Neural differentiation of periodontal ligament stem cells induced by neurotrophic Schwann cells factors

Xiaojie Li[1], Dapeng Liao[1], Ping Gong[2], Hua Tang[2]

[1]State Key Laboratory of Oral Diseases, Sichuan University, Chengdu, China
[2]Dental Implant Center, West China College of Stomatology, Sichuan University, Chengdu, China

Submitted: 1 December 2008
Accepted: 13 January 2009

Arch Med Sci 2009; 5, 3: 313-320

Corresponding author:
Ping Gong
No. 14, Third Section
Renmin Nan Road, Chengdu
Sichuan, 610041, China
Phone: +86 28 85503579
Fax: +86 28 85582167
E-mail:
dentistgong@hotmail.com

Abstract

Introduction: Recent reports showed allogenic neurotrophic Schwann-cell factors could induce neural differentiation of mesenchymal stem cells, so we want to investigate whether heterogeneic neurotrophic Schwann-cell factors could induce neural differentiation of periodontal ligament stem cells (PDLSCs).
Material and methods: We isolated and multiplied PDLSCs from periodontal ligament of 8 month old beagle dogs' teeth (n = 24), and harvested Schwann cells from sciatic nerves of 1- to 2-day-old Sprague-Dawley rat pups, performing indirect co-culture of heterogeneic Schwann cells and PDLSCs with trans-well culture dishes. At 1, 3, 7 and 14 day after co-culture, Quantitative Real-time PCR and immunochemistry were performed to investigate the neural differentiation of PDLSCs.
Results: Quantitative real-time PCR analysis disclosed the regularity of above differentiation: the mRNA expression level of S100 kept climbing from the 1st to the 14th day with a relative stable period from the 3rd to the 7th day; the mRNA expression level of Nestin continually decreased from the 1st to the 14th day with a relative stable period from the 3rd to the 7th day; the mRNA expression level of GFAP climbed from the 1st to the 3rd day, then maintained stable till the 14th day. Immunostaining results further confirmed the neural differentiation of PDLSCs from the protein level.
Conclusions: PDLSCs could be induced to differentiate into a Schwann cell phenotype by indirect co-culture with heterogeneic Schwann cells, such neural differentiation began as early as 24 h after co-culture, but the most apparent neural differentiation of PDLSCs appeared 14 days later.

Key words: periodontal ligament stem cells, heterogeneic Schwann cells, neural differentiation.

Introduction

In 2004, Seo *et al.* isolated the periodontal ligament stem cells (PDLSCs) and investigated its stem cell character for the first time [1]. There after, many studies further confirmed his conclusion. Studies showed about 3.9% of the periodontal ligament cells could be termed as PDLSCs, which expressed the early mesenchymal stem-cell markers STRO-1 and CD146/MUC18. Under appropriate conditions, PDLSCs could differentiate into cementoblast-like cells, adipocytes, and collagen-forming cells *et al*. When transplanted into immunocompromised rodents, PDLSCs showed the capacity to generate a cementum/PDL-like structure [2-4]. However, the neural differentiation potential of PDLSCs is still not very clear until now.

Since Woodbury *et al*. firstly induce bone marrow stromal cells (BMSCs) to differentiate into neuronal phenotype *in vitro* in 2000 [5], the possibility to obtain neural cells from BMSCs, as a new biological concept with potential clinical application, has been extensively investigated [6-11]. Recently, adipose-derived stem cells (ADSCs) showed neural differentiation potential *in vitro* too [12-14]. So we supposed that PDLSCs could also be induced to differentiate into neuro-like cells or a Schwann cell phenotype *in vitro*.

In all the methods of inducing BMSCs neurogenic differentiation *in vitro*, co-culture seems a simple and effective way [12]. After performing direct co-culture of Schwann cells (SCs) and BMSCs, Zurita *et al*. suggested that intercellular contact play an important role in the process of BMSCs neural differentiation [15]. However, according to former studies [16-19], the possibility of cell fusion could not be excluded as a cause of apparent neural differentiation of BMSCs. Therefore, Zurita *et al*. performed indirect co-culture of SCs and BMSCs with trans-well culture dishes, also proved neural differentiation of BMSCs even if without intercellular contact [20]. That means only by neurotrophic Schwann-cell factors, BMSCs can differentiate into neuro-like cells successfully. But until now, we still don't know whether PDLSCs could also be induced neural differentiation only by co-culture with allogenic SCs. Further, what will happen to PDLSCs if co-cultured with heterogeneic SCs? To investigate this problem, we harvested SCs from Sprague-Dawley rat pups, isolated and multiplied PDLSCs from average 8 month old beagle dogs' teeth, subsequently applied transparent polycarbonate membranes with pores of 1.0 μm to impede the contact between SCs and PDLSCs, performing an indirect co-culture. We found PDLSCs could also be induced neural differentiation even by co-culture with heterogeneic SCs, after that we further investigated the relative differences in gene expression of neurally differentiated PDLSCs at different time points (1, 3, 7, 14 day) after co-culture.

