (12 μg/lane) were subjected to electrophoresis on a 12% Tris·HCl gel (Bio-Rad) and transferred to a nitrocellulose membrane, which was stained with naphthol blue-black for confirmation of equal protein loading. The membranes were incubated in 5% dry milk for 1 h, incubated with primary p38 MAPK and phosphorylated p38 MAPK (Thr180/Tyr182) antibodies (Cell Signaling Technology, Beverly, MA) and then with secondary horseradish peroxidase-conjugated goat anti-rabbit IgG antibody, and detected using Supersignal West Pico stable peroxide solution (Pierce, Rockford, IL).

**Presentation of data and statistical analysis.** Values are means ± SE (n = 3). Data were compared using Student’s t-test. Two-tailed P < 0.05 was considered statistically significant.

**RESULTS**

**Effect of TNF on production of growth factors by hMSCs or hAPCs.** Over a 24-h period, TNF-stimulated hMSCs and hAPCs secreted a significant amount of VEGF, HGF, and IGF-I compared with non-TNF-stimulated cells (Fig. 1). TNF significantly increased production of growth factors: 30% increase of VEGF and 50% increase of HGF in hMSCs and 1.6-fold increase of VEGF, 2.2-fold increase of HGF, 2-fold increase of IGF-I in hAPCs.

**Activation of p38 MAPK.** TNF stimulation increased activation of p38 MAPK in hMSCs (10-fold) and hAPCs (−4-fold; Fig. 2).

**Regulation of growth factor secretion by p38 MAPK inhibition.** The p38 MAPK inhibitor SB-203580 (10 μM) decreased TNF-stimulated production of VEGF, HGF, and IGF-I in hMSCs and hAPCs (Figs. 3 and 4). The p38 MAPK inhibitor alone had no effect on growth factor production.

**DISCUSSION**

These results demonstrate, for the first time, that 1) TNF, as an important component in the wound milieu, stimulates production of VEGF, HGF, and IGF-I by hMSCs and hAPCs and 2) p38 MAPK is involved in TNF-induced production of VEGF, HGF, and IGF-I in hMSCs and hAPCs.

Bone marrow mesenchymal stem cells and adipose progenitor cells have been shown to be capable of positive remodeling and regeneration of healthy, functional tissue (10, 26). Although some studies have demonstrated that adult stem cells exert cardioprotection through transdifferentiation into cardiomyocytes, whether this cardioprotection actually occurs and the frequency with which it occurs remain controversial (39, 45). Indeed, some studies have failed to reproduce transdifferentiation of stem cells into functional cardiomyocytes in animal models (2, 23). In addition, other experiments have shown that the contribution of stem cells to target cell outcomes is
extremely rare, and this very low frequency of tissue contribution in most organs hardly constitutes protection (39). Our previous study indicated that infusion of hMSCs or hAPCs into myocardium immediately before global ischemia of isolated rat hearts significantly enhanced postischemic myocardial function, decreased proinflammatory cytokine production, and suppressed proapoptotic signaling (41). This study has shown that stem cells mediate acute myocardial protection through differentiation-independent mechanisms. Furthermore, it has been reported that administration of mesenchymal stem cells protected against ischemia-induced acute renal failure by decreasing proinflammatory cytokine production and increasing expression of antiapoptotic factors (37). Thus stem cells may mediate the beneficial effects on injured tissue via complex paracrine actions without differentiation into target cells. However, it is unclear how they may positively affect wound healing and what components of the wound milieu stimulate progenitor cells to produce growth factors.

