Novel Surface Marker for the Prospective Mesenchymal Stem Cell Characterization from Rabbit Visceral Adipose Tissue

Suryo Kuncorojakti[1,2]*, Ira Sari Yudaniayanti[3], Lina Susanti[3], Helen Susilawati[1], Ahmad Aswin[1], Diyantoro[1,4], Watchareewan Rodprasert[5]

[1]Research Centre for Vaccine Technology and Development, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia, [2]Division of Veterinary Anatomy, Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, [3]Division of Veterinary Clinical Studies, Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, [4]Faculty of Vocational Studies, Universitas Airlangga, Surabaya, Indonesia, [5]Veterinary Stem Cell and Bioengineering Innovation Center, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

*Corresponding author: suryokuncorojakti@fkh.unair.ac.id

Abstract

Rabbit as a laboratory animals play an important role in bridging basic research to clinical application. The exploration of rabbit mesenchymal stem cell (MSC) is still facing an obstacle regarding the standardization of characterization of MSC. This study is aimed to explore the novel candidate of rabbit MSC surface marker as an effort to establish the goal standardization of rabbit MSC. Three 2-month-old male White New Zealand rabbits weighing 2 kg were used as visceral rabbit adipose derived mesenchymal stem cell (Rab-ADMSC) donors. The cells were characterized according to their morphology characteristic, clonogenic and three-lineage differentiation capacity. Flow cytometry was used to evaluate the MSC surface markers of Rab-ADMSC against CD81, CD29, CD34 and CD45. The results of this study revealed that the Rab-ADMSC posses characteristic of MSC according to their morphology and differentiation capacity. It can be concluded that CD81 surface marker can be proposed as a stable alternative candidate marker for rabbit MSCs. This data is supported by other phenotypic characteristics of MSCs, both in morphology and in the ability of visceral Rab-ADMSCs to differentiate into adipogenic, osteogenic, and chondrogenic lineages.

Keywords: abdominal adipose tissue, anatomical site, *Oryctolagus cuniculus*, process innovation, regenerative medicine

Received: October 28, 2024 **Revised:** January 6, 2025 **Accepted:** February 9, 2025

INTRODUCTION

Laboratory animals play a crucial role in bridging in vitro research to clinical application studies in humans. Animal models are vital and necessary for evaluating various effects and safety aspects of new findings, such as biomaterials, medical devices, and new therapeutic modalities, including stem cell-based therapies. Choosing the right animal model can prevent interpretation errors that may hinder the translation process to clinical application (da Silva Morais *et al*., 2018). Rabbits are widely selected as an animal model in stem cell research for various diseases, such as neurological disorders (Bedar *et al*., 2023; Dar *et al*., 2023), cartilage damage (Wong *et al*., 2020; You *et al*., 2020), wound healing (Kuncorojakti *et al*., 2024; Susanti *et al*., 2024; Wicaksono *et al*., 2024), as well as trachea (Choi *et al*., 2021) and tendon regeneration (McClellan *et al*., 2022). Rabbits are chosen as animal models in human and veterinary medicine due to their ease of maintenance and relative affordability. Rabbits are ideal for surgical interventions and allow for the use of large number of sample sizes to improve statistical significance (Zomer *et al*., 2018).

The exploration of potential sources of mesenchymal stem cells from rabbits in biomedical and translational research has also been widely conducted (Calle *et al*., 2022; Koung Ngeun *et al*., 2023; Yang *et al*., 2021; Zomer *et al*., 2018). Adipose tissue is a potential candidate for rabbit adipose-derived mesenchymal stem cells (ADMSCs), although their characteristics depend on the anatomical location from which the

stem cells are isolated (Wang *et al*., 2020). These characteristic differences influence the selection of appropriate cell sources for biomedical and translational research. A comprehensive understanding of the extent to which characteristic differences in stem cells affect the acceleration of the translational process from biomedical research to clinical application is essential (Zomer *et al*., 2018). Although rabbit adipose-derived mesenchymal stem cells (Rab-ADMSCs) have been widely used, phenotypic characterization of specific surface markers for MSCs remains challenging (Calle *et al*., 2022). The identification of alternative specific surface markers for Rab-ADMSCs was conducted in this study. Additional phenotypic characteristics, such as differentiation capacity (osteogenic, chondrogenic, and adipogenic) and clonogenic assays, were also examined in this study as an effort to establish standardized phenotypic characteristics of Rab-ADMSCs.

MATERIALS AND METHODS

Ethical Approval

All of the protocol related with experimental animals were performed according to the guidelines and approved by Institutional Animal Care and Use Committee (IACUC) of The Faculty of Veterinary Medicine Universitas Airlangga, Surabaya Indonesia with the reference number 1.KEH.112.07.2024.

