Oncology and Translational Medicine
DOI 10.1007/s10330-019-0380-0

April 2020, Vol. 6, No. 2, P68–P71

ORIGINAL ARTICLE

The study of selective primary culture and determination of a breast cancer cell line in vitro*

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Abstract

Objective The successful establishment of a tumor cell bank is based on the premise that the target cells can be cultured by a legitimate approach. In this experiment, we used primary culture to select and detect breast cancer cells *in vitro*, which can provide experimental ideas and methods for the establishment of a living tumor tissue cell bank.

Methods Fifty-two specimens were collected over a two-year period from people with breast cancer who needed surgical treatment in our hospital. Cells were isolated and used to establish successful cell culture. Cell activity and cell purity were measured before liquid nitrogen cryopreservation.

Results (1) At the initial culture stage, cells grew with adherence. Cell multiplication could be seen after the cell medium was exchanged three times. Cell viability was above 86%, while the viability of the target cells was above 75%, as detected by hematoxylin and eosin (HE) staining. (2) The number of breast cancer cells decreased, while the number of fibroblasts increased after five rounds of passage. (3) The success rate was 73.08%, which did not include polluted cells and those that were not successfully cryopreserved.

Conclusion (1) breast cancer cells could be selected from primary culture *in vitro* through an appropriate method. (2) Exchange of the cell medium and further cell passage improved cell multiplication. (3) The experimental results could be monitored using trypan blue and HE staining. (4) The success of breast cancer cell culture *in vitro* could be used as a reference for other cell culture, so as to establish a tumor tissue cell bank.

Received: 26 December 2019
Revised: 20 January 2020
Accepted: 1 March 2020

Key words: breast cancer; primary culture; Trypan blue staining; hematoxylin and eosin (HE) staining

The incidence and mortality of malignant tumors have been on the rise for the past few years, resulting in a research hotspot [1]. Tumor cell lines with rapid propagation, a convenient source, and propagate have received more attention. However only 30%–40% of the original genetics has been retained [2]. Therefore, a way to keep the genome stabile and retain rapid propagation will provide better help for clinical research.

Breast cancer has reduced prevalence, but poor prognosis. As a malignant tumor, it seriously threatens the physical and mental health of humans [3–4]. There are many methods for the culture of breast cancer [5]. Our laboratory used enzyme digestion to culture breast cancer cells and summarized the experimental problems to lay the foundation for our future work, to establish a human breast cancer cell line and bank.

Materials and methods

General materials

Patients with breast cancer at the Inner Mongolia People's Hospital from February 2017 to January 2019 were recruited. All patients provided signed informed consent before treatment. Patients ranged in age from

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*Supported by a grant from the Science Foundation of Inner Mongolia Autonomous Region People's Hospital (No. 2016015).

28 to 74 years, with an average age of 47.67 ± 11.78 years. All patient samples were digitally coded to ensure anonymization.

Inclusion criteria

(1) First diagnosis of breast cancer;

(2) First surgical treatment of breast cancer;

(3) Never received radiation therapy;

(4) Never received chemotherapy.

Patients had to satisfy all the above requirements to be included in the study.

Experimental reagents

Experimental reagents for cancer cell culture

(1) Tissue separation system (CHI SCIENTIFIC, USA) for breast cancer cell culture;

(2) Digestive buffer for breast cancer tissues;

(3) Tissue washing liquid for breast cancer tissues;

(4) Basic culture medium for breast cancer cell culture;

(5) Culture medium supplements;

(6) Culture serum breast cancer cell.

Experimental reagents for detection of activity

Trypan blue reagent and Dulbecco's phosphate-buffered saline (DPBS) buffer solution.

Experimental reagents for hematoxylin and eosin (HE) staining

Xylene, anhydrous alcohol, sterile water for injection, hematoxylin, eosin, 1% hydrochloric acid alcohol, and neutral gum.

Experimental apparatus

VS-840K-U super-clean worktable (Suzhou Antai Air Tech Co., LTD., China), MCO-5AC carbon dioxide incubator (SANYO, Japan), OPTEC-BDS200 inverted biological microscope (Chongqing Optec Instrument Co., LTD., China), MD192 low temperature refrigerator (SANYO, Japan), MVE XC47/11-6 liquid nitrogen jar (MVE, USA), HW.SY21-K Electro-Thermostatic Water Bath (Beijing Changfeng Instrument Co., LTD., China), AL104-IC electronic balance (Shanghai Mettler Toledo Instrument Co., LTD., China), incubator shakers (Shanghai Kuangbei Industrial Co., LTD., China), HC-2062 high speed centrifuge (Anhui Zhongke Zhongjia Scientific Instrument Co., LTD., China), a liquid handler from Dragon (100–1000 μL), and a liquid handler from Eppendorf (10/20–200 μL).

Experimental methods

Cell culture

(1) Breast cancer tissue was collected during the operation and the specimens were sent to the laboratory under sterile conditions.

(2) The muscle and adipose tissue around the breast cancer tissue were removed on the super-clean worktable. Tissue was rinsed with sterile liquid for at least ten minutes, twice.

(3) Under sterile conditions, the tissue was cut into fragments with 0.2–0.5 mm^2 diameters.

(4) Tissue was placed in a 37 °C incubator shaker with digestion solution until the tissue mass could not be observed by the naked eye.

