Biomedical Science Letters 2013, 19(4): 295~302
eISSN : 2288-7415

Original Article

Isolation of Mesenchymal Stem-like Cells from a Pituitary Adenoma Specimen

Jin-Kyoung Shim[1], Seok-Gu Kang[2], Ji-Hyun Lee[2], Jong Hee Chang[2] and Yong-Kil Hong[1,†]

[1]Department of Neurosurgery, Seoul St. Mary's Hospital, The Catholic University of Korea College of Medicine, Seoul 130-701, Korea
[2]Department of Neurosurgery, Severance Hospital, Yonsei University College of Medicine, Seoul 120-752, Korea

Some of the pituitary adenomas are invasive and spread into neighboring tissues. In previous studies, the invasion of pituitary adenomas is thought to be associated with epithelial-mesenchymal transition (EMT). In addition to that, we thought that mesenchymal stem cells (MSCs) exist in relevant microenvironment in pituitary adenoma. However, it has been little known about the existence of MSCs from pituitary adenoma. So we investigated whether mesenchymal stem-like cells (MSLCs) can be isolated from the pituitary adenoma specimen. We isolated and cultured candidate MSLCs from the fresh pituitary adenoma specimen with the same protocols used in culturing bone marrow derived MSCs (BM-MSCs). The cultured candidate MSLCs were analyzed by fluorescence-activated cell sorting (FACS) for surface markers associated with MSCs. Candidate MSLCs were exposed to mesenchymal differentiation conditions to determine the mesenchymal differentiation potential of these cells. To evaluate the tumorigenesis of candidate MSLCs from pituitary adenoma, we implanted these cells into the brain of athymic nude mice. We isolated cells resembling BM-MSCs named pituitary adenoma stroma mesenchymal stem-like cells (PAS-MSLCs). PAS-MSLCs were spindle shaped and had adherent characteristics. FACS analysis identified that the PAS-MSLCs had a bit similar surface markers to BM-MSCs. Isolated cells expressed surface antigen, positive for CD105, CD75, and negative for CD45, NG2, and CD90. We found that these cells were capable of differentiation into adipocytes, osteocytes and chondrocytes. Tumor was not developed in the nude mice brains that were implanted with the PAS-MSLCs. In this study, we showed that MSLCs can be isolated from a pituitary adenoma specimen which is not tumorigenic.

Key Words: Pituitary adenoma, Mesenchymal stem-like cells, Epithelial-Mesenchymal transition, Cell separation

INTRODUCTION

Pituitary adenomas are common neoplasms in the sellar region that have wide range of biological behavior, in term of hormonal and proliferative activities (Asa and Ezzat, 1998). Confined to the sella turcica, pituitary adenomas are slow growing and they are demarcated from the adjacent normal pituitary tissue (Kovacs et al., 2001). However, several pituitary adenomas are invasive, exhibit a more rapid pace of growth, and spread into neighboring tissues. In other words, pituitary adenomas may be invasive, while still having a histologically benign feature (Martins et al., 1965; Scheithauer et al., 1986).

Epithelial-Mesenchymal Transition (EMT) is a cellular program through which differentiated epithelial cells could be transformed into mesenchymal cells. EMT is a process that allows polarized epithelial cells to present a mesenchymal cell phenotype whereby undergoes remodeling of the cytoskeleton and acquires migratory abilities and a mesenchymal-like gene expression program (Lekva et al., 2012; Mirantes et al., 2013). Tumor invasion and metastasis

*Received: October 15, 2013 / Revised: November 14, 2013
Accepted: November 14, 2013
†Corresponding author: Yong-Kil Hong. Department of Neurosurgery, Seoul St. Mary's Hospital, The Catholic University of Korea College of Medicine, Banpo-daero 222, Seocho-gu, Seoul 137-701, Korea.
Tel: +82-2-2258-6123, Fax: +82-2-594-4248
e-mail: hongyk@catholic.ac.kr
ⓒThe Korean Society for Biomedical Laboratory Sciences. All rights reserved.

are associated with signals responsible for EMT (Yang and Weinberg, 2008). A few examples exist where epithelial and mesenchymal states in the same cell line display dramatically different cancer activities (Thompson et al., 2005). The mesenchymal state possesses more motile and invasive characteristics *in vitro* and indicates more tumorigenic and often selectively metastatic state (Ackland et al., 2003; Donald et al., 2005). It is possible to think that the invasiveness of pituitary adenoma might be related to EMT.