Study Period and Location

This research was performed during June to September 2024 at the Research Center for Vaccine Technology and Development Institute of Tropical Disease, Universitas Airlangga (RCVTD–ITD Unair), Surabaya.

Cell Isolation and Culture

Rab-ADMSCs were isolated from the visceral adipose tissue (n = 3) of 2-month-old male White New Zealand rabbits weighing 2 kg obtained from local rabbit breeding in Malang – East Java. Briefly, visceral adipose tissue was collected from the abdominal cavity (at the mesenteric site) of the rabbits, then washed with PBS (Gibco, New York) and transferred to the laboratory under transport media (αMEM containing 3% Penicillin-Streptomycin and 3% Amphotericin B). The tissue then was subjected to mechanical and enzymatic dissociation using trypsin (Gibco, New York) for 1 hour at 37°C. The resulting cells were cultured in complete media containing Alpha Minimum Essential Medium (αMEM) (Gibco, Paisley, UK) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco, New York, USA), 1% Penicillin-Streptomycin (Gibco, New York, USA), and 1% Amphotericin B (Gibco, New York, USA) the medium was replaced every 48 hours. The cells were maintained at 37^{o}C in a humidified incubator with 5% CO_2. Cells at passages 4–6 were used in this study.

Morphological Observation

Morphological observations were conducted daily by examining the normal morphology of rabbit Rab-ADMSCs under an inverted microscope (Invitrogen™ EVOS™ XL Core Configured Cell Imager; Invitrogen, USA).

Clonogenic Assay

A colony-forming unit assay was conducted to determine the clonogenicity of ADMSCs. A total of $5x10^2$ cells were cultured in a 6-well plate (Nest, China) with complete media. On day 14, the cells were washed with PBS Gibco, New York, USA), fixed with 4% paraformaldehyde (Sigma-Aldrich, Netherlands), and stained with crystal violet (Sigma-Aldrich, Netherlands). Colony identification was based on the number of cells in each colony. A group of cells was counted as one colony if it contained fewer than 100 cells.

Three-Lineage Differentiation

Cells were cultured in a 24-well plate at a density of $5x10^4$ cells per well using standard basal media. Once the cells reached 80% confluency, the media was replaced with adipogenic media containing αMEM (Gibco, Paisley, UK) containing 10% FBS (Gibco, New York, USA), 10 mM isobutyl-methylxanthine (Thermo Scientific, NJ, USA), 100 mM indomethacin (Sigma Aldrich, MO, USA), 1 mM

dexamethasone (Sigma Aldrich, Darmstadt, Germany) and 10 μg/mL insulin (Sigma Aldrich, Darmstadt, Germany), osteogenic media containing αMEM (Gibco, Paisley, UK) supplemented with 10% FBS (Gibco, New York, USA) supplemented with 50 mg/mL L-ascorbic acid-2-phospate (Sigma Aldrich, Darmstadt, Germany), 10 mM β-glycerophosphate (Sigma Aldrich, Darmstadt, Germany), 100 nM dexamethasone (Sigma Aldrich, Darmstadt, Germany), and chondrogenic induction media containing medium αMEM (Gibco, Paisley, UK) 10% FBS (Gibco, New York, USA), supplemented with 1× insulin-transferrin-sodium selenite (ITS) (Sigma Aldrich, Darmstadt, Germany), 40 mg/mL L-proline (Sigma Aldrich, Darmstadt, Germany), 50 mg/mL L-ascorbic acid-2-phospate (Sigma Aldrich, Darmstadt, Germany), 1 ng/mL transforming growth factor-β1 (Thermo Scientific, CA, USA) and 100 nM dexamethasone (Sigma Aldrich, Darmstadt, Germany). The media was changed every two days, and on day 21, the cells were stained with oil red-O, alizarin red, and alcian blue to evaluate the osteogenic, chondrogenic, and adipogenic differentiation capabilities of the visceral Rab-ADMSCs.

Flow Cytometry

Characterization of rabbit visceral Rab-ADMSCs was performed based on the expression of surface proteins CD81, CD29, CD34, and CD45 (detail antibodies were described in Table 1) using Attune™ CytPix™ Flow Cytometer (Invitrogen, USA). Cell staining protocol and antibody dilutions used in this assay were carried out according to the manufacturer's instructions.

Data Analysis

The results of this study are presented descriptively in the form of images, bar charts and histograms (n=3).

