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RESEARCH ARTICLE

Hepatocyte growth factor-mediated attraction of mesenchymal stem cells for apoptotic neuronal and cardiomyocytic cells

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Abstract Human bone marrow-derived mesenchymal stem cells (MSC) home to injured tissues and have regenerative capacity. In this study, we have investigated in vitro the influence of apoptotic and necrotic cell death, thus distinct types of tissue damage, on MSC migration. Concordant with an increased overall motility, MSC migrated towards apoptotic, but not vital or necrotic neuronal and cardiac cells. Hepatocyte growth factor (HGF) was expressed by the apoptotic cells only. MSC, in contrast, revealed expression of the HGF-receptor, c-Met. Blocking HGF bioactivity resulted in significant reduction of MSC migration. Moreover, recombinant HGF attracted MSC in a dose-dependent manner. Thus, apoptosis initiates chemoattraction of MSC via the HGF/c-Met axis, thereby linking tissue damage to the recruitment of cells with regenerative potential.

Keywords Mesenchymal stem cells · Apoptosis · Tissue regeneration · Chemotaxis · Cell migration · HGF · c-Met

Introduction

Human mesenchymal stem cells (MSC) are adult multipotent stem cells isolated from bone marrow (BM) and several other tissues. Typically, they are plastic adherent, non-hematopoietic cells with fibroblastoid morphology, expressing CD73, CD90, and CD105 but not the lineage markers CD14, CD34, and CD45. They are capable of differentiating into various types of mesenchymal cells, including osteoblasts, adipocytes, and chondrocytes [1, 2]. Generation of other tissue types including cardiomyocytes [3], neuronal cells [4], and hepatocytes [5] has been reported as well. The multipotency of MSC and their beneficial effects on tissue repair, which may also be due to paracrine mechanisms without extensive engraftment and transdifferentiation, make MSC a promising tool in regenerative medicine [6, 7].

In addition, MSC have stromal activity, support hematopoiesis, and may improve hematopoietic engraftment after stem cell transplantation [8, 9]. Moreover, MSC have immunosuppressive activities [7] which have already been exploited therapeutically to reduce graft-versus-host disease after allogeneic hematopoietic stem cell transplantation [10, 11].

Although local transplantation of MSC under certain conditions, like in the treatment of bone defects, may show efficacy, homing to the respective tissues is key to the therapeutic potential of MSC. Tissue injury appears to generate a strong chemoattractive signal [12]. Irradiation damage [13], ischemia of heart [14] and brain [15], chemically induced renal failure [16], and allograft rejection [17] have been reported to attract MSC. Furthermore, MSC home to various tumors, an activity which can be increased by radiotherapy and which may allow for tumor site-directed delivery of cytotoxic therapeutics using MSC as vectors [18].

The mechanisms underlying homing of MSC to the various target tissues are not entirely understood. MSC express a multitude of receptors which have been implicated in chemo-attraction, including most chemokine receptors and receptors for growth factors like platelet-derived growth factor (PDGF), insulin-like growth factor

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(IGF), and hepatocyte growth factor (HGF) [12, 18]. This variety of receptors may allow MSC to respond to distinct signals and home to different tissues [12]. However, the events generating these signals initially in the injured tissues are less well characterized.

The receptor for HGF, c-Met, is expressed on MSC [19]. HGF has been reported to be produced following tissue damage [19], including ischemia of brain [20] and heart [21], and it may become proteolytically activated in response to tissue injury [22]. It exerts neuroprotective [23] and cardioprotective activities [24] which are at least partially due to anti-apoptotic signals. However, HGF has also been shown to attract MSC [19], and a beneficial effect of MSC after myocardial infarction or stroke has been established [25, 26], implying that attraction of MSC may contribute to the tissue protective effects of HGF after ischemic injury.

In the present study, we examined whether different modes of tissue cell death, apoptosis and necrosis, are involved in initiation of MSC homing to injured tissues, and whether they differently affect MSC migration. Furthermore, we analyzed the respective contribution of HGF.

Materials and methods

Mesenchymal stem cells

Bone marrow was obtained from volunteer donors after informed consent. BM mononuclear cells (BM-MNC) were isolated by Ficoll (Biochrom, Berlin, Germany) gradient separation followed by ammonium chloride lysis of residual red blood cells. 1×10^7 BM-MNC were plated in 75-cm^2 culture flasks (Costar/Corning, Wiesbaden, Germany) and cultured at 37°C and 5% CO_2 in a humidified atmosphere in DMEM medium (Lonza, Verviers, Belgium) supplemented with 30% fetal calf serum (FCS; GIBCO/Invitrogen, Karlsruhe, Germany), 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine (all from Lonza). Cells were left to adhere for 48 h and then the non-adherent fraction was removed. When adherently growing MSC reached 80% confluence, they were detached with 0.25% trypsin (Lonza) and replated at 1:3. All experiments were performed with cells from passages 3–9. All MSC preparations used showed a typical [1, 2] $CD10^+$, $CD13^+$, $CD29^+$, $CD44^+$, $CD71^+$, $CD73^+$, $CD90^+$, $CD105^+$, Lin^-, and $CD45^-$ immunophenotype (data not shown). Furthermore, they differentiated along osteogenic and adipogenic pathways upon induction [1, 2] (data not shown).

