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Effects of atelocollagen on neural stem cell function and its migrating capacity into brain in psychiatric disease model

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Abstract Stem cell therapy is well proposed as a potential method for the improvement of neurodegenerative damage in the brain. Among several different procedures to reach the cells into the injured lesion, the intravenous (IV) injection has benefit as a minimally invasive approach. However, for the brain disease, prompt development of the effective treatment way of cellular biodistribution of stem cells into the brain after IV injection is needed. Atelocollagen has been used as an adjunctive material in a gene, drug and cell delivery system because of its extremely low antigenicity and bioabsorbability to protect these transplants from intrabody environment. However, there is little work about the direct effect of atelocollagen on stem cells, we examined the functional change of survival, proliferation, migration and differentiation of cultured neural stem cells (NSCs) induced by atelocollagen in vitro. By 72-h treatment 0.01–0.05 % atelocollagen showed no significant effects on survival, proliferation and migration of NSCs, while 0.03–0.05 % atelocollagen induced significant reduction of neuronal differentiation and increase of astrocytic differentiation. Furthermore, IV treated NSCs complexed with atelocollagen (0.02 %) could effectively migrate into the brain rather than NSC treated alone using chronic alcohol binge model rat. These experiments suggested that high dose of atelocollagen exerts direct influence on NSC function but under 0.03 % of atelocollagen induces beneficial effect on regenerative approach of IV administration of NSCs for CNS disease.

Keywords Atelocollagen · Stem cell therapy · Neural stem cell · Regenerative medicine

Introduction

Cellular-based therapies using neural stem cells (NSCs) are being evaluated as promising treatment options for many disorders and injuries of the central nervous system (CNS). Recent studies have demonstrated successful engraftment and functional recovery in animal models of spinal cord injury (Yasuda et al. 2011; Tsuji et al. 2011; Salazar et al. 2010), cerebrovascular disease (Andres et al. 2011; Rota Nodari et al. 2010; Honmou et al. 2011), neurodegenerative disease (Lindvall et al. 2004; Mazzini et al. 2008; Blurton-Jones et al. 2009), and psychiatric disorders (Yoshinaga et al. 2007). Minimally invasive intravenous injection would present several benefits over intralesional, intrathecal, or intraventricular administration for the treatment of the CNS disease (Honmou et al. 2011). However, the intravenous injection has limited value in terms of the administered cell number in the lesion and the more effective cell delivery system is required for therapeutic application.

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Atelocollagen is a biocompatible and biodegradable component which is highly purified pepsin-treated type-1 collagen derived from calf dermis (Freyria et al. 2009). Since atelocollagen is known as a fibrous protein for maintaining tissue and organ morphology with low immunogenicity, it is used clinically for a wide range of purposes, such as wound-healing, vessel prosthesis, bone cartilage substitute and hemostatic agent (Ochiya et al. 2001). In addition, recent molecular neuroscientists reported that atelocollagen is a safe and useful application for an effective gene delivery method in vitro and vivo (Minakuchi et al. 2004; Hanai et al. 2006). Atelocollagen is also reported to display low toxicity and low immunogenicity when it is administrated in vivo.

In this study, to know the direct effect of atelocollagen on NSC function and proper concentration of atelocollagen for application of regenerative therapy of NSC treatment, we examined the influence of atelocollagen on proliferation, migration, differentiation, and survival functions of cultured NSCs in vitro, and analyzed the potential delivery of intravenously treated NSCs into the brain by complexing with atelocollagen using chronic alcohol binge model rat.

Materials and methods

Animals

For in vitro cell culture experiments, timed pregnant Wistar rats were purchased from Clea Japan, Inc. (Sapporo, Japan) and housed at 22 °C on a 12:12-h light/dark cycle with free access to food and water. All experimental procedures were approved by the institutional animal care committee and conducted following the Sapporo Medical University Guidelines for the Care and Use of Laboratory Animals.