Material and methods

All animal experiments described in this report were approved by the Ethical Guideline Committee for Animal care of West China College of Medical Sciences, Sichuan University.

Isolation, culture, identification of PDLSCs and SCs

Normal 8 month old beagle dogs' teeth (n = 24) from 4 individuals were collected under aseptic condition and placed into phosphate-buffered saline (PBS) solution supplemented with 1% (v/v) penicillin/streptomycin for 5 min. The periodontal ligament was gently separated from the surface of the tooth root and then dissociated with 0.25% trypsin (Sigma, UK) and 0.1% collagenase (Sigma, UK) for 90 min at 37°C. The solution was passed through a 75-μm filter to remove undissociated tissue, neutralized by the addition of alpha-Modified Eagle Medium (α-MEM; Hyclone, USA) containing 20% (v/v) fetal bovine serum (FBS, Hyclone, USA) and centrifuged at 1000 × g for 8 min. The cell pellet was resuspended in α-MEM containing 20% (v/v) FBS (Hyclone, USA) with 1% (v/v) penicillin/streptomycin solution and cultured in an incubator at 37°C with 5% carbon dioxide. Culture medium was first changed 7d later, then regularly changed every 3d. Cells were passaged with trypsin/EDTA (Sigma, UK) when required. To isolate purified PDLSCs, we applied the method of single-colony selection [1]. Briefly, single-cell suspensions of periodontal ligament cells (1-2 cells/200 μl) were seeded into 96-well culture plates at 1-2 cells/well and then incubated at 37°C with 5% carbon dioxide. Medium was changed every 3d. After two weeks, aggregates of more than 50 cells were scored as colonies and digested, transferred to 6-well culture plates for propagation. The morphology of these PDLSCs was observed under phase contrast microscope. To identify the stem cell surface markers of PDLSCs, we performed immunocytochemistry with antibodies against Stro-1, Cytokeratin, Vimentin (1 : 100, Santa Cruz, CA, USA) and CD146/MUC18 (1 : 100, Zymed Laboratories, USA) as described in former researches [1, 4, 13, 21]. Then cover slips were examined using an Olympus IX 70 inverted phase contrast fluorescence microscope.

SCs were isolated from the bilateral sciatic nerves of 1- to 2-day-old Sprague-Dawley rat pups according to former reports [20, 22], and cultured in α-MEM containing 20% (v/v) FBS (Hyclone, USA) with 2 μM forskolin (Sigma, UK), 20 ng/ml basic fibroblast growth factor (bFGF; PeproTech Ltd., UK) and 1% (v/v) penicillin/streptomycin solution. The culture medium was changed every 3d. SCs were identified in living cultures on the basis of cell soma and nuclear morphology. In fixed cultures, SCs were identified by immunocytochemical labeling for S100 protein, a specific cell marker of SCs, with antibody against S100 (1 : 100, Santa Cruz, CA, USA) as described in former studies [14, 20]. The cover slips were examined using an Olympus IX 70 inverted fluorescence microscope and the number of immuno-positive cells was counted from a minimum total of 100 cells per experiment.

Co-culture of PDLSCs and SCs

To co-culture PDLSCs and SCs, we use trans-well culture dishes with polycarbonate membrane (Milipore, USA).These culture dishes are commonly used to produce a cell culture environment simulating

the *in-vivo* state. The newest kind of porous membrane of the insert is optically transparent, and the pore size of membranes in our experiment is 1.0 μm. In our experimental conditions, the pore size is much smaller than the size of the PDLSCs body, which greatly inhibits the migration of PDLSCs into the lower chamber. Schwann cells ($6 \times 10^4/cm^2$, in 3 ml culture medium) were cultured in the well, and PDLSCs (3×10^4 cells/cm^2) were cultured on the permeable membrane support, so that both types of cells were exposed to the same culture medium conditions without cell contact [20]. Meanwhile, PDLSCs cultured on the permeable membrane support without SCs in the lower chamber were taken as control groups. During the course of the following 2 weeks, morphology of the co-cultured PDLSCs were observed, and at different time points (1, 3, 7, 14 d) after co-culture, the membrane were cut down from the insert, and mounted on the glass cover slips for study with immunocytochemistry techniques. So did the cultured PDLSCs of control groups.