Induction and detection of apoptosis and necrosis in neuronal and cardiac cells

HT-22 murine hippocampal neurons [27] were cultured in DMEM medium supplemented with 10% FCS, antibiotics, and L-glutamine. HL-1 murine cardiac myocytes [28] were cultured in Claycomb medium (Sigma–Aldrich, Taufkirchen, Germany) supplemented with 10% FCS, antibiotics, L-glutamine and 0.1 mM norepinephrine (Sigma–Aldrich). All cultures were performed at 37°C and 5% CO_2 in a humidified atmosphere.

To induce apoptosis, cells were treated with 300 nM staurosporine (Calbiochem, Bad Soden, Germany) [29] or 10 mM sodium azide (Sigma–Aldrich) for 3, 6 and 10 h or with 100 µg/ml poly (I:C) (Sigma–Aldrich) for 10 and 24 h. Necrotic cell death was initiated by incubation with 40 µM H_2O_2 (Sigma–Aldrich) for 10 h [29], 25% ethanol (Sigma–Aldrich) for 1 h, or by incubation at 56°C for 30 min. To confirm apoptosis/necrosis, annexin V/propidium iodide (PI) staining and flow cytometry were performed as recommended by the manufacturer (Beckman-Coulter, Krefeld, Germany), using a FACS Canto flow cytometer with Diva software (BD Biosciences, Heidelberg, Germany). For detection of DNA fragmentation, a terminal-deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL)-kit was used (Fluorescein In Situ Cell Death Detection Kit; Roche Applied Science, Mannheim, Germany) and data evaluated on a FACS Canto flow cytometer.

After induction of apoptosis or necrosis, cells were washed with PBS (Lonza) and incubated with fresh culture medium for 12 h to produce conditioned medium (CM). Alternatively, cells were used directly for experiments.

Scratch assay

After MSC had grown to confluence in 6-well plates (Costar/Corning), a scratch was made in the cellular layer with a sterile pipette tip over the total diameter of each well. Migration of adjoining MSC into this ‘wound’ was documented photographically after 0, 6, 12, and 24 h. Scratch assays were performed in the absence or presence of 100% CM derived from apoptotic or necrotic HT-22 and HL-1 cells.

Under-agarose chemotaxis assay

Migration of MSC towards apoptotic or necrotic brain and cardiac cells was analyzed in an under-agarose chemotaxis assay [29, 30]. 0.8% agarose (Eurogentec, Cologne, Germany) in DMEM medium was boiled, mixed with 0.5% bovine serum albumin (BSA; Roth, Karlsruhe, Germany) after cooling and poured into the wells of a 6-well plate. After the agarose had solidified, three 2-mm-wide and 5-mm-long slots 5 mm apart from each other were cut in the agarose of each well using a specifically designed stamp. Amounts of 8×10^4 MSC were then added to the central slot of each well and incubated for 2 h at 37°C and 5% CO_2

in a humidified atmosphere. During that time, left and right slots of each well were filled with 0.5% BSA/DMEM to prevent the agarose from drying-out; 70 µl of chemoattractant were then added to the left slots. CM (100%) of apoptotic and necrotic HT-22 or HL-1 cells, the cells themselves (4×10^4 cells) or 20–60 ng/ml recombinant HGF (R&D Systems, Wiesbaden, Germany) served as chemoattractants. For certain experiments, 2 µg/ml of neutralizing anti-mHGF goat polyclonal IgG antibody (AF2207; R&D Systems) or normal goat IgG (Santa Cruz, Heidelberg, Germany) were added; 0.5% BSA/DMEM in the right slot of each well served as negative control. After addition of chemoattractants, plates were incubated at 37°C and 5% CO_2 in a humidified atmosphere and migration of MSC documented after 4, 8, and 12 h. For counting migrated cells, the agarose between the center slot and the left slot was divided into 4 equally sized segments, numbered 1–4 starting at the center slot. The total number of migrated cells and the number of migrated cells in each segment subtracted by the number of cells migrating to the negative control slot/segments were documented. Only those cells were counted as migrating cells that were completely under the agarose.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Reverse transcription was carried out with 1 µg of RNA using Transcriptor high fidelity reverse transcriptase (Roche Applied Science) and oligo-dT primers. Reverse transcription reactions without the addition of enzyme served as negative controls. RT reactions were carried out for 30 min at 50°C followed by 5 min incubation at 85°C. Resulting cDNA fragments were amplified using Taq DNA polymerase (Qiagen) according to the supplier's instructions. The cycle profile was: 3 min of denaturation at 94°C, 35 cycles of 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C, followed by a final elongation step for 7 min at 72°C and cooling to 4°C. Reactions were carried out in a GeneAmp PCR System 9700 (Applied Biosystems, Darmstadt, Germany). The following forward and reverse PCR primers were used [29]: 5′-GCACTGC TTTAATAGGACACT-3′ and 5′-CCACAACCTGCATGA AGCG-3′ for human c-Met (215 bp fragment), 5′-CAT CAGCAATGCCTCCTGC-3′ and 5′-GTTCAGCTCAGGG ATGACC-3′ for human GAPDH (238 bp fragment), 5′-GTGGACAAGATTGTTATCGTG-3′ and 5′-GTGTAG TATCTCCTTCACAAC-3′ for mouse HGF (264 bp fragment) and 5′-GCAGTGGCAAAGTGGAGATTG-3′ and 5′-ATTTGCCGTGAGTGGAGTCAT-3′ for mouse GAPDH (96 bp fragment). Results were evaluated after agarose gel electrophoresis and ethidium bromide staining. HepG2 cells served as positive control for c-Met.

