第 49 卷 第 5 期
2022 年 5 月

湖南大学学报（自然科学版）
Journal of Hunan University (Natural Sciences)

Vol. 49 No. 5
May 2022

Open Access Article https://doi.org/10.55463/issn.1674-2974.49.5.16

The Use of Patient-Derived Explant Cultures for Predicting Breast Cancer Cell Migration Potential In Vitro

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Abstract: Breast cancer ranks as one of the leading causes of death globally, while mortality rates due to breast cancer continue to rise. In Indonesia, breast cancer is the most common type of cancer, with women dying from breast cancer at a higher rate than men. Primary cultures play a significant role in examining the behavior of breast cancer cells. Because explant cultures can be performed directly from the primary tumor, they constitute a promising new tool for observing cell migration in cancer, including breast cancer. Nevertheless, the use of explant cultures to predict the migration of breast cancer cells has yet to be investigated. This study aims to assess the potential of the explant culture method to predict the migration ability of breast cancer cells in vitro. Tumor explants from two different patients were evaluated in this study. The explant cultures were observed for 14 days until passage, and the results were examined using a microscope. We found that BC02 cells took less than seven days to migrate from the primary tumor, while BC01 cells took 21 days. Furthermore, a mammosphere was observed in the BC02 sample. The rate of cell migration from the tissue depends on the malignant status of the tissue. In conclusion, this study suggests that explant cultures can be used to study the characteristics of cancer cell migration and its correlation with the malignancy of the original tissue.

Keywords: explant culture, cell migration, breast cancer, in vitro.

使用源自患者的外植体培养物预测体外乳腺癌细胞迁移潜力

摘要：乳腺癌是全球主要的死亡原因之一，而乳腺癌导致的死亡率继续上升。在印度尼西亚，乳腺癌是最常见的癌症类型，女性死于乳腺癌的比例高于男性。原代培养在检查乳腺癌细胞的行为方面发挥着重要作用。因为外植体培养可以直接从原发肿瘤中进行，它们构成了一种有前途的新工具，用于观察癌症（包括乳腺癌）中的细胞迁移。然而，使用外植体培养物来预测乳腺癌细胞的迁移还有待研究。本研究旨在评估外植体培养方法预测乳腺癌细胞体外迁移能力的潜力。在本研究中评估了来自两名不同患者的肿瘤外植体。观察外植体培养

Received: February 18, 2022 / Revised: March 21, 2022 / Accepted: April 13, 2022 / Published: May 30, 2022

Fund Project: The Faculty of Mathematics and Natural Sciences, Universitas Indonesia (The FMIPA Grant No. NKB-008/UN2.F3/HKP.05.00/2021)

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物14天直至传代，并使用显微镜检查结果。我们发现公元前02细胞从原发性肿瘤迁移不到7天，而公元前01细胞则需要21天。此外，在公元前O2样品中观察到一个乳腺球。细胞从组织迁移的速度取决于组织的恶性状态。总之，本研究表明外植体培养可用于研究癌细胞迁移的特征及其与原始组织恶性程度的相关性。

关键词：外植体培养，细胞迁移，乳腺癌，体外。

1. Introduction

Breast cancer (BC) has recently surpassed lung cancer as the leading cause of cancer death globally, representing 11.7% of all cancer cases, with an estimated 2.3 million new cases in 2020 [1–2]. Indonesia is a country with a high number of cases of BC in women [3]. Based on GLOBOCAN 2020, BC in Indonesia ranks highest at 16.6% of new cases per 100,000 people, with the second-highest mortality rate after lung cancer, at about 9.6%.

More than 90% of BC deaths are due to metastasis, which occurs when tumor cells detach from the primary tumor site and migrate to the closest lymph system or blood vessels, spreading to other organs [4]. During metastasis, tumor cells acquire motile phenotypes through a differentiation process known as the epithelial-mesenchymal transition (EMT) [5]. Although the EMT is linked to embryonic development and the differentiation of several tissues and organs, recent studies have implied that it is the central mechanism in tumor invasion and metastasis [6].

