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СЛУЧАЙ ПОЛУЧЕНИЯ ПЕРВИЧНОЙ КУЛЬТУРЫ КАРЦИНОМЫ МОЛОЧНОЙ ЖЕЛЕЗЫ ПОЛОЖИТЕЛЬНОГО ЛЮМИАЛЬНОГО – В ПОДТИПА

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THE CASE OF OBTAINING THE PRIMARW CULTURE OF BREAST CANCER CELLS OF A POSITIVE LUMINAL-B SUBTYPE

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Аннотация. В статье рассмотрен случай культивирования клеток карциномы молочной железы положительного люминального – В-подтипа. Описанвариантполучениямаммосфер.

Annotaion. The article discusses the case of culturing breast carcinoma cells of a positive luminal - B subtype. An option for producing mammospheres is described.

Ключевыеслова: карциномамолочнойжелезы, маммосферы, первичнаякультура.

Keywords: carcinoma of the breast, mammospher, primary culture.

Introduction

There are various protocols for culturing breast cancer cells. Although it is a relatively simple method of study, it can be difficult to use. There are methodological and analytical aspects of the assay which require careful consideration when interpreting the results [1-3]. The articles describe the preparation of both primary epithelial cell cultures and the production of mammospheres [1-4].

Multicellular tumor spheroids are widely used in tumor research. Because of their three-dimensional organization they can simulate avascular tumor areas comprising proliferative and necrotic cells [4].

In this study, the case of obtaining epithelial cells and mammospheres according to a specific protocol will be described.

Material and methods

Tumor samples were obtained from 1 patient with a diagnosis of breast carcinoma T4N1M0 luminal-B positive subtype. The sample was collected by a surgeon in aseptic conditions and transferred into Hank's Balanced Salt Solution supplemented with gentamycin. Tissue sections were treated no later than 3–4 hours following their resection.

Mammary tissue was placed in DMEM/F-12 with 15 mM HEPES supplemented with 2% FBS and collagenase/hyaluronidase solution. This flask was placed on the rotary shaker at 37°C for 16 hours (overnight without CO2).The flasks were sealed with Parafilm®. Then, the dissociated tissue was placed to 50 mL conical tubes, and centrifuged at 80 x g for 30 seconds. Pellet 1 was obtained. This pellet was expected to be enriched with terminal ductal lobular unit (TDLU) epithelial fragments. The supernatant was transferred to a new 50 mL conical tube and centrifuged at 200 x g for 3 minutes. Pellet 2 was obtained. This pellet was expected to contain variable numbers of epithelial cells, stromal cells, and red blood cells. The supernatant from the second centrifugation shall contain human mammary fibroblasts (pellet 3). It was then transferred to a new 50 ml conical tube and centrifuged at 350 g for 5 minutes to get pellet 3.

1 - 5 mL of pre-warmed Trypsin-EDTA (0.25%) was added to these pellets obtained after dissociation with Collagenase/Hyaluronidase and it was resuspended. It was gently pipetted up and down using with a 1 mL pipettor for 1 -3 minutes and centrifuged at 350 x g for 5 minutes. Then 2 mL of pre-warmed Dispase (5 U/mL) and 200 µL of DNase I Solution (1 mg/mL) was added. The sample was pipetted for 1 minute with a 1 mL pipettor to further dissociate pellets and centrifuge at 350 x g for 5 minutes, the supernatant was discarded. The resulting pellet was diluted in the complete medium of mammoculture on the non-treated Petri dishes (pellet 1) for creating mammospheres and on the collagen coated vials to get epithelial fraction (pellet 1, 2, 3).

Results and discussion

Mammospheres were formed on day 5 and had a size of 40 µm, numbered 5 or 6 per power field. After a week, the mammosphere reached a size of 60 µm and had a brown center. According to the protocol, it was necessary to transfer them. However, it was noted that some mammospheres attach to the dish and stop moving. Two weeks later, almost all mammospheres attached to the dish, cells migrated and formed a monolayer with an epithelial-like phenotype.

On day 5, fibroblast-like cells were observed in pellet 1. On day 2, epithelial islets (30%) were observed in pellet 2. In pellet 3, 7-8 mammospheres sized 40-50 µm per power field and a few islets of fibroblast-like cells were observed. After a week, in pellet 1, fibroblast-like cells were observed; in pellet 2, epithelial islets began to occupy more than 50% of the vial; in pellet 3, mammospheres attached to fibroblast-like cells.

Conclusion

The primary culture of breast carcinoma derived from three pellets shows heterogeneity and different growth rates of cells. Cells obtained from pellet 1 more

actively form mammospheres and a monolayer on a non-adhesive Petri dish. Pellet 2 revealed fewer cells with a fibroblast-like phenotype compared to pellet 1.

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СРАВНЕНИЕ РОСТА ПЕРВИЧНОЙ КУЛЬТУРЫ КАРЦИНОМЫ МОЛОЧНОЙ ЖЕЛЕЗЫ В РАЗЛИЧНЫХ УСЛОВИЯХ ДЛЯ КУЛЬТИВИРОВАНИЯ

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COMPARISON OF THE GROWTH OF THE PRIMARY CULTURE OF BREAST CARCINOMA IN VARIOUS CONDITIONS FOR CULTIVATION

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Аннотация. В статье представлены данные по культивированию карциномы молочной железы в специфической среде на разных подложках и произведено сравнение роста клеточных культур, полученных от двух осадков.