Detection of c-Met expression and production of HGF

Expression of c-Met on MSC was determined by flow cytometry. MSC were labeled with anti-c-Met monoclonal antibody (5 µg/ml; clone 95106, IgG1; R&D Systems) followed by FITC-conjugated F(ab)2-goat-anti-mouse IgG + M (Beckman-Coulter). Flow cytometric analysis was performed on a FACS Canto flow cytometer.

HGF levels in conditioned media derived from vital HT-22 and HL-1 cells or harvested 12 h after induction of apoptosis or necrosis (see above) were determined using an ELISA kit (Gentaur, Brussels, Belgium).

Statistical analysis

All data are presented as mean ± SEM for $n \geq 3$ unless stated otherwise. Statistical significance was determined with the Student's t test using Graph Pad Prism software (GraphPad, San Diego, CA, USA).

Results

Apoptotic but not necrotic neuronal and cardiac cells increase overall motility of MSC

To investigate the influence of apoptotic and necrotic brain and cardiac tissues on overall MSC motility, a scratch assay was used. After a scratch was made in a confluent layer of MSC, cultures were continued in the presence of CM derived from either staurosporine-induced apoptotic or H_2O_2-induced necrotic neuronal HT-22 or cardiac HL-1 cells. Recolonization of the scratch by adjoining MSC was already observed after 6 h in the presence of CM derived from apoptotic cells (data not shown), and after 24 h, MSC completely covered the scratch (Fig. 1a, c). In contrast, MSC cultured in the presence of CM derived from necrotic HT-22 (Fig. 1b) or HL-1 cells (Fig. 1d) for 24 h failed to recolonize the scratch.

MSC migrate towards apoptotic but not vital or necrotic neuronal and cardiac cells

Using an under-agarose chemotaxis assay, the specific target-directed migration of MSC was assessed. Treatment of either HT-22 or HL-1 cells with staurosporine to induce apoptosis resulted in a strong chemoattractive activity for MSC (Fig. 2a). In contrast, CM derived from vital or necrotic HT-22 and HL-1 cells obtained after treatment with H_2O_2 induced no target-directed migration of MSC

