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Original Article

Characterization of Adipose-derived Stem Cells from Breast Cancer Cell Transplanted Mice

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Abstract: Mesenchymal stem cells (MCSs) from adipose tissue are reported to have a pronounced impact on tumor growth or suppression. However, how cancer cells affect the stem cells *in vivo*, conditions has not been clarified. In this study, we evaluated the impact of breast cancer cells on the characteristics of adipose-derived stem cells (ADSCs) under *in vivo* conditions. ADSCs were obtained from Balb/c mice three consecutive weeks after transplantation with 1×10^6 MCF7 cells. The results showed that the amount of ADSCs per one gram of fat reduced over time, but that reduction was not significantly different. In fact, isolated ADSCs presenting specific MSC surface markers increased at the second passage, compared to the time of isolation. Particularly, they were highly positive for CD90 (a MSC indicator) and negative for CD45 (a hematopoietic marker). Moreover, in *in vitro* culture, ADSCs were successfully differentiated into adipocytes, detected by Oil Red O staining. This present experiment gives initial assessments of ADSC characteristics; however, further investigations are necessary to fully evaluate the differences in stem cell potency and immunophenotype caused by cancer cell transplantation.

Keywords: Adipose Stem cell, Breast cancer, transplantation, potential, mouse.

1. Introduction

Adipose tissue is identified as the most abundant source of MSC. MSCs from adipose tissue have a strong, stable ability to proud and maintain a higher differentiation ability than MSCs from bone marrow [1]. Specifically, according to Yamamoto et al., [2], one gram of adipose tissue produces about 5000 MSCs, while bone marrow-derived MSC production is 100 to 1000 cells per milliliter of the marrow. In mesotherapy, stem cells in adipose tissue have been separated using an enzyme method; in particular, adipose tissue is digested with collagenase to release MSCs [3]. It has been widely recognized that multipotent MSCs, with

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their renewable and differentiation abilities, such as angiogenetic, anti-apoptotic, and proliferative capability, inside the transferred fat, mainly contribute to the quality of recovery and regeneration of autologous fat transplantation [4].

In 2020, breast cancer was ranked the most common cancer in women and the fifth deadliest cancer worldwide, with 685,000 deaths reported [5]. The incidence of breast cancer in the world, in general, and Vietnam, in particular, has increased in recent years. Recently, stem cell therapy has been evaluated as one of the most hopeful solutions for breast cancer patients. Stem cell transplantation is assessed to not only help during the therapeutic phase but also possible to regenerate breast tissue for cosmetic purposes. To have a suitable source of stem cells for transplantation, the isolation of stem cells on the patient is considered the most optimal resolution in this research direction. However, the potential of adipose stem cells from patients with breast cancer remains unclear. Indeed, some studies suggest that MSCs can stimulate the growth, progression, and metastasis of the mass for breast cancer [4, 6] or increase the malignant properties of cancer cells for cervical cancer [6].

In this study, we used a Balb/c mouse injection model that was injected directly with the MCF7 breast cancer cell into the mammary fat cushion to characterize ADSCs under the influence of cancer cells. Mouse fat stem cells are isolated, cultured, and evaluated for several properties and differentiation potential at different periods.

2. Material and Methods

2.1. Materials

MCF7 cells from ATCC are cultured in low glucose DMEM environments, 10% FBS (Merk, Germany), 37 °C, and 5% CO_2. Before being transplanted into mice, they are assessed as non-bacterial, normal growth.

Balb/c mice 6-8 weeks old, mass 20-25 gr, are raised by the biological laboratory, Institute of Biotechnology. The animal experiments described in this study were approved by the Scientific Committee of the Institute of Biotechnology (VAST), and All animals were maintained and treated following animal experiment guidelines of the Institute for Laboratory Animal Research (USA) [7].

2.2. Methods

2.2.1. Cell Transplantation into Mice

Mice were weighed, stabilized, and then sterilized in the abdominal area with 70% ethanol. For the experiment, 1×10^6 MCF7 cells/100 µl in PBS were subcutaneously injected into the second breast, starting from the tail.