Latest research identified that the invasive pituitary adenoma cells acquire different patterns: mesenchymal, and leucocyte/amoeboid patterns (del Pliego et al., 2013). In addition, several brain tumors have been studied about the presence of cells which are similar to mesenchymal stem cells (Kim et al., 2013; Kwak et al., 2013; Lim et al., 2013). Accordingly, we have taken profound interest in tumor microenvironment, especially mesenchymal stem-like cells (MSLCs) which are similar to bone marrow-mesenchymal stem cells (BM-MSCs) (Kim et al., 2011; Kim et al., 2013).

However, it is unknown that mesenchymal stem cells exist in pituitary adenoma. The purpose of this study is to address whether mesenchymal stem like cells can be isolated from pituitary adenoma in patients associated with the invasiveness of the tumor. We isolated pituitary adenoma stroma mesenchymal stem like cells (PAS-MSLCs) and studied this based on cell morphology, differential potential, surface antigens, and non-oncogenic potential.

MATERIALS AND METHODS

Single cell isolation and PAS-MSLCs culture

Specimen from a patient with human pituitary adenoma was freshly obtained from operating room. Approval was obtained from the Institutional Review Boards of our institutes (KC10SNSI0466). Informed consent was provided according to the Declaration of Helsinki. For isolation of PAS-MSLCs from pituitary adenoma specimen, we followed proven methods for MSC isolation from bone marrow (Lennon and Caplan, 2006; Mareschi et al., 2001), normal brain (Kang et al., 2010) and gliomas (Kim et al., 2013). We performed the isolation procedure within 60 minutes of pituitary adenoma removal using a mechanical dissociation method. Surgical specimens were minced and dissociated with a scalpel in minimal essential medium-α (MEMα; Mediatech, Herndon, VA, USA) and then passed through a series of cell strainers with 100-μm nylon mesh (BD Falcon, Franklin Lakes, NJ, USA). Cell suspensions were washed twice in MEMα, and single-cell suspensions were placed in a 10-cm^2 cell culture dish at a density of 2×10^6 cell/cm^2. These cells were cultured in complete MSC medium consisting of MEMα, 10% fetal bovine serum (FBS; Lonza, Basel, Switzerland), 2 mM L-glutamine (Mediatech), and antibiotic-antimycotic solution (100x, Gibco, Invitrogen Korea, Seoul, Korea). After 24 hours, non-adherent cells were removed by washing with phosphate-buffered saline (PBS; Mediatech) twice, and the adherent cells were cultured until they reached confluence. The cells were then trypsinized (0.25% trypsin with 0.1% EDTA) and sub-cultured at a density of 5,000 cells/cm^2. The cells were cultured continuously through 3~4 passages, consistent with their role as progenitor/stem cells. Cell cultures were observed with an inverted phase-ontrast microscope (IX71 Inverted Microscope; Olympus, Tokyo, Japan) to determine their morphology. Photographs of cells were obtained with a digital camera (DP70 Digital Microscope Camera; Olympus), using DP Controller software (Olympus) at each passage.

Flow cytometry analysis

To investigate the surface antigen expression profile, candidate PAS-MSLCs were counted, washed in PBS (Mediatech), and pellets were resuspended in fluorescent-activated cell sorting (FACS) buffer (PBS with 10% FBS) at a concentration of 5×10^5 cells per 100 μl. This single-cell suspensions was incubated at 4℃ for 30 minutes with phycoerythrin, fluorescein isothiocyanate (FITC), Alexa Fluor 647, or allophycocyanin-conjugated antibodies against CD105 at a concentration of 0.25 μg/100 μl FACS buffer (eBioscience, San Diego, CA, USA), CD45 at a concentration of 5 μg/100 μl FACS buffer (BD Pharmingen, San Diego, CA, USA), CD73 at a concentration of 5 μg/100 μl FACS buffer (BD Pharmingen), CD90 at a concentration of 0.25 μg/100 μl FACS buffer (eBioscience), CD31 at a concentration of 0.5 μg/100 μl FACS buffer (eBiosciece), and nerve/glial antigen 2 (NG2) at a concentration of 2.5

μg/100 μl FACS buffer (R&D Systems, Minneapolis, MN, USA). For the detection of NG2 proteoglycan, an FITC-conjugated secondary antibody (Millipore, Billerica, MA, USA) was used following primary antibody incubation (NG2 antibody; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). FACS analysis was performed using a FACS vantage SE (BD Biosciences) flow cytometry system equipped with Flowjo software (Tree Star, Inc., Ashland, OR, USA), and 30,000 events were recorded for each sample. For isolation of MSLCs among heterogeneous cell population, we assumed positive for surface antigen analysis as more than 10% of cells in FACS and negative as less than 5% in FACS (Kim et al., 2013) because we merely try to show presence of MSLCs among heterogeneous cells, instead of identical MSCs isolation (Dominici et al., 2006).