Monolayer culture of NSCs

NSCs were cultured from rat embryos on gestational day 13.5 by previously described methods with minor modifications (Tateno et al. 2005, 2006). Briefly, the telencephalon was isolated from the whole brain and trimmed in ice-cold Hank's balanced salt solution (HBSS; Invitrogen Corp., Carlsbad, CA, USA) microscopically. Cells were dissociated by pipetting and plated in dishes coated with poly-L-ornithine/fibronectin in neurobasal medium (NBM; Invitrogen) supplemented with 2 % B27 (Invitrogen), 0.5 mM L-glutamine (Wako, Tokyo, Japan) and 20 ng/ml recombinant human fibroblast growth factor-2 (FGF-2; PeproTech, London, UK) at the density of 3×10^6 cells per 100-mm dish. Cells were incubated under 5 % CO_2 at 37 °C for 7 days followed by the each experiment. Almost all the cells in this culture condition were nestin-positive (Anti-Nestin, Millipore, Temecula, CA, USA) NSCs (>95 %).

MTT assay evaluating cell survival

Cultured NSCs were re-plated in 24-well assay plates at the density of 5×10^4 cells/well and incubated with 0–0.05 % (0–500 μg/ml) atelocollagen (3 % atelocollagen in phosphate-buffered saline, Koken, Co., Ltd, Tokyo, Japan) for 72 h under the same culture condition.

The mitochondrial dehydrogenase activity that cleaved 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA) was used to measure viability of cells in a quantitative colorimetric assay (Sieuwerts et al. 1995). MTT solution (0.5 mg/ml) was added to each well, and the plate was incubated for 2 h at 37 °C, producing blue formazan cleaved by the mitochondrial enzyme in living cells. 20 % sodium dodecyl sulphate (SDS)/50 % *N*,*N*-dimethylformamide (DMF) solution was added to solubilize the formazan, and the OD of each well was measured at 570 nm.

Bromodeoxyuridine (BrdU)-ELISA evaluating cell proliferation

For evaluating NSC proliferation, cell proliferation ELISA (Amersham Biosciences, Little Chalfont Buckinghamshire, UK) was performed on re-plated NSCs to detect the alteration of incorporation of 5-bromo-2′-deoxyuridine as a marker of NSC proliferation by the atelocollagen (0–0.05 %) treatment for 72 h. Briefly, cultured cells in 96-well microplate was labeled with BrdU for 24 h at 37 °C. The labelling medium removed and cells were fixed for 30 min at room temperature (RT) followed by the incubation with blocking reagent for 30 min at RT. After removing blocking buffer, cells were incubated with peroxidase-labelled anti-BrdU solution for 90 min. Then the microplate washed three times and incubated with substrate for 15 min followed by the measurement of optical density at 450 nm (Muir et al. 1990). As a background data we observed that almost 100 % of cultured cells were positive with anti-BrdU antibody detected by diaminobenzidine tetrahydrochloride (DAB) stainning.

Chemotaxis assay evaluating cell migration

The Chemotaxi cell Chamber (Kurabo, Osaka, Japan) was used for measuring cell migration by the modified method of Glaser et al. (2007) and Liu et al. (2011). The incubation culture plates were composed of two chambers. The base of the upper chamber was a polycarbonate filter with 8-μm pores. We used 24-well plates for the lower chamber. The NSC suspension (0.25×10^5 cells/well) were loaded into

the upper chamber and incubated for 12 h in the same culture condition treated with atelocollagen (0–0.05 %). Then, the cells on the top of the filter were washed with PBS and the remaining cells on the bottom side of the filter were fixed in 4 % formalin (Wako). The filters were mounted onto microscope slides, and cells were counted at 200× magnification in two fields per filter. In this examination, the culture medium contained FGF-2, so that we can evaluate basic migrating capacity of NSCs especially through the promotive effect of FGF-2 signaling mechanism in NSCs.

