Analysis_of_ALDH1A1_and_ALDH1A3_gene_mRNA_expressi

by Purnamawati Purnamwati

Submission date: 09-Jan-2021 06:30PM (UTC+0700)

Submission ID: 1484949567

File name: Analysis_of_ALDH1A1_and_ALDH1A3_gene_mRNA_expressi.pdf (1.23M)

Word count: 4260

Character count: 23180

DOI: 10.18585/inabj.v10i3.477

RESEARCH ARTICLE

Analysis of *ALDH1A1* and *ALDH1A3* Gene mRNA Expressions in Adipose-Derived Stem Cells (ASCs) and Umbilical Cord Stem Cells (UCSCs)

Septelia Inawati Wanandi[1,*], Purnamawati[2], Alice Tamara[3], Karina Teja Putri[3], Daniel Marcellius Simadibrata[3]

[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya, Kenari, Jakarta, Indonesia
[2]Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya, Kenari, Jakarta, Indonesia
[3]Undergraduate Program, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya, Kenari, Jakarta, Indonesia

*Corresponding author. E-mail: septelia@gmail.com

Received date: Jun 13, 2018; Revised date: Sep 4, 2018; Accepted date: Sep 14, 2018

Abstract

BACKGROUND: *ALDH1A1* and *ALDH1A3*, the most renowned isozymes of aldehyde dehydrogenase (ALDH)1, are important in regulating the pluripotency of human mesenchymal stem cells (MSCs) and cancer stem cells (CSCs). The study aimed to analyze the mRNA expression of *ALDH1A1* and *ALDH1A3* genes in adipose stem cells (ASCs) and umbilical cord stem cells (UCSCs) along with their correlations to *Oct-4* mRNA expression. Additionally, the interaction between these proteins was also investigated using *in silico* study to confirm the pluripotency of both MSCs compared to human breast ALDH+ CSCs.

METHODS: This research focused on determining mRNA levels of *ALDH1A1*, *ALDH1A3* and *Oct-4* in ASCs and UCSCs using one-step qRT-PCR. The data were then normalized to those in human breast CSCs and *18S* rRNA. *Oct-4* gene expression was also analyzed to determine the pluripotency of ASCs and UCSCs. The protein-protein interactions were *in silico* analyzed using String 9.1 software.

RESULTS: Relatively, *ALDH1A3* was expressed at similar level in ASCs and UCSCs, while *ALDH1A1* expression level was significantly higher in ASCs compared to UCSCs. In contrast to *ALDH1A3*, the expressions of *ALDH1A1* in both MSCs were significantly lower than breast CSCs similar to *Oct-4* expressions, as also revealed by the *in silico* data showing the interaction between these proteins. This suggests the role of *ALDH1A1* on pluripotency.

CONCLUSION: *ALDH1A1* and *ALDH1A3* were distinctly expressed in UCSCs and ASCs, which might be associated with unique properties of ASCs and UCSCs. This study may contribute to further research in terms of implication of *ALDH1A1* and *ALDH1A3* expressions towards the properties of MSCs and its application in stem cell therapy.

KEYWORDS: *ALDH1A1*, *ALDH1A3*, UCSC, ASC, breast ALDH+ CSC, *Oct-4*

Indones Biomed J. 2018; 10(3): 290-6

Introduction

Human aldehyde dehydrogenase (ALDH), a superfamily consisting of 19 isoforms, plays important role in irreversible oxidation of acetaldehyde.(1,2) *ALDH1A1* and *ALDH1A3* belong to ALDH1A subfamily which have been suggested to play important role in retinoic signaling pathway.(1,3) Acting as a detoxification enzyme, both *ALDH1A1* and *ALDH1A3* catalyze the oxidation of retinal to retinoic acid, contributing to the characteristic of self-protection in stem cells.(1,4) Moreover, they also influence the stemness regulation in terms of cell proliferation and differentiation.(3,4)

Mesenchymal stem cells (MSCs) can be abundantly obtained from human adult tissues (5-7) and share common

CC BY NC

characteristics which include being plastic-adherent when maintained in standard culture condition, have multipotent capacity to differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro*, while still express cluster of differentiation (CD)73, CD90, and CD105, with lack of CD14, CD19, CD45, CD34 and human leukocyte antigen-DR isotype (HLA-DR) expression.(8) Recently, MSCs have been widely used in regenerative medicine. Among various kinds of MSCs, there are adipose-derived stem cells (ASCs) and umbilical cord stem cells (UCSCs) which are recently reported as preferable options for stem cell therapy.(6,9,10) This fact is due to their advantages over bone marrow-derived stem cells such as being able to be obtained non-invasively with minimal immunological and ethical issues.(6,11)