Quantitative real-time PCR analysis

Total RNA was extracted using the Trizol Reagent (Invitrogen, USA) according to the manufacturer's instruction. The yield and quality of the RNA were assessed by measuring the absorbance at 260 and 280 nm followed by electrophoresis on 3% (w/v) agarose gels. Total RNA (500 ng) was reverse-transcribed into complementary DNA (cDNA) with PrimeScriptTM RT Reagent Kit (Perfect Real Time, Takara, Japan) according to the manufacturer's guideline. Primers of S100, GFAP, Nestin, and the internal calibrator-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (synthesized by Takara Biological Technology Co. Ltd, Dalian, China) and related GeneBank accession nos were listed in Table I. A 25 μl PCR reaction solution (2 × SYBR Premix Ex TaqTM 12.5 μl; 10 μmol/l forward primer 0.5 μl; 10 μmol/l reverse primer 0.5 μl; 50 × ROX Reference Dye1 0.5 μl, cDNA 2 μl; dH_2O 9 μl) was amplified in Applied Biosystems 7300 Real-Time PCR System (ABI, USA). The thermocycle of PCR reaction was as follows: after an initial denaturation at 95°C for 10 s, 45 cycles of denaturation at 95°C for 5 s, annealing at 60°C for 31 s were performed. PCR products went through agarose-gel electrophoretic analysis (BIO-RAD, USA). During the real-time PCR process, fluorescence signal was collected during annealing steps. Ct (cycle of threshold) was calculated for further statistical analysis. In order to examine the efficiency of real-time PCR, standard curves were established with serial dilution of sample RNA (500 ng; S100, GFAP, Nestin, GAPDH, 10× dilution). Total RNA of uPDLSCs was taken as a non-template control, which was included in all experiments and performed with RT- PCR to evaluate DNA contamination of reagents used for amplification. None of the experiments resulted in a positive signal from the non-template control.

Immunocytochemistry

For immunocytochemical analysis, cells were cultured on glass cover slips for 24 h and fixed in 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS, pH 7.5) for 30 min, washed with PBS and treated with 3% (v/v) H_2O_2 for 5 min, incubated for 1 h with blocking buffer [5% (w/v) normal goat serum (Sigma, UK)/0.1% (w/v) TritonX-100 in PBS, pH 7.5], then incubated with primary antibodies, including S100 (1 : 100, Santa Cruz, USA), glial fibrillary acidic protein (GFAP) (1 : 200, Santa Cruz), Nestin (1 : 100, Santa Cruz), Stro-1 (1 : 100, Santa Cruz), Cytokeratin (1 : 100, Santa Cruz), Vimentin (1 : 100, Santa Cruz), CD146/MUC18 (1 : 100, Zymed Laboratories, USA), respectively, at 4°C overnight. After thorough washing, the cover slips were incubated with biotinylated secondary antibodies and then horseradish peroxide conjugated streptavidin. The peroxidase reaction was developed using 3, 30-diaminobenzidine tetrahydrochloride (DAB) as chromogens. The cells were counterstained with hematoxylin, and then examined using an Olympus IX 70 inverted phase contrast microscope (Olympus, JAPAN). Stro-1 was incubated with fluorescein isothiocyanate (FITC)-conjugated secondary antibody (1 : 100, Santa Cruz), and the fluorescence signals were detected at emission

Table I. Primer sets for real-time PCR analysis

Gene	Primer sequences (5'-3')	Product size (bp)	Annealing temperature [°C]	GenBank accession number
S-100	For: ctcgacgtgttccagcagta Rev: ctgtccagtgtctccatgacc	155	60.03	XM548737
NESTIN	For: gggaagaatgtggtcaggact Rev: catcagactccctatccctgtc	165	60.16	XM547531
GFAP	For: ctggatctggagaggaagattg Rev: ttctcttagggctgctgtgag	162	60.00	XM843285
GAPDH	For: gatgctggtgctgagtatgttg Rev: agaaggagcagagatgatgacc	111	60.28	NM001003142

For. and Rev. indicate forward, reverse primer, respectively. All primers were purchased from Takara Biological Technology Co. Ltd, (Dalian, China)

wavelength of 488/522 nm (FITC, green) with Olympus IX 70 fluorescence microscope (Olympus, JAPAN).