Immunocytochemistry

Following fixation in 4 % formalin (Wako) neutral buffer solution for 15 min at room temperature, cultured cells were washed with PBS, fixed in 0.2 % Triton X-100 (Wako) in PBS for 5 min and again rinsed with PBS. After incubation with 10 % normal goat serum (Vector Laboratories, Burlingame, CA, USA) in PBS for 30 min, cells were incubated with the solution including antibodies of anti-microtubule-associated protein 2 (MAP2; 1:2,000) (Sigma-Aldrich) or anti-glial fibrillary acidic protein (GFAP; 1:2,000) (Sigma-Aldrich) and Zenon IgG labeling reagent (Invitrogen, Carlsbad CA, USA) for 45 min and washed with PBS. Nuclei were stained with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) (Invitrogen).

MAP2/GFAP-ELISA evaluating cell differentiation

For measuring cell differentiation function, cultured NSCs were re-plated in 24-well assay plates at the density of 1.5×10^5 cells/well and cultured in FGF-2 removed differentiation condition with atelocollagen (0–0.05 %) for 72 h. ELISA for each NSC, neuron and glial cell marker protein was performed using the avidin–biotin-peroxidase complex system (Vectastain ABC Kit; Vector Laboratories, Burlingame, CA). The primary antibodies used were mouse monoclonal anti-MAP2 (1:2,000) (Sigma-Aldrich) and rabbit monoclonal anti-GFAP (1:2,000) (Sigma-Aldrich). The secondary antibody used was biotinylated anti-mouse or rabbit IgG (Vector Laboratories). We have previously indicated that value of MAP2-ELISA and number of MAP2 positive cell, and positive correlation between value of GFAP-ELISA and number of GFAP-positive cell are also indicated (Röhl et al. 2001; Tateno et al. 2005) in NSC cultures. During low serum induction of neural cell differentiation context (growth factor withdrawal and low serum media), the number of active caspase three positive cells is indicated to have dramatically increased without PARP cleavage which is the apoptosis indicator downstream of caspase activation (Fernando et al. 2005). For detection of cell apoptosis during NSC differentiation, we performed TUNEL staining analysis of specific labeling of nuclear DNA fragmentation using an in situ Apoptosis Detection Kit (Takara Bio, Tokyo, Japan) according to the manufacturer's instructions. In brief, fixed and permeabilized cells were incubated with TUNEL reaction mixture containing terminal deoxynucleotidyl transferase (TdT) enzyme and fluorescein-dUTP for 1 h at 37 °C in a humidified chamber, followed by washing with PBS 3 times. TUNEL-positive nuclei (green fluorescence) and total nuclei (DAPI-positive, blue fluorescence) were identified by fluorescence microscopy using Olympus IX71 (Olympus, Tokyo, Japan) with image processing by Meta Morph software (Molecular Devices, Sunnyvale, CA, USA).

Chronic ethanol treatment

For in vivo experiments, Wistar rats (3-month old) were purchased and administered chronic binge ethanol ($4\ g\ kg^{-1}$, estimated mean blood concentration: $200\ mg\ dl^{-1}$) or an equivalent volume of physiological saline via intragastric catheter every 12 h for 4 days as described by Nixon (Nixon et al. 2002).

NSC preparation and [^{35}S]-methionine labeling

For intravenous administration procedure, NSCs were obtained as described in methods. After 7 days of cell expansion, culture cells were labeled with 8.25 μM of [^{35}S]-methionine for 24 h, before administration.

NSC administration and quantitation of brain delivery

At 24 h after the last ethanol administration, a NSC suspension (5×10^6) mixed with equal volumes of saline or 0.02 % atelocollagen dissolved in saline in 0.5 ml total fluid volume was injected slowly into the rats' tail vein over 1 min. All rats received cyclosporine orally (0.2 mg/ml in their drinking water, corresponding to a blood concentration of 15 mg/kg daily i.p. (Barzilay et al. 2011)). Two weeks after NSC administration, all rats were deeply anesthetized with isoflurane and transcardially perfused with heparinized saline (0.5 %). Rat brains were removed, and divided into cortex, hippocampus, striatum and subventricular zone (SVZ). Each brain fraction was analyzed by liquid scintillation counter (Beckman LS6500, Brea, CA, USA).

