Hepatocyte Growth Factor/c-MET Axis-mediated Tropism of Cord Blood-derived Unrestricted Somatic Stem Cells for Neuronal Injury*

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An under-agarose chemotaxis assay was used to investigate whether unrestricted somatic stem cells (USSC) that were recently characterized in human cord blood are attracted by neuronal injury in vitro. USSC migrated toward extracts of post-ischemic brain tissue of mice in which stroke had been induced. Moreover, apoptotic neurons secrete factors that strongly attracted USSC, whereas necrotic and healthy neurons did not. Investigating the expression of growth factors and chemokines in lesioned brain tissue and neurons and of their respective receptors in USSC revealed expression of hepatocyte growth factor (HGF) in post-ischemic brain and in apoptotic but not in necrotic neurons and of the HGF receptor c-MET in USSC. Neuronal lesion-triggered migration was observed in vitro and in vivo only when c-MET was expressed at a high level in USSC. Neutralization of the activity of HGF with an antibody inhibited migration of USSC toward neuronal injury. This, together with the finding that human recombinant HGF attracts USSC, document that HGF signaling is necessary for the tropism of USSC for neuronal injury. Our data demonstrate that USSC have the capacity to migrate toward apoptotic neurons and injured brain. Together with their neural differentiation potential, this suggests a neuroregenerative potential of USSC. Moreover, we provide evidence for a hitherto unrecognized pivotal role of the HGF/c-MET axis in guiding stem cells toward brain injury, which may partly account for the capability of HGF to improve function in the diseased central nervous system.

Endogenous as well as transplanted stem cells have the capacity to migrate toward lesions in the adult central nervous system and may have the therapeutic potential to enhance regeneration after brain injury. Therefore, cell replacement therapy has received considerable attention as a future treatment for brain injury such as stroke (1–5). Various cellular transplantation strategies have shown some efficacy in treating different types of central nervous system injuries in animal models (6–8). The mechanisms underlying the observed beneficial effects of these therapies have not been elucidated. The most straightforward idea is that stem cells differentiate into mature cell types and simply replace the lost tissue. However, there is increasing evidence that transplanted cells may secrete neurotrophic or neuroprotective factors that can counteract degeneration or promote regeneration (9–13).

Concerning stem cell-based therapies various kinds of stem cells, i.e. embryonic, fetal, and adult stem cells, are under investigation. Although embryonic and fetal stem cells may have the broadest differentiation potential (1, 14), their use raises serious biological, ethical, and legal questions limiting a widespread clinical use of these cells at present. Moreover, allogeneic transplantation of embryonic stem cells was shown to produce highly malignant teratocarcinomas at the site of implantation in mice (15). Faced with these difficulties, investigators identified and evaluated alternative stem cell sources for therapeutic use.

During the past decade human umbilical cord blood has emerged as a great interest as a novel valuable source for stem cells, which possess several unique characteristics. Various studies have shown how subsets of cord blood cells differentiate under defined conditions into neurons, astrocytes, and microglia. Moreover, it has been reported that cord blood stem cells secrete trophic factors that initiate and maintain the process of repair (13, 16–19).

Recently, we reported the characterization of a novel stem cell population that has been identified in human umbilical cord blood. The cell type termed unrestricted somatic stem cell (USSC) has been shown to be pluripotent with intrinsic potential to develop into mesodermal, endodermal, and ectodermal

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2 The abbreviations used are: USSC, unrestricted somatic stem cell(s); HGF, hepatocyte growth factor; MCA, middle cerebral artery; TUNEL, terminal transferase biotinylated-UTP nick end labeling; PBS, phosphate-buffered saline; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide; CHAPS, 3-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; RT, reverse transcription.
Generation of USSC—Multipotent adult progenitor cells are largely unknown, the goal of this study was to investigate whether USSC are attracted by neuronal injury and to analyze their underlying molecular mechanisms.