Mesenchymal differentiation

To determine the mesenchymal differentiation potential of candidate PAS-MSLCs, we used a proven trilineage differentiation test identical to that described previously (Kim et al., 2013; Lennon and Caplan, 2006; Mareschi et al., 2001). Briefly, we tested the capacity of candidate PAS-MSLCs to differentiate along adipogenic, osteogenic, and chondrogenic lineages. For adipogenic differentiation, PAS-MSLCs were seeded in a six-well plate at a density of 4×10^4 cells/cm^2 in complete MSC medium. At confluence, cell differentiation was induced with adipogenic differentiation medium from the adipogenic differentiation BulletKit (Lonza Walkersville, Walkersville, MD, USA). This cell was fed with fresh medium every 3~4 days for 3 weeks. In control experiments, cells were incubated for the same period of time in complete MSC medium. On day 21, the cells were washed in PBS (Mediatech) and fixed in 10% formalin (Fisher Scientific, Fair Lawn, NJ, USA) for 1 hour at room temperature. After fixation, the cells were rinsed with deionized water several times, followed by the addition of 60% isopropanol (Pharmco-AAPER, Brook-field, CT, USA), and were allowed to sit for 5 min. Oil red O solution (Sigma) was then added to each well. After 5 minutes, the cells were rinsed with deionized water and briefly counter-stained with hematoxylin (Sigma).

For osteogenic differentiation, candidate PAS-MSLCs was plated at a density of 3×10^4 cells/cm^2 in a six-well plate. The next day, the medium was replaced with osteogenic differentiation medium from the osteogenic differentiation BulletKit (Lonza Walkersville). These were fed with fresh medium every 3~4 days for 3 weeks. In control experiment, cells were incubated for the same period of time in complete MSC medium. On day 21, cell culture was washed twice with PBS (Mediatech) and fixed in 70% ice-cold ethanol (Pharmco-AAPER) for 1 hour, followed by washing with deionized water. The cells were stained with 40 mM Alizarin red (pH 4.2; Sigma) for 10 min at room temperature with rotation, followed by washing with deionized water five times.

For chondrogenic differentiation, a modification in previous protocols was used. Candidate PAS-MSLCs was trypsinized and washed in serum-containing medium. Aliquots of 250,000 cells suspended in 0.5 ml of medium were placed in 15-ml conical polypropylene tubes (SPL, Pocheon, Gyeonggi, Korea). The cells were then gently centrifuged for 5 min at $150 \times g$ and left at the bottom of the tubes, which were placed in an incubator with caps loosened to permit gas exchange. The cells formed small pellets that were cultured for 3 weeks in chondrogenic differentiation medium from the chondrogenic differentiation BulletKit (Lonza Walkersville) and 20 μg/ml of TGF-β3 (Ontogeny Research Products, Cambridge, MA, USA). Every 3~4 days, the cells were fed with fresh medium. In control experiments, the cells were incubated for the same period of time in complete MSC medium. These pellets were fixed in 10% formalin for 1 hour at room temperature and then embedded in paraffin sections, there after stained with toluidine blue (Sigma) for proteoglycans and glycosaminoglycans.

Animal subjects

4- to 8-week-old male athymic nude mice (Central Lab. Animal Inc., Seoul, Republic of Korea) were used to check tumorigenesis of PAS-MSLCs. Mice were housed in micro-isolator cages under sterile conditions and observed for at least 1 week before study initiation to ensure proper health. Lighting, temperature, and humidity were controlled centrally. All experimental procedures were approved by our In-

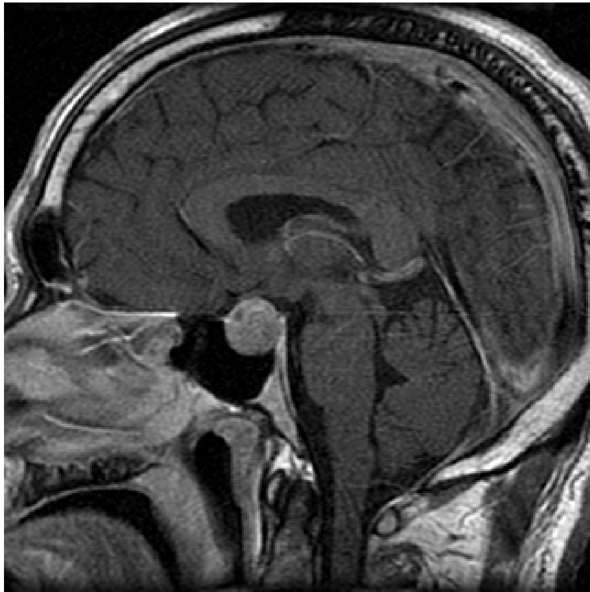

Fig. 1. Magnetic resonance image (MRI) of a patient. Sagittal MRI showed that there was about 1.6 × 1.2 × 1.3 cm sized hypoenhancing intra and suprasella mass. The mass was abutting to optic chiasm with mild compression.

stitutional Animal Care and Use Committee. Every day, we checked the body weight of the mice. If the body weight decreased more than 15% compared with the original body weight, mice were euthanized according to protocol. The brain was dissected and placed in formalin for pathological study.