Adult progenitor cells may secrete protective factors that limit inflammation and apoptosis. The transplanted adult progenitor cells face a foreign, inflammatory environment and may produce factors that limit local inflammation to enhance their survival. Adipose progenitor cells have been shown to increase VEGF and HGF secretion in hypoxic conditions (27). Furthermore, it has been reported that mesenchymal stem cells mediate acute protective effects on ischemic myocardium through elevated VEGF (11, 35). These studies suggest that progenitor cells may exert protection by regulating local inflammation and repair mechanisms through secretion of protective factors such as VEGF and HGF (42, 43). The significance of these growth factors in the reduction of tissue injury is as follows: 1) VEGF and HGF have been observed to improve cell growth and reduce cell apoptosis (27, 34); 2) VEGF and HGF exert a beneficial effect on neovascularization and tissue remodeling (8, 35, 42); and 3) VEGF, HGF, and IGF-I mobilize circulating progenitor cells and cardiac stem cells from the surrounding myocardium to the dead tissue (8, 31, 38).

On the other hand, TNF is induced in various injury conditions and is mainly responsible for localized responses, such as inflammation and apoptosis. Cardiomyocytes and monocytes/mast cells produce substantial amounts of TNF in response to myocardial infarction (7, 14). Recent evidence has shown that TNF promotes migration of embryonic stem cells (4) and increases homing of mesenchymal stem cells to the heart (30). However, it is unclear whether locally produced TNF stimulates hMSC or hAPC production of growth factors. Indeed, mesenchymal stem cells (15) or adipose progenitor cells (16) express TNF receptors, through which TNF induces its biological function. TNF inhibits adipocyte differentiation through a TNF receptor 1-activated pathway in cultured preadipocyte cell lines (24). In addition, it has been shown that TNF affects adipose tissue expansion through paracrine action (16). In this study, we confirmed the release of VEGF, HGF, and IGF-I by hMSCs or hAPCs. Furthermore, TNF, as a local proinflammatory mediator, may contribute to the neovascularization and tissue remodeling processes.

Fig. 3. Production of VEGF (A), HGF (B), and IGF-I (C) in hMSCs in response to TNF with or without p38 MAPK inhibitor [10 μM SB-203580 (p38MKI)]. p38 MAPK inhibitor significantly decreased release of VEGF, HGF, and IGF-I in hMSCs exposed to TNF. Values are means ± SE (n = 3). *P < 0.05 vs. control. #P < 0.05 vs. TNF.

Fig. 4. Production of VEGF (A), HGF (B), and IGF-I (C) in hAPCs in response to TNF with or without 10 μM SB-203580 (p38MKI). p38 MAPK inhibitor significantly decreased release of VEGF, HGF, and IGF-I in hAPCs exposed to TNF. Values are means ± SE (n = 3). *P < 0.05 vs. control. #P < 0.05 vs. TNF.
Reference cytokine, stimulates hMSCs or hAPCs to produce these growth factors. By what mechanisms does TNF influence hMSCs or hAPCs to release growth factors? p38 MAPK is an important mediator in the production of cytokines (13, 29, 32). Activation of p38 MAPK is required for VEGF production in fibroblast cell lines (6). In addition, a role for MAPK in the regulation of VEGF expression has been reported in mouse embryonic stem cells (28) and mesenchymal stem cells (40). Furthermore, p38 MAPK has been shown to have a synergistic effect on HGF production in human dermal fibroblasts (22, 33). However, it is not clear whether p38 MAPK is involved in TNF-induced production of growth factors in hMSCs or hAPCs. In the present study, we have demonstrated that the increase in production of VEGF, HGF, and IGF-I by hMSCs or hAPCs was associated with the increase in p38 MAPK activation by exposure to TNF. Administration of the p38 MAPK inhibitor decreased release of growth factors by TNF-stimulated hMSCs and hAPCs.

This study highlights the effect of TNF, as an important local factor, on progenitor cell release of growth factor and further demonstrates the involvement of p38 MAPK in TNF-induced growth factor production in hMSCs and hAPCs. This study brings us one step closer to understanding progenitor cell release of growth factor and local factor, on progenitor cell release of growth factor and accessory cells. Cell 124: 175–189, 2006.


GRANTS

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