**EXPERIMENTAL PROCEDURES**

**Focal Cerebral Ischemia**—The experiments were carried out in male C57 Black/6J mice weighing 20–25 g. The animals were housed under controlled diurnal light conditions and allowed access to water and food *ad libitum*. Anesthesia was induced by 1.5% halothane in 30% O2 and 70% N2O. Transient focal cerebrovascular occlusion was performed as previously described (23). An 8.0 nylon monofilament was advanced to a point of occlusion into the common carotid artery and was advanced to a point of occlusion under light halothane anesthesia into the contralateral cerebral artery at coordinates 0 mm from bregma, 1 mm lateral, and 1.5 mm below the dorsal surface. After reperfusion of the ischemic hemisphere, the brains were removed. Ischemic brain tissue was dissected from the brain, and the tissue of several animals was pooled. For control experiments without ischemia, the same method was used.

**Detection of Human DNA by PCR**—To detect USSC migration to ischemic brain tissue in *vivo*, we studied the presence of human DNA in murine ischemic brain tissue after contralateral intracerebral USSC transplantation. Ischemic brain tissue was dissected from the brain, and the tissue of several animals was pooled. For control experiments without ischemia, the same area of the brain as in ischemic animals was pooled. DNA was extracted from pooled tissue with a Qiagen DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions and analyzed for the presence of a human α-satellite chromosome sequence by PCR using human-specific primers D7a and D7B of chromosome 7 Locus D7Z1. The cycling conditions were 35 × 94 °C for 30 s, 48 °C for 30 s, 72 °C for 30 s, and 72 °C for 5 min. The following primer sequences were used: upstream primer 7A, agc gat ttg agg aca gca gta c; and downstream primer 7B, cca cct gaa aat gcc aca gc (25).

**In Vitro Model of Neuronal Apoptosis, Necrosis, and Hypoxia**—Clonal murine hippocampal neurons (HT22) (26) were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 5% fetal calf serum (Biochrom, Berlin, Germany) at 5% CO2 and 37 °C. Apoptosis was induced by incubation with staurosporine (300 nM; Calbiochem, Bad Soden, Germany) (27), whereas necrotic cell death was initiated by incubation with H2O2 (40 μM; Sigma-Aldrich). Cholestatic-induced hypoxia was initiated by incubation with sodium azide (10 mM; Sigma-Aldrich).

**Generation and Expansion of USSC**—Cord blood was collected from the umbilical cord vein with informed consent of the mother. The mononuclear cell fraction was obtained by Ficoll (Biochrom, Berlin, Germany) gradient separation followed by ammonium chloride lysis of red blood cells. The cells were plated at 5–7 × 10⁶ cells/ml in 25-cm² culture flasks. For
HGF-mediated Stem Cell Migration to Brain Injury

initial generation of USSC low glucose Dulbecco modified Eagle’s medium (Cambrex, Verviers, Belgium) with 30% fetal calf serum (Perbio, Bonn, Germany), dexamethasone (100 nM; Sigma), penicillin (100 units/ml; Cambrex), streptomycin (0.1 mg/ml; Cambrex), and L-glutamine (2 mM; Cambrex) were used (20). Expansion of the cells was performed in the same medium but in the absence of dexamethasone. The cells were incubated at 37 °C in 5% CO2 in a humidified atmosphere. When the cells reached 80% confluency, they were detached with 0.25% trypsin (Cambrex) and replated 1:3.

In Vitro Differentiation of USSC into Osteoblastic Cells—USSC were plated at 8000 cells/cm² in 24-well plates. At 70% confluence the expansion medium was supplemented with 100 nM dexamethasone, 50 μM ascorbic acid-2-phosphate, and 10 mM β-glycerol phosphate (28). The medium was changed every 3 days. For Alizarin Red staining, the cells were fixed for 5 min with 70% ethanol at 4 °C and subsequently stained with Alizarin Red (Sigma; 1% in distilled water, pH 4.2) to determine calcium deposition within the extracellular matrix.

TUNEL—Cryosections were fixed in ice-cold 4% paraformaldehyde in PBS, pH 7.4, for 15 min. Subsequently the sections were washed twice in 70% ethanol (1 min), once in PBS (3 min), once in 0.3% hydrogen peroxide/PBS (5 min), and then again in PBS (5 min). After preincubation for 15 min in TDT buffer (100 mM potassium cacodylate, 2 mM cobalt chloride, 0.2 mM diithiothreitol), 50 μl of reaction mix (10 μM biotin-16-dUTP; Roche Applied Science) containing 150 units/ml terminal deoxynucleotidyl transferase (Invitrogen) in TDT buffer were added to the sections, which were covered with a coverslip. Following incubation for 60 min at 37 °C, the reaction was terminated by washing the sections for 15 min in TB buffer (300 mM sodium chloride, 30 mM sodium citrate). Incorporated biotin was visualized using the avidin-biotin-peroxidase complex method according to the instructions of the manufacturer (Vector Laboratories, Burlingame, CA). Finally, the sections were dehydrated and embedded in Eukitt (Kindler, Feiburg, Germany). For determination of unsppecific staining, the incubation was carried out without terminal deoxynucleotidyl transferase.