The control mice were injected with 100 µl PBS.

2.2.2. Fat Collection and Cell Isolation

We isolated and collected multipotent cells (ADSCs) from the adipose tissue-derived stromal vascular fraction cultured cells (ADSVF cells) according to the protocol of Yamamoto et al., [2].

Mice were operated on weeks 1, 2, and 3 to collect fat and isolate ADSCs. Each session was performed on one control (PBS buffer injection only, raised simultaneously) and three breast cancer cell-injected mice. Mice were killed by breaking the cervical. Next, they were whole-body asepticized by ethanol 70% and fixed on the operating table. Using scissors to cut the abdominal skin, subcutaneous fat was removed immediately and put into PBS 1X. Fat tissues were then washed with a wash environment (low glucose DMEM (Gibco, USA), contains 1% antibiotics (Sigma, USA)) about 3-5 times before being divided into small pieces of tissue around 1-2 mm^2 and incubated in collagenase (Gibco, USA) at 37 °C for about 15-40 minutes and monitored until the tissue fragments were dissipated. After that, the suspension was centrifuged at 3000 rpm for 10 min and then rinsed again with the washing environment. The process was repeated 3-5 times to collect the pellet cells in the aqueous phase. Half of the isolated cells were read by a flow machine, and the other half were fed in a culture medium of low-glucose DMEM (Sigma, USA), 10% FBS (Sigma, USA), and 1% antibiotic at 37 °C, 5% CO_2.

Cultured cells were observed and evaluated on the time that cells were attached to the plastic disk and the cell growth rate. After about 2-3 days, the culture medium was replaced, or if the cell density reached 80-100% confluence, cells were transferred to new plates.

2.2.3. Cell Proliferation

When the cell density covered about 70-80% of the surface of the disk, cells were subcultured by following steps: the old medium was removed; the cells were washed with PBS; treated and incubated with Trypsin/EDTA 0.25%/ 0.02% in PBS (PAN Biotech, England) at 37 ^{o}C, 5% CO_2 in 5 minutes; after that, the same volume of trypsin of low-glucose DMEM solution was added to inactivate trypsin; the cells were transferred to the centrifugal tube and centrifuged at 3000 rpm in 3 min; cell pellets were collected and resuspended by 1000 µl of the culture medium; the cells were seeded into wells and put them into the incubator. The cell growth was checked regularly to evaluate the cell morphology and density.

2.2.4. Cell Counting

The number of cells was determined by the Neubauer counting chamber. After cells were treated with Trypsin EDTA 0.25%, the suspension of cells was mixed gently with trypan blue 0.4% in a ratio of 1:1. 10 µl of the mix was loaded into the counting chamber and counted.

2.2.5. Flow Cytometry

The identity of mouse ADSCs was verified by surface markers (CD - Cluster of differentiation) using fluorescent activation cell classification (Flow Cytometer, model NovoCyte Acea Agilent). The procedure was followed step by step in the product's manual (Miltannyl, USA). First of all, cells were mixed with 1:50 (v/v) in antibodies solution and then incubated with primary antibodies against the following cell surface markers: CD29 (FITC), CD44 (PE Clone), CD90 (APC), CD45 (PerCP). After that, the mixture was incubated in the dark for 10 min at 2-8 °C. Then, cells were washed twice with PBS buffer before analysis.

2.2.6. Induction of Adipogenesis

We set up two experimental groups, with or without adipogenic induction, with three replications in each group. Cells were seeded in 4-well plates at a seeding density of $1x10^4$ cells/well. Cells were cultured in a medium supplemented with 10 µg/L insulin, 0.5 µM IBMX, and 1 µM Dexamethasone (all from Sigma) to induce adipogenic differentiation. In contrast, the medium was replaced with low glucose DMEM in the controls. The differentiation medium was changed every 3 days. After 3 weeks, cells were stained with Oil Red O staining (Bio Basic Canada Inc.).