Table 1. List of antibodies

Antibody	Clone	Clonality	Isotype	Conjugated Fluorophore	Brand and Catalog Number
CD81	M38	Monoclonal	Mouse IgG1	FITC	Invitrogen (A15753)
CD29	P4G11	Monoclonal	Mouse IgG1	FITC	Sigma-Aldrich (MAB1951F)
CD34	581	Monoclonal	Mouse IgG1, k	FITC	Biolegend (304006)
CD45	H130	Monoclonal	Mouse IgG1, k	FITC	Biolegend (304006)

RESULTS AND DISCUSSION

Based on microscopic observations to evaluate morphology, visceral Rab-ADMSCs showed a fibroblastic morphology and adhered to the bottom of the container (Figure 1). The clonogenicity of visceral Rab-ADMSCs was assessed using a colony-forming unit assay. The quantification of colony numbers indicated that visceral Rab-ADMSCs could form an average of 6.67 ± 1.52 colonies (Figure 2). The differentiation ability to resemble adipocyte, osteocyte, and chondrocyte lineages, or adipogenic, osteogenic, and chondrogenic differentiation capabilities, was evaluated microscopically for phenotypic characteristics (Figure 3). Rabbit visceral Rab-ADMSCs were cultured in three different induction media: osteogenic, adipogenic, and chondrogenic induction media. The results showed that histochemical staining using oil red-O, alizarin red, and alcian blue demonstrated that visceral Rab-ADMSCs could differentiate into adipocytes, osteocytes, and chondrocytes, as indicated by red droplet-like structures within the cells representing lipid droplets, brick-red calcium deposits in osteogenic differentiation, and blue staining indicating glycosaminoglycan deposition, which is a primary component of chondrocyte extracellular matrix.

The use of mesenchymal stem cells derived from rabbit tissue has been widely reported in various biomedical studies however, there is still no standardized characterization for Rab-ADMSCs, particularly concerning specific markers for rabbit MSC surface markers (Calle *et al*., 2022). In this study, visceral Rab-ADMSCs were isolated using a standard protocol involving

enzymatic digestion with trypsin, as used in numerous previous studies (Ajit *et al*., 2023; Calle *et al*., 2022; Delaiah *et al*., 2024; Kuncorojakti *et al*., 2024). Phenotypic evaluation based on morphology indicated that Rab-ADMSCs exhibited the required MSC characteristics as set by the International Society for Cellular Therapy (ISCT), which include fibroblastic shape and adherence to the container surface. Similar findings were reported by Calle *et al*. (2022), which noted that mesenchymal stem cells isolated from adipose tissue of the Wild European rabbit also had fibroblastic morphology and adherence (Calle *et al*., 2022).

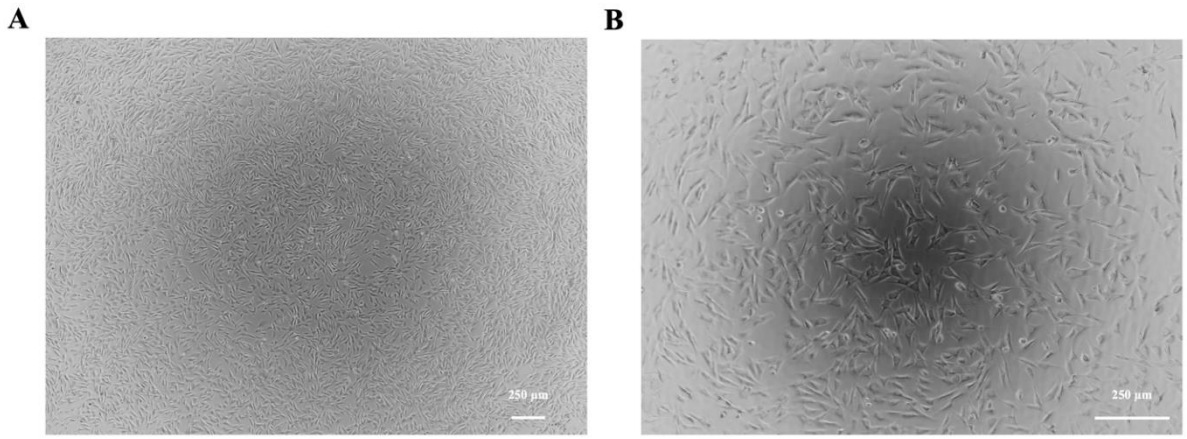

Figure 1. Photomicrograph of Visceral Rab-ADMSC under the light microscope with (A) low (40×) and (B) high magnification (100×).