Cytotoxicity Assay—Cell viability was determined using the 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT) assay. This method to determine viability respectively cell death is based on the transformation of the MTT dye to blue formazan crystals by the mitochondria enzyme succinate dehydrogenase. HT22 cells grown in 96-well microtiter plates (3500 cells in 100 μl medium/well) were incubated with vehicle (dimethyl sulfoxide), staurosporine (300 nM), sodium azide (10 mM), or H2O2 (40 μM) were homogenized in cell lysis buffer (50 mM HEPES, 100 mM NaCl, 0.1% CHAPS, 10 mM dithiothreitol, 0.1 mM EDTA, pH 7.4). Homogenates were centrifuged at 100,000 × g for 10 min at 4 °C, and the supernatant was stored at −70 °C. After determination of the protein concentration of the extracts using standard methods, the cell lysate was incubated in assay buffer with 200 μM of the caspase-3 substrate Ac-DEVD-pNA (Biomol, Hamburg, Germany). The reaction was monitored by reading the absorbance at 405 nm in a microtiter plate reader, and readings were taken at 1–10–min intervals for 240 min. To test specificity of the reaction, the caspase-3 inhibitor Ac-DEVD-CHO (0.1 μM; Biomol, Hamburg, Germany) was included in selected experiments. The statistics were performed with the Sigma Plot program.

Under-agarose Chemotaxis Assay—The under-agarose chemotaxis assay was performed as described by Laevsky and Knecht (30) with modifications. 3 ml of agarose (1%) in RPMI medium containing 0.5% bovine serum albumin were poured into 35-mm plastic Petri dishes. After the agarose was allowed to solidify, three 2-mm-wide parallel wells were cut 5 mm apart with a standard razor blade using a template. USSC suspension (the cell number should allow the formation of a confluent layer) was then added to the central well and allowed to attach in a humidified incubator for 2 h at 37 °C. 70 μl of the chemoattractant was added to the right well, and migration buffer (RPMI medium containing 0.5% bovine serum albumin) serving as control was added to the left well. Petri dishes were incubated in a humidified incubator for 6 h at 37 °C, and after fixation (methanol for 30 min at room temperature followed by 37% formaldehyde for 30 min at room temperature) USSC that migrated toward the chemoattractant or the migration buffer were counted. Only those cells were counted that migrated at least 100 μm into the agarose. The number of cells that migrated toward the control buffer was subtracted from the number of those cells that migrated toward the target. The data are expressed as number of specifically migrated USSC and represent the means ± S.E. from at least three independent experiments. As targets for USSC migration the following conditions were used: (a) conditioned medium of rat primary fibroblasts; (b) conditioned medium of HT22 cells treated for 0, 3, 6, and 16 h with staurosporine (300 nM); (c) conditioned medium of HT22 cells treated for 0, 3, 6, and 16 h with H2O2 (40 μM); (d) conditioned medium of HT22 cells treated for 16 h with sodium azide (10 mM); (e) conditioned medium of HT22 cells treated for 0, 6, and 16 h with staurosporine (300 nM) that was incubated for 2 h with an anti-HGF antibody (AF-294-NA; R & D Systems, Wiesbaden, Germany) at the concentrations indicated in the figure; as a control unspecific antibodies at the same concentrations were used, and conditioned media were concentrated by filter centrifugation and diluted in migration buffer; (f) human recombinant HGF (R & D Systems) in migration buffer at concentrations indicated in the figure; (g) extracts that were generated from post-ischemic and contralateral brain hemispheres of mice 24 h following 1 h of MCA occlusion; extracts were generated by homogenization of post-ischemic or contralateral brain hemispheres in RPMI on ice (100 mg of
tissue/ml of buffer) and subsequent centrifugation; and (h) extracts that were generated from post-ischemic brain hemispheres of mice 24 h following 1 h of MCA occlusion and that were incubated for 2 h with an anti-HGF antibody (AF-294-NA; R & D Systems) at concentrations indicated in the figure. As a control unspecific antibodies at the same concentrations were used. In selected experiments USSC were pretreated for 2 h with the Rho kinase inhibitor hydroxyfasudil (30 μM; Sigma).