UCSCs are stem cells which can be obtained from total umbilical cord or various sections of umbilical cord, comprising Wharton's jelly, and subendothelial layer of both umbilical arteries and umbilical vein excluding its inner blood vessel walls.(9,12) On the other hand, ASCs are stem cells which are retrieved from adipose tissue.(7,13) Aside from having the common characteristics of MSCs, UCSCs and ASCs can be differentiated in terms of proliferation ability and differentiation capability. It has been reported that UCSCs have higher proliferation ability compared to ASCs, while ASCs are more adipogenic than UCSCs.(14)

Cancer stem cells (CSCs) have been reported to be responsible for initiation and maintenance of cancer due to their self-renewal, pluripotency, and tumorigenic properties, similar to normal stem cells.(15) Regarding the fact that *ALDH1A1* and *ALDH1A3* are found in both normal and cancer stem cells, the specific role of these genes in the aspect of stemness, especially pluripotency, remains unclear. Therefore, this study aimed to analyze the expression of *ALDH1A1* and *ALDH1A3* genes in ASCs and UCSCs relatively to human breast ALDH$^+$ CSCs; along with the confirmation of its pluripotency when being compared to *Oct-4* relative expression in both stem cells.

Methods

Stem Cell Specimens

This study has been approved by Health Research Ethics Committee of Faculty of Medicine Universitas Indonesia – Dr. Cipto Mangkunkusumo Hospital (No. 205/UN2.F1/ETIK/2016). After informed consent, three specimens of ASCs were obtained from healthy women of reproductive age who underwent cosmetic liposuction, after informed consent, at HayandraLab, while three UCSC specimens were obtained from human placenta of women who underwent normal delivery. After being characterized and tested for its differentiation at HayandraLab and Stem Cell Medical Technology Integrated Service Unit (FKUI-RSCM), ASCs and UCSCs were cultured in Alpha-MEM medium supplemented with 10% fetal bovine serum, 1% Penicillin-Streptomycin, 1% Gentamycin, 1% Fungizone, and 1% Glutamine under standard conditions (37°C, 20% O_2, 5% CO_2). Meanwhile, three samples of primary human breast CSCs (ALDH$^+$) were obtained from Cancer Stem Cell Laboratory, Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia. These samples were sorted using ALDEFLUOR™ Assay (STEMCELL Technologies, Vancouver, Canada) to obtain ALDH+ breast CSCs. Breast CSCs were cultured in serum-free DMEM/F12 medium supplemented with 1% Penicillin-Streptomycin, 1% Gentamycin and 1% Fungizone, under standard conditions, as described previously.(16)

Primer Design

Primers for *ALDH1A1*, *ALDH1A3* and *Oct-4* were obtained from previous study done by Purnamawati, *et al.*(17) The primers are listed in Table 1.

RNA Isolation and One-step Quantitative Real-time Polymerase Chain Reactionq (RT-PCR)

Total RNA samples were extracted with Tripure Isolation Reagent® (Roche, Mannheim, Germany) according to manufacturer's instructions. Total RNA concentration was then quantified by spectrophotometry at λ 260 nm. One-step qRT-PCR was performed using KAPA SYBR® FAST One-Step qRT-PCR Kit (Kapa Biosystems, Wilmington, United States of America) in the Exicycler™ 96 (Bioneer, Daejeon, Republic of Korea). A total of 200 ng RNA was diluted in nuclease free water, added to a reaction mixture containing 10 µL of Master Mix, 0.4 µL of 10 µM forward primer, 0.4 µL of 10 µM reverse primer and 0.4 µL of 50X RT Mix, and adjusted to 20 µL of solution.

The qRT-PCR is done in triplicate in the following sequence: cDNA synthesis for 5 minutes at 42°C, inactivation of reverse transcriptase for 5 minutes at 95°C, 40 cycles consisting of denaturation of double stranded DNA for 30 seconds at 95°C and gene annealing for 20 seconds at different optimized temperatures for each genes (Table 1), and elongation for 20 seconds at 72°C.

The measured gene expression was recorded in Cq, and the values were then normalized to the respective gene expression in ALDH$^+$ breast CSCs and *18S* rRNA gene as

DOI: 10.18585/inabj.v10i3.477
Indones Biomed J. 2018; 10(3): 290-6

Table 1. Primers, amplicons and annealing temperature of *ALDH1A1*, *ALDH1A3*, *Oct-4* and *18S* genes.