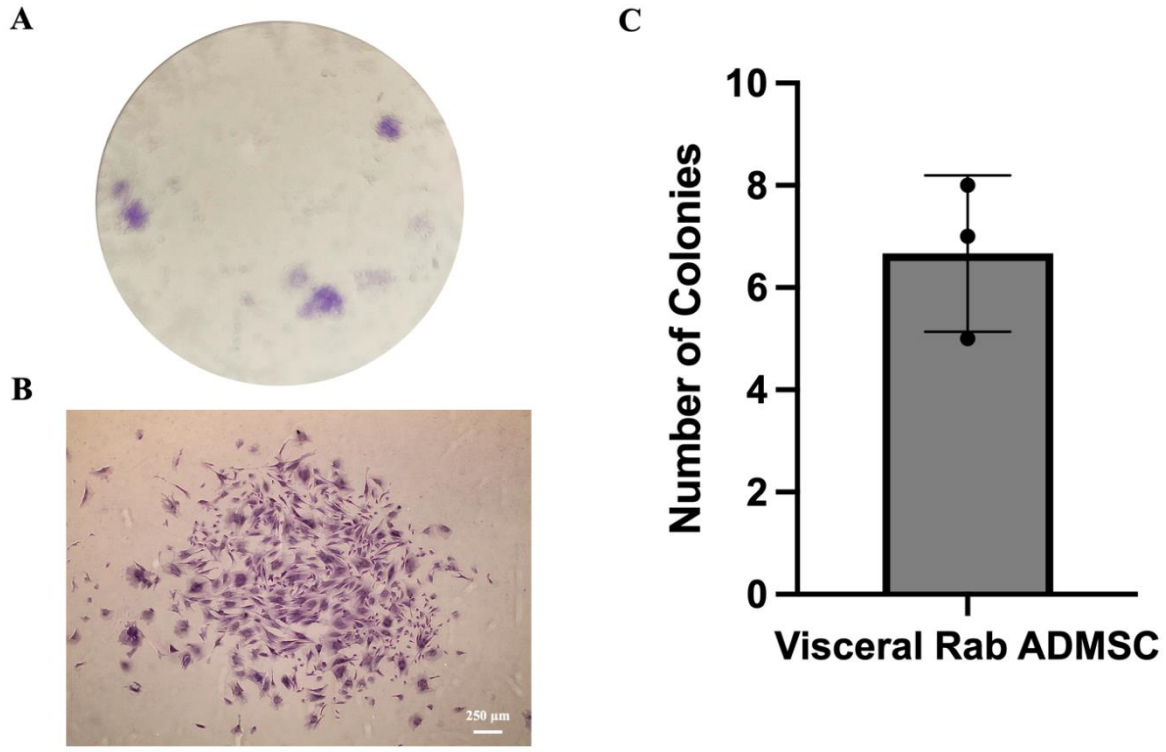

Figure 2. Colony-forming unit assay of Visceral Rab-ADMSC. (A) Macroscopical finding of CFU-assay, (B) photo microscopy of visceral Rab-ADMSC stained with crystal violet, and (C) the number of colony.

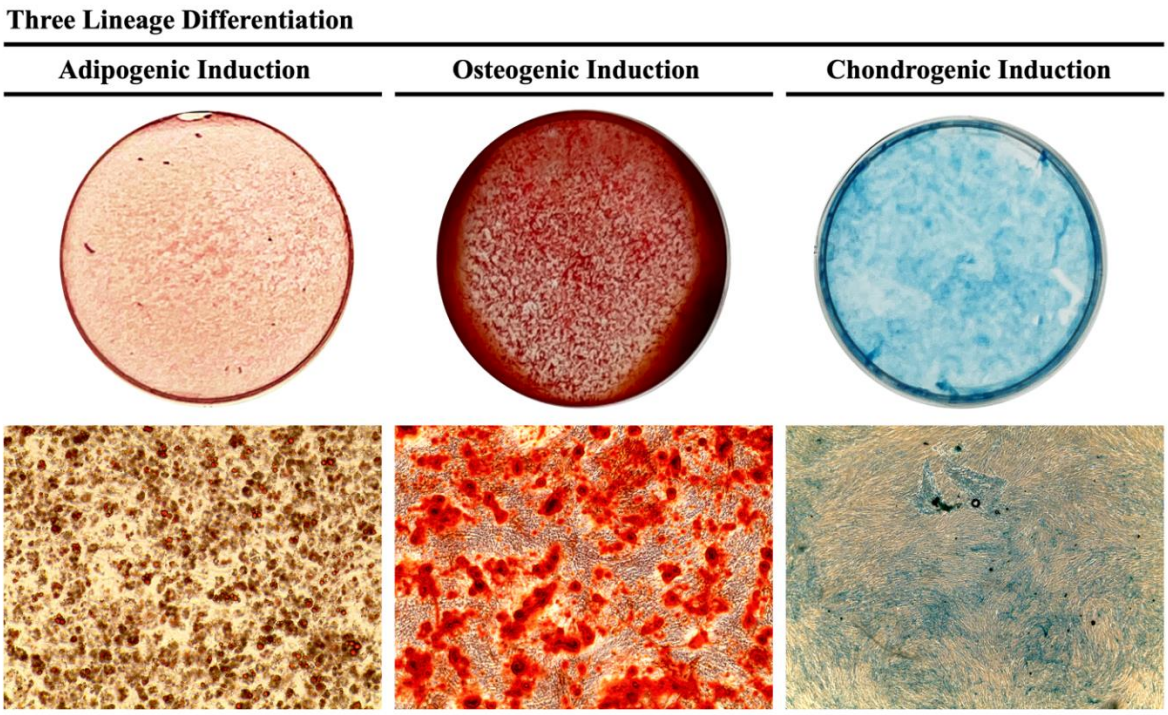

Figure 3. Differentiation capacity of visceral Rab-ADMSC. Visceral Rab-ADMSC after induction with adipogenic induction media (left), osteogenic induction media (center), and chondrogenic induction media (right).