Xenografting of candidate PAS-MSLCs

Mice were anesthetized with a solution of Zoletil® (30 mg/kg; Virbac Korea, Seoul, Korea) and xylaxine (10 mg/kg; Bayer Korea, Seoul, Korea) delivered intraperitoneally. Candidate PAS-MSLCs were implanted into the right frontal lobe of nude mice using a guide-screw system within the skull, as described previously (Lal et al., 2000). Mice received 5 × 10^5 candidate PAS-MSLCs via a Hamilton syringe (Dongwoo Science Co., Seoul, Korea) inserted to a depth of 4.5 mm. Each candidate MSLCs were injected into three mice simultaneously using a multiple micro infusion syringe pump (Harvard Apparatus, Holliston, MA, USA) at a speed of 0.5 μl/min as previously described (Kang et al., 2010; Kim et al., 2011; Kim et al., 2013; Lal et al., 2000; Nakamizo et al., 2005). At least 180~200 days after injection, we carefully removed mice brains to see whether tumorigenesis occurred with H/E staining.

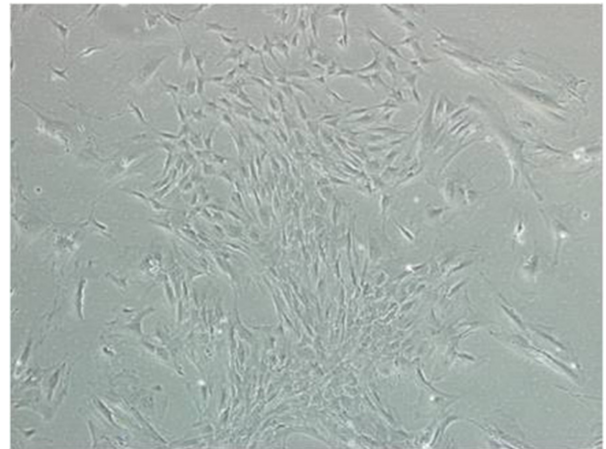

Fig. 2. Morphology of pituitary adenoma stroma mesenchymal stem-like cells (PAS-MSLCs) from specimen of pituitary adenoma. Isolated cells grew as spindle-shaped cells in MSC culture conditions and were resembled bone marrow-derived MSCs (BM-MSCs). Original magnification ×100.

RESULTS

Patient information

The tumor specimen was obtained from a 62-year-old female patient. Preoperative magnetic resonance image (MRI) revealed hypoenhancing intra- and supra sella mass (Fig. 1). The tumor was resected by transsphenoid approach. Pathologic diagnosis revealed pituitary adenoma. Immunohistochemistry stain showed negative for ACTH, prolactin, GH, TSH, estrogen and positive for β-HCG.

Isolation of candidate MSLCs from a pituitary adenoma

To isolate PAS-MSLCs from a pituitary adenoma specimen, fresh pituitary adenoma specimen was obtained and then cultured. Adherent cells were cultured discarding non-adherent cells in MSCs media, and grew as spindle-shaped cells (Fig. 2).

Surface antigen expression of candidate MSLCs

Adherent cells were analyzed by flow cytometry for mesenchymal (CD105, CD90, and CD73), leukocyte (CD-45), pericyte (NG2) surface marker that were associated