Statistical analysis

All data were expressed as the mean ± SEM. Statistical calculations were performed using the SPSS software (SPSS, Chicago, IL, USA). Inter-group comparisons were made by a one-way analysis of variance (ANOVA), and then Student-Newman-Keuls range test was adopted to perform multiple inter-group comparisons. *P* values less than 0.05 were considered to be significant.

Results

Under phase contrast microscope, the morphology of PDLSCs mainly appeared polygon with nuclear in the center. Immunocytochemical assessment of cell surface markers of PDLSCs showed positive for Stro-1, CD146/MUC18 and vimentin, but negative for cytokeratin (Figure 1). The results indicated that our cultured PDLSCs showed some characteristics of adult stem cell morphology, expressing two early mesenchymal stem-cell markers Stro-1 and CD146/MUC18. Expression of vimentin also indicated that our cultured PDLSCs were mesenchymal stem cells derived from embryonic mesoderm. No expression of cytokeratin further excluded the possibility of cell mixture from ectoderm. These results were consistent with other reports [1, 4, 13, 21]. The morphology of Schwann cells under phase contrast microscope appeared typical spindle elongated shape, and immunocytochemical assessment showed positive for S100 protein with purity as high as 98% (Figure 1).

We performed Real-time-PCR to investigate the relative differences in gene expression of S100, Nestin and GFAP at different time points (1, 3, 7, 14 d). For S100 and Nestin gene expression relative quantities, statistic analysis showed no statistical difference between 3d and 7d ($p > 0.05$), but the results of 3d or 7d had significant statistical difference comparing to those of 1d or 14d respectively ($p < 0.05$). These meant S100 gene expression relative quantities were the highest at 14d and the lowest at 1d after co-culture, but were of no differences between 3d and 7d; while Nestin gene expression relative quantities were the highest at 1d and the lowest at 14d, without differences between 3d and 7d (Tables II, III). For GFAP gene expression relative quantities, there were no statistical differences at 3d, 7d and 14d respectively ($p > 0.05$), but each of them had statistical difference with 1d ($p < 0.05$), that meant GFAP gene expression relative quantity maintained a stable level from 3 days to 14 days without apparent fluctuation (Table IV). To make the results clear and direct, we used the bar graph to show the relative quantity differences at different time points (1, 3, 7, 14 d) for gene S100, Nestin and GFAP (Figure 2).

Under phase contrast microscope, the morphology of the co-cultured PDLSCs began to change as early as 24 h after co-culture, showing a spindle elongated shape characteristic of Schwann cells, and with the time prolonged, these changes became more and more apparent. Nestin protein is a neural progenitor cell marker, while both S-100 and GFAP protein are glial markers; they are all cell markers for identifying SCs [1, 13, 14]. Our immunocytochemical assessment of co-cultured PDLSCs showed positive in different degrees for S-100, Nestin and GFAP protein at different time points (1, 3, 7, 14 d) (Figure 3).

Both real-time PCR and immunostaining data demonstrated differentiation of PDLSCs into neuronal phenotype. The real-time PCR data suggested the differentiation process as S100 and GFAP gene transcription was increased during the first two weeks whereas stem cell marker Nestin mRNA expression was decreased starting day 1 indicating the initiation of differentiation process starting within 24 h. The immunostaining results further confirmed the real-time PCR data as both S100 and GFAP protein expression increased during the first two weeks following the increase in the mRNA levels. While the Nestin protein expression was decreased starting from day 1, which is consistent with its mRNA level too.

Discussion

The neural differentiation potential of PDLSCs has never been investigated before. In our previous studies, we have successfully induced PDLSCs to differentiate into a Schwann cell phenotype with certain growth factors such as BDNF, NGF, bFGF and PDGF *et al*., proving the neural differentiation potential of PDLSCs for the first time [23]. Adult Schwann cells can secret BDNF and NGF [24], which provides the basis for neural differentiation of co-culturing stem cells and SCs. Indirect co-culture of stem cells and SCs with trans-well culture dishes provides a unique and powerful opportunity to demonstrate neural stem cells differentiation, excluding the possibility of cell fusion. Zurita *et al*. previously reported the neural differentiation of BMSCs by indirect co-culture with allogenic SCs [20]. In our present study, we demonstrated that PDLSCs could differentiate into a Schwann cell phenotype by indirect co-culture with heterogeneic SCs. Although some researches reported that allogenic SCs transplantation could promote nerve regeneration and spinal cord remyelination without apparent immunological rejection [25, 26], there were seldom reports about