Gene	Forward Primer	Reverse Primer	Amplicons (bp)	Annealing Temperature (°C)
ALDH1A1 (16)	5'-TTGGAAGATAGGGCCTGCAC-3'	5'-GGAGGAAACCCTGCCTCTTTT-3'	117	60
ALDH1A3 (16)	5'-CGACCTGGAGGGCTGTATTA-3'	5'-TGGTGAAGCACACGACGTT-3'	104	55
Oct-4 (16)	5'-GAGGAGTCCCAGGACATCAAA-3'	5'-AGCTTCCTCCACCCACTTCT-3'	234	57
18S (11)	5'-AAACGGCTACCACATCCAAG-3'	5'-CCTCCAATGGATCCTCHTTA-3'	155	59

bp: base pairs.

reference. Furthermore, melting curve analysis was also performed for each sample to evaluate the authenticity of the products.

Electrophoresis

To verify the PCR products, 2% agarose gel electrophoresis was performed and visualized using GelRed stain (Biotium, Hayward, California, USA).

Bioinformatic Analysis

To further explore the gene expression data, we conducted *in silico* protein-protein interaction analysis using the Search Tool for the Retrieval of Interacting Genes (STRING 9.1) database. This software associates high throughput experiment data from database mining and literature, as well as from predictions based on genomic context analysis.

Statistical Analysis

Data are shown in relative expression±standard error. Statistical differences between two groups were analyzed using SPSS Software Version 20.0.0.0 by Independent T-Test, considering the p-value<0.05 is statistically significant.

Results

***ALDH1A1* mRNA Expression Level in ASCs and UCSCs**

To investigate the expression of *ALDH1A1*, qRT-PCR was conducted in ASCs and UCSCs. Single-peak melting curve was obtained from this PCR, suggesting that the amplified gene is indeed the gene of interest (*ALDH1A1*). Figure 1 depicts that *ALDH1A1* gene was expressed significantly higher in ASCs than UCSCs when being normalized to breast CSCs (p=0.01). Furthermore, this result also points out the lower expression of *ALDH1A1* in MSCs (ASCs and UCSCs) compared to ALDH$^+$ breast CSCs.

***ALDH1A3* mRNA Expression Level in ASCs and UCSCs**

Similar to *ALDH1A1*, *ALDH1A3* gene expression was measured by conducting qRT-PCR in both ASCs and UCSCs samples. The quality of *ALDH1A3* RT-PCR was measured using its single-peak melting curve. In the study, it was found that *ALDH1A3* was expressed at similar level in ASCs and UCSCs (p=0.275), as depicted in Figure 2. Additionally, in contrast to *ALDH1A1*, *ALDH1A3* expression in both MSCs was higher than those expressed in ALDH$^+$ breast CSCs.

***Oct-4* mRNA Expression Level in ASCs and UCSCs**

To explore whether there were any differences between ASCs and UCSCs in the aspect of pluripotency, *Oct-4* expression was also measured by RT-PCR. The melting curve analysis of *Oct-4* RT-PCR results points out a single peak, suggesting that the amplified gene si indeed *Oct-4*. Figure 3 shows that there was no significant difference between ASCs and UCSCs in terms of *Oct-4* expression (p=0.827). Moreover, *Oct-4* is expressed significantly lower in MSCs compared to CSCs, in line with the expression of ALDH1A1.

***ALDH1A1*, *ALDH1A3* and *Oct-4* Protein-Protein Interactions**

The result of the *in silico* analysis, as depicted in Figure 4, indicates that there is a potential protein-protein interaction between *ALDH1A1* and pluripotency markers (POU class 5 homeobox 1 (*POU5F1*) (*Oct-4*), sex determining region Y-box 2 (*SOX2*), nanog homeobox (*NANOG*), and Kruppel-like factor 4 (*KLF4*)). *ALDH1A3* interacts with *ALDH1A1* through alcohol dehydrogenases (ADH4 and ADH1A). This result is in accordance to the fact that these four proteins belong to group of proteins which functions in intracellular detoxification mechanisms. However, the interaction between ALDH1A3 and aforementioned pluripotency markers remains unclear.

