ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research paper Acta Neurobiol Exp 2011, 71: 24–35

Migratory capabilities of human umbilical cord blood-derived neural stem cells (HUCB-NSC) *in vitro*

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Many types of neural progenitors from various sources have been evaluated for therapy of CNS disorders. Prerequisite for success in cell therapy is the ability for transplanted cells to reach appropriate target such as stroke lesion. We have established neural stem cell line from human umbilical cord blood neural stem (HUCB-NSC). In the present study we evaluated migratory capabilities of cells (HUCB-NSC) and the presence of various migration-related receptors. Immunocytochemical analysis revealed abundant expression of CXCR4, PDGFRα, PDGFRβ, c-Met, VEGFR, IGF-1R and PSA-NCAM receptors in non-adherent population of HUCB-NSC cultured in serum free (SF) conditions (SF cells). Biological activity of selected receptors was confirmed by HUCB-NSC in vitro migration towards SDF-1 and IGF-1 ligands. Additionally, rat brain-derived homogenates have been assessed for their chemoattractive activity of HUCB-NSC. Our experiments unveiled that brain tissue was more attracted for HUCB-NSC than single ligands with higher potency of injured than intact brain. Moreover, adherent HUCB-NSC cultured in low serum (LS) conditions (LS cells) were employed to investigate an impact of different extracellular matrix (ECM) proteins on cell motility. It turned out that laminin provided most permissive microenvironment for cell migration, followed by fibronectin and gelatin. Unexpected nuclear localization of CXCR4 in SF cells prompted us to characterize intracellular pattern of this expression in relation to developmental stage of cells cultured in different conditions. Continuous culture of LS cells revealed cytoplasmatic pattern of CXCR4 expression while HUCB-NSC cultured in high serum conditions (HS cells) resulted in gradual translocation of CXCR4 from nucleus to cytoplasm and then to arising processes. Terminal differentiation of HUCB-NSC was followed by CXCR4 expression decline.

Key words: cord blood, CXCR4, fibronectin, IGF-1, laminin, migration, neural stem cells, SDF-1, stroke, transwell

INTRODUCTION

The concept of stem cells and their therapeutic use was one of the most exciting fields of science in recent years (Gokhale and Andrews 2006, Muller 2006, Tataria et al. 2006, Giannoudis et al. 2008). Neurological disorders remain one of the greatest challenges of medicine with little or no effective treatment available. As such they are considered the prime application for stem cell technology (Lindvall and Kokaia 2006). Numerous research groups evaluated potential of cell-based therapy in animal models of neurological disorders (Walczak et al. 2008, Janowski and Date 2009, Joers and Emborg 2009). Unfortunately clinical trials did not show robust effect indicating the need for more studies in animal models directed at better understanding of the role of transplanted cells (Freed et al. 2001, Olanow et al. 2003, Bersano et al. 2010, Boncoraglio et al. 2010).

Among neurological disorders stroke has a major disease burden on the society as it is the most frequent cause of serious disability (Horner 1998). The success of cell-based therapy of stroke relies on appropriate delivery of cells to the lesion site and proper function of the graft i.e., differentiation to specific phenotype or release of growth factors. Limited positive effects of cell therapy in stroke, at least partially can be attributed to insufficient or inappropriate delivery of the cells (Hicks and Jolkkonen 2009, Zawadzka et al. 2009). Optimization of cell migration and homing is

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Received 20 January 2011, accepted 03 March 2011

intuitively essential and needs to be established first, before advancing to studies addressing graft functionality.

Cord blood has been considered attractive source of stem cells as it is widely available and is not plagued in ethical controversy (Arcese et al. 1998). Safety and therapeutic potential of cord blood-derived cells have already been proven in hematological disorders (Kline and Bertolone 1998). Neural differentiation of cord blood-derived progenitors has also been achieved (Sanchez-Ramos et al. 2001, Buzanska et al. 2002) and their application in neurological disorders is increasingly appealing (Ali and Bahbahani 2010).

In our laboratory neural stem cell line derived from human umbilical cord blood (HUCB-NSC) was established (Buzanska et al. 2006b). Long-term culture and numerous passages over several years proved its stability and lack of tumorogenic properties (Buzanska et al. 2006a, Jurga et al. 2006). Subsequently our group demonstrated that HUCB-NSC transplanted in the vicinity of the stroke lesion display tropism towards infarcted area of the brain (Kozlowska et al. 2007). Control over migration of transplanted cells is of paramount importance to tailor better cell therapies, thus in this study we decided to characterize in detail migratory capabilities of HUCB-NSC *in vitro,* focusing primarily on their response to chemoattractive signals observed in stroke.

Based on data from literature several receptors were selected to search leading molecular pathway involved in neural progenitor cell migration process: c-Met (for HGF; Son et al. 2006, Garzotto et al. 2008), PDGFRβ (Habisch et al. 2008), PSA-NCAM (Franceschini et al. 2004, Battista and Rutishauser 2010), PDGFRα (Andrae et al. 2004), Flk-1 (for VEGF, Barkho et al. 2008), IGF-1R (Schlenska-Lange et al. 2008, Annenkov 2009) and CXCR4 (for SDF-1; Janowski 2009, Carbajal et al. 2010). Among them CXCR4 and IGF-1 were most frequently referred in publications thus with matching ligands were studied in more detailed manner.