Statistical analysis

Data are presented as the mean ± standard error of the mean. The statistical significance between experimental observations was determined by the one-way ANOVA, and

post hoc comparisons were using Turkey's HSD test. In addition, differences between brain samples using atelocollagen and not were evaluated by means of unpaired t test. In all cases, statistical significance was set at $p < 0.05$. Statistical analyses were performed using SPSS 11.0 for Windows (SPSS Japan Inc., Tokyo, Japan).

Results

Atelocollagen does not affect on NSC survival and migration

Recent study suggested that survival of the administered NSCs is responsible for the functional recoveries of cognition and motor abilities observed in traumatic brain injury model (Shear et al. 2011). To examine the effect of atelocollagen on NSC survival function, we treated NSCs with 0–0.05 % atelocollagen for 72 h followed by the cell survival evaluation using MTT metabolism assay. As shown in Fig. 1a, there was no significant difference in viabilities of NSCs, exposed by 0–0.05 % atelocollagen. The OD570 of control cells was 0.612 ± 0.018 and approximate cell number was 1.5×10^5/well, increased from 5×10^4/well for 72 h. While, the targeted migration of the administered NSCs was reported in ischemic model, with many cells migrating long distances toward the impaired lesion (Kelly et al. 2004). To assess the effect of atelocollagen on migration of NSCs, we evaluated the change of migrating function of NSCs by atelocollagen using modified Boyden chamber assay. As shown in Fig. 1b, 0.01–0.05 % atelocollagen did not influence on the chemotactic activities of NSCs (140 ± 12.7 cells/field in control cells).

Effect of atelocollagen on NSC proliferation and differentiation

Regulating the proliferation/differentiation of transplanted NSCs is reported to be the potential strategy in a various cell replacement therapies of CNS disease such as spinal cord injury (Kim et al. 2011). To define the reliable concentration of atelocollagen used for efficient cell delivery system, we tested the potential of atelocollagen on the proliferation and differentiation functions of NSCs. For assessing the proliferative function change of NSCs by atelocollagen, we treated NSCs with 0.01–0.05 % atelocollagen for 72 h and evaluated cell proliferation by using BrdU-proliferating ELISA. There was no significant change in proliferative capacity (BrdU absorbance) of NSCs in all concentration groups of atelocollagen. The OD450 of control cells was 0.861 ± 0.052 and approximate cell number was 1×10^4/well (Fig. 1c). For assessing the NSC differentiation function change, we treated NSCs with atelocollagen for 72 h in a differentiation medium withdrawal of FGF-2, and performed ELISA assay with anti-MAP2 or GFAP antibodies (markers for neurons and astrocytes, respectively). As shown in Fig. 2a, d, 0.01 and 0.02 % of atelocollagen did not display any effect on the neuronal differentiation. While, 0.03, 0.04 and 0.05 % atelocollagen exposures significantly reduced the neuronal differentiation and showed reduction of MAP2 ELISA value to 51, 23 and 19 % of control cells, respectively. The OD492 of control cells was 0.482 ± 0.009 and approximate cell number was 1×10^5/well that was 70 % of total cells. In detection of astrocytic differentiation, we found that 0.01, 0.02 and 0.03 % of atelocollagen showed no significant influence but 0.04 and 0.05 % atelocollagen exposures significantly increased astrocytic differentiation and showed increase of GFAP ELISA value to 111 and 111 % of control cells, respectively. The OD492 of control cells was 0.518 ± 0.026 and approximate cell number was 1.5×10^4/well that was 10 % of total cells (Fig. 2b, e). In this experiment, NSC differentiation was induced under nonapoptotic context. By the cell apoptosis detection assay, differentiating cells (control) were negatively labeled with DNA strand breaks in TUNEL analysis (Fig. 2f).

Effect of atelocollagen on NSC migration into brain

Although stem cell treatment has potential benefits for severe incurable neuropsychiatric disorders, there may be direct risks including tumor formation, immune rejection of administered stem cells, hemorrhage during neurosurgery and postoperative infection (Master et al. 2007). To minimize invasiveness and risk of tumor formation by the NSC treatment, we are trying to analyze the possible approach of intravenous administration. The tumor risk outcome depends on the degree of histocompatibility of the cells and circumstances (cell number, injection site, disease model, etc.) and a very low risk of teratomas was reported in NSC treatment studies using intravenous administration (Dressel 2011).