Isolation and Reverse Transcription (RT)-PCR of RNA—RNA was isolated from USSC and HT22 cells that were treated as indicated in the figures using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instruction. RT reactions were performed at 50 °C with Omniscript (Qiagen) and the 3 ‘ primers following the supplier’s instruction. The PCRs were performed with the HotStar Taq Master Mix (Qiagen) following the supplier’s instruction. The cycling conditions were 93 °C for 5 min, 35 × 93 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and 72 °C for 5 min. Expression of the following transcripts was detected by RT-PCR: human c-MET, upstream, gca ctt cta tag gac act, and downstream, cca caa cct gca tga agc g; human HGF, upstream, cag tgt tca gaa gtt gac tgc, and downstream, gtc tca ttc ata gta tgt tca g; human CXCR4, upstream, gca gat ata cac ttc aga taa c, and downstream, gtc ggc cac tga cag gtc; human SDF-1α, upstream, atg aac ggc aag gtc gtc g, and downstream, cag ccc ggc tac aat ctg a; human GAPDH, upstream, cat cag caa tgc ctc ctg c, and downstream, gtc ggc ctc agg gat gac c; murine HGF, upstream, gtc gac aat gtt atc gtc, and downstream, gtc tag ttc ctc ccc cac; and murine GAPDH, upstream, cat gac cac atg tcc atc ggc, and downstream, ctt gat gtc atc ata ctt ggc.

Western Blot Analysis—Brain tissue, cells, or conditioned media that were concentrated by filter centrifugation according to the manufacturer’s instructions (Amicon Ultra-15 centrifugal filter unit; Millipore, Schwalbach, Germany) were boiled in loading buffer containing 20% glycerol, 3% SDS, 3% 2-mercaptoethanol, and bromphenol blue for 10 min. After measurement of total protein concentration, proteins (20 μg/lane) were separated on a 12% SDS-polyacrylamide gel. Following tank blotting onto a nitrocellulose membrane (GE Healthcare), the blots were washed and developed using the Amersham Biosciences ECL system at room temperature. The membranes were incubated with the respective horseradish peroxidase-coupled secondary antibodies (1:2000; GD Healthcare) at room temperature for 1 h. Once more, the membranes were washed and developed using the Amersham Biosciences ECL system. As controlled by marker proteins, all of the protein bands that were presented in the figures correspond to the expected size of the protein that the antibody should detect.

Statistical Analysis—Significance of changes were determined by Students t test. The data are represented as the means ± S.E.

RESULTS

Ischemic Brain Tissue Attracts Cord Blood-derived USSC—Experimental stroke was induced in mice by occlusion of the MCA for 1 and 24 h of reperfusion. The effects of MCA thread occlusion on general physiological parameters have been described previously (22, 27, 31). In this study the reduction of the regional cerebral blood flow was measured by laser Doppler flowmetry and as shown in Fig. 1A the mean ischemic laser Doppler flowmetry values declined to below 35% of the basic level and returned to the control level within 15 min, indicating efficient occlusion and reperfusion. To detect cellular damage in this stroke model, we visualized DNA fragmentation by TUNEL staining, which was restricted to the ischemia-damaged hemisphere and could not be detected in the healthy hemisphere (Fig. 1B). As documented in Fig. 1C, the TUNEL staining covered a huge area of the affected hemisphere, and injury can be detected in the striatum as well as in the cortex. By Western blot analysis using an antibody that specifically detects cleaved and thereby activated caspase-3, we were able to demonstrate that proapoptotic signal transduction is activated in the ischemia-damaged hemisphere in the stroke model used (Fig. 1D).