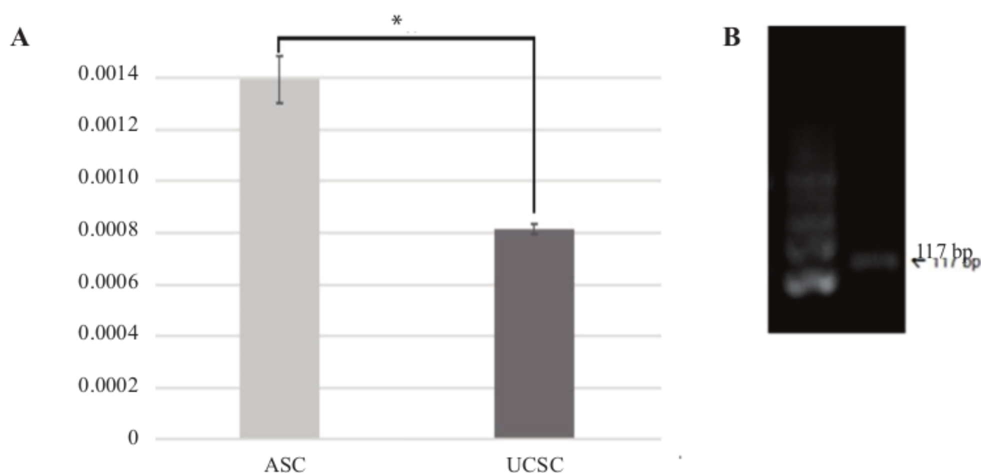

Figure 1. *ALDH1A1* gene expression in ASCs and UCSCs. The results of qRT-PCR, represented as Cq, were normalized by those in ALDH+ breast CSCs. A: Relative expression of *ALDH1A1* was significantly higher in ASCs (mean:1.4×10^{-3}) than in UCSCs (mean: 8.1×10^{-4}) with $p<0.01$. B: Electrophoresis result with 100 bp DNA ladder shows that cDNA with a length of 117 bp was synthesized and amplified using PCR, confirming *ALDH1A1* as PCR product. *=significance $p<0.01$ (tested by independent T-Test).

Discussion

ALDH1A1 and *ALDH1A3*, the isozymes of ALDH1 family, have been known to be the marker of stem cells and cancer stem cells. These enzymes are crucial to detoxify both endogenous and exogenous aldehyde substrates through NAD(P)+-dependent oxidation, emphasizing its capability in self-renewal, differentiation, and self-protection.(3,4) Nevertheless, little is understood on the specific role of these genes in the pluripotency of MSCs from various sources.

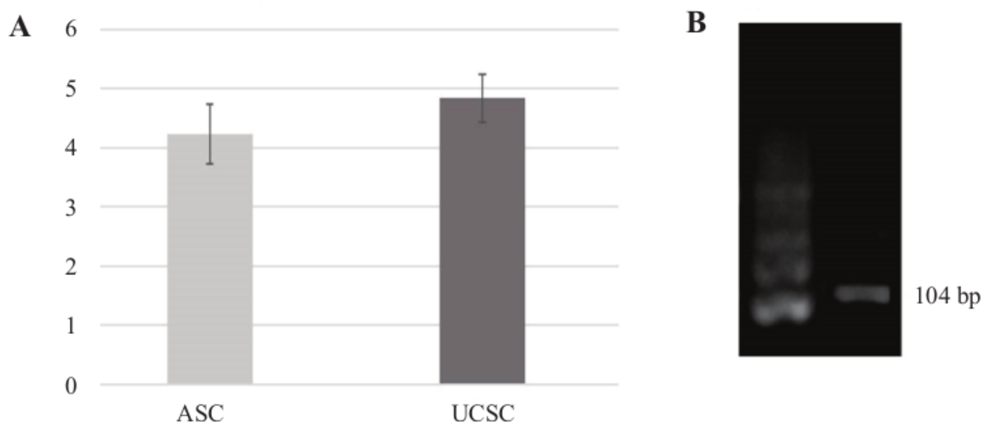

Figure 2. *ALDH1A3* gene expression in ASCs and UCSCs relative to those in CSCs. ASC and UCSC samples underwent RNA isolation. RT-PCR was then conducted and the Cq results were normalized by those in CSCs. A: ASCs (mean: 4.23) and UCSCs (mean: 4.83) expressed similar level of *ALDH1A3*. No significant difference was found between both cells ($p>0.05$). B: Electrophoresis result with 100 bp DNA ladder shows that cDNA with a length of 104 bp was synthesized and amplified using PCR. This also confirms ALDH1A3 as PCR product.

