Cytotherapy, 2018; ■■: ■■–■■

International Society for Cellular Therapy ISCT®

Comparison of trophic factors secreted from human adipose-derived stromal vascular fraction with those from adipose-derived stromal/stem cells in the same individuals

YUJIRO HIROSE[1,2], YASUHITO FUNAHASHI[1], YOSHIHISA MATSUKAWA[1], TSUYOSHI MAJIMA[1], MASAYA YAMAGUCHI[2], SHIGETADA KAWABATA[2], MOMOKAZU GOTOH[1] & TOKUNORI YAMAMOTO[1,3]

[1]Department of Urology, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]Department of Oral and Molecular Microbiology, Osaka University Graduate School of Dentistry, Suita, Japan, and [3]Laboratory for Clinical Application of Adipose-Derived Regenerative Cells, Nagoya University Graduate School of Medicine, Nagoya, Japan

Key Words: *adipose-derived stromal/stem cells, cell therapy, stromal vascular fraction, trophic factors*

Introduction

Cell therapy for several different diseases has been experimentally investigated for regeneration of damaged tissues. Adipose tissue in the human body is abundant, and can be easily and safely harvested in large quantities, thus it has attracted attention as a source for cell therapy. Stromal vascular fraction (SVF) is currently a major source for cell therapy after isolation from adipose tissues without the need for culture using a Celution System (Cytori Therapeutics) [1,2]. In our previous studies, we found that periurethral injection of autologous SVFs may represent a safe and feasible treatment modality for male stress urinary incontinence [3,4]. However, characterization of the SVFs used in our experiments has yet to be reported.

Mesenchymal stromal cells (MSCs) are well known as a potential source for cell therapy and their therapeutic effect reportedly occurs through secretion of trophic factors [5,6]. SVFs are composed of heterogeneous cell populations, including adipose-derived stromal/stem cells (ASCs), granulocytes, monocytes, lymphocytes, endothelial cells and pericytes [7]. Unfortunately, there is scant information available from comprehensive cytokine or chemokine quantitative analyses of conditioned medium samples isolated from SVFs. In this letter, we show trophic factors released by SVFs and compared them with those released by ASCs in the same individuals.

SVFs were previously isolated from tissues obtained from three male subjects (77, 69 and 75 years old) with a Celution System and used in our clinical research [3]. For the present study, SVFs were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; AusGenex) for isolation of ASCs and SVFs were also cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma-Aldrich) supplemented with 10% FBS as culture of SVFs in 24-well plates. ASCs were detached after reaching 60–70% confluence by incubation with 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA; Life Technologies) and subcultured at a 1:4 dilution under the same conditions. Furthermore, after reaching 60–70% confluence following the second passage (Figure 1A), the culture medium was switched to DMEM without serum and medium samples were collected 24 h later. For SVFs, on day 2 of the primary culture, floating cells and culture media were collected and centrifuged, then the cell pellets were suspended in RPMI 1640 without serum and re-plated in 24-well plates containing adherent cells (Figure 1B), then medium samples were collected after 24 h. Collected medium samples from both ASCs and SVFs were concentrated approximately 20-fold by use of an Amicon Ultra-15 centrifugal filter unit with an Ultracel-3 membrane (Millipore). The concentrated samples were placed in 96-well plates for analyses of

Correspondence: **Tokunori Yamamoto**, MD, PhD, Laboratory for Clinical Application of Adipose-Derived Regenerative Cells, Nagoya University Graduate School of Medicine, Nagoya, Japan. E-mail: toku@med.nagoya-u.ac.jp

(Received 19 January 2018; accepted 5 February 2018)