A

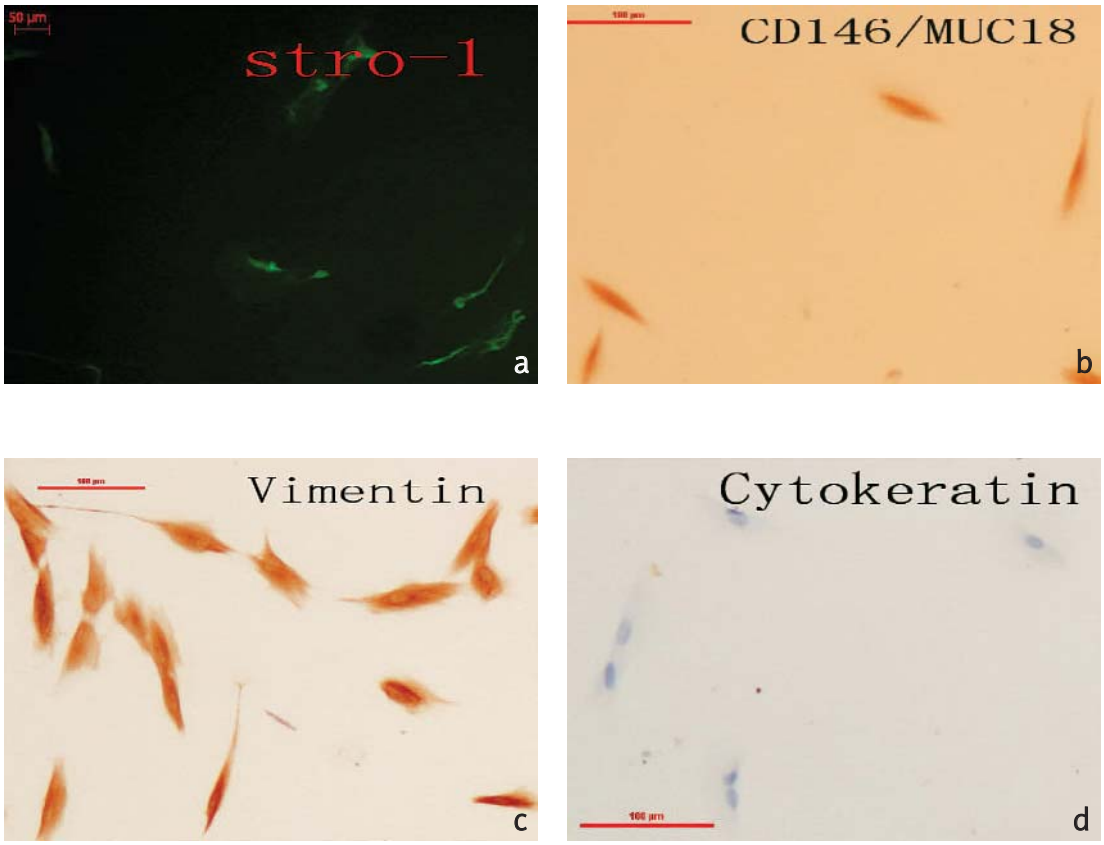

Figure 1. A – Immunocytochemical assessment of PDLSCs cell markers: a – stro-1 (+) Scale bar = 50 μm, b – CD146 (+), c – vimentin (+), d – cytokeratin (–), e – PDLSCs show little polygon morphology. Scale bar = 100 μm. **B** – Immunocytochemical assessment of Schwann cells shows positivity for S100 protein (left), while the morphology of Schwann cells under phase contract microscopy is typical spindle elongated shape (right). Scale bar = 100 μm