DOI: 10.18585/inabj.v10i3.477
Indones Biomed J. 2018; 10(3): 290-6

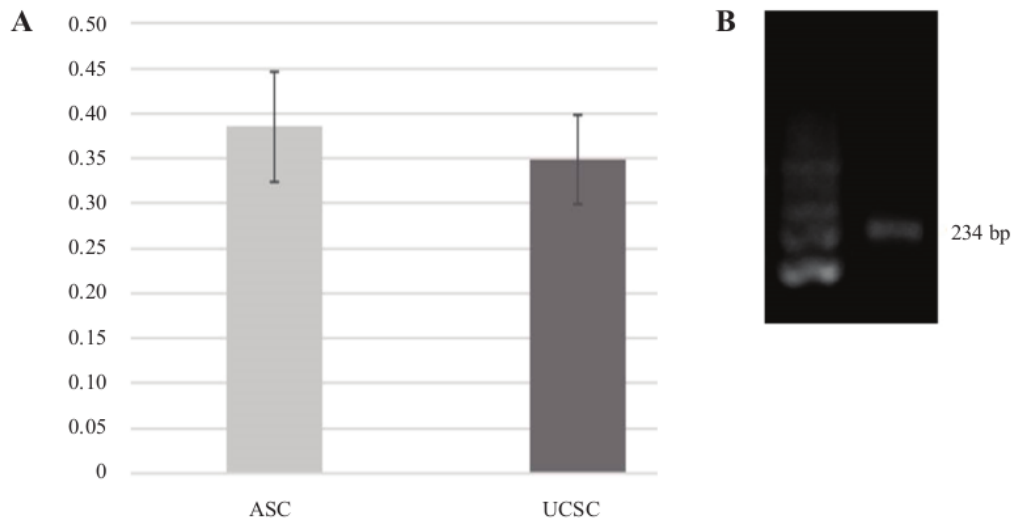

Figure 3. *Oct-4* gene expression in ASCs and UCSCs relative to those in CSCs. The mRNA of ASCs and UCSCs were isolated to undergo RT-PCR. The Cq gained were then normalized by those in CSCs. A: Relative expression of *Oct-4* was expressed similarly between ASCs (mean: 0.39) and UCSCs (mean: 0.35). No significant difference was found between both cells (p>0.05). B: Electrophoresis result with 100 bp DNA ladder shows that cDNA with a length of 234 bp was synthesized and amplified using PCR. This also confirms *Oct-4* as PCR product.

The present study measures mRNA levels of *ALDH1A1* and *ALDH1A3* to investigate the synthesis of these genes. We observed higher expression level of *ALDH1A1* in ASCs compared to UCSCs. This finding supports the previous study performed by Kiefer FW, *et al.*, which stated that *ALDH1A1* expression can be dominantly found in adipose tissue, especially White Adipose Tissue (WAT).(18) Greater *ALDH1A1* expression in ASCs suggests that *ALDH1A1* might contribute to certain properties which differ ASCs from UCSCs such as proliferation and adipogenic differentiation capability.(19,20) In contrast to *ALDH1A1*, *ALDH1A3* was expressed in ASCs and UCSCs at similar levels. Albeit previous studies have reported that both *ALDH1A1* and *ALDH1A3* are expressed in MSCs (2,3), our results, for the first time, showed that *ALDH1A1* expression is related to different sources of MSCs while *ALDH1A3* expression is not.

Interestingly, *ALDH1A1* expression in both ASCs and UCSCs is much lower than in human ALDH$^+$ breast CSCs. Different expressions in MSCs and CSCs might be due to lower pluripotency of MSCs compared to CSCs which is caused by uncontrolled proliferation capability acquired by CSCs after repeated gene mutations. Nevertheless, *ALDH1A1* contributes in a similar way to the pluripotent of both MSCs and CSCs, through Wnt pathway.(21-23)

Similarly, *Oct-4* mRNA was expressed greater in CSCs than MSCs. This finding emphasized similar trend of *ALDH1A1* and *Oct-4* gene expressions, which is best expressed by positive correlation between both genes. Hence, since this research studied the regulation of mRNA synthesis of each gene, this parallel findings between *ALDH1A1* and *Oct-4* mRNA expression propose the possibility these genes could be expressed due to similar regulatory factors as the stimulation.

In contrast to *ALDH1A1* and *Oct-4*, our study emphasizes that *ALDH1A3* is found in a higher level in MSCs than in CSCs. This suggests that *ALDH1A3* plays a more significant role in MSCs than in CSCs and has little or no contribution to the pluripotency of stem cells and CSCs. Purnamawati, *et al.*, found that the expression of *ALDH1A3* is consistent with TGF–β1 which plays a role in inhibiting cell proliferation, cell motility, invasion, and metastasis.(24) This finding is also supported by previous study by Crocker, *et al.*, which stated that unlike *ALDH1A1*, *ALDH1A3* is found to have no effect on the proliferation of human breast CSCs.(25) Our result obtained from *in silico* analysis confirms that in contrast to *ALDH1A1*, *ALDH1A3* was found not to interact directly to the pluripotency markers, *Oct-4*, *SOX2*, *NANOG*, and *KLF4*. Further studies are required to elaborate the specific role of *ALDH1A3* in MSCs.