To investigate migration of cord blood-derived USSC in vitro, we used an under-agarose chemotaxis assay. In contrast to cell motility assays, this assay allows the formation of a stable gradient of a chemoattractant, and therefore it is well suitable for analyzing stem cell migration. As shown in Fig. 1E USSC are strongly attracted by extracts from ischemia-damaged murine brain hemispheres, whereas extracts made from contralateral murine brain hemispheres only initiate a moderate migration of USSC, and conditioned medium of rat primary fibroblasts serving as negative control did not attract USSC at all. These results indicate that ischemia-damaged brain tissue produces factors that attract cord blood-derived USSC.

Apoptotic but Not Necrotic Hippocampal Neurons Initiate Migration of USSC—Because a multitude of different cell types including invading cells of the immune system that secrete several chemokines are present in ischemia-damaged brain tissue, we were interested to analyze whether neurons themselves secrete factors that attract stem cells. Moreover, we investigated apoptotic as well as necrotic neuronal signal transduction in their capability to initiate USSC migration because both types of cell death occur in the chosen animal model of cerebral ischemia. To induce apoptotic or necrotic cell death, clonal...
murine hippocampal neurons were treated with staurosporine or H$_2$O$_2$, respectively. Concentrations were chosen to obtain comparable rates of neuronal cell death as demonstrated by the MTT assay (Fig. 2A). Determination of caspase-3 activity in staurosporine- or H$_2$O$_2$-treated HT22 cells revealed that staurosporine but not H$_2$O$_2$ initiates the proapoptotic signal transduction, resulting in caspase-3 activity (Fig. 2B).

To reflect the cellular situation of cerebral ischemia in a better way, we also made use of a model of chemical-induced hypoxia (32). HT22 cells were treated with sodium azide, which resulted in ~50% cell death as demonstrated by the MTT assay with HGF Secretion of Injured Brain Tissue and Neurons—For therapeutic purposes it is very important to know which factor or factors are responsible for stem cell migration. To answer this question we determined expression of chemokines and growth factors and their respective receptors in USSC (Fig. 4A). With this approach we found that USSC strongly expressed c-MET but not its ligand HGF. In contrast, the most prominent factor known to initiate migra-
tion of stem cells toward stroke tissue, SDF-1α (33, 34), is already expressed by USSC.

As a first step to investigate whether HGF is responsible for USSC migration toward neuronal injury, we analyzed the expression of HGF in the used paradigms. As shown in Fig. 4B by Western blot, expression of HGF in the post-ischemic hemisphere could be clearly detected, whereas in the contralateral hemisphere only a basal expression of HGF was visible. These expression levels of HGF correlate very well with the potency of the respective extracts to attract USSC (Fig. 1E).

We also determined HGF mRNA and protein by RT-PCR and Western blot analysis, respectively, in neuronal necrosis and apoptosis. In untreated and necrotic neurons, no expression could be detected, whereas in apoptotic neurons a prominent production of HGF was visible, showing a peak of HGF expression at 6 h after staurosporine treatment. To be sure that HGF is not only expressed but also secreted by the cell, we determined the HGF protein in conditioned media by Western blot and were able to detect HGF in conditioned media of cells that were treated with staurosporine or sodium azide. HGF was not detected in conditioned media of untreated or H2O2-treated HT22 cells (Fig. 4D). Therefore, the level of HGF correlates very well with the capacity of the conditioned media to initiate stem cell migration in these paradigms, indicating that secretion of HGF by neuronal injury is responsible for attracting USSC.

c-MET Level of USSC Correlates with Migration toward Apoptotic Neurons in Vitro and Ischemia-damaged Brain Tissue in Vivo—Next, we were interested to study whether the HGF receptor c-MET, a tyrosine kinase that is known to regulate multiple cellular events ranging from cell motility to morphological differentiation (35), is important for migration of USSC. As already shown by RT-PCR (Fig. 4A) c-MET is expressed in USSC on the level of mRNA. To study the expression of c-MET in more detail, we determined the protein level of c-MET in different USSC lines by Western blot analysis (Fig. 5A). Most USSC lines produced high amounts of c-MET. Only one cell line (USSC line C; Fig. 5A) showed a very weak signal for c-MET in the Western blot. When we studied those cell lines in the under-agarose chemotaxis assay, it was evident that USSC lines with high c-MET expression showed a strong...
migratory capacity toward conditioned media of apoptotic neurons, whereas minimal migration was observed by USSC line C, which expressed c-MET at a low level (Fig. 5B). To investigate whether the level of c-MET may also influence USSC migration in vivo, we transplanted cells of USSC lines A and C stereotactically into the healthy brain hemisphere of mice in which focal cerebral ischemia had been induced (15). Three weeks after transplantation, human DNA indicative of the presence of USSC could only be detected in the damaged brain hemisphere of mice in which USSC with a high level of c-MET were transplanted (Fig. 5C). No human DNA was found in the left hemisphere when USSC were transplanted into the right hemisphere of uninjured murine brains (Fig. 5C). These results indicate that USSC may have the capacity to migrate toward neuronal injury in vivo and that c-MET plays an important role in USSC migration toward neuronal injury.