2.2.7. Data Analysis

Data in Table 1 and Table 2 presented the mean ± SEM of the weight of isolated fat (gr), the number of cultured cells at passage 0, and cell density (cell/gr). Differences between groups were used Student's t-test for statistical significance, in which $p < 0.05$ was chosen to indicate the significant difference.

3. Results and Discussion

3.1. Cell Density from the Isolated Fat Tissue

The weight of fat tissue and the number of cultured cells are used to calculate the cell density in fat tissue, shown in Table 1. During three weeks, the measurements of control mice changed insignificantly; hence we collated their data in one group, as demonstrated in Table 1. Although the mean of weight fat is similar in the four groups, the highest cell number per gram of fat tissue is week 1 group ($2.11\pm1.56\times10^6$ cell/gr), and the lowest is week 2 group ($1.20\pm1.26\times10^6$ cell/gr). The density of cells in fat tissue was not significantly different.

The number of stromal cells per gram and their differentiation capacity depended on many factors. In humans, the body mass index (BMI) was identified as having a relationship with the density of stem cells in fat tissue [9]. In this present, the number of cells per fat gram was similar of them can be isolated from human subcutaneous liposuction aspirates, which were approximately $0.5\text{-}2.0 \times 10^6$ cells per gram of adipose tissue [10]. In Swiss mice, the number of cells per gram was from $3{,}93 \pm 0{,}38\times10^5$

cells/gr to 4,77 ± 0,31×10^5 cells/gr [11], it was the same as reported by Luna et al., [12], an amount of 4.2 ± 1.1×10^5 cells/gr.

Table 1. The density of cells collected in three weeks

Groups	Weight of fat (gr)	Cells obtained (x10^6)	Cell density (x10^6 cells/gr)
Control	1.44±0.65[a]	2.37±0.86[b]	1.86±0.92[c]
Week 1	1.77±0.89[a]	2.76±1.19[b]	2.11±1.56[c]
Week 2	1.58±1.05[a]	1.83±1.04[b]	1.20±1.26[c]
Week 3	1.83±0.81[a]	2.07±0.82[b]	1.26±0.45[c]

[a,b,c] superscripts within a column indicate that there was no significant difference (P>0.05).

3.2. Morphology and Growth Rate of ADSCs

The morphology of ADSCs after isolation from fat tissue injected with cancer cells is shown in Figure 1. As observed, cells start attaching to the well surface on the first day of the culture process (Figure 1 A). On day 2, the adhesive spindle-shaped cells appear (Figure 1 B). On day 6, the spindle-shaped cells reached about 60-80% confluence (Figure 1 C).

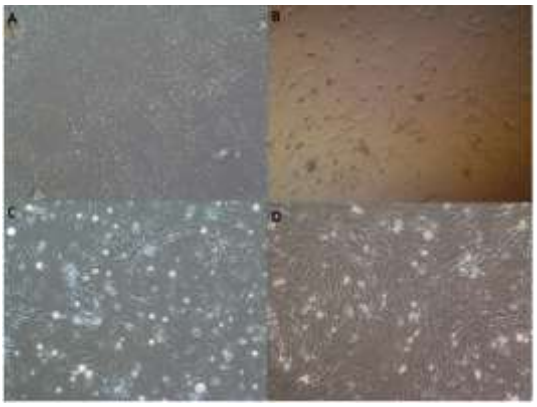

Figure 1. Morphological observation of isolated ADSCs during culture. (A) the cells after 12 h seeding, (B) the cells after 2-day seeding, (C) the cells on day 6 after seeding, (D) the cell on day 3 of passage 2 (x10 objective).

Three days after passage 2 (Figure 1 D), the cells begin to thrive, spread rapidly, cover the disk's surface and be more homogeneous toward fibroblast cell shape (rhombus-shaped, oblong, and pointed on both ends, with a large circular nucleus). Cell adhesion time and growth rate are similar to a report of Thi-Phuong and Huu-Hung [13]. They successfully isolated and cultured MSCs from adipose tissue by a non-enzymatic method. At the time of digestion, the morphology and size of the cells are not uniform; the ratio of large/small cell size can be varied depending on factors such as sex and age of the mouse [12].