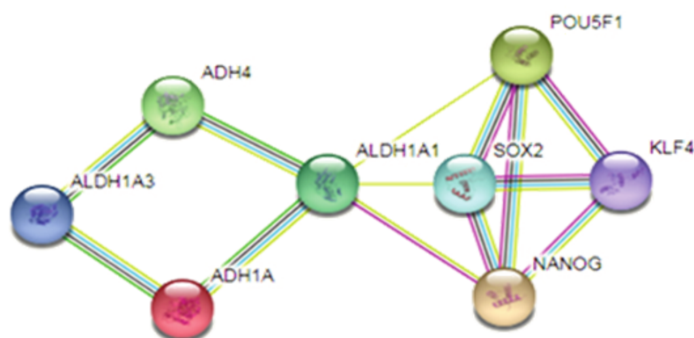

Figure 4. Analysis of ALDH interaction with pluripotency genes from STRING database. *In silico* study was conducted using String 9.1 Software to analyze the protein-protein interactions between ALDH1 and other pluripotency markers. Interactions between each protein was analyzed from several categories including from known interactions (curated databases and experimentally determined), predicted interactions (gene neighborhood, gene fusions, and gene co-occurrence), and others such as textmining, co-expression and protein homology.

From these findings, it can be inferred that ASCs and UCSCs express different levels of *ALDH1A1* and *ALDH1A3*. This implies on the application of MSCs, such as in the use of ASCs in fat grafting of breast reconstruction after breast cancer surgery (26) and UCSCs in tissue engineering.(5,27) Different usage of stem cells in clinical settings might be due to various differentiation capacities that they possess. ASCs show excellent differentiation and clonal ability to adipose tissue.(28,29) Meanwhile, UCSCs present outstanding capability in differentiating to cardiomyocyte, fibrocartilage tissue, or even collagen, making it suitable to be used in tissue engineering.(9,12)

Nevertheless, several reports have shown the possibility of cancer recurrence post-ASCs fat grafting.(30,31) Mazur has reported the cancer recurrence in 3.7% patients treated with ASC-enriched fat. Although it is not differ significantly to the control group (4.13%), the recurrence might be caused by the pro-angiogenesis and adipogenesis capabilities from ASCs, which support the survival of neighboring malignant cells.(32,33) Knowing these facts, it is prudent to consider other type of MSCs to be used in fat grafting of breast reconstruction after breast cancer surgery. However, little studies have been performed to study the benefits of UCSCs in fat grafting. This condition might be due to the difficulty in provision of appropriate MSCs number, which is not usually adequate to be used in major clinical disorders.(34)

This study measures the mRNA expression to investigate the synthesis of the respective genes. Thus, to further ensure the pluripotency and specific roles of each gene in stem cells, it is recommended to conduct further research to investigate more on the role of the proteins of these genes in ASCs and UCSCs.

Conclusion

Taken together, we conclude that *ALDH1A1* and *ALDH1A3* were distinctly expressed in UCSCs and ASCs which might be associated with unique properties of the aforementioned stem cells. The distinct properties of ASCs and UCSCs are important to be taken into account in the application of both of the MSCs, particularly in stem cell therapy.

Acknowledgement

This study was funded by Ministry of Research, Technology and Higher Education Indonesia (Hibah PITTA 2018). Special thanks are given to dr. Karina F. Moegni, SpBP-RE (HayandraLab), Dr. dr. Reza Y. Purwoko, SpKK, and Stem Cell Medical Technology Integrated Service Unit (FKUI RSCM) for their contributions in providing the samples for the stem cells.

References

1. Duan J, Cai J, Guo Y, Bian X, Yu S. ALDH1A3, a metabolic target for cancer diagnosis and therapy. Int J Cancer. 2016; 139: 965-75.
2. Yassin F. Aldehyde dehyderogenase (ALDH1A1) delineating the normal and cancer stem cells in spectral lung lesions: an immunohistochemical appraisal. Pathol Res Pract. 2016; 212: 398-409.
3. Tomita H, Tanaka K, Tanaka T, Hara A. Aldehyde dehydrogenase 1A1 in stem cells and cancer. Oncotarget. 2016; 7: 11018-32.
4. Ma I, Allan AL. The role of human aldehyde dehydrogenase in normal and cancer stem cells. Stem Cell Rev and Rep. 2011; 7: 292-306.
5. Weiss ML, Troyer DL. Stem cells in the umbilical cord. Stem Cell Rev. 2006; 2: 155-62.