Clonogenicity is also an important characteristic of mesenchymal stem cells. In this study, Rab-ADMSC clonogenicity was evaluated using the colony-forming unit (CFU) assay. The results were consistent with previous studies reporting that Rab-ADMSCs possess adequate clonogenic potential (Zolocinska *et al*., 2020; Zomer *et al*., 2018). Another ISCT standard required for Rab-ADMSCs is their ability to differentiate into adipogenic, osteogenic, and chondrogenic lineages. All visceral Rab-ADMSC lines in this study showed differentiation potential into adipocytes, osteocytes, and chondrocytes based on simple evaluation using histochemical methods to identify phenotypic changes in Rab-ADMSCs. Studies on tri-lineage differentiation of Rab-ADMSCs have also been widely conducted and used as a characteristic marker to meet the criteria for MSC classification. All studies involving Rab-ADMSCs have reported that mesenchymal stem cells isolated from rabbit adipose tissue across different anatomical depots have the capacity to differentiate into adipogenic, osteogenic, and chondrogenic lineages (Calle *et al*., 2022; Zolocinska *et al*., 2020; Zomer *et al*., 2018).

Phenotypic analysis using flow cytometry to determine specific surface markers for mesenchymal stem cells showed varied results for rabbit visceral Rab-ADMSCs. The specific marker CD81 was highly expressed (98.19 ± 0.45%), while the specific marker CD29 showed moderate expression at 65.74 ± 0.77%. The specific markers CD34 and CD45 were not expressed in visceral Rab-ADMSCs (0.17 ± 0.07% and 1.34 ± 0.42%) (Figure 4).

One major challenge in characterizing Rab-ADMSCs is determining specific MSC surface markers. According to ISCT, mesenchymal stem cells must have positive expression of at least 95% for markers like CD90, CD73, and CD105, and negative expression for CD45, CD34, CD11b, CD19, CD14, CD79a, and HLA-DR (Viswanathan *et al*., 2019). The MSC surface markers CD73 and CD90 are widely reported to be expressed on MSCs isolated from various sources, such as adipose, umbilical, bone marrow, dermis, and muscle, and from various species, including humans, dogs, cats, and pigs (Zomer *et*

al., 2018). However, these markers show different expressions in rabbit mesenchymal stem cells. The expression of CD90 in rabbit MSCs has been reported at only 40.5%, while CD73 and CD105 are not expressed at all (Martínez-Lorenzo *et al*., 2009). In this study, exploration was conducted to identify new candidate of surface markers that could serve as alternatives for Rab-ADMSC characterization. Flow cytometry analysis of specific MSC surface markers, CD81 and CD29, showed a strong expression of CD81 but only moderate expression of CD29. Similar results were reported by Rajagopal and Maduri (2021), who found that rabbit MSCs isolated from fat pads, periosteum, and bone marrow also exhibited strong CD81 expression (Rajagopal and Madhuri, 2021).