METHODS

Study Design

For initial experiments non-adherent HUCB-NSC cultured in serum free media (SF cells) were characterized immunocytochemically in term of migration-re-

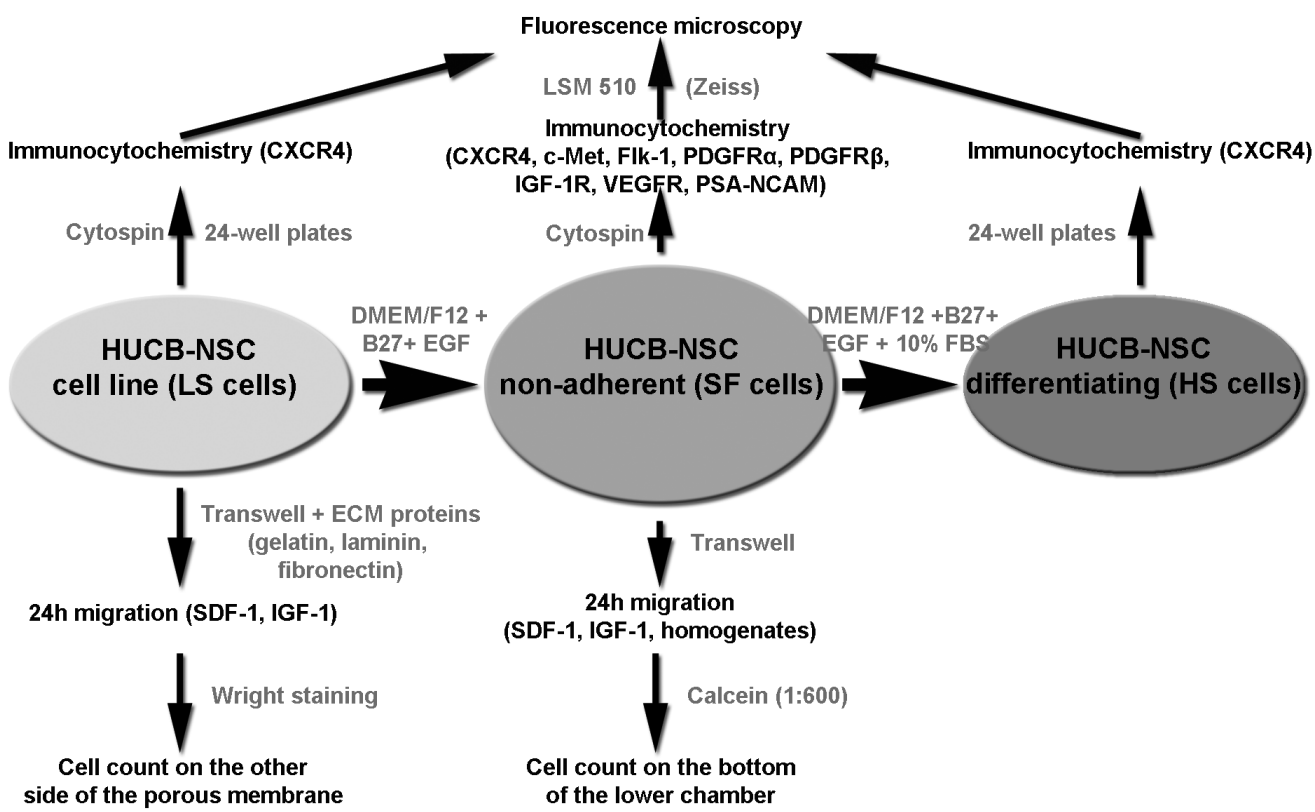

Fig. 1. Schematic drawing of the study design.

lated receptor expression. To assess migratory capabilities of HUCB-NSC *in vitro* transwell-based migration assay was performed (Kucia et al. 2006). Different chemoattractants selected for the migration study i.e., brain-derived homogenates (intact and infarcted tissue), chemokine (SDF-1) and growth factor (IGF-1) were used. For evaluation of extracellular matrix (ECM) protein involvement in the process of migration, adherent subset of HUCB-NSC (LS cells) have been investigated in the presence of gelatin, fibronectin and laminin (Bilozur and Hay 1988).

Additionally, the localization of CXCR4 molecule in the different compartments of HUCB-NSC cultured in SF, LS or high serum (HS) conditions was analyzed. The entire study design is shown in scheme (Fig. 1).

Culture of HUCB-NSC

Three populations of HUCB-NSC maintained in different culture conditions were evaluated.

Population of LS cells was established by HUCB-NSC culture in low serum (LS) medium contained of Dulbecco's modified Eagle medium (DMEM) and F12 (Gibco) supplemented with 2% fetal bovine serum (FBS, Gibco), insulin, transferrin, and selenium (ITS, 1:100, Gibco), and antibiotic antimycotic solution (AAS, 1:100, Sigma, Buzanska et al. 2006a). Under these conditions HUCB-NSC grew as heterogeneous populations of floating and adherent cells.

To undifferentiated cells, HUCB-NSC were cultured in 25cm^2 flasks in serum free (SF) medium consisted of DMEM/F12 supplemented with B27 (1:50, Gibco) and epidermal growth factor (EGF 20 ng/ml; Sigma). The population of SF cells contained floating, morphologically round-shaped, cells were taken for immunocytochemical analysis and migration experiments. Of note, long-term SF cell culture results with neurosphere formation (Sarnowska et al. 2009).