To test the hypothesis of useful characteristic of atelocollagen for reducing immune rejection and increase entry of administrated NSCs into brain, we compared the conditions of pre-treated of NSCs with or without atelocollagen recently developed means of promoting gene delivery. Two weeks after the NSC treatment labeled with [^{35}S]-methionie, we achieved about a twofold higher migrating ratio of NSCs into brain including areas of cortex, hippocampus, striatum and SV in response to intravenous administration on chronic alcohol binge model (Fig. 3).

Discussion

In this study, we investigated the effect of atelocollagen on survival, proliferation, migration and differentiation of NSCs to clarify the useful concentration of atelocollagen

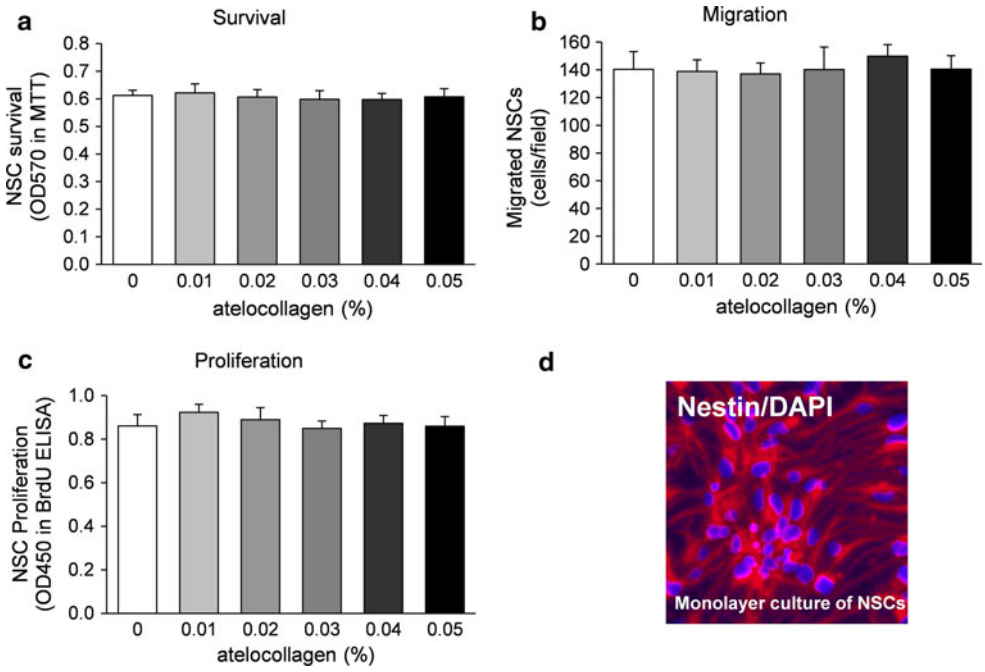

Fig. 1 Effects of atelocollagen on NSC survival, migration and proliferation. NSCs were treated with 0.01–0.05 % atelocollagen for 72 h (for survival and proliferation) or 12 h (for migration). Cell viability was determined by MTT metabolism assay ($n = 6$) (**a**), cell migration was assessed by Chemotaxis assay ($n = 4$) (**b**), and cell proliferation was estimated by BrdU-ELISA ($n = 4$) (**c**). Exposure of NSCs with atelocollagen (0.01–0.05 %) did not induce any change either on survival, migration and proliferation of NSCs. Fluorescence image of monolayer culture of NSCs for each assay (**d**). Data were expressed as mean ± SEM