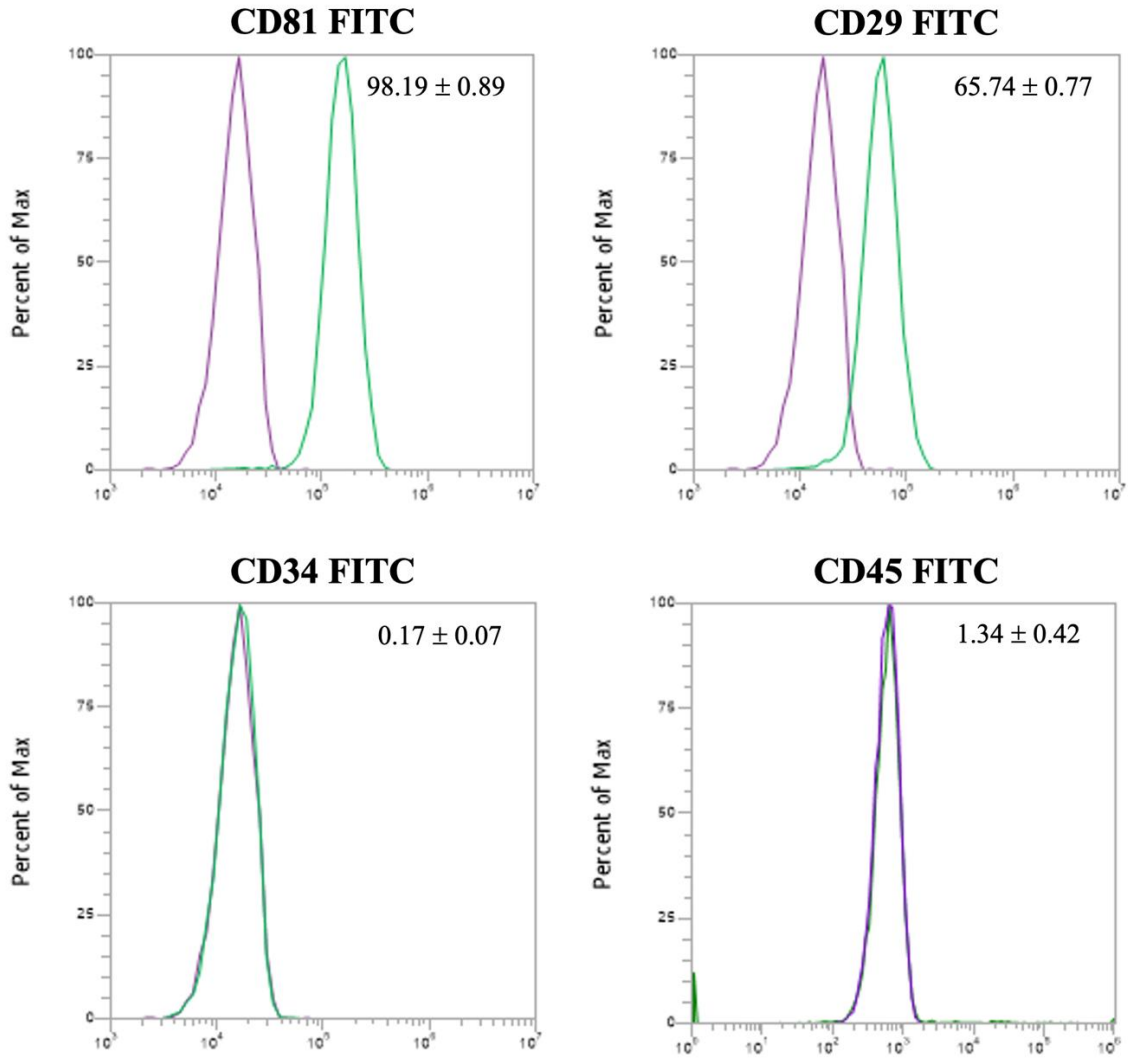

Figure 4. Immunophenotyping of Visceral Rab-ADMSC. Visceral Rab-ADMSC were characterized against MSC surface markers (CD81 and CD29) showing positive results while hematopoietic surface markers (CD34 and CD45) were negative.

Another study on MSCs derived from rabbit hard palatal tissue also showed high CD81 expression (Kim *et al*., 2019). CD81 is part of the tetraspanin, widely recognized as a specific biomarker for exosomes, distributed across the cell membranes of various cell types (Andreu and Yáñez-Mó, 2014). Tetraspanin CD81 is reported to play a vital role in controlling growth and regulating energy balance through FAK signaling (Oguri *et al*., 2020). In contrary, CD29 was

moderately expressed. As reported by previous study, CD29 together with CD39 and CD90 are adult stem cell marker obtained from adipose tissue (Samadi *et al*., 2022), But in rabbit MSC the expression of this marker was inconsistent. Previous study reported that the expression of CD9, CD29, CD44, CD90 and CD105 tend to decrease after the cryopreservation, unfortunately the scientific reasons of this finding was not clearly explained (Koung Ngeun *et al*., 2023), thus the utilization of this marker should be performed together with other MSC surface markers. In this study, two hematopoietic stem cell surface markers, CD45 and CD34, were also evaluated. Results from this study revealed that neither CD45 nor CD34 were expressed in Rab-ADMSCs. These findings are consistent with previous studies (Calle *et al*., 2022; Kim *et al*., 2019; Martínez-Lorenzo *et al*., 2009; Rajagopal and Madhuri, 2021; Zomer *et al*., 2018). Based on the findings of this study, CD81 may be proposed as an alternative surface marker for rabbit mesenchymal stem cells.

CONCLUSION

This study highlights the need for comprehensive research to establish various new alternatives for standardizing surface markers of rabbit mesenchymal stem cells. CD81 surface marker can be proposed as a stable alternative candidate for characterizing rabbit MSCs. This data is supported by other phenotypic characteristics of MSCs, both in morphology and in the ability of visceral Rab-ADMSCs to differentiate into adipogenic, osteogenic, and chondrogenic lineages.