Population of HS cells was obtained by HUCB-NSC culture in high serum medium cosisted of Dulbecco's modified Eagle medium (DMEM) and F12 (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), insulin, transferrin, and selenium (ITS, 1:100, Gibco), and antibiotic antimycotic solution (AAS, 1:100, Sigma, Buzanska et al. 2006a). These cells grow as adherent population.

To assess the expression of migration-related receptors on HUCB-NSC: SF, LS or HS cells were pipetted into glass bottom 24-well plates (20 000 cells/cm^2) and cultured for 7 days in serum free, low serum (2% FBS) or high serum (10% FBS) media, respectively.

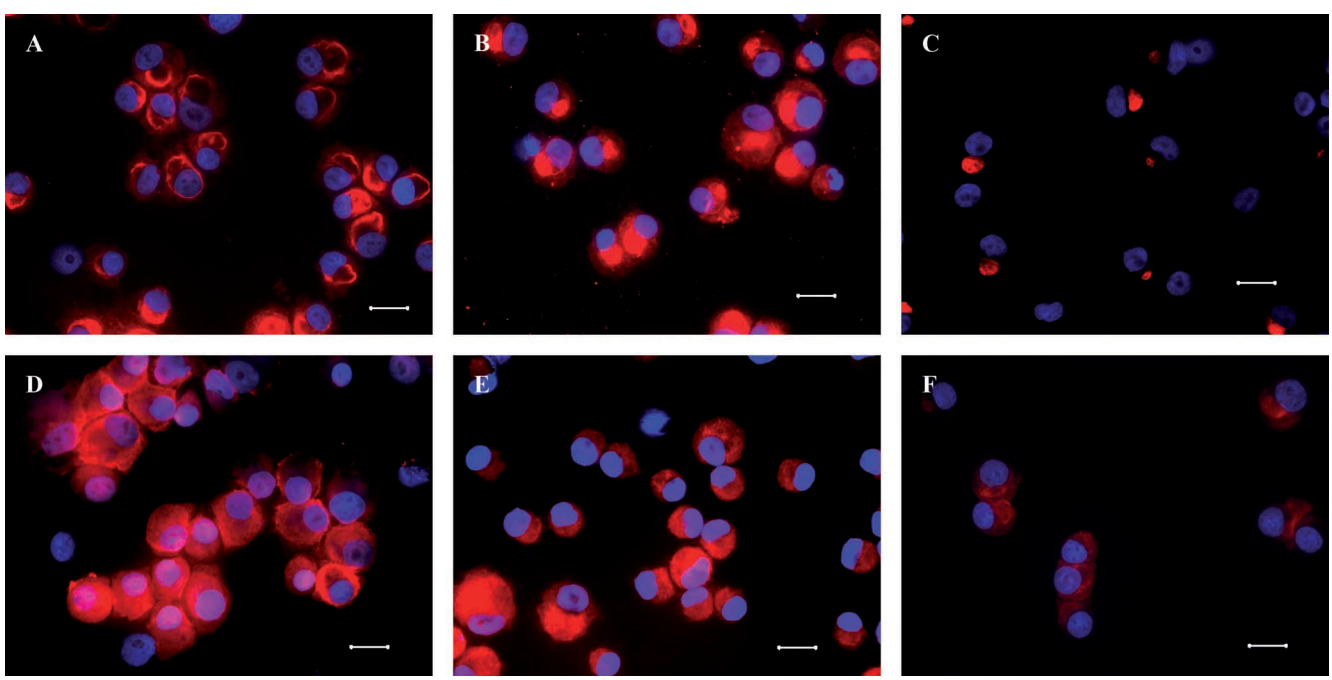

Fig. 2. Subcellular localization of selected receptors related to the process of cell migration in SF cells: (A) c-Met (receptor for HGF), (B) PDGFRB, (C) PSA-NCAM, (D) PDGFRA, (E) Flk-1 (VEGF-R1), (F) IGF-1R. Nuclei are stained in blue (Hoechst 33342) and respective receptors in red. Scale bar = 20 μm.

Immunocytochemistry

The cell migration-related receptors were assessed on HUCB-NSC cultured in serum free (SF cells), low serum (LS) or high serum (HS) conditions by immunofluorescence using specific antibodies. Slides with adhered cells were air-dried, washed thoroughly 3×5 min in PBS and fixed in 4% PFA for 15 min. Following fixation cells were blocked and permeabilized (10% NGS or 5% BSA, in 0.25% Triton). Then the primary antibodies were used to determine the presence of selected receptors: goat polyclonal anti-PDGFRβ (1:100, Santa Cruz), rabbit polyclonal anti-PDGFRα (1:100, Santa Cruz), mouse monoclonal anti-Flk-1 (1:100, Santa Cruz), mouse monoclonal anti-c-Met (1:50, Novocastra), rabbit polyclonal anti-CXCR4 (1:200, Chemicon), mouse monoclonal anti-PSA-NCAM (1:200, Chemicon) and mouse monoclonal anti-IGF-1R (1:100, Oncogene). After rinsing with PBS the slides were exposed to Alexa Fluor 546 secondary antibodies (1:500, Invitrogen) for 60 min at RT. Additionally cell nuclei were stained with Hoechst 33342 (1:100, 15 min). All the procedures for negative controls were processed in the same manner, except omission of primary antibodies. Zeiss LSM 510 confocal laser scanning microscope was used for image acquisition and processing.