Differentiation of USSC Results in Loss of c-MET Expression and Migrational Potency—USSC are pluripotent and can be differentiated into cell types of all germ layers (20). However, osteogenic differentiation of USSC seems to be a default pathway, because USSC tend to differentiate into osteoblastic cells spontaneously at very high passages (data not shown). To analyze the influence of differentiation on the migrational potency osteogenic differentiation of USSC was induced, and differentiated cells were investigated at different time points. In Fig. 6A efficient differentiation into osteoblasts is demonstrated by Alizarin red staining that determines calcium deposition. When we studied c-MET expression by Western blot analysis,
down-regulation of c-MET during osteogenic differentiation was observed (Fig. 6B). Investigation of the migrational capacity of USSC that were differentiated osteogenically for 0, 3, and 7 days toward conditioned media of apoptotic neurons revealed a loss of migration during osteogenic differentiation (Fig. 6C). Once more, the expression of c-MET in USSC could be coupled successfully with their migrational potency. Moreover, these data imply that migration and differentiation are competing events for USSC.

HGF Signaling Is Necessary for the Migration of USSC toward Neuronal Injury—The results shown above strongly suggest that HGF is the responsible factor for the tropism of USSC for neuronal injury. To verify this hypothesis, we used a neutralizing antibody to inhibit HGF bioactivity in conditioned media of apoptotic neurons. Using the under-agarose chemotaxis assay, we demonstrated that conditioned medium of HT22 cells treated for 6 or 16 h with 300 nM staurosporine strongly attracted USSC, whereas incubation of these conditioned media with an anti-HGF antibody inhibited migration dose-dependently (Fig. 7A). When an unspecific antibody instead of the anti-HGF antibody was used, USSC migration was unaltered, indicating the specificity of these results (Fig. 7A). Fig. 7B demonstrates that homogenate of ischemia-damaged murine brain tissue strongly attracted USSC, whereas incubation of the homogenate with an anti-HGF antibody inhibited migration. Once more the use of an unspecific antibody instead of the anti-HGF antibody was without effect on USSC migration (Fig. 7B).
HGF-mediated Stem Cell Migration to Brain Injury

Finally, we made use of recombinant human HGF in the under-agarose chemotaxis assay and were able to demonstrate that HGF attracts USSC in a dose-dependent manner (Fig. 7C). Taken together these results demonstrate that HGF signaling is necessary for the migration of USSC toward neuronal injury.

DISCUSSION

Our data indicate that USSC are strongly attracted by HGF that is secreted by ischemia-damaged brain tissue and by apoptotic neurons. Necrotic neurons do not secrete HGF and have no potential to initiate migration of USSC. In all of the paradigms used in this study, the secretion of HGF by target tissue and the expression of the HGF receptor c-MET in USSC directly correlated to migrational potency of USSC, strongly indicating that the HGF/c-MET axis is the driving force for USSC migration toward the investigated targets. Because migration of USSC toward apoptotic neurons can be blocked completely by a neutralizing antibody that inhibits the bioactivity of HGF, it can be concluded that HGF is necessary to attract USSC toward neuronal damage.

Interestingly, only apoptotic but not necrotic neurons secrete HGF and thereby attract USSC. This difference can be explained when USSC are seen as circulating repair cells that have a function in regenerating small lesions in the fetus because multiple apoptotic processes occur in fetal development, whereas necrosis is not a physiological event.