ISSN 1465-3249
https://doi.org/10.1016/j.jcyt.2018.02.001

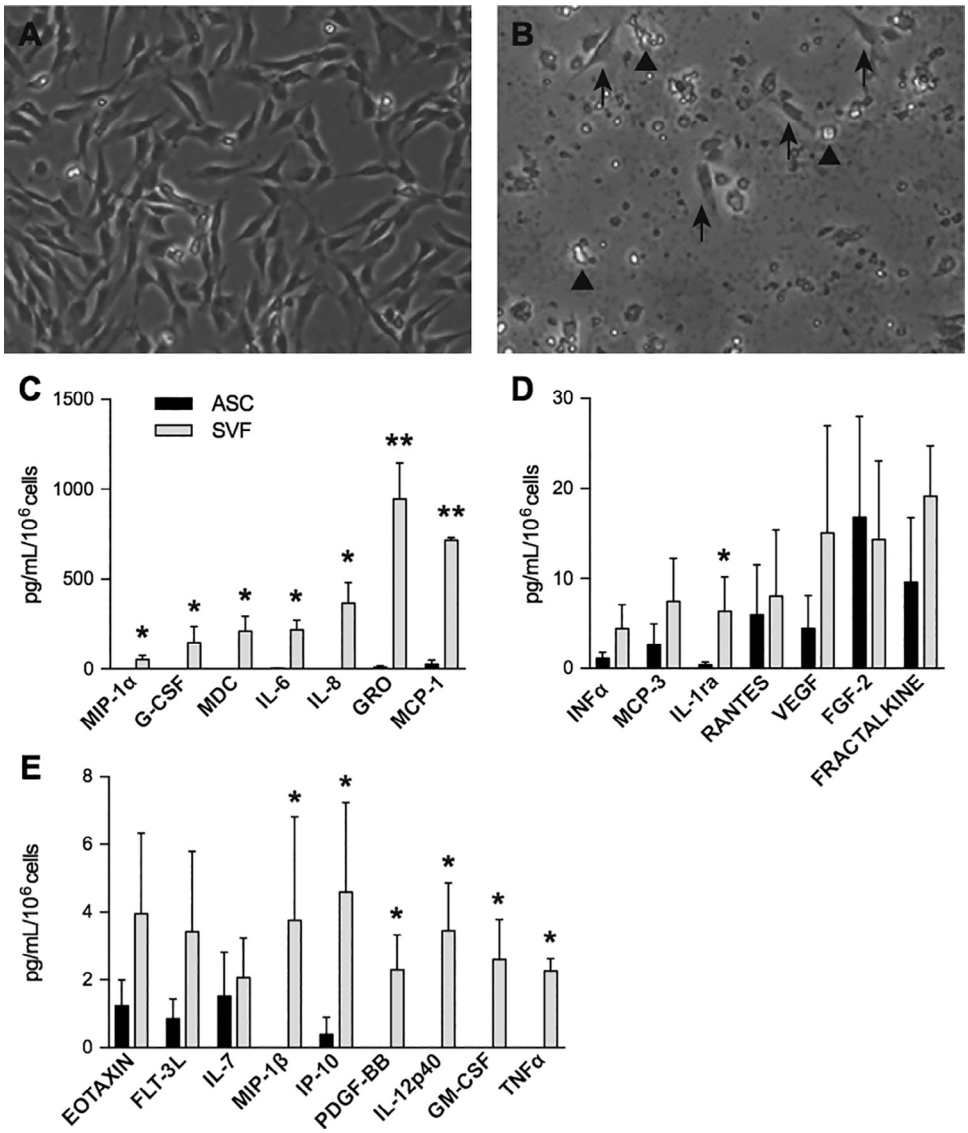

Figure 1. Trophic factors secreted from ASCs and SVFs. (A) ASCs after the second passage on day 2 showed a stellate or spindle shape (arrow). (B) SVF after 2 days of culture were composed of adherent (arrow) and floating (triangle) cells. (C, D and E) SVFs secreted a greater variety of soluble proteins as compared with ASCs. $*P < 0.05$, $**P < 0.01$, as compared with ASCs. MIP, macrophage inflammatory protein; G-CSF, granulocyte colony-stimulating factor; MDC, macrophage-derived chemokine; IL, interleukin; GRO, growth-related oncogene; MCP, monocyte chemoattractant protein; INF, interferon; IL-1ra, IL-1 receptor antagonist; RANTES, regulated on activation, normal T cell expressed and secreted; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; FLT3L, FMS-like tyrosine kinase 3 ligand; IP, interferon-inducible protein; PDGF, platelet-derived growth factor; GM-CSF, granulocyte macrophage colony-stimulating factor; TNF, tumor necrosis factor.

cytokine and chemokine expressions (Milliplex MAP; Human Cytokine/Chemokines Panel-Premixed 41 Plex; Millipore), in accordance with the manufacturer's guidelines. Secreted factors were quantified using a Bio-Plex 200 reader and Bio-Plex Pro wash station (Bio-Rad). Findings regarding the secreted factors were analyzed using Bio-Plex Manager Version 3.0 software. All measurements were normalized to pg/mL/10^6. The present study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine, and by the committee of the Japanese Ministry of Health, Labor and Welfare according to the Guidelines on Clinical Research using Human Stem Cells. Written informed consent was obtained from the patients.