Chemoattractant preparation

Supernatants from rat brain tissue homogenates as well as SDF-1 and IGF-1 were used as chemoattractants. Fetal bovine serum (30%) was selected as a positive control and human albumin (500 µg/ml) as a negative control. The experiments were approved by First Local Ethics Committee in Warsaw. Supernatants were obtained from tissue homogenates derived from intact and infarcted adult (250 g) Wistar rat brains. Brain infarcts (striatum and thalamus) were induced by stereotactic injection of ouabain (5 nmol, 1µl, Janowski et al. 2008) then brain tissue was harvested 6 h, 48 h, 7 days after surgery. Striatum and thalamus were dissected from both intact and infarcted rat brains, frozen on dry ice and subsequently stored in -70ºC. Directly after thawing, tissue was homogenized on ice in DMEM/F12 with addition of protease inhibi-

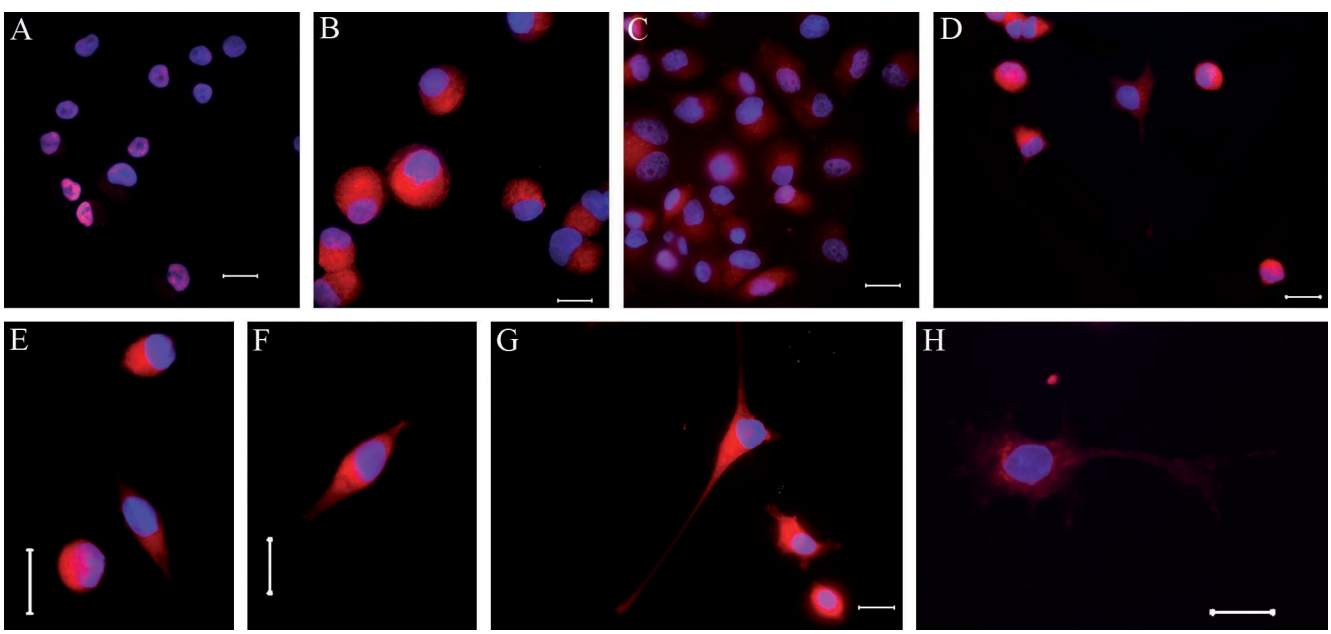

Fig. 3. Intracellular localization of CXCR4 receptors in SF cells, (B) LS cells (the whole population of floating and adherent cells), (C) adherent subset of LS cells, and adherent population of HS cells (D). Lower panel of photographs show localization of CXCR4 receptors in HUCB-NSC during their culture in HS conditions. The expression of CXCR4 in different cell compartments is found to be related to the different developmental cell stage of HS cells. Round-shape cell revealed both: nuclear and cytoplasmic expression of CXCR4 and more differentiated oval-shape cells showed exclusively cytoplasmic localization of CXCR4 with translocation of CXCR4 towards arising processes subsequently following HS cell elongation (E, F, G). Terminal cell differentiation as characterized by the presence of many processes resulted in gradual decline of CXCR4 expression (H). CXCR4 molecules are stained in red and nuclei in blue (Hoechst 33342). Scale bar = 20 µm.

tors. Homogenates of brain tissues were spun down at 4000 rpm for 20 min and supernatant was carefully collected. Protein concentration in the supernatant was measured, adjusted to 500 µg/ml and frozen until was used for cell migration experiments. SDF-1 and IGF-1 were dissolved in DMEM/F12 at 1, 10 and 100 ng/ml (SDF-1) and 30, 100, 500 ng/ml (IGF-1).

