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

Tran Thi Huong Giang¹,², Nguyen Van Anh¹, Nguyen Thi Hiep¹, Nguyen Thi Nhung¹, Bui Van Ngoc¹, Nguyen Van Hanh¹,²,*

¹Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
²Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

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Abstract: Mesenchymal stem cells (MSCs) from adipose tissue are reported to have a pronounced impact on tumor growth or suppression. However, so far, how the 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 in three consecutive weeks after transplantation with $1 \times 10^6$ MCF7 cells. The results showed that the amount of ADSCs per one gram 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, which were 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...

¹ Tác giả liên hệ.
Địa chỉ email: nvhanh@ibt.ac.vn
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collagenase to release MSCs [3]. It has been widely recognized that multipotent MSCs with their renewable and differentiation abilities, such as angiogenic, 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 tended to increase 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 is 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 direction of research. However, the potential of adipose stem cells from patients who have had breast cancer have so far 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, to characterize ADSCs under the influence of cancer cells, we used a Balb/c mouse injection model that was injected directly with the MCF7 breast cancer cell into the mammary fat cushion. 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, 5% CO2. 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 in accordance with animal experiment guidelines of Institute for Laboratory Animal Research (USA) [7].

2.2. Methods

2.2.1. Cell Transplantation into Mice

Mice were weighed, sterilized, and then stabilized in the abdominal area with 70% ethanol. For the experiment, 1x10⁶ MCF7 cells/100 µl in PBS were subcutaneously injected into the second breast, starting from the tail.

For the control mice, they 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 at week 1, 2, and 3 to collect fat and isolate ADSCs. Each session was performed on one control (PBS buffer injection only, raised at the same time) 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 were 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 mm² 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 in 10 min and then rinse again with the washing environment. Repeating the process 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% CO₂.

Cultured cells were observed and evaluated on the time that cells were attached to the plastic disk and on the rate of cell growth. 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 °C, 5% CO₂ in 5 minutes; after that, the same volume of trypsin of low-glucose DMEM solution was added to inactivate trypsin; the cells was transferred to the centrifugal tube and centrifuged at 3000 rpm in 3 min; cell pellets was collected and resuspended by 1000 µl of the culture medium; the cells was 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%, suspension of cells was mixed gently with trypsin blue 0.4% in a ratio 1:1. 10 µl of the mix were loaded to 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, three replications in each group. Cells were seeded in 4-well plates at a seeding density of 1x10⁴ cells/well. To induce adipogenic differentiation, cells were cultured in a medium supplemented with 10 µg/L insulin, 0.5 µM IBMX and 1 µM Dexamethasone (all from Sigma). 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 cell at passage 0 and cell density (cell/gr). Differences between groups were used Student’s t-test for statistical significance, which p < 0.05 was chosen to indicate the significant difference.

3. Results and Discussion

3.1. Cell Density from the Isolated Fat Tissue

Weight of fat tissue and number of cultured cells are used to calculate the cell density in fat tissue, which is shown in Table 1. During three weeks, the measurements of control mice change insignificantly, hence we collated their data in one group as demonstrated in Table 1. Although the mean of weight fat is similar in four groups, the highest cell number per gram of fat tissue is week 1 group (2.11±1.56×10⁶ cell/gr) and the lowest is week 2 group (1.20±1.26×10⁶ cell/gr). The density cell 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 were similar of them can be isolated from human subcutaneous liposuction aspirates, which were approximately 0.5-2.0 × 10⁶ cells per gram of
adipose tissue [10]. In swiss mice, the number of cell per gram was from 3.93 ± 0.38×10^5 cells/gr to 4.77 ± 0.31×10^5 cells/gr [11], it was the same as reported by Luna et al., [12], amount 4.2 ± 1.1×10^4 cells/gr.

Table 1. The density of cells collected in three weeks

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight of fat (gr)</th>
<th>Cells obtained (x10^6)</th>
<th>Cell density (x10^4 cells/gr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.44±0.65\textsuperscript{a}</td>
<td>2.37±0.86\textsuperscript{b}</td>
<td>1.86±0.92\textsuperscript{c}</td>
</tr>
<tr>
<td>Week 1</td>
<td>1.77±0.89\textsuperscript{a}</td>
<td>2.76±1.19\textsuperscript{b}</td>
<td>2.11±1.56\textsuperscript{c}</td>
</tr>
<tr>
<td>Week 2</td>
<td>1.58±1.05\textsuperscript{a}</td>
<td>1.83±1.04\textsuperscript{c}</td>
<td>1.20±1.26\textsuperscript{c}</td>
</tr>
<tr>
<td>Week 3</td>
<td>1.83±0.81\textsuperscript{a}</td>
<td>2.07±0.82\textsuperscript{b}</td>
<td>1.26±0.45\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{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, on the first day of the culture process, cells start attaching to the well surface (Figure 1 A). On day 2, the adherent 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 at day 3 of the passage 2 (x10 objective).](image)

Three days after passage 2 (Figure 1 D), the cells begin to thrive, spread rapidly, cover the surface of the disk 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 succeeded in isolating and culturing MSCs from adipose tissue by a non-enzymatic method. At the time of digestion, the morphological 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 were shown in Table 2, Figure 2. At the isolation, the percentage of cells that are positive for the CD45 marker (a blood stem cell specific marker) in control group was lower than that in experiment group (10.79% and 66.94%, respectively). The rate of cells positive of specific markers for MSCs (CD29, CD44, and CD90) were no significant differentiation in the control and experiment groups. In the second week, in the experiment group, the percentage of CD45 and CD90 was 66.94% and 26.88%, respectively.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group (%)</th>
<th>Two-week experiment group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45+ (%)</td>
<td>10.79±8.69\textsuperscript{a}</td>
<td>66.94±5.52\textsuperscript{b}</td>
</tr>
<tr>
<td>CD44+ (%)</td>
<td>9.55±7.03\textsuperscript{b}</td>
<td>6.51±5.62\textsuperscript{b}</td>
</tr>
<tr>
<td>CD90+ (%)</td>
<td>6.98±4.61\textsuperscript{c}</td>
<td>26.88±1.85\textsuperscript{b}</td>
</tr>
<tr>
<td>CD29+ (%)</td>
<td>13.41±0.61\textsuperscript{d}</td>
<td>7.33±2.95\textsuperscript{d}</td>
</tr>
</tbody>
</table>

\textsuperscript{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 is 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 marker 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].

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, in the experimental group, cells also 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.

3.4. Cell Differentiation Potential

On day 21 of exposure to differentiation medium, as shown in Figure 4 A, adipocyte-like cells appear with lipid-rich vacuoles in MCF7-injected mouse sample, 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 publish of Thuy et al., [11] and others [13].

4. Conclusion

The cell density in adipose 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 cell after the second passage and showed the 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