3.3. Immunophenotypical Profile of Isolated ADSCs

The characteristics of cells after isolation are shown in Table 2, Figure 2. At the isolation, the percentage of positive cells for the CD45 marker (a blood stem cell specific marker) in the control group was lower than that in the experiment group (10.79% and 66.94%, respectively). The rate of cells positive of specific markers for MSCs (CD29, CD44, and CD90) showed no significant differentiation in the control and experiment groups. In the second week, the percentage of CD45 and CD90 in the experiment group was 66.94% and 26.88%, respectively.

Table 2. Flow cytometric analysis of the isolated cell from fat tissue

Parameters	Control group (%)	Two-week experiment group (%)
CD45+ (%)	10.79±8.69[a]	66.94±5.52[b]
CD44+ (%)	9.55±7.03[b]	6.51±5.62[b]
CD90+ (%)	6.98±4.61 [c]	26.88±1.85[c]
CD29+ (%)	13.41±0.61[d]	7.33±2.95[d]

[a,b,c,d] superscripts within a row indicate that there was no significant difference (P>0.05)(n=3).

In the experimental mice of Luna et al., [12], the cell percentage that was positive for the CD45 indicator ranged from 5.6 ± 0.9% to 24.5 ± 2.8%, and the percentage positive for CD90 ranged from 51.5 ± 4.2% to 75.8 ± 3.7% depending on the group, and there was no change in culture.

In this study, the expression of specific MSCs markers was lower than that in other

reports. According to Taha and Hedayati [14], cells showed 99% positive for CD29 and 98% for CD44, the cells do not respond to CD31, and only 1% of the cells are CD11b and CD45 positive. Likewise, Maddox et al., showed that the expression level of CD44 was 98%; meanwhile, CD90 was 2% at passage 3 [15].

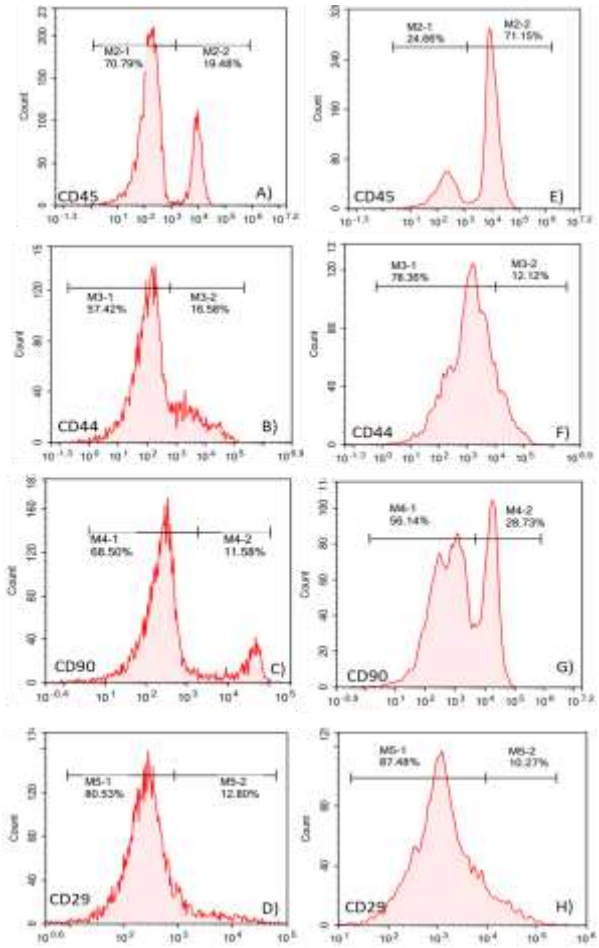

Figure 2. Expression of selected surface markers of the sorted cell after isolation. The A, B, C, D in the control group and the E, E, G, and H in the two-week experiment group.