Cell migration assay

Cell migration assay was performed using SF and LS cells in 24 well transwells (Costar) equipped with inserts dividing wells into upper and lower chamber. The chambers used for LS and SF cells were separated by porous membrane (diameter of pores = 8 µm). For each chemoattractant 6 wells were used per assay with two independent experiments. For adherent LS cells porous membrane was coated by ECM (gelatin, laminin or fibronectin; 30 min, 37ºC) while for non-adherent SF cells porous membrane was left uncoated. Hundred µl of cell suspension (10^6/ml) was loaded into upper chamber and 600 µl of chemoattractant into lower chamber. Transwells were incubated for 24 h at standard conditions (5% CO_2, 37º C) to allow migration of the cells. Following this step the number of cells migrated to lower chamber was evaluated and expressed as number of cells per well.

Non-adherent SF cells were counted at the bottom of lower chamber after addition of calceine (1:600) and removal of upper chamber (no cells adhered to the lower side of membrane), while adherent LS cells were counted on the lower side of the porous membrane after cell staining with Wright method (Merck) and wiping out cells from upper side of the porous membrane. Cell counting process was manual.

Statistical analysis

For statistical analysis lowest means square (LMS) test incorporated into PROC MIXED software (SAS) was used. The level of statistical significance was set up at p=0.05. Results were presented as means and confidence intervals (CI). To clarify graphic representation of the results the general rule that CI of one variable may not include a mean of other variable to get statistically significant difference can be employed.

RESULTS

Expression of migration-related receptors in HUCB-NSC cultured in different conditions

Immunocytochemistical analysis of SF cells revealed abundant expression of migration-related receptors. Various pattern of localization of these receptors have been observed. c-Met, PDGFRβ and PSA-NCAM were localized paranuclearly, likely to be associated with endoplasmatic reticula or Golgi apparatus. Among them c-Met was characterized by rather membranous

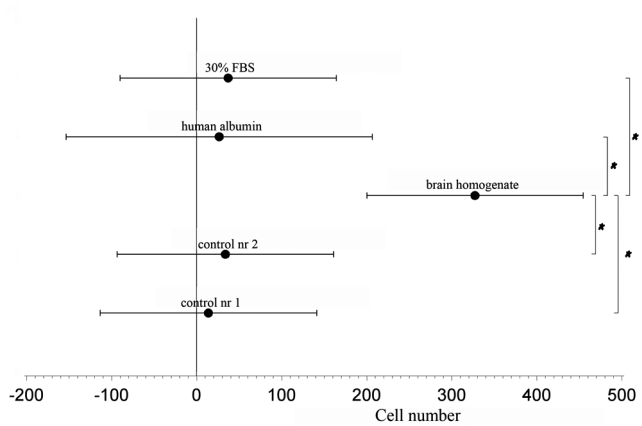

Fig. 4. SF cell migration towards brain-derived homogenate, in comparison with multiple controls. Means are marked with black points and confidence intervals (CI) are shown as horizontal lines. Vertical lines and leaning stars display most relevant statistical significance, n=6 (SAS, PROC MIXED, LMS, p<0.05).

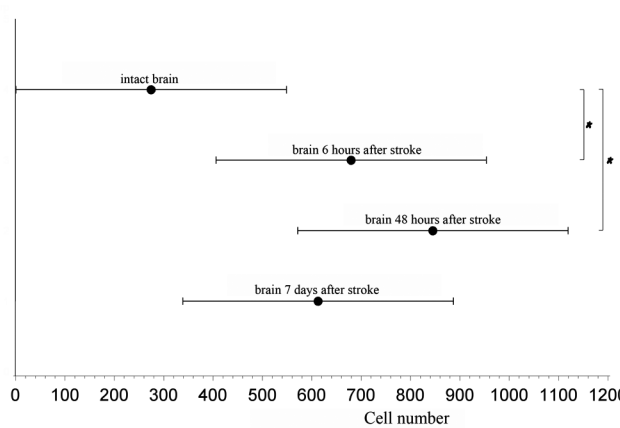

Fig. 5. SF cell migration towards brain-derived homogenate obtained in certain time sequence after stroke induction. Means are marked with black points and confidence intervals (CI) are shown as horizontal lines. Vertical lines and leaning stars display most relevant statistical significance, n=6 (SAS, PROC MIXED, LMS, p<0.05).

distribution (Fig. 2A), while PDGFRβ and PSA-NCAM were packed more densely (Fig. 2B, C). PDGFRα was present prevailingly on cell membrane and Flk-1 and IGF-1R were deployed homogenously within cytoplasm (Fig. 2D-F, respectively).

Surprisingly CXCR4 expression on SF cells was found exclusively in nuclei, mostly in the nuclear periphery (Fig. 3A). In term of unexpected nuclear localization of CXCR4 molecules in SF cells we decided to follow this expression in HUCB-NSC appearing in the different developmental stage due to their culture in different media conditions. We observed that heterogeneous population of floating and adherent LS cells revealed cytoplasmic expression of CXCR4 molecules (Fig. 3B). The adherent subset of LS cells also showed cytoplasmic pattern of CXCR4 distribution, except of few cells with predominantly nuclear expression within nests of high cellularity (Fig. 3C). We observed that initiation of cell adherence following HUCB-NSC culture in differentiating medium (10% FBS; HS cells) resulted in gradual translocation of CXCR4 molecules from nucleus towards cytoplasm. HUCB-NSC cultured in HS media revealed different morphology. The population of HS cells contained round-shape cells expressing CXCR both in the nucleus and cytoplasm and oval-shape showing complete shift of CXCR4 from nucleus to the cytoplasm (Fig. 3D, E). Further cell differentiation caused translocation of CXCR4 towards arising processes subsequently following their elongation (Fig. 3D, F, G). Terminal cell differentiation as characterized by the presence of many processes resulted in gradual decline of CXCR4 expression (Fig. 3D, H).