As compared with ASCs, SVFs released a greater variety of cytokines (Figure 1C, 1D and 1E), including macrophage inflammatory protein 1 alpha (MIP-1α), granulocyte colony-stimulating factor (G-CSF), macrophage-derived chemokine (MDC), interleukin (IL)-6, IL-8, growth-related oncogene (GRO), monocyte chemoattractant protein-1 (MCP-1), IL-1 receptor

antagonist (IL-1ra), macrophage inflammatory protein 1 beta (MIP-1β), interferon-inducible protein (IP-10), platelet-derived growth factor BB (PDGF-BB), IL-12p40, granulocyte macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor alpha (TNF-α). The distribution of secreted factors from ASCs was similar to that noted from dental pulp stem cells, as detailed in our previous study [8].

Fibroblast growth factor 2 (FGF-2), vascular endothelial growth factor (VEGF), G-CSF and GM-CSF have been clinically used for wound healing [9]. FGF and VEGF, secreted from both ASCs and SVFs, are well-known trophic factors that have angiogenesis and anti-apoptosis effects [5,6]. On the other hand, G-CSF and GM-CSF are multifunctional cytokines [9] secreted by SVFs alone. We also found that SVFs secreted many cytokines or soluble protein at significantly higher amounts as compared with ASCs, indicating that SVFs are a more multifunctional source for cell therapy. In other words, factors secreted from SVFs may harbor various paracrine effects, such as immunomodulation, anti-apoptosis, angiogenesis, support of growth and differentiation of local stem cells and progenitor cells, anti-scarring and chemoattraction.

Acknowledgments

This study was supported in part by Nagoya University Hospital and a Health Labor Science Research Grant for Research on incurable disease and cancer from the Ministry of Health, Labor and Welfare of Japan, Japan Society for the Promotion of Science (JSPS) KAKENHI (17K11181, 17K11666), and Japan Agency of Medical Research and Development (AMED) (JP17fk0108117). The funders had no role in study design, data collection or analysis, decision to publish or preparation of the manuscript. Yujiro Hirose was a recipient of an Iwadare Scholarship from the Iwadare Educational Association for Dental Graduate Students.

Disclosure of interests: The authors of this article have no conflicts of interest to declare.

References

[1] Fraser JK, Hicok KC, Shanahan R, Zhu M, Miller S, Arm DM. The celution® system: automated processing of adipose-derived regenerative cells in a functionally closed system. Adv Wound Care (New Rochelle) 2014;3:38–45.

[2] Lin K, Matsubara Y, Masuda Y, Togashi K, Ohno T, Tamura T, et al. Characterization of adipose tissue-derived cells isolated with the Celution™ system. Cytotherapy 2008;10:417–26.

[3] Yamamoto T, Gotoh M, Kato M, Majima T, Toriyama K, Kamei Y, et al. Periurethral injection of autologous adipose-derived regenerative cells for the treatment of male stress urinary incontinence: Report of three initial cases. Int J Urol 2012;19:652–9.

[4] Gotoh M, Yamamoto T, Kato M, Majima T, Toriyama K, Kamei Y, et al. Regenerative treatment of male stress urinary incontinence by periurethral injection of autologous adipose-derived regenerative cells: 1-year outcomes in 11 patients. Int J Urol 2014;21:294–300.

[5] Bronckaers A, Hilkens P, Martens W, Gervois P, Ratajczak J, Struys T, et al. Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis. Pharmacol Ther 2014;143:181–96.

[6] da Silva Meirelles L, Fontes AM, Covas DT, Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 2009;20:419–27.

[7] Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy 2013;15:641–8.

[8] Hirose Y, Yamamoto T, Nakashima M, Funahashi Y, Matsukawa Y, Yamaguchi M, et al. Injection of dental pulp stem cells promotes healing of damaged bladder tissue in a rat model of chemically induced cystitis. Cell Transplant 2016;25:425–36.

[9] Barrientos S, Brem H, Stojadinovic O, Tomic-Canic M. Clinical application of growth factors and cytokines in wound healing. Wound Repair Regen 2014;22:569–78.