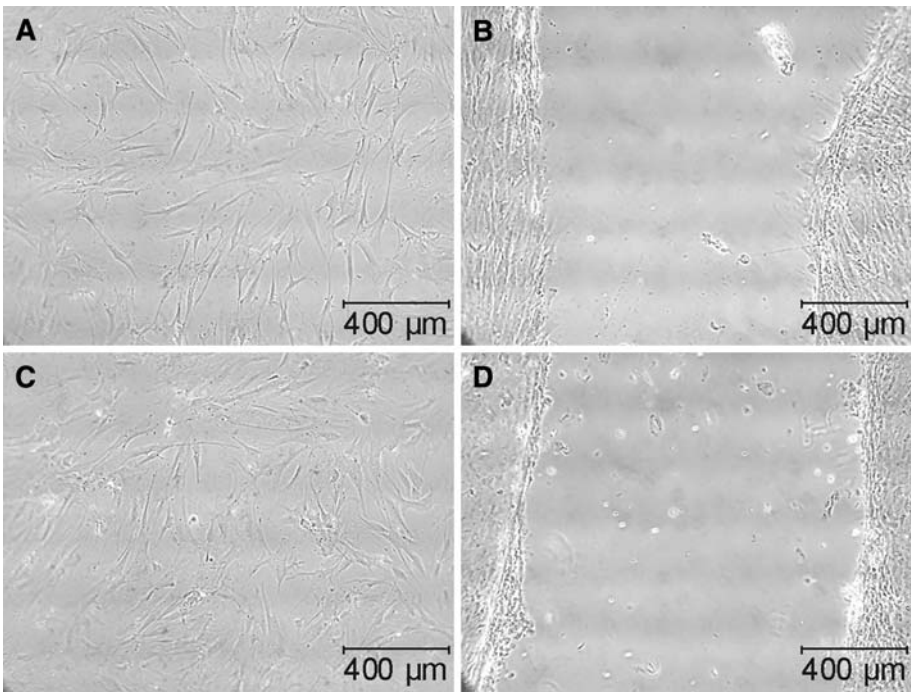

Fig. 1 Motility of MSC in response to apoptotic and necrotic cells. A scratch was made in a confluent monolayer of MSC, and cells cultured in the presence of conditioned media derived from apoptotic (300 nM staurosporine, 10 h; **a**,**c**) or necrotic (40 µM H_2O_2, 10 h; **b**,**d**) HT-22 hippocampal neurons (**a**,**b**) or HL-1 cardiac myocytes (**c**,**d**). Recolonization of the scratch by adjoining MSC was documented after 24 h

(Fig. 2a). Similar results were obtained when sodium azide or poly (I:C) were used to induce apoptosis and ethanol or incubation at 56°C to induce necrosis: only CM derived from apoptotic HT-22 and HL-1 cells constituted a chemoattractant for MSC (Fig. 2a). When the influence of the length of induction of apoptosis on the migratory response of MSC was evaluated, there was no difference between 3-, 6- or 10-h treatment with staurosporine or sodium azide (Fig. 2b) or 12- and 24-h treatment with poly (I:C) (data not shown) of HT-22 and HL-1 cells.

Total numbers of MSC migrating specifically towards the chemoattractant continuously increased over the 12-h investigation period (Fig. 2c). There was no difference whether CM or the apoptotic cells themselves were used as chemoattractant, neither in the total number of migrating cells (Fig. 2c) nor in the fraction of cells covering a certain distance within the 12-h period (Fig. 2d), indicating a comparable speed of migration.

To assess the influence of passage number on the migratory capacity of MSC, cells from passages 3, 6, and 9 were compared regarding their migration towards staurosporine-induced apoptotic HT-22 cells. There was a decrease in the total number of migrating MSC associated with increasing passage number. After 3 passages, the number of migrated MSC (1.330 ± 54 cells) was significantly higher than after 9 passages (1.010 ± 62 cells, $n = 4$; $p = 0.008$). Furthermore, the number of migrated MSC in distant agarose segments (segments 3 and 4) was higher for MSC with lower passage number. After 3 passages, 184 ± 22 MSC reached segment 4 compared to 91 ± 23 MSC ($n \geq 3$; $p = 0.049$) after 9 passages.

Extent of apoptosis and necrosis of HT-22 and HL-1 cells

To evaluate the extent of induction of apoptosis and necrosis by the various procedures, annexin V/PI and TUNEL staining were performed (Fig. 3). Induction of apoptosis in HT-22 and HL-1 cells by staurosporine or sodium azide for 10 h or by poly (I:C) for 24 h revealed Annexin V^+/PI^- early apoptotic cells as well as a smaller population ranging from 12.7 to 18.3% of Annexin V^+/PI^+ secondary necrotic cells. These secondary necrotic cells were not observed at earlier time points of induction (data not shown). At all time points analyzed, TUNEL staining detected DNA fragmentation in the majority of apoptotic cells, ranging from 90.0 to 97.3%.

Induction of necrosis by H_2O_2, ethanol or heat resulted in Annexin V^-/PI^+ necrotic cells (range 96.7–97.4%). DNA fragmentation was only detected in a minor population of the necrotic cells (range 0.3–7.1%).

The HGF/c-Met pathway mediates chemoattraction of MSC to apoptotic brain and cardiac cells

To analyze the molecular mechanism underlying MSC migration towards apoptotic brain and cardiac tissue, we determined expression of HGF in HT-22 and HL-1 cells by RT-PCR and HGF protein levels in CM derived from vital, apoptotic, and necrotic cells. Only apoptotic but not vital or necrotic HT-22 and HL-1 cells revealed HGF expression (Fig. 4a). Consistent with this observation, significant levels of HGF protein were detected only in CM of HT-22