Migration of SF cells

The first assay was performed to evaluate cell migration toward homogenate from intact brain with special emphasis on appropriate set up of controls. Control 1 was medium only (without chemoattractant), control 2 consisted of the same concentration of homogenate in both chambers. Negative and positive control was composed of albumin and 30% FBS, respectively. Homogenates from intact brain tissues induced statistically significant migration in comparison to all controls (Fig. 4). Unexpectedly 30% FBS (used as positive control) failed to stimulate cell migration.

The second assay was designed to compare chemotactic activity of homogenates from intact and infarcted brains obtained at 3 different time points after stroke insult (6 h, 48 h and 7 days) (Fig. 5). The assay revealed the highest chemoattractive potential of the middle time point (48 h), followed by early time point (6 h) and late time point (7 days). Both early and middle time point revealed statistically significant increase of cell migration comparing to intact brain (p=0.04 and p=0.005, respectively). Tendency toward increase of cell migration was observed in case of late time point (p=0.09).

The third assay evaluated chemoattractive capabilities of soluble factors: IGF-1 (Fig. 6) and SDF-1 (Fig. 7). Both factors induced cell migration in dose-

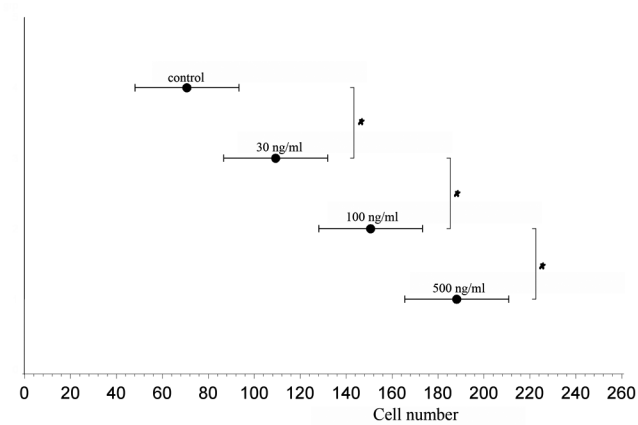

Fig. 6. SF cell migration towards various concentrations of IGF-1. Means are marked with black points and confidence intervals (CI) are shown as horizontal lines. Vertical lines and leaning stars display most relevant statistical significance, n=6 (SAS, PROC MIXED, LMS, p<0.05).

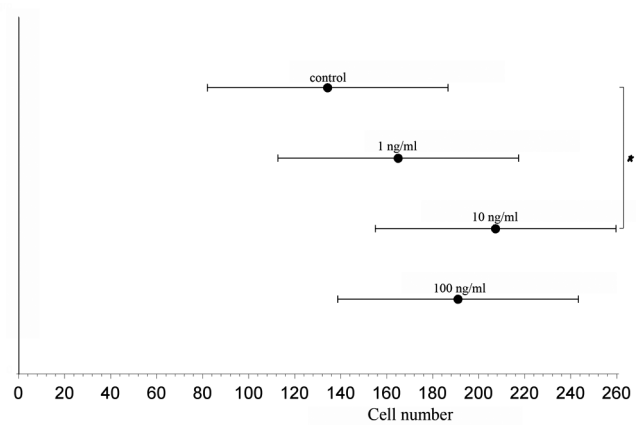

Fig. 7. SF cell migration towards various concentrations of SDF-1. Means are marked with black points and confidence intervals (CI) are shown as horizontal lines. Vertical lines and leaning stars display most relevant statistical significance, n=6 (SAS, PROC MIXED, LMS, p<0.05).

dependent manner with saturation effect observed in case of SDF-1. However efficiency of cell migration induction by single factors was substantially lesser as compared to brain tissue homogenates.

Migration of LS cells

Adherent cells (LS cells) were employed to assess the influence of ECM proteins on cell migration process. Porous membrane coated by gelatin revealed similar dose-dependent migration characteristics toward SDF-1 with the same saturation effect at the dose of 100 ng/ml, as in case of SF cells (Fig. 8). However basal motility of LS cells was substantially higher as compared to SF cells. Use of fibronectin and laminin ECM molecules revealed that migration was much more pronounced in comparison to gelatin (Fig. 9). Further investigations showed dramatic effect of laminin and fibronectin on cell migration even without application of chemoattractant (only culture medium in lower chamber; Fig. 10). Chemokine SDF-1 caused an additional increase of cell migration only on laminin-coated membranes while producing no effect on fibronectin-coated ones.