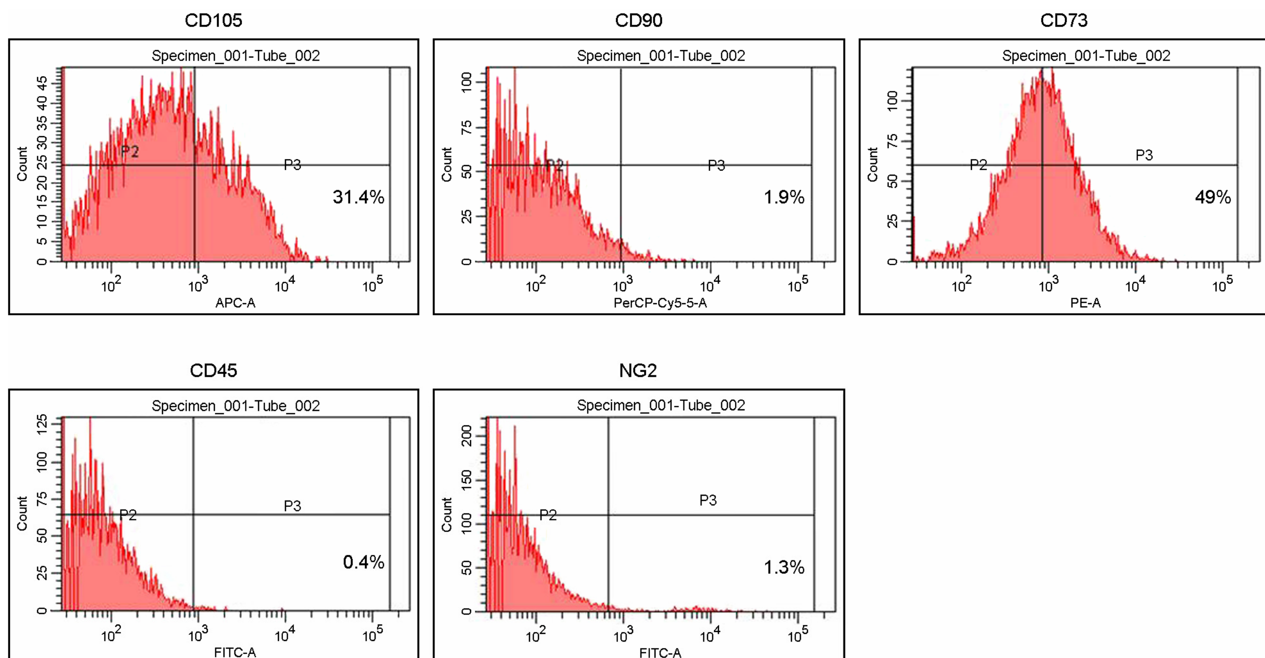

Fig. 3. PAS-MSLCs analyzed by flow cytometry for MSCs surface antigens. We identified mesenchymal (CD105, CD90, and CD73), leukocyte (CD45), and pericyte (NG2) surface antigen expression. PAS-MSLCs presented positive for CD105/CD73 and negative for CD90/CD45/NG2.

with human MSCs. Although there is no pathognomonic marker for MSC, it is generally agreed that human MSCs are positive for CD105, CD90, and CD73, and negative for CD45. In this study, isolated candidate PAS-MSLCs expressed a bit similar surface antigen pattern for MSCs, positive for CD105 (31.4%), CD73 (49%), and negative for CD45 (0.4%), NG2 (1.3%) , and CD90 (1.9%) (Fig. 3).

Mesenchymal differentiation of candidate PAS-MSLCs

To identify whether PAS-MSLCs have the mesenchymal differentiation capacity, PAS-MSLCs were cultured in the mesenchymal trilineage-inducing media. During osteogenic induction of PAS-MSLCs, cells filled with calcium deposits were confirmed by Alizarin Red staining 3 weeks after the initiation of the induction (Fig. 4B). In the control experiments, we showed that cells did not have calcium deposits in non-inducing medium. After cells were cultured in adipogenic differentiation condition, lipid droplets formed in cells were detected by Oil Red O staining (Fig. 4E). In the control experiments, adipogenesis was not detected. Finally, PAS-MSLCs were cultured and pelleted in chondrogenic differentiation medium for 3 weeks. Then these cells were stained by toluidine blue for detection of proteoglycans and glycosaminoglycans in the pellet matrix (Fig. 4F). Chondrogenesis was not detected in control culture.

No tumorgenicity of PAS-MSLCs

PAS-MSLCs were tested for lack of tumorigenesis capacity *in vivo*. Nude mice intracranially implanted with pituitary adenoma cells were monitored for about 6 months and sacrificed upon weight loss of 15% and over as compared to the maximal weight. All mice that implanted with were PAS-MSLCs survived more than 6 month. According to pathologic result, we found no tumorigenesis from which we could conclude the absence of tumorigenesis characteristics in PAS-MSLCs (Fig. 5).

DISCUSSION

In this study, we showed that MSLCs were isolated in pituitary adenoma specimen. Immunohistochemistry for ACTH, prolactin, GH, TSH, estrogen were all negative