6. Rusu E, Necula LG, Neagu AI, Alecu M, Stan C, Albulescu R. Current status of stem cell therapy: opportunities and limitations. Turk J Biol. 2016; 40: 955-67.
7. Bunnell BA, Flaat M, Gagliardi C, Patel B, Ripoll C. Adipose-derived stem cells: isolation, expansion and differentiation. Methods. 2008; 45: 115-20.
8. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause DS, *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8: 315-7.
9. Nagamura-Inoue T, He H. Umbilical cord-derived mesenchymal stem cells: their advantages and potential clinical utility. World J Stem Cells. 2014; 6: 195-202.
10. Frese L, Dijkman E, Hoerstrup SP. Adipose tissue-derived stem cells in regenerative medicine. Transfus Med Hemother. 2016; 43: 268-74.
11. Wanandi SI, Yustisia I, Neolaka GMG, Jusman SWA. Impact of extracellular alkalinization on the survival of human CD24-/CD44+ breast cancer stem cells associated with cellular metabolic shifts. Brazilian J Med Biol Res. 2017; 50: e6538. doi: 10.1590/1414-431X20176538.
12. Arutyunyan I, Elchaninov A, Makarov A, Fatkhudinov T. Umbilical cord as prospective source for mesenchymal stem cell-based therapy. Stem Cells International. 2016; 2016: 6901286. doi: 10.1155/2016/6901286.
13. Witkowska-Zimny M, Walenko K. Stem cells from adipose tissue. Cell Mol Biol Lett. 2011; 16: 236-57.
14. Hu L, Hu J, Zhao J, Liu J, Ouyang W, Yang C, *et al.* Side-by-side comparison of the biological characteristics of human umbilical cord and adipose tissue-derived mesenchymal stem cells. Int J Mol Med. 2013; 37: 115-25.
15. Minteer D, Marra KG, Rubin JP. Adipose-derived mesenchymal stem cells: biology and potential applications. Adv Biochem Eng Biotechnol. 2013; 129: 59-71.
16. Hosea R, Hardiany NS, Ohneda O, Wanandi SI. Glucosamine decreases the stemness of human ALDH+ breast cancer stem cells by inactivating STAT3. Oncology Letters. 2018; 16: 4737-44.
17. Purnamawati, Pawitan JA, Rachman A, Liem IK, Wanandi SI. Secretomes of adipose and umbilical cord-derived stem cells affect ALDH1A1 expression in breast cancer stem cells. Adv Sci Lett. 2017; 23: 6701-4.
18. Kiefer FW, Vernochet C, O'Brien P, Spoerl S, Brown JD, Nallamshetty S, *et al.* Retinaldehyde dehydrogenase 1 regulates a thermogenic program in white adipose tissue. Nat Med. 2012; 18: 918-25.
19. Itoh H, Nishikawa S, Haraguchi T, Arikawa Y, Hiyama M, Eto S, *et al.* Aldehyde dehydrogenase activity identifies a subpopulation of canine-adipose-derived stem cells with higher differentiation potential. J Vet Med Sci. 2017; 79: 1540-4.
20. Choudehery MS, Badowski M, Muise A, Harris DT. Comparison of human mesenchymal stem cells derived from adipose and cord tissue. Cytotherapy. 2013; 15: 330-43.
21. Cojoc M, Peitzsch C, Kurth I, Trautmann F, Kunz-Schughart LA, Telegeev GD, *et al.* Aldehyde dehydrogenase is regulated by β-catenin/TCF and promotes radioresistance in prostate cancer progenitor cells. Cancer Res. 2015; 75:1482-94.
22. Merrill BJ. Wnt pathway regulation of embryonic stem cell self-renewal. Cold Spring Harb Perspect Biol. 2012; 4: a007971. doi: 10.1101/cshperspect.a007971.
23. Ring A, Kim YM, Kahn M. Wnt/catenin signaling in adult stem cell physiology and disease. Stem Cell Rev. 2014; 10: 512-25.
24. Purnamawati, Pawitan JA, Rachman A, Wanandi SI. Effects of umbilical cord- and adipose-derived stem cell secretomes on ALDH1A3 expression and autocrine TGF-β1 signaling in human breast cancer stem cells. F1000Res. 2018; 7: 249. doi: 10.12688/f1000research.13609.1
25. Croker AK, Rodriguez-Torres M, Xia Y, Pardhan S, Leong HS, Lewis JD, *et al.* Differential functional roles of ALDH1A1 and ALDH1A3 in mediating metastatic behavior and therapy resistance of human breast cancer cells. Int J Mol Sci. 2017; 18: pii: E2039. doi: 10.3390/ijms18102039.
26. Gardani M, Bertozzi N, Grieco MP, Pesce M, Simonacci F, Santi P, *et al.* Breast reconstruction with anatomical implants: A review of indications and techniques based on current literature. Ann Med Surg. 2017; 21: 96-104.
27. Maslova O, Novak M, Kruzliak P. Umbilical cord tissue-derived cells as therapeutic agents. Stem Cells Int. 2015; 2015: 150609. doi: 10.1155/2015/150609.
28. Choudhery MS, Badowski M, Muise A, Harris DT. Comparison of human mesenchymal stem cells derived from adipose and cord tissue. Cytotherapy. 2013; 15: 330-43.
29. Chi Y, Han Z, Xu F, Wang Y, Feng X, Chen F, *et al.* Adipogenic potentials of mesenchymal stem cells from human bone marrow, umbilical cord and adipose tissue are different. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2014; 22: 588-94.
30. Brett EA, Aitzetmuller MM, Sauter MA, Machens HG, Duscher D. Breast cancer recurrence after reconstruction: know thine enemy. Oncotarget. 2018; 9: 27895-906.
31. Mazur S, Zolocinska A, Siennicka K, Janik-Kosacka K, Chrapusta A, Pojda Z. Safety of adipose-derived cell (stromal vascular fraction – SVF) augmentation for surgical breast reconstruction in cancer patients. Adv Clin Exp Med. 2018; 2018: 70798. doi: 10.17219/acem/70798.
32. Orecchioni S, Gregato G, Martin-Padura I, Reggioani F, Braidotti P, Mancuso P, *et al.* Complementary populations of human adipose CD34+ progenitor cells promote growth, angiogenesis, and metastasis of breast cancer. Cancer Res. 2013; 73: 5880-91.
33. Martin-Padura I, Gregato G, Marighetti P, Mancuso P, Calleri A, Corsini C, *et al.* The white adipose tissue used in lipotransfer procedures is a rich reservoir of CD34+ progenitors able to promote cancer progression. Cancer Res. 2012; 72: 325-34.
34. Nowacki M, Kloskowski T, Pietkun K, Zegarski M, Pokrywczyńska M, Habib SL, *et al.* The use of stem cells in aesthetic dermatology and plastic surgery procedures. A compact review of experimental and clinical applications. Adv Dermatol Allergol. 2017; 34: 526-34.