DISCUSSION

In this study we have shown abundant expression of various migration-related receptors on SF cells what is in agreement with reports of robust *in vivo* migration of transplanted HUCB-NSC (Kozlowska et al. 2007, Jablonska et al. 2010). However this fortune made the identification of one leading receptor involved in HUCB-NSC migration process impossible. Biological activity of selected receptors has been confirmed by dose-dependent cell migration induced by their ligands: SDF-1 and IGF-1. SF cells migrated to the lower chamber of transwell without adhesion on the lower surface of the porous membrane. This pattern directly resembles leukocyte trafficking (Lambert et al. 2011). Of note, both SF cells and leukocytes are non-adherent cell populations. Although statistically significant, induction of HUCB-NSC (SF cells) migration by single soluble factor was modest (0,2%) comparing to values reported for leukocytes (up to 80%; Bleul et al. 1996, Wang et al. 2010). However juxtaposition of terminally differentiated cells such as leukocytes programmed for fast migratory response in liquid environment, and neural stem cells naturally residing in solid tissue niche may be not appropriate. The literature search for non-adherent pattern of *in vitro* migration of NSC did not identify any such study. Among stem cells only leukocyte (hematopoietic) and endothelial progenitors reveal the same pattern (Peichev et al. 2000, Netelenbos et al. 2002) described by us as hematopoietic-like pattern of cell migration. The extent of hematopoietic/stem cell migration was found to be lower than their differentiated derivatives, but much higher than our SF cells, and was approximately 20 % of cells (Takizawa et al. 2006). In fact, in relative units, the soluble factor-induced increase of our SF cell migration by several times is similar to results of other stem cell migration studies (Zou et al. 2010). The major difference lies in very low basal (under control conditions) SF cell motility. This may be attributed to the very low level of SF cell differentiation. It was revealed that side population of glioma cells representing putative cancer stem cells exhibited decreased cell migration in comparison to the rest of cells (Weber et al. 2010). In agreement with this hypothesis was slow proliferation rate and increased expression of pluripotency genes in SF cells, comparing to LS cells (Aleksandra Habich, personal communication). Thus very limited SF cell motility may depend on their primitive character, a result of quiescent stem cell niche in serum free conditions.

Rat brain-derived homogenates were shown to be relatively very potent inducers of SF cells migration (up to 1%). In terms of total cell count these chemoat-

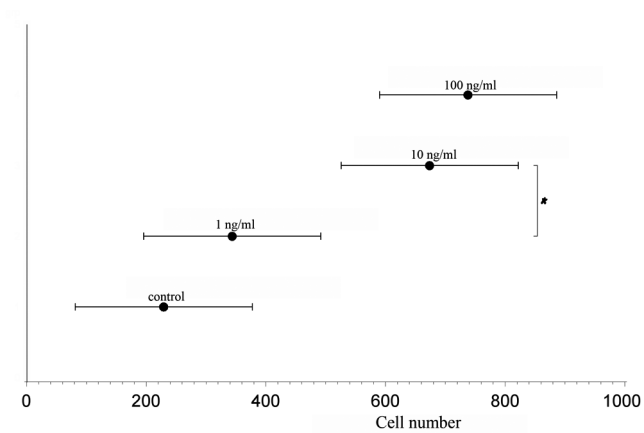

Fig.8. LS cell migration throughout gelatin-coated porous membranes in various gradients of SDF-1. Means are marked with black points and confidence intervals (CI) are shown as horizontal lines. Vertical lines and leaning stars display most relevant statistical significance, n=6 (SAS, PROC MIXED, LMS, p<0.05).

tractants were much more efficient than single soluble factors, in particular those obtained from infarcted brains. It may be credited for a riot of soluble biologically active molecules present in homogenates. Interestingly serum which is rich in a variety of chemokines and growth factors failed to induce SF cell migration. It may also be attributed to inhibitory effect of soluble factors such as SDF-1 at higher concentrations (Dziembowska et al. 2005). Negative effect of serum on dental pulp stem cell migration was reported as well (Howard et al. 2010). Nevertheless the mechanism of homogenate-induced migration should rather be evaluated based on differences between brain homogenates and serum. The high content of ECM proteins in homogenates may be essential. In fact, it was shown that collagen fragments derived from proteolysis are strong chemoattractants (Mauney et al. 2010). In the same study it was also revealed that cellular stress resulted in increased protease activity and this well explains stronger chemotactic activity of homogenates derived from infarcted than intact brains. Homogenates derived from brain 48 h following stroke induction revealed higher chemoattractive potential than at earlier and later time points. It is in accordance with others (Wang et al. 2002, Newman et al. 2005) and may constitute an invaluable advice on time window for clinical application of cell therapy in stroke patients.

The impact of ECM proteins on proliferation and differentiation of HUCB-NSC has already been established (Szymczak et al. 2010). In addition to the role of ECM proteins on chemotaxis induction we explored their impact on the process of haptotaxis, which is inherent in cell migration in solid tissues such as brain. Haptotaxis depends on cell adhesion to extracellular matrix thus it was indispensable to include adherent LS cells for that experiment. Virtually no migration was observed for LS cells with uncoated porous membrane (data not shown). Membrane coating with gelatin led to significantly greater migration as compared to SF controls. Chemoattractant (SDF-1) had a dose-dependent additive effect on LS cell migration. The dose-dependent increase of NSC migration toward SDF-1 gradient over porous ECM coated membrane has been previously reported (Liu et al. 2008, Yu et al. 2010). However presence of migrated cells on the lower side of the porous membrane is typical for mesenchymal stem cell migration studies thus we call it mesenchymal-like pattern of cell migration.