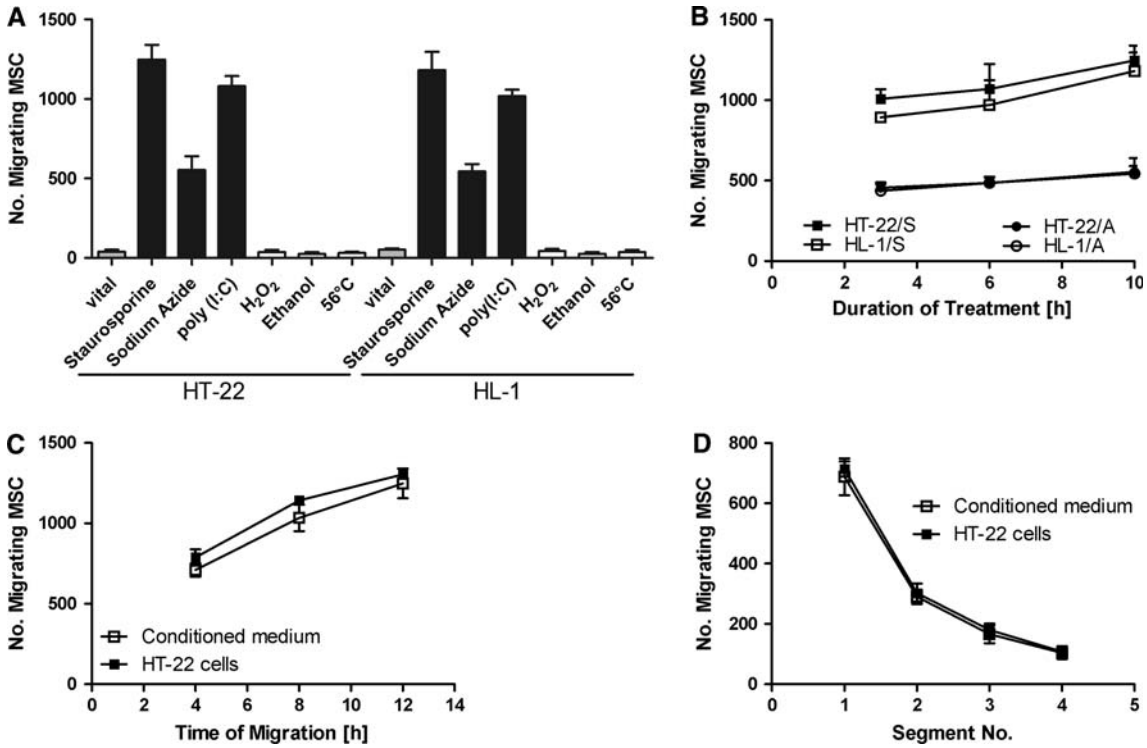

Fig. 2 Chemoattractive activity of apoptotic and necrotic neuronal and cardiac cells for MSC. Conditioned media derived from vital, apoptotic [300 nM staurosporine, 10 h; 10 mM sodium azide 10 h; 100 μg/ml poly (I:C), 24 h] or necrotic (40 μM H_2O_2, 10 h; 25% ethanol, 1 h; 56°C, 30 min) HT-22 hippocampal neurons (**a**–**d**) or HL-1 cardiac myocytes (**a**,**b**) or the cells themselves (**c**,**d**) were used as targets in an under-agarose chemotaxis assay. The total number of specifically migrating cells (**a**–**c**) and the number of cells reaching the migration segments 1–4 (**d**) were determined after 12 h (**a**,**b**,**d**) or over a 12-h period (**c**). Data are presented as mean ± SEM for $n \geq 3$. Vital, apoptotic, and necrotic cells are indicated by *grey*, *black*, and *white columns*, respectively

and HL-1 cells after induction of apoptosis with staurosporine, sodium azide, or poly (I:C), but not for vital and necrotic cells (Fig. 4b). Moreover, HGF levels only slightly increased with increasing length of apoptosis-inducing treatment (data not shown), and HGF levels measured were in good agreement with the extent of migration observed (compare Figs. 2a, 4b).

The respective receptor of HGF, c-Met, was detected in MSC by RT-PCR (Fig. 5a) as well as by flow cytometry (Fig. 5b).

Thus, there is a correlation between expression of HGF/c-Met and the migratory response of MSC to apoptotic cells. To confirm that this axis is responsible for chemoattraction of MSC to apoptotic cells, neutralization studies were performed. In the presence of a neutralizing anti-HGF polyclonal antibody, migration of MSC towards CM derived from apoptotic HT-22 and HL-1 cells was inhibited significantly ($p < 0.001$; $n \geq 3$) irrespective of whether staurosporine (78.4 ± 4.1 and 78.5 ± 5.6% inhibition, respectively), sodium azide (80.1 ± 2.6 and 80.5 ± 2.7% inhibition, respectively), or poly (I:C) (81.9 ± 2.2 and 81.7 ± 2.6% inhibition, respectively) were used to induce apoptosis (Fig. 6a). Furthermore, CM derived from apoptotic cells could be replaced by recombinant HGF. Graded doses of HGF stimulated a dose-dependent migratory response of MSC (Fig. 6b).

Discussion

MSC were shown to migrate towards apoptotic but not necrotic brain and cardiac cells in vitro. Migration correlated with c-Met expression on MSC and induction of HGF by apoptosis in the target tissues. Vital and necrotic neurons or cardiomyocytes showed no HGF expression. Moreover, blocking of HGF with a neutralizing antibody inhibited migration of MSC nearly completely. These data indicate that the HGF/c-Met axis is a key pathway involved in attracting MSC to damaged neuronal and cardiac tissues, and that tissue apoptosis constitutes a requirement to initiate these processes.