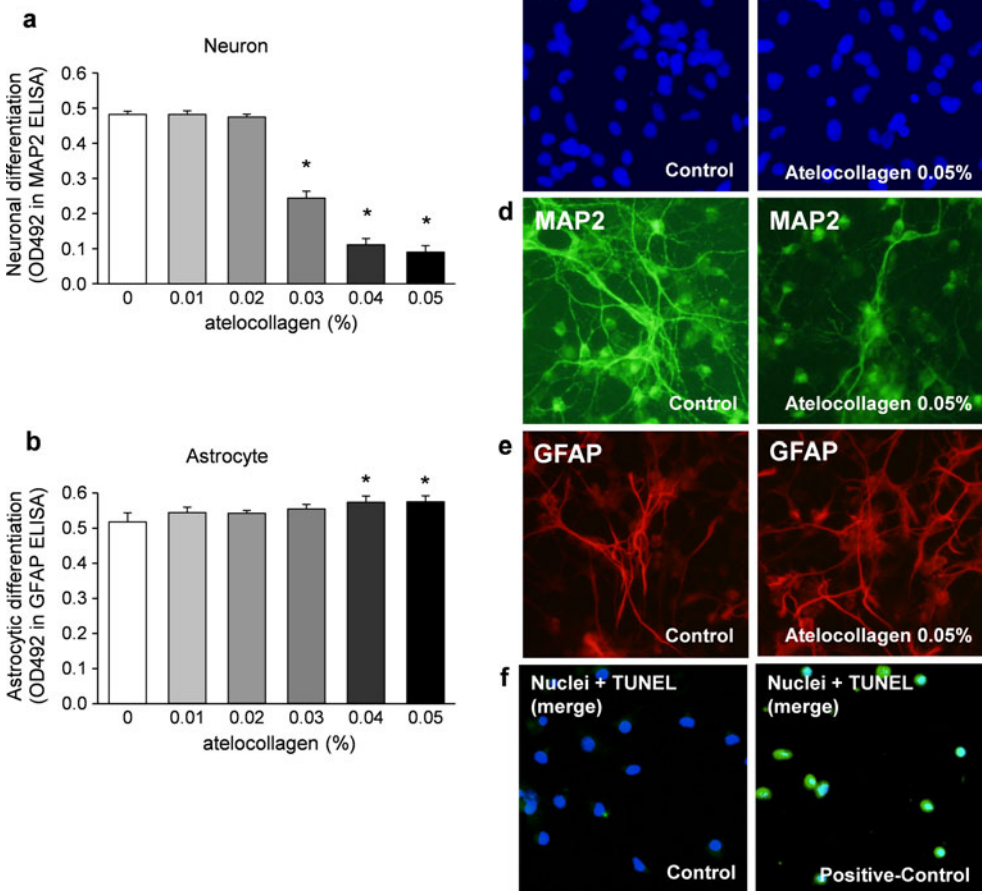

Fig. 2 Effects of atelocollagen on NSC differentiation. NSCs were induced to differentiate by FGF-2 deprivation with exposure of 0–0.05 % atelocollagen for 72 h. Atelocollagen (0.03–0.05 %) significantly reduced neuronal differentiation of NSCs which was evaluated by MAP2-ELISA ($n = 4$) (**a**). Atelocollagen also showed increased glial differentiation evaluated by GFAP-ELISA ($n = 6$) (**b**). Immunofluorescence of microscopies of MAP2 and GFAP positive cells, and total cells of DAPI staining are shown in (**d**), (**e**) and (**c**), respectively. Fluorescence images of control NSCs differentiated for 72 h and differentiated cells with DNase 1 exposure (2,000 U/ml in PBS for 15 min) are shown in (**f**). Data were expressed as mean ± SEM