Furthermore we revealed distinct effect of various ECM proteins on LS cell migration toward SDF-1. Laminin provided most permissive microenvironment for cell migration, followed by fibronectin and gelatin. The prominent role of laminin in supporting cell migration process was also observed in other studies (Rooprai et al. 1999, Strachan and Condic 2004, Ziu et

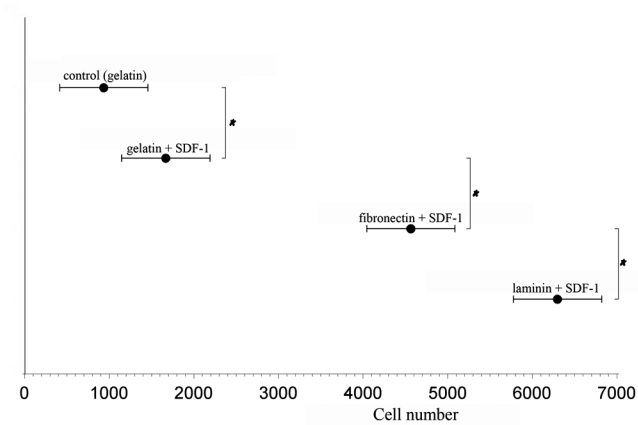

Fig. 9. The influence of porous membranes coated by various ECM proteins (laminin, fibronectin and gelatin) on LS cell migration induced by SDF-1 gradient (0/10 ng/ml). Coating membrane by gelatin was used as a sole control. Means are marked with black points and confidence intervals (CI) are shown as horizontal lines. Vertical lines and leaning stars display most relevant statistical significance, n=6 (SAS, PROC MIXED, LMS, p<0.05).

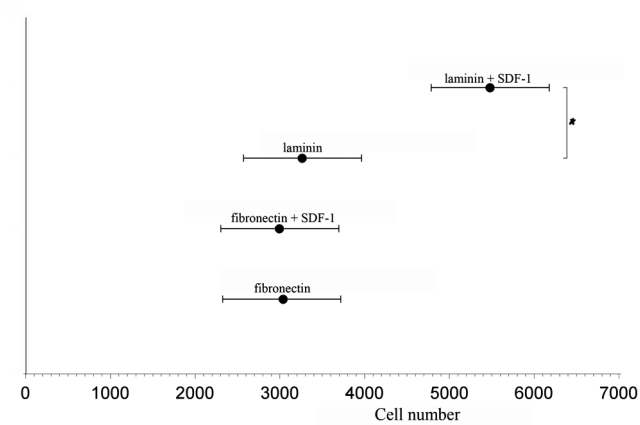

Fig. 10 The influence of ECM proteins, namely fibronectin and laminin, on LS cell migration toward defined SDF-1 gradient (10 ng/ml). Coating membrane by respective ECM protein was used as a control. Means are marked with black points and confidence intervals (CI) are shown as horizontal lines. Vertical lines and leaning stars display most relevant statistical significance, n=6 (SAS, PROC MIXED, LMS, p<0.05).

al. 2006). Laminin and fibronectin binding to a specific β1-integrin receptor on cell surface as well as increase of MMPs activity may be responsible for such robust effect (Tate et al. 2004, Sypecka et al. 2009). Thus ECM proteins seem to play a key role in NSC migration process and soluble factors may accomplish an additional effect (Strachan and Condic 2008).

Unexpected, nuclear localization of CXCR4 in SF cells requires also a comment. CXCR4 is involved in migration process and to fulfill its function it needs to be located on cell membrane (Wysoczynski et al. 2005) or in the cytoplasm after internalization of active form (Altenburg et al. 2010). To date nuclear location of CXCR4 was only observed in tumor cells. However opposed effects on patient outcome and prognosis were reported (Spano et al. 2004, Na et al. 2008, Speetjens et al. 2009, Wagner et al. 2009). Recently nuclear localization sequence (NLS) was identified within CXCR4 (Wang et al. 2010) and renal cancer responded to high concentration of SDF-1 by nuclear translocation of CXCR4 (Wang et al. 2009). On the contrary in our experiments CXCR4 nuclear translocation was a result of serum withdrawal. To our knowledge this is the first report on nuclear location of CXCR4 in non-tumor cells. In addition we demonstrated for the first time translocation of CXCR from nucleus to cytoplasm. Biological rationale for nuclear deposition of CXCR4 receptor however still needs to be solved.

CONCLUSIONS

We confirmed *in vitro* migratory capabilities of HUCB-NSC. ECM proteins seems to play a dominant role in HUCB-NSC migration. The highest chemoattractive potential of brain homogenates derived 48 hours after stroke induction may have implications for clinical translation of cell therapy. Cell migration pattern of HUCB-NSC depends on their developmental stage. Biological rationale for unexpected nuclear localization of CXCR4, its translocation toward cytoplasm and down-regulation during differentiation is unclear and requires further investigation.

ACKNOWLEDGEMENT

This work was supported by the Polish Ministry of Scientific Research and Higher Education grants: No. 142/B/P01/2008/35 and no. 3326/B/P01/2009/36.

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