ACKNOWLEDGEMENTS

Authors would like to thanks to the Directorate of Research, Technology and Community Service, Ministry of Education, Culture, Research and Technology Republic of Indonesia through Regular – Fundamental Research Grant (Penelitan Fundamental – Reguler) 2024 with grant number 1712/B/UN3.LPPM/PT.01.03/2024.

AUTHORS' CONTRIBUTIONS

SK: designed, conceptualized, supervised, analyzed data and wrote the manuscript, AA and WR: performed the experiment, analyzed data and wrote the manuscript, ISY and LS performed adipose tissue isolation and conceptualized the study, HS and DD: performed visceral Rab-ADMSC processing, maintained cell culture work and analyzed the data. All authors have read and approve the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Ajit, A., Kumar, T. R. S., Harikrishnan, V. S., Anil, A., Sabareeswaran, A., & Krishnan, L. K. (2023). Enriched adipose stem cell secretome as an effective therapeutic strategy for in vivo wound repair and angiogenesis. *Biotechnology*, 13(3), 83.

Andreu, Z., & Yáñez-Mó, M. (2014). Tetraspanins in extracellular vesicle formation and function. *Frontiers Immunology*, 5, 442.

Bedar, M., van Wijnen, A. J., & Shin, A. Y. (2023). Safety of Allogeneic Mesenchymal Stem Cell Seeding of NeuraGen Nerve Guides in a Rabbit Model. *Tissue Engineering Part C Methods*, 29(2), 43–53.

Calle, A., Zamora-Ceballos, M., Bárcena, J., Blanco, E., & Ramírez, M. (2022). Comparison of Biological Features of Wild European Rabbit Mesenchymal Stem Cells Derived from Different Tissues. *International Journal of Molecular Science*, 23(12).

Choi, J. S., Lee, M. S., Kim, J., Eom, M. R., Jeong, E. J., Lee, M., Park, S. A., Jeong, J. H., & Kwon, S. K. (2021). Hyaluronic Acid Coating on Hydrophobic Tracheal Scaffold Enhances Mesenchymal Stem Cell Adhesion and Tracheal Regeneration. *Tissue*

Engineering Regenerative Medicine, 18(2), 225–233.

da Silva Morais, A., Oliveira, J. M., & Reis, R. L. (2018). Small Animal Models. *Advance Experimental Medical Biology*, 1059, 423–439.

Dar, E. R., Gugjoo, M. B., Farooq, F., Nazir, T., Shah, S. A., Ahmad, S. M., Shah, R. A., Ahmad, R. A., Dar, S. H., & Makhdoomi, D. M. (2023). Mesenchymal stem cells derived from bone marrow and adipose tissue for repairing acute sciatic nerve injury in a rabbit model. *Tissue Cell*, 84, 102162.

Delaiah, D., Aswin, A., Susilowati, H., Wijaya, A. Y., Maulana, F. K., Rodprasert, W., Puspitasari, Y., Dhamayanti, Y., & Kuncorojakti, S. (2024). Epidermal growth factor promotes E6 and CCL-81 Vero cells proliferation under serum-free condition. *Jurnal Medik Veteriner*, 7(1), 1–6.

Kim, D. H., Kim, B. Y., Kim, D. H., Hur, J., & Baek, C. H. (2019). Rabbit palatum-derived mesenchymal progenitor cells tri-lineage differentiation on 2D substrates and 3D printed constructs. *Journal of Applied Biomaterial Function Material*, 17(3), 2280800019834520.

Koung Ngeun, S., Shimizu, M., & Kaneda, M. (2023). Characterization of Rabbit Mesenchymal Stem/Stromal Cells after Cryopreservation. *Biology*, 12(10), 1312.

Kuncorojakti, S., Pratama, A. Z. A., Antujala, C. A., Harijanto, C. T. B., Arsy, R. K., Kurniawan, P. A., Tjahjono, Y., Hendriati, L., Widodo, T., Aswin, A., Diyantoro, D., Wijaya, A. Y., Rodprasert, W., & Susilowati, H. (2024). Acceleration of wound healing using adipose mesenchymal stem cell secretome hydrogel on partial-thickness cutaneous thermal burn wounds: An in vivo study in rats [Article]. *Veterinary World*, 17(7), 1545–1554.

Martínez-Lorenzo, M. J., Royo-Cañas, M., Alegre-Aguarón, E., Desportes, P., Castiella, T., García-Alvarez, F., & Larrad, L. (2009). Phenotype and chondrogenic differentiation of mesenchymal cells from adipose tissue of different species. *Journal of Orthopadic Research*, 27(11), 1499–1507.