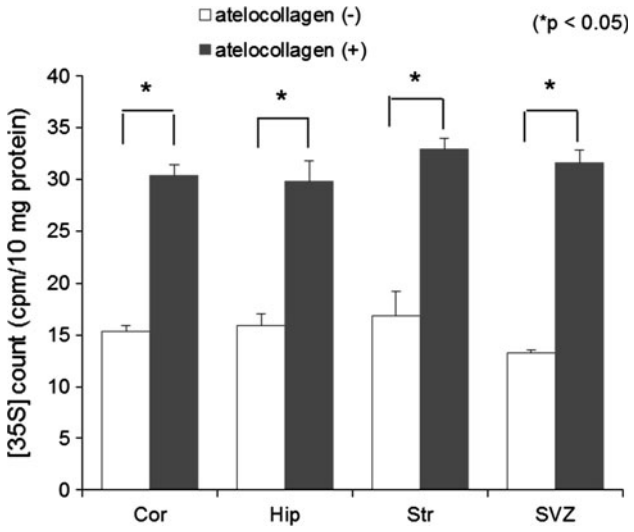

Fig. 3 Effect of atelocollagen on NSC migration into brain. NSCs pre-labeled with [^{35}S]-methionine were combined with or without atelocollagen (0.02 %) and injected intravenously in chronic alcohol binge model rats. Two weeks after administration, NSCs migrated into brain were quantitated by the radioactivity of homogenizes of several brain region tissue samples including cortex, hippocampus, striatum, and SVZ ($n = 3$). NSCs combined with atelocollagen showed about twofold higher migrating capacity into each divided region of the brain. Data were expressed as mean ± SEM

for NSC treatment without direct influence on NSC function. We found that treatment with less than 0.03 % atelocollagen did not induce any change in survival, proliferation and migration function of NSCs. However, over 0.03 % of atelocollagen produced significant repression of neuronal differentiation of NSCs and significant promotion of astrocytic differentiation at over 0.04 % concentration. Atelocollagen (0.03–0.05 %) caused reduction of immunoreactivity of MAP2 and increase of immunoreactivity of GFAP in a dose-dependent manner, respectively. We demonstrated that neuronal/glial differentiation paralleled MAP2/GFAP immunoreactivity using an established MAP2- and GFAP-ELISA. We confirmed the reliability of this method by comparison of the immunoreactivities of MAP2/GFAP with the number of neurons/astrocytes in cultured cells previously (Tateno et al. 2005, 2006).

Currently, stem cell-based approaches have received much hype as potential treatments for CNS disorders, such as spinal cord injury, ischemic/injured brain, and neurodegenerative disease (e.g., Parkinson's disease, Amyotrophic lateral sclerosis, Alzheimer's disease). For example, it might be possible to replace or make up for lost/deficit neurons by treatment of stem cell-derived cells that have been pre-differentiated in vitro to various maturation stages, even in a field of psychiatric disorders (Maroof et al. 2010; Tanaka et al. 2011). Among several methods for admiring NSCs treating CNS disease, minimally invasive intravenous administration would carry most benefits. Furthermore, the intravenous NSC administration might overcome the difficulties of cell address to diffuse or undefined cerebral pathologies in psychiatric illness, such as depression, schizophrenia and developmental disorders. However, studies have reported poor cell delivery to the brain by intravenous infusion (Takahashi et al. 2011), and successful method is needed for effective cell migration and for exerting satisfied functional recovery.

In this experiment, we demonstrated that intravenously injected NSCs complexed with atelocollagen showed about twofold higher migration capacity into brain of chronic alcohol binge model comparing with simple injection of NSCs alone. Excessive alcohol intake reported to produce structural and functional deficits in corticolimbic pathways that are thought to underlie cognitive deficits in the alcohol use disorders. Animal models of binge alcohol administration support the direct link of high levels of alcohol consumption and neurotoxicity in the hippocampus and surrounding cortex including functions of cell proliferation and cell death (Leasure et al. 2010). In addition, using the intravenous NSC administration method, we have recently reported that intravenously administered NSCs could migrate across blood–brain barrier and delivered to cortical and limbic areas in the brain and survive at least for 4 months in fetal alcohol spectrum disorder model rat (Shirasaka et al. 2012). Notable findings in the study were that NSC treatment utilizing atelocollagen displayed potential therapeutic efficacy against memory and social recognition deficits demonstrated in the psychiatric model.