The growth of ADSCs in the control groups and after one week is shown in Figure 3. In the control group, cells rapidly increased in number from day 2 to day 5, then tended to stop. Meanwhile, cells in the experimental group increased in number like the control group but slower. As a result, cells from the experimental group proliferated more slowly than the cells derived from the control group.

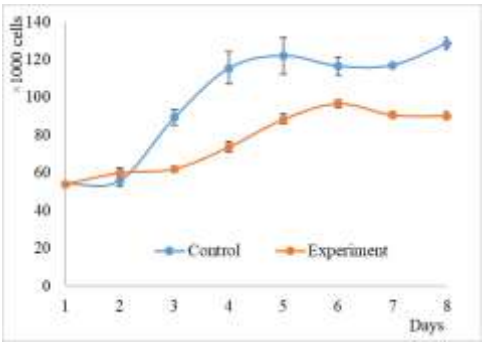

Figure 3. The total cell number was recorded every day for a total of 7 days. Values are expressed the mean ± SEM (n=3 in each group).

3.4. Cell Differentiation Potential

On day 21 of exposure to the differentiation medium, as shown in Figure 4 A, adipocyte-like cells appear with lipid-rich vacuoles in MCF7-injected mouse samples, which are stained red with Oil red O (Figure 4 A). In contrast, the control cells retain their morphology and have no lipid accumulation in their cytoplasm (Figure 4 B). Our results are in concordance with a publication of Thuy et al., [11] and others [13].

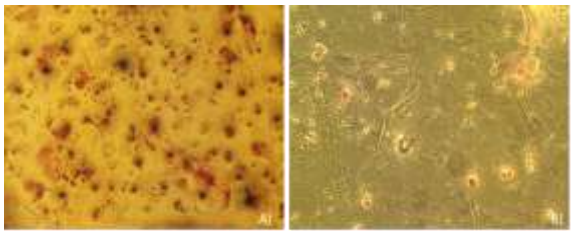

Figure 4. Adipogenic differentiation of mouse ADSCs stained with Oil red O (A) Mouse ADSCs induced in differentiation medium after 21 days, (B) Mouse ADSCs at passage 2 after 21 days in culture medium refreshing (negative control) (20x objective).

4. Conclusion

The cell density in adipoose tissue witnessed no differentiation between transplanted mice and control mice. The isolated cells from fat tissue injected with cancer cells exhibited signature

morphology of adipose-derived stem cells after the second passage and showed potent differentiation into adipose cells. However, to thoroughly evaluate the characteristics and the potency as well as specific surface marker expression of ADSCs in response to cancer cell transplantation, further experiments need to be conducted.

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References

[1] C. Y. Li, X. Y. Wu, J. B. Tong, X. X. Yang, J. L. Zhao, Q. F. Zheng, G. B. Zhao, Z. J Ma, Comparative Analysis of Human Mesenchymal Stem Cells from Bone Marrow and Adipose Tissue Under Xeno-Free Conditions for Cell Therapy, Stem Cell Research and Therapy, Vol. 6, No. 1, 2015, pp. 55, https://doi.org/10.1186/s13287-015-0066-5.

[2] N. Yamamoto, H. Akamatsu, S. Hasegawa, T. Yamada, S. Nakata, M. Ohkuma, E. Miyachi, T. Marunouchi, K. Matsunaga, Isolation of Multipotent Stem Cells from Mouse Adipose Tissue, Journal of Dermatological Science, Vol. 48, No. 1, 2007, pp. 43-52, https://doi.org/10.1016/j.jdermsci. 2007. 05.015.

[3] N. V. Andreeva, A. Dalina, A. V. Belyavsky, Isolation and Expansion of Mesenchymal Stem Cells from Murine Adipose Tissue, Bio-Protocol, Vol. 7, No. 16, 2017, pp. e2516, https://doi.org/10.21769/ BioProtoc.2516.