McClellan, P., Ina, J. G., Knapik, D. M., Isali, I., Learn, G., Valente, A., Wen, Y., Wen, R., Anderson, J. M., Gillespie, R. J., & Akkus, O. (2022). Mesenchymal Stem Cell Delivery via Topographically Tenoinductive Collagen Biotextile Enhances Regeneration of Segmental Tendon Defects. *American Journal Sports Medicine*, 50(8), 2281–2291.

Oguri, Y., Shinoda, K., Kim, H., Alba, D. L., Bolus, W. R., Wang, Q., Brown, Z., Pradhan, R. N., Tajima, K., Yoneshiro, T., Ikeda, K., Chen, Y., Cheang, R. T., Tsujino, K., Kim, C. R., Greiner, V. J., Datta, R., Yang, C. D., Atabai, K., & Kajimura, S. (2020). CD81 Controls Beige Fat Progenitor Cell Growth and Energy Balance via FAK Signaling. *Cellular*, 182(3), 563–577.

Rajagopal, K., & Madhuri, V. (2021). Comparing the chondrogenic potential of rabbit mesenchymal stem cells derived from the infrapatellar fat pad, periosteum & bone marrow. *Indian Journal Medical Research*, 154(5), 732–742.

Samadi, Y., Egro, F. M., Rodriguez, R., & Ejaz, A. (2022). Chapter 12 - Adipose-derived stem cells for wound healing and fibrosis. In L. Kokai, K. Marra, & J. P. Rubin (Eds.), *Scientific Principles of Adipose Stem Cells* (pp. 225–255). Academic Press.

Susanti, L., Kuncorojakti, S., Oktaviani, S., & Simarmata, M. G. (2024). Lower lip avulsion: Surgery and management using stem cell metabolites preparation in a domestic cat [Article]. *Open Veterinary Journal*, 14(8), 2092–2096.

Viswanathan, S., Shi, Y., Galipeau, J., Krampera, M., Leblanc, K., Martin, I., Nolta, J., Phinney, D. G., & Sensebe, L. (2019). Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature. *Cytotherapy*, 21(10), 1019–1024.

Wang, T., Hill, R. C., Dzieciatkowska, M., Zhu, L., Infante, A. M., Hu, G., Hansen, K. C., &

Pei, M. (2020). Site-Dependent Lineage Preference of Adipose Stem Cells. *Frontiers Cellular Developmental Biology*, 8, 237.

Wicaksono, S., Nugraha, A. P., Rahmahani, J., Rantam, F. A., Kuncorojakti, S., Susilowati, H., Riawan, W., Arundina, I., Lestari, P., Masya, R. N., Surboyo, M. D. C., & Ernawati, D. S. (2024). Adipose Mesenchymal Stem Cell Metabolites Oral Gel Enhance Pro-Angiogenic Factors Expression, Angiogenesis, and Clinical Outcome of Oral Ulcer Rat Model [Article]. *European Journal of Dentistry*, 18(1), 117–123.

Wong, K. L., Zhang, S., Wang, M., Ren, X., Afizah, H., Lai, R. C., Lim, S. K., Lee, E. H., Hui, J. H. P., & Toh, W. S. (2020). Intra-Articular Injections of Mesenchymal Stem Cell Exosomes and Hyaluronic Acid Improve Structural and Mechanical Properties of Repaired Cartilage in a Rabbit Model. *Arthroscopy*, 36(8), 2215–2228.

Yang, G., Shao, J., Lin, J., Yang, H., Jin, J., Yu, C., Shen, B., Hu, X., Si, H., Li, X., Niu, Y., & Wu, Z. (2021). Transplantation of Human Umbilical Cord-Derived Mesenchymal Stem Cells Improves Cartilage Repair in a Rabbit Model. *BioMed Research International*, 2021, 6380141.

You, Q., Liu, Z., Zhang, J., Shen, M., Li, Y., Jin, Y., & Liu, Y. (2020). Human Amniotic Mesenchymal Stem Cell Sheets Encapsulating Cartilage Particles Facilitate Repair of Rabbit Osteochondral Defects. *American Journal Sports Medicine*, 48(3), 599–611.

Zolocinska, A., Siennicka, K., Debski, T., Gut, G., Mazur, S., Gajewska, M., Kaminski, A., & Pojda, Z. (2020). Comparison of mouse, rat and rabbit models for adipose - Derived stem cells (ASC) research. *Current Research Translational Medicine*, 68(4), 205–210.

Zomer, H. D., Roballo, K. C., Lessa, T. B., Bressan, F. F., Gonçalves, N. N., Meirelles, F. V., Trentin, A. G., & Ambrósio, C. E. (2018). Distinct features of rabbit and human adipose-derived mesenchymal stem cells: implications for biotechnology and translational research. *Stem Cells Cloning*, 11, 43–54.

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