Homing of MSC to injured tissues is well established [12]. Following ischemia of heart and brain, MSC migrate to the injured tissue and contribute to tissue regeneration, although the regenerative capacity may be rather due to paracrine mechanisms than to a direct

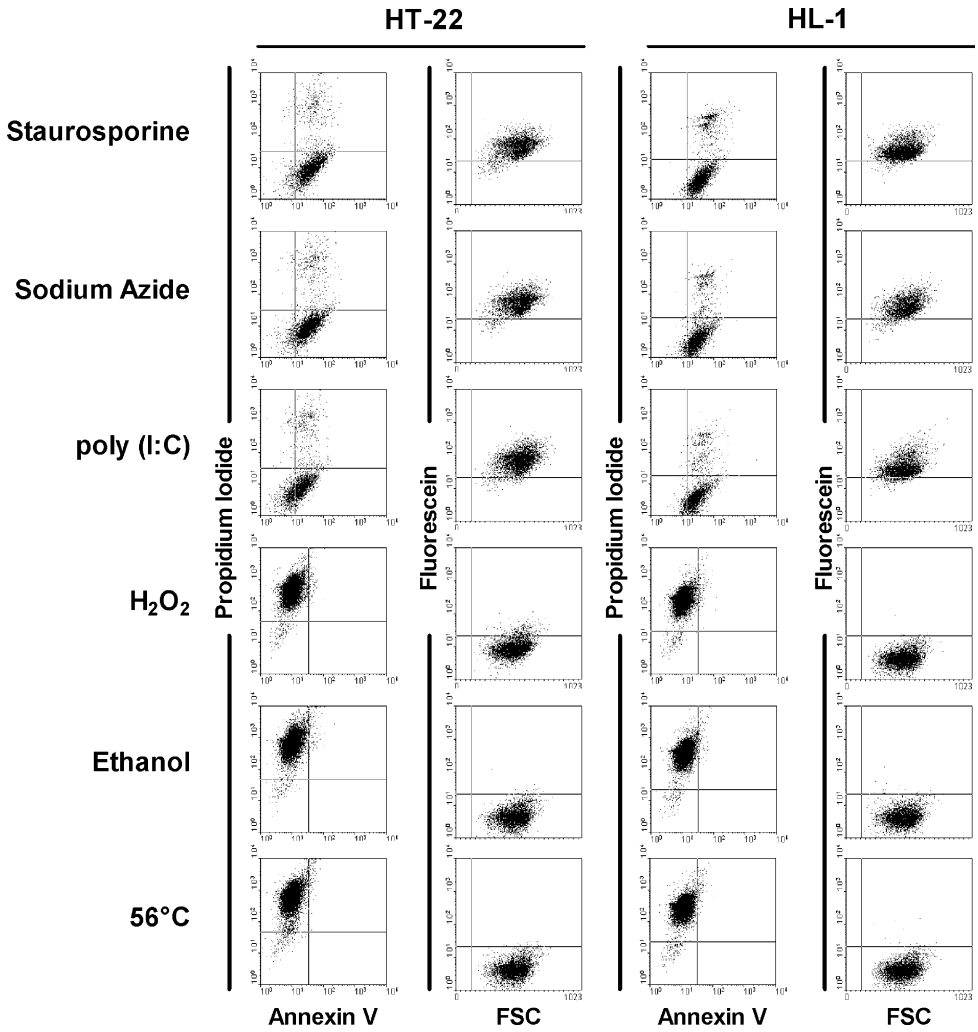

Fig. 3 Extent of apoptosis and necrosis in HT-22 and HL-1 cells. After induction of apoptosis [staurosporine, 10 h; sodium azide, 10 h; poly (I:C), 24 h] or necrosis (H_2O_2, 10 h; ethanol, 1 h; 56°C, 30 min), the frequencies of apoptotic (annexin V^+/PI^-), necrotic (annexin V^-/PI^+), and secondary necrotic cells (annexin V^+/PI^+) as well as of cells showing DNA fragmentation (fluorescein$^+$) were determined by flow cytometry. Quadrants were set according to isotype controls or to untreated cells. *FSC* Forward scatter

differentiation of MSC to the target tissue cell types [6, 14, 15, 25, 26]. HGF is produced and proteolytically activated in response to tissue injury [19, 22]. During cardiac damage, a rapid increase in HGF plasma levels has been observed [31], and cardioprotective properties of HGF have been attributed to anti-apoptotic and pro-angiogenic activities [24, 32]. Similarly, HGF production in the ischemic brain [20, 29] and neuroprotective activity of HGF due to the protection against apoptotic death of cerebral endothelial cells [33] have been reported, and this may also be true for other tissues [19, 34]. However, HGF is a potent chemo-attractant for MSC [19], and the c-Met/HGF axis is also used by other cells contributing to tissue regeneration, including endothelial progenitor cells [35], neural stem cells [36], and cord blood-derived unrestricted somatic stem cells [29]. Moreover, up-regulation of the HGF receptor c-Met on MSC, e.g., by hypoxia, is associated with increased migration towards and accelerated restoration of hind limb injury [37]. Therefore, the HGF-mediated guiding of MSC towards sites of tissue damage may also contribute to the cytoprotective activity of HGF.