[4] Y. Chen, Y. He, X. Wang, F. Lu, J. Gao, Adipose-Derived Mesenchymal Stem Cells Exhibit Tumor Tropism and Promote Tumorsphere Formation of Breast Cancer Cells, Oncology Reports, Vol. 41, 2019, pp. 2126-2136, https://doi.org/doi: 10.3892/or.2019.7018.

[5] WHO, Breast Cancer, https://www.who.int/news-room/fact-sheets/detail /breast-cancer.

[6] F. Rey, E. Lesma, D. Massihnia, E. Ciusani, S. Nava, C. Vasco, G. A. Haj, G. Ghilardi, E. Opocher, A. Gorio, S. Carelli, A. M. D. Giulio, Adipose-Derived Stem Cells from Fat Tissue of Breast Cancer Microenvironment Present Altered Adipogenic Differentiation Capabilities, Hindawi Stem Cells International, Vol. 2019, pp. 15, https://doi.org/10.1155/2019/ 1480314.

[7] Committee for the Update of the Guide for the Care and Use of Laboratory Animals, National Research Council Guide for the Care and Use of Laboratory Animals, Eighth Edition, National Academies Press, USA, 2010.

[8] R. C. Oropeza, K. V. Santillan, C. D. Gastelum, J. M. Zajgla, C. Zampedri, E. F. Osorio, A. C. Oropeza, R. K. V. Santillan, C. D. Gastelum, J. M. Zajgla, C. Zampedri, E. F. Osorio, A. R. González, L. A. Pizano, V. Maldonado, Adipose-Derived Mesenchymal Stem Cells Promote the Malignant Phenotype of Cervical Cancer, Science Report, Vol. 10, 2020, pp. 14205, https://doi.org/10.1038/ s41598-020-69907-x.

[9] J. Zhang, Y. Liu, W. Yin, X. Hu, Adipose-derived Stromal Cells in Regulation of Hematopoiesis, Cellular & Molecular Biology Letters, Vol. 25, 2020, pp. 16, https://doi.org/10.1186/s11658-020-00209-w.

[10] P. C. Baer, H. Geiger, Adipose-Derived Mesenchymal Stromal/stem Cells: Tissue Localization, Characterization, and Heterogeneity, Stem Cells International, Vol. 2012, 2012, pp. 812693, https://doi.org/10.1155/2012/812693.

[11] N. T. Thuy, T. N. Vu, C. T. C. Ha, Isolation and Culture of Adipose - Derived Mesenchymal Stem Cells from Mice (Swiss) Adipose Tissue, Can Tho Journal of Medicine and Pharmacy, Vol. 6, No. 2, 2020, pp. 372-379 (in Vietnamese).

[12] A. C. Luna, M. E. Madeira, T. O. Conceição, J. A. Moreira, R. A. Laiso, D. A. Maria, Characterization of Adipose-derived Stem Cells of Anatomical Region from Mice, BMC Research Notes, Vol. 7, 2014, pp. 552, https://doi.org/10.1186/1756-0500-7-552.

[13] N. T. Phuong, N. H. Hung, A Simple Non-Enzymatic Protocol for Isolation and Culture of Mesenchymal Stem Cells from Adipose Tissues, Journal of Science and Technology, Vol. 2, 2018, pp. 12-16.

[14] M. F. Taha, V. Hedayati, Isolation, Identification and Multipotential Differentiation of Mouse Sdipose Tissue-derived Stem Cells, Tissue Cell, Vol. 42, No. 4, 2010, pp. 211-216, https://doi.org/10.1016/j.tice. 2010.04.003.

[15] J. R. Maddox, X. Liao, F. Li, C. Niyibizi, Effects of Culturing on the Stability of the Putative Murine Adipose Derived Stem Cells Markers, The Open Stem Cell Journal, Vol. 1, 2009, pp. 54-61, https://doi.org/10.2174/1876893800901010054.