(5) After digestion, an aliquot of cells was used for cell counting while another aliquot was used to start the cell culture.

(6) Cells were examined by regular microscopic examination during cell passage.

(7) Once cells were determined to be viable and the cell purity coefficient was determined, they were cryopreserved in liquid nitrogen.

Detection of cancer cell viability

Trypan blue (0.4%) was added to the cell suspension, and the living and dead cells were counted after 3 minutes. The living cell rate (%) was calculated as the total number of living cells/(total number of living cells + total number of dead cells) ×100%.

Purity test of cancer cells

Cells were smeared onto a microscope slide and HE staining was performed. The HE stained cells were used for pathological diagnosis and to test the purity of the cancer cells by microscopic examination.

Results

Number of cancer cells obtained

The size range of tissue samples was 0.25–1.2 cm^3, with an average of 0.68 ± 0.25 cm^3. The number of cancer cells obtained by digestion was 4.03–14.00 × 10^5 cells/mL, with an average of 8.76 ± 2.35 × 10^5 cells/mL. The digestion time was 10–24 h, with an average of 14.62 ± 1.81 h.

Cell culture results

(1) Most of the cells grew with adherence after 24 h (Fig. 1a). After 48 h, all of the cell lines grew with adherence (Fig. 1b).

(2) Cell started to rapidly propagate with the appropriate shape of cells after 9–10 days and three rounds of passage (Fig. 1c). The cells were tested for viability before cryopreservation; all cells were above 86% viability, with HE stained cells above 75% (Fig. 1d–1e).

(3) After five rounds of passage, a large number of dead tumor cells were observed and they stopped multiplying (Fig. 1f).

(4) Of the 52 patients, 38 cell lines were successfully cryopreserved with the number off cells at least 3 × 10^6 cells/mL. Of the remaining samples, eight had too low a cell population, seven cases were contaminated with

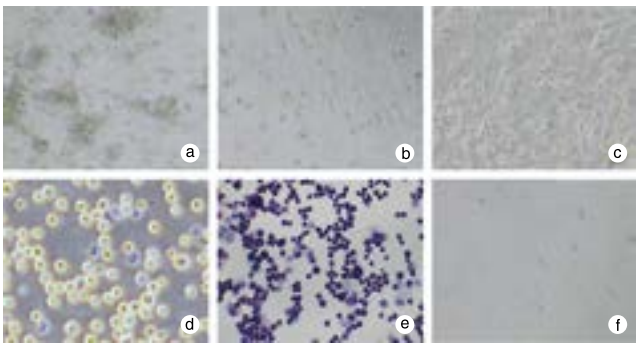

Fig. 1 Primary culture and determination. (a) Cell culture after 24 hours (×100); (b) Cell culture after 48 h (× 400); (c) Cell culture after 9 days (× 400); (d) Cell viability, (× 400, 90.30%); (e) HE consequence, (× 400,81.90%); (f) After 5 times of passages(× 400)

bacteria, one case had an fungal infection, and six were not successfully cryopreserved. The success rate was 73.08%.

Discussion

A tumor tissue bank refers to an institution that systematically collects and stores tumor tissues after surgical resection [6]. Traditionally, tumor tissues were stored under low temperature conditions with a temperature from -195°C to -80°C. However, research shows that after a long time at low temperature, the biological activity of macromolecules in tumor tissues decreases with the passage of time [7–8]. The muscle and adipose tissue which surrounds the tumor tissue also influences the obtained cells. In this experiment, tumor cells were cultured and frozen *in vitro*, which ensured a high number of tumor cells and reduced the difference between the cultured and the original cells.

Cell culture refers to a culture technique in which cells are extracted from tissues in the body, which mimics the environment in which cells grow in the body, and where cells are cultured and passaged in the proper conditions to maintain the original structure and function of the cells [9]. As a rapidly developing experimental technology in modern biological science, cell culture has been applied in various experimental studies.

The basis of successful primary cell culture is to acquire specific target cells. Understanding the growth characteristics of cancer cells guides the time to change the culture medium and when to passage cells [10–12]. Cell activity can be influenced if the cells contact experimental reagents for too long a time [13–15]. Thus, determining the correct time to combine the cells with experimental reagents seems particularly important. How to prepare frozen stock solution is as important as when to cryopreserve the cell culture [16–17].

Deficiencies in the experiment

It is necessary to explore the possible causes of contamination and then strengthen the awareness of aseptic technique after cells have been contaminated. Additionally, it is necessary to determine better culture methods to improve the growth and proliferation ability of cells to avoid the cessation of growth after the fifth cell passage.

In summary, the establishment of a tissue bank can not only provide necessary materials for follow-up experiments, but also provides certain guiding significance for the progress of clinical work. Our laboratory used enzyme digestion to culture breast cancer cells *in vitro*. We not only obtained a high enough number of tumor cells for cryopreservation, but also reduced the genetic difference between the cultured and the original cells. The success of primary culture of breast cancer cells *in vitro* provides an idea for subsequent primary culture of other cells, and at the same time, provides the basis for establishment of a living tumor tissue cell bank.

Conflicts of interest

The authors indicate no potential conflicts of interest.

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DOI 10.1007/s10330-019-0380-0
Cite this article as: Ren M, Xu HX, Lu XJ, *et al*. The study of selective primary culture and determination of a breast cancer cell line in vitro. Oncol Transl Med, 2020, 6: 68–71.