Only apoptotic cells produced HGF and induced HGF-dependent migration of MSC. Necrotic cell death failed to result in HGF production and no migration of MSC towards necrotic neurons or cardiomyocytes was observed. Thus, the type of cell death, i.e., the type of tissue damage, may allow for distinct cellular response patterns to occur. Apoptosis resulted in production of HGF which on the one side has anti-apoptotic activities and on the other side attracts cells with regenerative potential, providing the basis for limiting apoptosis and initiating tissue repair.

Different cellular responses on apoptotic versus necrotic cell death have also been reported for the immune system. Necrotic cell death constitutes an immunostimulatory signal resulting in activation of dendritic cells (DC) and induction of immunity whereas apoptotic cell death appears to be associated with immunological tolerance although the microenvironment may modulate this outcome [38]. HGF [39] and MSC [7]

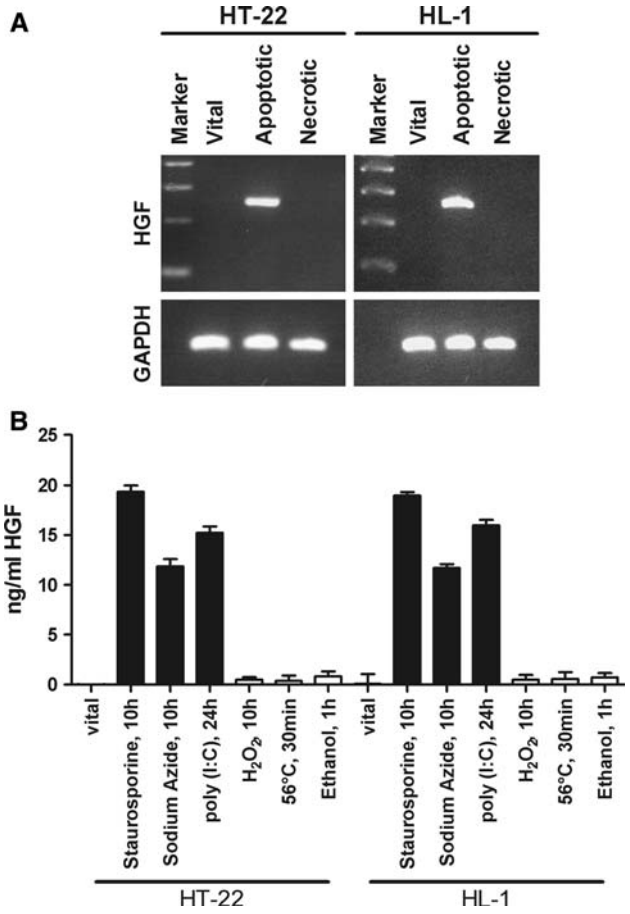

Fig. 4 Expression of HGF in HT-22 and HL-1 cells. mRNA expression of HGF in vital, apoptotic (300 nM staurosporine, 10 h) and necrotic (40 μM H_2O_2, 10 h) HT-22 hippocampal neurons and HL-1 cardiac myocytes was determined by RT-PCR (**a**). GAPDH served as positive control, reactions without reverse transcriptase (all negative, data not shown) served as negative controls. A 100-bp ladder was used as size marker. HGF levels in conditioned media derived from vital, apoptotic [300 nM staurosporine, 10 h; 10 mM sodium azide 10 h; 100 μg/ml poly (I:C), 24 h] or necrotic HT-22 and HL-1 cells (40 μM H_2O_2, 10 h; 25% ethanol, 1 h; 56°C, 30 min) were measured by ELISA (**b**). Vital, apoptotic, and necrotic cells are indicated by *grey*, *black*, and *white columns*, respectively. Data are expressed as mean ± SEM for $n \geq 2$

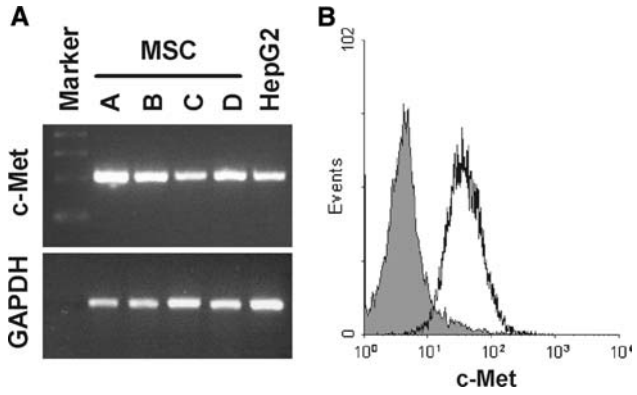

Fig. 5 Expression of the HGF receptor c-Met in MSC. Expression of c-Met in 4 MSC lines (MSC *A–D*) was detected by RT-PCR (**a**). GAPDH and RNA from the cell line HepG2 served as positive controls and reactions without reverse transcriptase (all negative, data not shown) served as negative controls. A 100-bp ladder was used as size marker. Surface expression of c-Met on MSC (**b**) was detected by staining with a c-Met specific monoclonal antibody and flow cytometry (*open histogram*). Negative control staining is indicated by a *gray* histogram

have immunosuppressive properties and affect dendritic cell as well as T-cell activation and function. Therefore, apoptosis appears not only to set off processes to limit apoptosis and to favor tissue regeneration but also immunological tolerance. In contrast, necrosis is associated with pro-inflammatory signals which generate an immunostimulatory microenvironment and result in recruitment of dendritic cells, thereby laying the path to immunity.