B

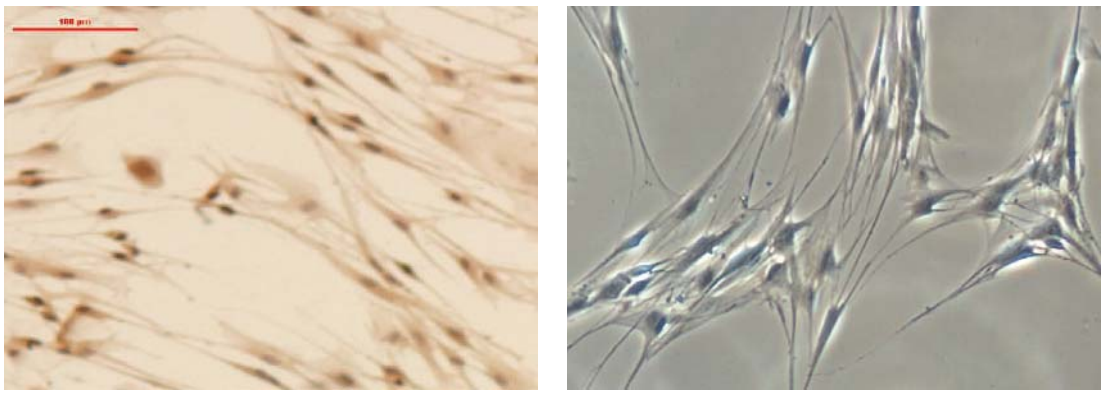

Table II. Relative differences in the S100 gene expression

	Time point (d)	N	Subset for α = 0.05		
			1*	2*	3*
Student-Newman-Keuls**	1	3	0.0000		
	3	3		7.6832	
	7	3		8.7711	
	14	3			19.7011
	Sig.		1.000	0.698	1.000

Means for groups in homogeneous subsets are displayed
***Uses harmonic mean sample size = 3.000*

Table III. Relative differences in the Nestin gene expression

	Time point (d)	N	Subset for α = 0.05		
			1*	2*	3*
Student-Newman-Keuls**	14	3	0.0000		
	7	3		2.3585	
	3	3		2.3624	
	1	3			3.4751
	Sig.		1.000	0.987	1.000

Means for groups in homogeneous subsets are displayed
***Uses harmonic mean sample size = 3.000*

Table IV. Relative differences in the GFAP gene expression

	Time point (d)	N	Subset for α = 0.05	
			1*	2*
Student-Newman-Keuls**	1	3	0.0000	
	3	3		5.3412
	7	3		6.2268
	14	3		7.4064
	Sig.		1.000	0.136

Means for groups in homogeneous subsets are displayed
***Uses harmonic mean Sample size = 3.000*

the function of heterogeneic SCs. Our results support a role for heterogeneic SCs factors in the biological phenomenon of PDLSCs neural differentiation, but the internal molecule mechanism needs further investigation.

In our co-cultures, PDLSCs expressing S100, Nestin and GFAP appeared as early as 24 h according to immunocytochemical staining and RT-PCR analysis. While quantitative real-time PCR further disclosed the above three gene expression relative quantity differences. The gene expression level of Nestin was the highest at the 1st day and the lowest at the 14th day; as to S100, its gene expression level climbed to the highest at the 14th day; for GFAP, its gene expression level climbed from the 1st to the 3rd day, then kept stable till the 14th day. Nestin, as a neural progenitor cell marker, mainly appeared in immature proliferating neural progenitor cells [27]; it was reported that its gene expression level down-regulated in co-cultured BMSCs with the time prolonged [20], our co-cultured PDLSCs showed the same biological phenomenon. S100 is a special cell surface marker of SCs. Meanwhile, both S100 and GFAP are glial markers. Their strong gene expressions represent the differentiation toward a Schwann cell phenotype [14, 28]. Our co-cultured PDLSCs showed the highest gene expression level of S100 and GFAP at the 14th day, so we thought that in co-culture system with SCs, PDLSCs could be induced to

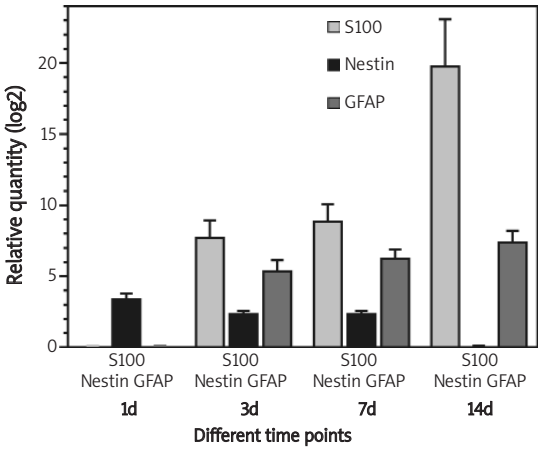

Figure 2. In the graph, bar height represents the gene expression quantity (mean ± SEM). For S100 and Nestin gene expression relative quantities, statistic analysis show no statistical difference between 3d and 7d ($p > 0.05$), but the results of 3d and 7d have significant statistical differences comparing to those of 1d or 14d respectively ($p < 0.05$). For GFAP gene expression relative quantities, there are no statistical difference at 3d, 7d and 14d respectively ($p > 0.05$), but the results of each of them has statistical difference with of 1d ($p < 0.05$)