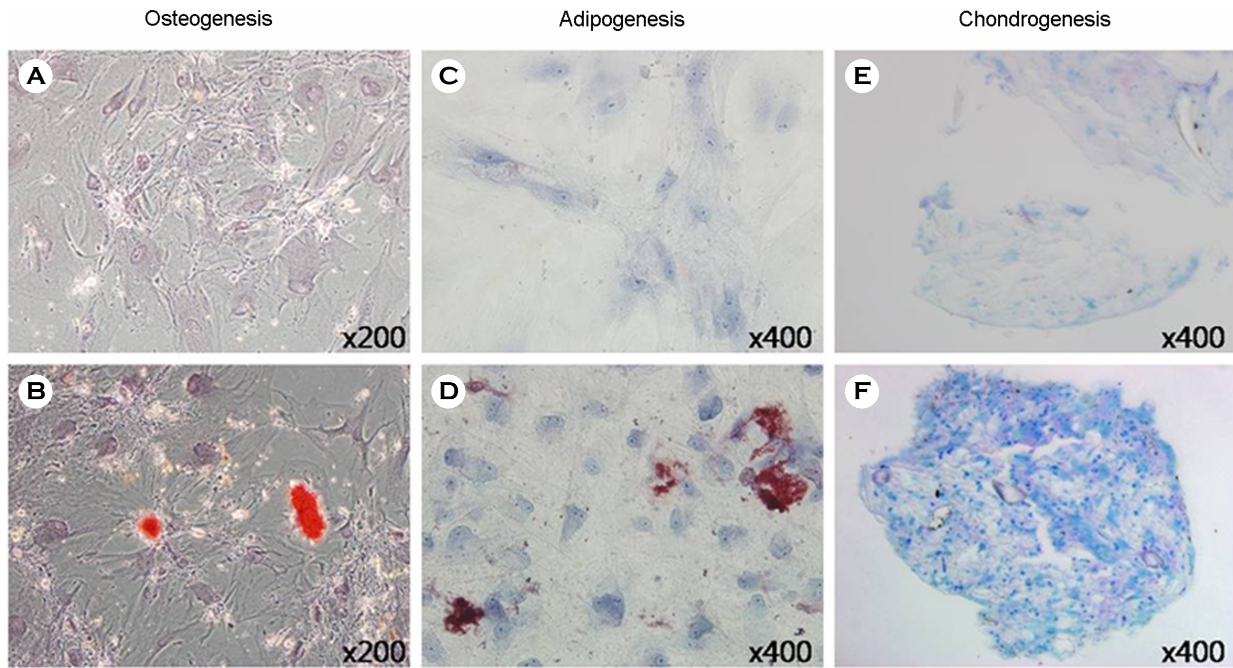

Fig. 4. PAS-MSLCs identified for trilineage mesenchymal differentiation capacity. (A) In non-induced medium, PAS-MSLCs were negative for osteogenesis. (B) Osteogenic differentiation of PAS-MSLCs was seen calcium deposition. (C) In control medium, PAS-MSLCs were negative for adipogenesis. (D) Adipogenic differentiation of PAS-MSLCs was seen intracellular lipid droplets. (E) PAS-MSLCs were negative for toluidine blue staining in non-induced medium. (F) Chondrogenic differentiation of PAS-MSLCs was stained proteoglycans and glycosaminoglycans by toluidine blue.

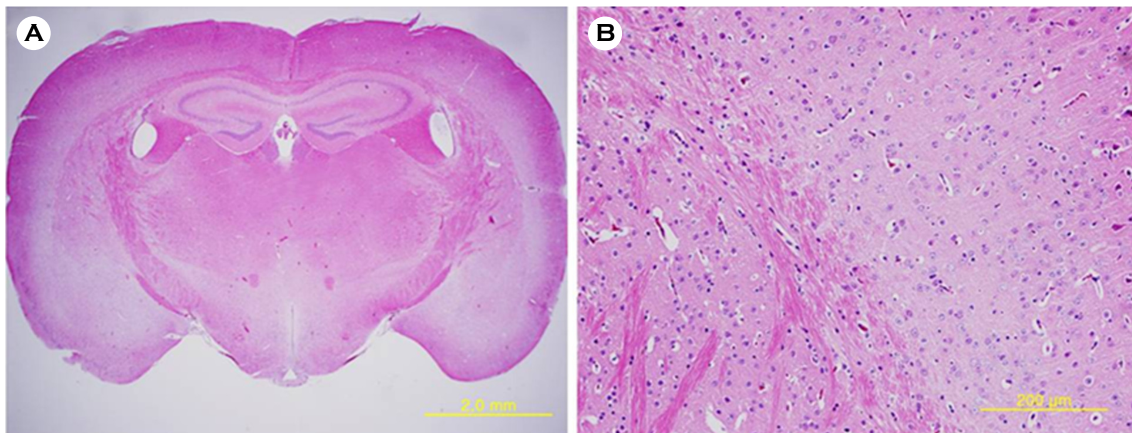

Fig. 5. No tumorigenesis capacity of PAS-MSLCs. Nude mice brains implanted with PAS-MSLCs were sectioned and showed no tumorigenesis. (A) Original magnification ×20 (H&E) (B) Original magnification ×200 (H&E).