Our findings shed important light on the role of the HGF/c-MET axis in neural regeneration. HGF is a multifunctional cytokine that was originally characterized as a mitogen for hepatocytes. Meanwhile, HGF is known to evoke a multitude of cellular responses in various cell types and organs. The function of HGF is mediated via its receptor c-MET. Binding of the ligand leads to autophosphorylation of multiple tyrosine residues located in the cytoplasmatic domain of c-MET activating cellular cascades that lead to angiogenesis, cellular motility, growth, invasion, morphological differentiation, embryonic development, and tissue regeneration. Moreover, a deregulated activation of c-MET has been shown to be crucial for the acquisition of tumorigenic properties, and the involvement of c-MET in different human tumors has been established (36–43). HGF has also been shown to play a role in migration of gonadotropin hormone-releasing hormone-1 secreting neurons during embryonic development and in mediating angiopoietin-induced smooth muscle cell recruitment. Moreover, it was described that a gradient of recombinant HGF attracts mesenchymal stem cells from bone marrow and cord blood (44–46). In addition to all the functions that are already attributed to the HGF/c-MET axis, the results of this study demonstrate that the interplay of HGF and its receptor is crucial for the cross-talk between neuronal injury and USSC. Although we focused on the migrational potential of USSC in this study, it is well possible that also other stem cell populations that express functional c-MET (e.g. mesenchymal stem cells from bone marrow) show HGF-mediated tropism for neuronal injury. Because migration is a complex biochemical process, it is likely that in addition to the HGF/c-MET axis, other signal transduction pathways exert function on neuronal lesion-triggered USSC migration. USSC also express the SDF-1 receptor CXCR4, and therefore USSC may also respond to this potent chemokine under certain conditions.

Interestingly, different studies have clearly demonstrated that infusion or overexpression of HGF improved function in the diseased central nervous system. Although it has been documented that an antiapoptotic function of HGF may be responsible for the neuroprotective action of the HGF/c-MET axis, other underlying mechanisms are discussed (47–51). Our results provide evidence that in addition to the published mechanisms, HGF might improve function in the diseased brain via attracting stem cells toward neuronal injury. Those attracted stem cells might act via differentiating directly into required cell types or via secreting neuroprotective factors that trigger neuronal survival or promote regeneration. Therefore, the hitherto unrecognized role of the HGF/c-MET axis in guiding stem cells toward neuronal injury may partly account for the proven capability of HGF to improve function in the diseased brain.

Together with their proven neural differentiation potential (20), the prominent tropism for apoptotic neurons makes USSC a serious candidate for cell replacement therapies of different neurological diseases. In a number of neurological diseases, proapoptotic signal transduction is activated, and therefore it is very possible that USSC are attracted by brain lesions resulting from those diseases. Moreover, USSC can potentially be used as vehicles because their tropism for neuronal injury might deliver neuroprotective factors in a targeted way toward a lesion. Because we demonstrate in this study that differentiation of USSC results in a down-regulation of c-MET accompanied by a loss of migrational potency, it is evident that migration and differentiation are competing events for USSC, and these results argue that for transplantational purposes that aim at transport USSC toward lesions, only USSC with a high c-MET level and a low differentiation grade should be used. Taken together our results demonstrate prominent tropism of USSC for stroke-damaged tissue and apoptotic neurons and provide evidence for a hitherto unrecognized pivotal role of the HGF/c-MET axis in attracting stem cells toward the injured central nervous system.

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REFERENCES

1. Kelly, S., Bliss, T. M., Shah, A. K., Sun, G. H., Ma, M., Foo, W. C., Masel, J., Yen, E. M., Weissman, I. L., Uchida, N., Palmer, T., and Steinberg, G. K. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 11839–11844
2. Bang, O. Y., Lee, J. S., Lee, P. H., and Lee, G. (2005) Ann. Neurol. 57, 874–882
3. Peterson, D. A. (2004) J. Clin. Investig. 114, 312–314
4. Haas, S., Weidner, N., and Winkler, I. (2005) Curr. Opin. Neurol. 18, 59–64
5. Hermann, A., Gastl, R., Liebau, S., Popa, M. O., Fiedler, J., Boehm, B. O., Maisel, M., Lerche, H., Schwarz, J., Brenner, R., and Storch, A. (2004) J. Cell Sci. 117, 4411–4422
6. Hoehn, M., Kustermann, E., Blunk, J., Wiedermann, D., Trapp, T., Wecker, S., Focking, M., Arnold, H., Hescheler, J., Fleischmann, B. K., Schwindt, W., and Buhre, C. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 16267–16272