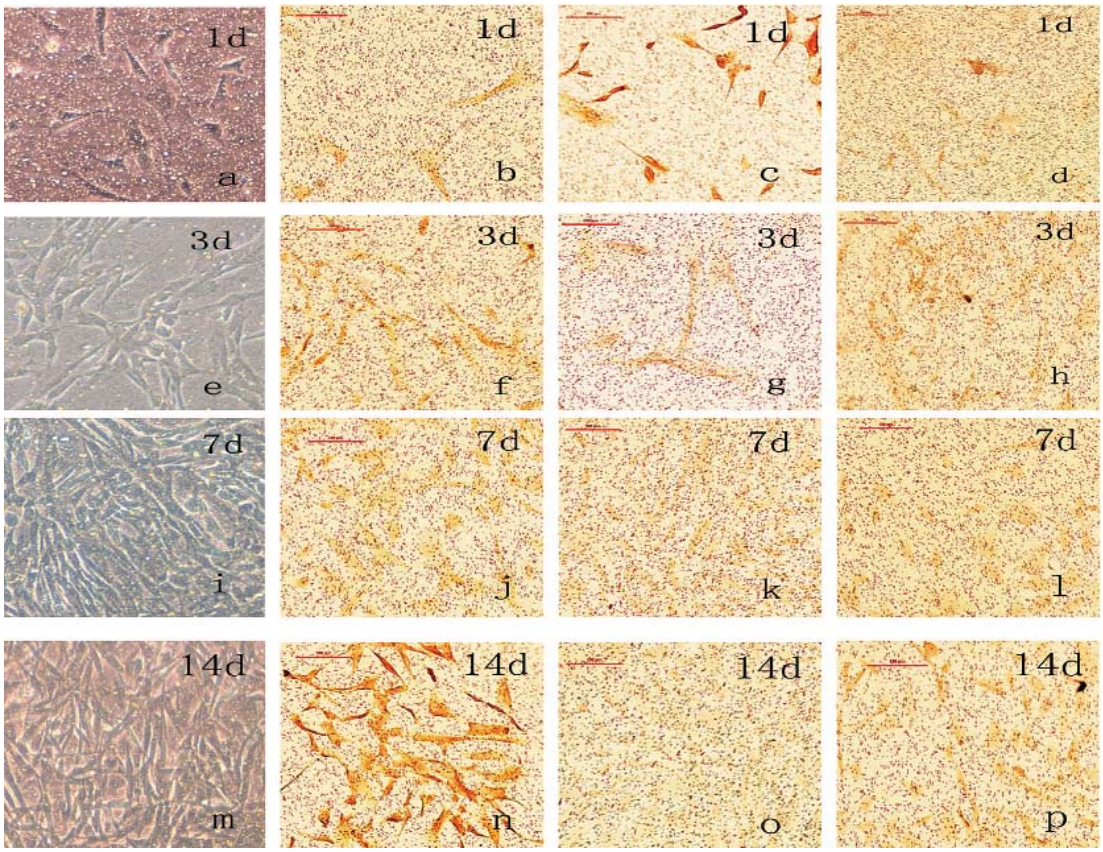

Figure 3. The morphology of the co-cultured PDLSCs gradually show a spindle elongated shape characteristic of Schwann cells with the time passing by (a, e, i, m). Immunocytochemical assessment of co-cultured PDLSCs show positive in different degrees for s-100 (b+, f++, j++, n+++), Nestin (c+++, g++, k++, o+) and GFAP (d+, h++, 1++, p++) at different time points (1, 3, 7, 14d) respectively. Scale bar = 100 μm

differentiate into an apparent Schwann cell phenotype at least two weeks later.

Based on above analyses, we draw a preliminary conclusion: PDLSCs could be induced to differentiate into a Schwann cell phenotype by indirect co-culture with heterogeneic Schwann cells, such neural differentiation began as early as 24 h after co-culture, but the most apparent neural differentiation of PDLSCs appeared 14 days later.

Acknowledgments

This study is supported by funds from National Natural Science Foundation of China (NO.30772448).