The reason why the combination with atelocollagen elevates stem cell delivery into brain over blood–brain barrier (BBB) has not yet been understood. However, a recent report has indicated that atelocollagen can stimulate a signal transduction pathway which acts as a permeability modulator of endothelial cells similar to those triggered by thrombin, histamine, TNF-alpha, and VEGF to effective deliver molecule such as oligonucleotide over tight junctions. To date, no report has indicated the investigation for the ability of atelocollagen to effectively deliver NSCs from blood to brain. One of the objectives of this study was to determine the proper concentration of atelocollagen in vitro as combined material for successful delivery with intravenously administered NSCs into brain and to our knowledge, this is the first report to describe the direct effect of atelocollagen on the basic cellular functions, such as proliferation, migration, differentiation, and survival of NSCs. Over 0.03 % of atelocollagen showed inhibition of neuronal differentiation and increase of astrocytic differentiation of NSCs. These results suggested that atelocollagen may have a specific influence on NSC differentiation mechanisms at over 0.03 % concentration and the values are reasonable when considering the report of atelocollagen concentration (0.008 %) used for siRNA delivery method

in vitro (Minakuchi et al. 2004). However, when all the in vitro data considering, we cannot lead complete explanation upon the specific substantial reduction of neuronal differentiation by atelocollagen. The possibility should be analyzed precisely that atelocollagen alters growth pattern of neurons differentiated from NSCs which could influence the values of MAP2-ELISA. Although the molecular mechanism of direct effect of atelocollagen on cell function change is totally unknown, recent report suggesting the possible cellular DNA synthesis change induced by the treatment of collagen in rabbit nucleus pulposus cells (Lee et al. 2012), so that the gene expression changes might be included in the cell morphological change induced by atelocollagen.

Although almost no study has been done about the precise influence of atelocollagen on brain NSC functions, atelocollagen has been used as the scaffold material for cell culture, especially in the regenerative medicine for other kind of disease. For inducing osteoblastic differentiation, mesenchymal stem cells were cultured on atelocollagen honeycomb scaffold (George et al. 2006). The $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins are the major collagen binding integrins, with $\alpha 2\beta 1$ having a higher affinity for the fibrillar type I collagen, the major protein constituent of bone. The $\alpha 2\beta 1$ integrin interaction with type I collagen is a crucial signal for the induction of osteoblastic differentiation and matrix mineralization (Mizuno et al. 2000).

In the in vivo studies, the peripheral nerve regeneration was performed by using neuronal progenitor cells embedded in collagen gel. In this report, NSCs derived from fetal rat hippocampus had shown to have ability to differentiate into neurons, Schwann-like supportive cells, astrocytes and oligodendrocytes, respectively in atelocollagen gel (Murakami et al. 2003). For brain function, atelocollagen has revealed to increase permeability of tight junction of endothelial cells (Hanai et al. 2012). Recently, the utility of atelocollagen is widely spreading in the clinical field. Therefore, measurement of various cell activity changes by atelocollagen is essential for proceeding clinically safety investigations. Our findings may be especially critical for stem cell therapy wherein the effective cell migration is needed safely.

In conclusion, the current studies have demonstrated that 0.03–0.05 % of atelocollagen did not influence NSC survival, proliferation and migration, but repressed neuronal differentiation and promote differentiation to astrocytes. Therefore, the proper atelocollagen concentration which does not induce morphological change of NSCs was estimated at under 0.03 %. In addition, pre-treatment of NSCs with atelocollagen (0.02 %) can provide superior migrating capacity into brain demonstrated using chronic alcohol binge rat, one of the kinds of psychiatric disease model. For advanced practical use of intravenous NSC treatment in CNS disease, more effective ideas to induce neuronal differentiation might be required. To address this issue, we previously indicated the promotion of neuronal differentiation by antipsychotics through the NSC gene expression regulator (i.e., Repressor element-1 silencing transcription/neuron-restrictive silencer factor; REST/NRSF) of cell fate choice for neurogenesis (Ishii et al. 2008). Based on these results, therefore, further investigation into the effect of NSC treatment on psychiatric symptoms by using atelocollagen in combination with antidepressants or gene induction may lead to the possible future treatment option for the regenerative medicine in CNS disease.

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Conflict of interest The authors declare that they have no conflict of interest.

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