Interestingly, although in this study no migration towards necrotic cells was detected, Meng et al. [40] reported migration of MSC towards high mobility group box 1 (HMGB-1), a nuclear protein passively released upon necrotic cell death [41]. Whether this reflects heterogeneity of MSC, subsets of cells responding to distinct signals, or unique tissue-specific signals is currently unknown. c-Met was expressed on all cells homogenously and a negative subset was not identified. Nevertheless, contribution of other factors cannot be ruled out completely, because neutralization of HGF bioactivity inhibited migration of MSC only by about 75%. Other reports indicated the CXCL12/CXCR4 axis to be involved in homing of MSC to the injured heart [42] and brain [43]. However, blocking the CXCL12–CXCR4 interaction did not inhibit homing completely either [42], or it caused no inhibition at all [44]. Homing to tissue injuries is a complex process which may use different pathways for distinct tissues, and there may also be redundancy in chemoattractants guiding MSC to individual tissues. MSC express a multitude of chemokine and growth factor receptors involved in chemo-attraction [12, 18], some of which are expressed on subsets of cells only like CXCR4, CCR1, and CX3CR1 [45]. Other molecules including ligands of selectins may also contribute to tissue-specific homing [46]. Furthermore, culture-dependent differences in expression of receptors as well as in migration of MSC [47] have been reported, and the source of cells, e.g., bone marrow, placenta and cord blood, influences migratory potential [48]. In the present study after longer culture periods, MSC showed the same chemotaxis pattern towards apoptotic neurons and cardiomyocytes. However, a decline in migration with increasing passage number was observed.

In conclusion, we have identified a mechanism, apoptosis, which initiates migration of MSC after neuronal and

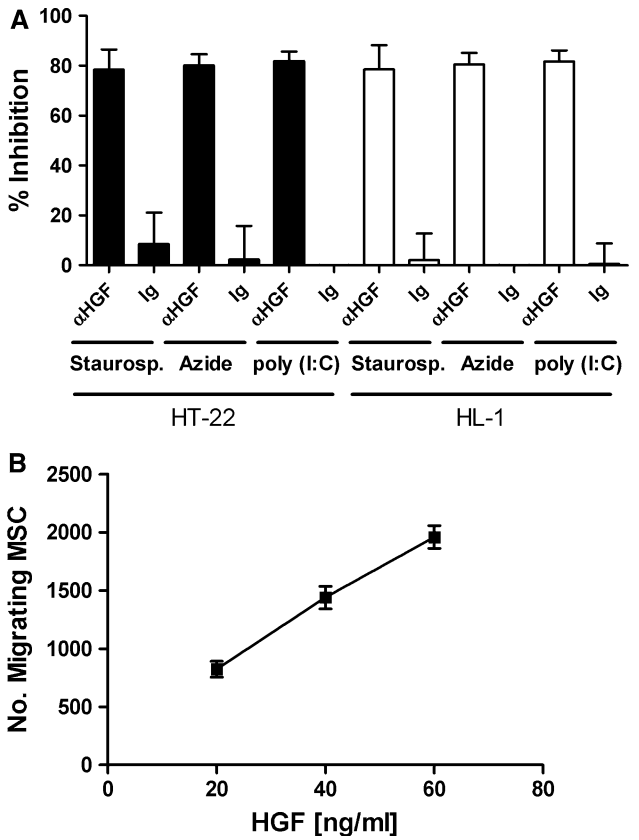

Fig. 6 Role of HGF/c-Met in MSC migration towards apoptotic neuronal and cardiac cells. Conditioned media of apoptotic HT22 hippocampal neurons (*black columns*) or HL-1 cardiac myocytes (*white columns*) treated with 300 nM staurosporine for 3 h, 10 mM sodium azide for 3 h, or 100 μg/ml poly (I:C) for 10 h with or without the addition of 2 μg/ml anti-HGF neutralizing antibody or control antibody (**a**) or graded doses of recombinant HGF (**b**) served as targets for MSC in an under-agarose chemotaxis assay. Total numbers of specifically migrating cells were counted after 12 h. Data (mean ± SEM for $n \geq 3$) are presented as % inhibition calculated from the number of migrating cells in the absence of antibodies set to 0% (**a**) or as number of migrating cells (**b**)

cardiac tissue injury and could show that the HGF/c-Met axis is the key pathway involved.

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