References

1. Seo BM, Miura M, Gronthos S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004; 9429: 149-55.
2. Ivanovski S, Gronthos S, Shi S, Bartold PM. Stem cells in the periodontal ligament. Oral Dis 2006; 4: 358-63.
3. Kawanabe N, Murakami K, Takano YT. The presence of ABCG2-dependent side population cells in human periodontal ligaments. Biochem Biophys Res Commun 2006; 4: 1278-83.
4. Nagatomo K, Komaki M, Sekiya I, et al. Stem cell properties of human periodontal ligament cells. J Periodontal Res 2006; 4: 303-10.
5. Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 2000; 4: 364-70.
6. Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. Lancet Neurol 2002; 2: 92-100.
7. Hermann A, Gastl R, Liebau S, et al. Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. J Cell Sci Pt 2004; 19: 4411-22.
8. Mahmood A, Lu D, Chopp M. Marrow stromal cell transplantation after traumatic brain injury promotes cellular proliferation within the brain. Neurosurgery 2004; 5: 1185-93.
9. Zurita M, Vaquero J. Functional recovery in chronic paraplegia after bone marrow stromal cells transplantation. Neuroreport 2004; 7: 1105-8.
10. Bossolasco P, Cova L, Calzarossa C, et al. Neuro-glial differentiation of human bone marrow stem cells in vitro. Exp Neurol 2005; 2: 312-25.
11. Zurita M, Vaquero J. Bone marrow stromal cells can achieve cure of chronic paraplegic rats: functional and morphological outcome one year after transplantation. Neurosci Lett 2006; 1-2: 51-6.
12. Liao DP, Li XJ, Gong P, Tan Z, Yuan Q. Co-culture with Schwann cells is an effective way for adipose-derived stem cells neural transdifferentiation. Arch Med Sci 2008; In press.
13. Ning HX, Lin GT, Lue TF, Lin CS. Neuron-like differentiation of adipose tissue-derived stromal cells and vascular smooth muscle cells. Differentiation 2006; 9-10: 510-8.
14. Kingham PJ, Kalbermatten DF, Mahay D, Armstrong SJ, Wiberg M, Terenghi G. Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. Exp Neurol 2007; 2: 267-74.
15. Zurita M, Vaquero J, Oya S, Miguel M. Schwann cells induce neuronal differentiation of bone marrow stromal cells. Neuroreport 2005; 5: 505-8.
16. Ying QL, Nichols J, Evans EP, Smith AG. Changing potency by spontaneous fusion. Nature 2002; 6880: 545-8.
17. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, et al. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003; 6961: 968-73.
18. Wang X, Willenbring H, Akkari Y, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 2003; 6934: 897-901.
19. Chen KA, Laywell ED, Marshall G, Walton N, Zheng TS, Teindler DA. Fusion of neural stem cells in culture. Exp Neurol 2006; 1: 129-35.
20. Zurita M, Vaquero J, Oya S, Bonilla C, Aguayo C. Neurotrophic Schwann-cell factors induce neural differentiation of bone marrow stromal cells. Neuroreport 2007; 16: 1713-7.
21. Seo BM, Miura M, Sonoyama W, Coppe C, Stanyon R, Shi S. Recovery of stem cells from cryopreserved periodontal ligament. J Dent Res 2005; 10: 907-12.
22. Yuan Q, Gong P, Li XY, et al. Enhanced proliferation and differentiation of osteoblasts induced by co-culturing with Schwann cells. Arch Med Sci 2008; 4: 242-8.
23. Liao DP, Li XJ, Gong P. Neural differentiation potential of periodontal ligament stem cells in vitro. Unpublished data.
24. Yamamoto M, Sobue G, Li M, et al. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and low-affinity nerve growth factor receptor (LNGFR) mRNA levels in cultured rat Schwann cells; differential time-and dose-dependent regulation by cAMP. Neurosci Lett 1993; 152: 37-40.
25. Lankford KL, Imaizumi T, Honmou O, Kocsis JD. A quantitative morphometric analysis of rat spinal cord remyelination following transplantation of allogenic Schwann cells. J Comp Neurol 2002; 3: 259-74.
26. Udina E, Rodriguez FJ, Verdu E, Espejo M, Gold BG, Navarro X. FK506 enhances regeneration of axons across long peripheral nerve gaps repaired with collagen guides seeded with allogeneic Schwann cells. Glia 2004; 2: 120-9.
27. Dahlstrand J, Lardelli M, Lendahl U. Nestin mRNA expression correlates with the central nervous system progenitor cell state in many, but not all, regions of developing central nervous system. Brain Res Dev Brain Res 1995; 84: 109-29.
28. Caddick J, Kingham PJ, Gardiner NJ, Wiberg M, Terenghi G. Phenotypic and functional characteristics of mesenchymal stem cells differentiated along a Schwann cell lineage. Glia 2006; 54: 840-9.