Analysis_of_ALDH1A1_and_ALDH1A3_gene_mRNA_expressi

ORIGINALITY REPORT

16%	15%	13%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

#	Source	Type	%
1	Submitted to iGroup	Student Paper	3%
2	f1000research.com	Internet Source	2%
3	Stem Cells in Aesthetic Procedures, 2014.	Publication	1%
4	id.123dok.com	Internet Source	1%
5	www.scribd.com	Internet Source	1%
6	hdl.handle.net	Internet Source	1%
7	libmast.utm.my	Internet Source	1%
8	indonesia.digitaljournals.org	Internet Source	1%
9	www.mjms.usm.my	Internet Source	1%

10	journals.sagepub.com Internet Source	1 %
11	Erizal Sugiono, Andi Wijaya, Anwar Santoso, Ferry Sandra, Ilham Jaya Patellongi, Irawan Yusuf. "Frequencies of CYP1A2 Single Nucleotide Polymorphism in Indonesian and Its Effect on Blood Pressure", The Indonesian Biomedical Journal, 2018 Publication	1 %
12	www.mdpi.com Internet Source	1 %
13	www.oncotarget.com Internet Source	1 %
14	www.pnas.org Internet Source	1 %
15	Hiroyuki Tomita, Kaori Tanaka, Takuji Tanaka, Akira Hara. "Aldehyde dehydrogenase 1A1 in stem cells and cancer", Oncotarget, 2016 Publication	1 %
16	www.spandidos-publications.com Internet Source	<1 %
17	Cerecedo, Doris, Ivette Martínez-Vieyra, Deneb Maldonado-García, Enrique Hernández-González, and Steve J. Winder. "Association of Membrane/Lipid Rafts With the Platelet Cytoskeleton and the Caveolin PY14:	<1 %

Participation in the Adhesion Process : ROLE OF RAFTS IN ADHERED PLATELETS", Journal of Cellular Biochemistry, 2015.
Publication

18	www.jlr.org Internet Source	<1%

Exclude quotes	On	Exclude matches	< 15 words
Exclude bibliography	On		