except β-HCG in this pituitary adenoma tissue. Cells from PA specimen were successfully isolated and cultured in common MSCs culture conditions, as described previously (Kim et al., 2013; Lennon and Caplan, 2006; Mareschi et al., 2001). These cells exhibited adherence to a plastic surface (Fig. 1). Isolated cells in pituitary adenoma were analyzed of the presence of the surface antigens CD105/CD90/CD73/CD45, which are typical surface markers of MSLCs used in other studies (Kim et al., 2013; Kwak et al., 2013; Lim et al., 2013). These cells exhibited positive for

CD105, CD73, and negative for CD45, NG2, and CD90 surface antigen expression profile (Fig. 2). PAS-MSLCs had a similar surface antigen profile to MSLCs, except negative expression about CD90. In addition, these cells were capable of trilineage diffenentation to adipogenic, osteogenic and chodrogenic (Fig. 3), and lack of tumorigenesis (Fig. 4). Although the obtained cells do not completely equated to MSCs, some features can be seen similar to the MSCs (Dominici et al., 2006; Chamberlain et al., 2007). Accordingly, isolated cells in pituitary adenoma were called mesenchymal stem-like cells.

This study have an obvious limitation due to the small number of PA specimens (n=1). However, our hypothesis that PAS-MSLCs could be isolated from specimen was substantiated. We did not identify EMT the specimens from the patients with pituitary adenoma. However, a few research present that pituitary adenoma are invasive (Kovacs et al., 2001; Martins et al., 1965; Scheithauer et al., 1986). Previously, it was known that the invasion was associated with EMT (Guarino et al., 2007). Because there is no single marker to define the MSCs, we adapted the 2006' International Society for Cellular Therapy (ISCT)'s minimal requirement to define the MSCs (Dominici et al., 2006).

Recent studies have reported the presence of MSC or MSLCs in the stroma of brain and other tumor (El-Haibi and Karnoub, 2010; Kang et al., 2010; Karnoub et al., 2007), but little information is known about MSLCs in pituitary adenoma (PA). PAS-MSLCs are a kind of stromal cells in tumor microenvironment and no tumorigenic cells unlike cancer stem cells (Kim et al., 2013; Kwak et al., 2013; Lim et al., 2013), so no tumorigenic capability of PAS-MSLCs is one of most important characteristics which are property of tumor stromal cells although these cells were obtained from tumor. In case of invasive PA, we thought that the invasiveness of PA were from EMT that is the characteristic of mesenchyme (Lekva et al., 2012; Mirantes et al., 2013). In addition, because the EMT is the characteristic of mesenchyme, we thought that there is much chance of existence of MSLCs in invasive PA. We will continue to research for mesenchymal transition of pituitary adenomas and the consequential invasion mechanism from the relationship between mesenchymal molecular signature of pituitary adenomas and PAS-MSLCs, which was not directly addressed in the present study. Future studies should clarify the biological relationship between PAS-MSLCs and pituitary adenomas invasion, the experiments of which is about to be undertaken in our institute.

Recently, the isolation (Kim et al., 2011; Kim et al., 2013) and function (Kong et al., 2013) of MSCs/MSLCs from gliomas were reported. Pituitary tumor cells were acquired mesenchymal patterns in previous study (del Pliego et al., 2013). Our study has demonstrated the existence of MSLCs from specimen of pituitary adenoma. This study will lead to the development of therapeutic strategies for PA patients because it represents a small step in understanding the pituitary adenoma.

Acknowledgement

This research was supported by grants from the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education Science and Technology (2013R1A1A2006427, and 2013R1A1A2055597) and a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI13C1509).

REFERENCES

Ackland ML, Newgreen DF, Fridman M, Waltham MC, Arvanitis A, Minichiello J, Price JT, Thompson EW. Epidermal growth factor-induced epithelio-mesenchymal transition in human breast carcinoma cells. Lab Invest. 2003. 83: 435-448.

Asa SL, Ezzat S. The cytogenesis and pathogenesis of pituitary adenomas. Endocr Rev. 1998. 19: 798-827.

Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007. 25: 2739-2749.

del Pliego MG, Aguirre-Benitez E, Paisano-Ceron K, Valdovinos-Ramirez I, Rangel-Morales C, Rodriguez-Mata V, Solano-Agama C, Martin-Tapia D, de la Vega MT, Saldoval-Balanzario M, Camacho J, Mendoza-Garrido ME. Expression of Eag1 K+ channel and ErbBs in human pituitary adenomas: cytoskeleton arrangement patterns in cultured cells. Int J Clin Exp Pathol. 2013. 6: 458-468.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006. 8: 315-317.

Donald F, Newgreen, Sonja J, McKeown. Rise and Fall of Epithelial Phenotype: Concepts of Epithelial-Mesenchymal Transition. Plenum. 2005. 3: 29-39.

El-Haibi CP, Karnoub AE. Mesenchymal stem cells in the pathogenesis and therapy of breast cancer. J Mammary Gland Biol Neoplasia. 2010. 15: 399-409.

Guarino M. Epithelial-mesenchymal transition and tumour invasion. Int J Biochem Cell Biol. 2007. 39: 2153-2160.

Kang SG, Shinojima N, Hossain A, Gumin J, Yong RL, Colman H, Marini F, Andreeff M, Lang FF. Isolation and perivascular localization of mesenchymal stem cells from mouse brain. Neurosurgery. 2010. 67: 711-720.

Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature. 2007. 449: 557-563.

Kim SM, Kang SG, Park NR, Mok HS, Huh YM, Lee SJ, Jeun SS, Hong YK, Park CK, Lang FF. Presence of glioma stroma mesenchymal stem cells in a murine orthotopic glioma model. Childs Nerv Syst. 2011. 27: 911-922.

Kim YG, Jeon S, Sin GY, Shim JK, Kim BK, Shin HJ, Lee JH, Huh YM, Lee SJ, Kim EH, Park EK, Kim SH, Chang JH, Kim DS, Kim SH, Hong YK, Kang SG, Lang FF. Existence of glioma stroma mesenchymal stemlike cells in Korean glioma specimens. Childs Nerv Syst. 2013. 29: 549-563.

Kong BH, Shin HD, Kim SH, Mok HS, Shim JK, Lee JH, Shin HJ, Huh YM, Kim EH, Park EK, Chang JH, Kim DS, Hong YK, Kim SH, Lee SJ, Kang SG. Increased *in vivo* angiogenic effect of glioma stromal mesenchymal stem-like cells on glioma cancer stem cells from patients with glioblastoma. Int J Oncol. 2013. 42: 1754-1762.

Kovacs K, Horvath E, Vidal S. Classification of pituitary adenomas. J Neurooncol. 2001. 54: 121-127.

Kwak J, Shin HJ, Kim SH, Shim JK, Lee JH, Huh YM, Kim EH, Park EK, Chang JH, Kim SH, Hong YK, Kim DS, Lee SJ, Kang SG. Isolation of tumor spheres and mesenchymal stem-like cells from a single primitive neuroectodermal tumor specimen. Childs Nerv Syst. 2013.

Lal S, Lacroix M, Tofilon P, Fuller GN, Sawaya R, Lang FF. An implantable guide-screw system for brain tumor studies in small animals. J Neurosurg. 2000. 92: 326-333.

Lekva T, Berg JP, Fougner SL, Olstad OK, Ueland T, Bollerslev J. Gene expression profiling identifies ESRP1 as a potential regulator of epithelial mesenchymal transition in somatotroph adenomas from a large cohort of patients with acromegaly. J Clin Endocrinol Metab. 2012. 97: E1506-1514.

Lennon DP, Caplan AI. Isolation of human marrow-derived mesenchymal stem cells. Exp Hematol. 2006. 34: 1604-1605.

Lim HY, Kim KM, Kim BK, Shim JK, Lee JH, Huh YM, Kim SH, Kim EH, Park EK, Shim KW, Chang JH, Kim DS, Kim SH, Hong YK, Lee SJ, Kang SG. Isolation of mesenchymal stem-like cells in meningioma specimens. Int J Oncol. 2013. 43: 1260-1268.

Mareschi K, Biasin E, Piacibello W, Aglietta M, Madon E, Fagioli F. Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood. Haematologica. 2001. 86: 1099-1100.

Martins AN, Hayes GJ, Kempe LG. Invasive pituitary adenomas. J Neurosurg. 1965. 22: 268-276.

Mirantes C, Espinosa I, Ferrer I, Dolcet X, Prat J, Matias-Guiu X. Epithelial-to-mesenchymal transition and stem cells in endometrial cancer. Hum Pathol. 2013.

Nakamizo A, Marini F, Amano T, Khan A, Studeny M, Gumin J, Chen J, Hentschel S, Vecil G, Dembinski J, Andreeff M, Lang FF. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. Cancer Res. 2005. 65: 3307-3318.

Scheithauer BW, Kovacs KT, Laws ER, Jr., Randall RV. Pathology of invasive pituitary tumors with special reference to functional classification. J Neurosurg. 1986. 65: 733-744.

Thompson EW, Newgreen DF, Tarin D. Carcinoma invasion and metastasis: a role for epithelial-mesenchymal transition? Cancer Res. 2005. 65: 5991-5995; discussion 5995.

Yang J, Weinberg RA. Epithelial-Mesenchymal Transition: At the Crossroads of Development and Tumor Metastasis. Dev Cell. 2008. 14: 818-829.