The current development of specific targeted therapies is due to a better understanding of the basic concepts in tumor biology. In recent decades, cell lines have been one of the most commonly used models for understanding the various mechanisms in tumor biology. However, this model does not accurately reproduce the microenvironment of the tumor cells and exhibits heterogeneity in tumor evolution and patient-related information [7]. For this reason, primary cell culture has been recognized as a more appropriate model closer to the parental tissue. Therefore, primary tissue culture provides patient-related information related to various biological processes, including cell migration, which can be deregulated and contribute to many pathological processes, such as inflammation and metastatic cancer [8–9].

Primary cultures can be developed using several methods, including mechanical and enzymatic disaggregations and explant cultures. Compared to other methods, explant culture has been shown to reflect molecular representations in vivo and maintain the characteristics and microenvironment of the native tissue [10]. Moreover, a previous study reported the potential of explant cultures to predict the migration capabilities of intestinal tumor cores [11]. Nevertheless, the use of explant cultures to predict the migration of BC cells has yet to be investigated. In this study, we assessed the potential of using explant cultures to predict the migration capacity of a BC biopsy and reported the critical finding of the mammosphere released from BC tissues.

2. Subjects and Methods

2.1. Ethical Statement

The present study was approved by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia (KET-1058/UN2.F1/ETIK/PPM.00.02/2021). The recruited subjects provided their written informed consent for biopsy donation (biobanking) and the use and publication of their data for research purposes. All participants signed an informed consent form before participating in the study.

2.2. Sample Collection

The samples were obtained from two BC patients who underwent surgery and incision biopsies at the Cipto Mangunkusumo Hospital. Biopsies were collected after obtaining the patients' approval for sample donation. The biopsies were then transferred to a transport medium containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with a 5% antibiotic–antimycotic solution. In addition, clinical profiles, such as the patients' histopathology examinations and estrogen receptor (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2) status, were collected from the electronic health records kept by the Cipto Mangunkusumo Hospital.

2.3. Explant Tissue Culture

The debris and fat were removed from the tissues using scissors and a scalpel. The tissue was later cut into smaller pieces of approximately 1–3 mm^3. The samples were washed with 0.1% povidone-iodine in Dulbecco's Modified Eagle Medium for 20 seconds. Every two or three small pieces of tissue were placed on a 24-well plate and let dry for approximately 20–30 minutes. Mammocult medium (STEMCELL

Technologies, Vancouver, Canada) was added afterward. The explant was grown at 37°C with 5% CO_2 for approximately three weeks to achieve a 70–80% confluence. The migrated cells in the 24 wells were observed using an inverted microscope, and images were collected at 10 × 40 magnifications.

3. Results and Discussion

3.1. The Migration Rate of the Explant Culture Is Related to the Malignancy Status of the Patient's Tissue

Next, two samples were collected from two distinct patients with different characteristics, as summarized in Table 1. BC01 was collected from a nonmalignant sample, while BC02 was collected from a sample with high proliferative indexes. According to the histology evaluation performed by the pathologist, BC01 was not malignant, while BC02 was NST (no special type/invasive breast cancer) grade III with a KI67 high proliferation index. The migration capacities of these cultures were also different: BC01 required 21 days, while BC02 required seven days to migrate (in other words, the BC01 specimen required three times longer than BC02 to migrate). According to the clinical profiles, BC01 was collected from nonmalignant tissues, while BC02 was collected from an NST Grade II sample that expressed tumor markers, including ER-, PR-, and Ki-67, indicating that the corresponding BC tissues had a highly proliferating status.

Table 1 Clinical trial overview

Sample	The time it took for the cells to migrate	Clinical assessment	
		Histological assessment (EHR results from pathology)	Tumor marker expression
BC01	21 days	Canalicular fibroadenoma mammae: did not appear malignant.	N/A
BC02	7 days	Grade III invasive carcinoma of no special type (NST)	ER -, PR -, HER2+ 20%, Ki-67+ 30% (high proliferation)

Explant culture is a technique that can help us understand the dynamic behavior of cells in homeostasis and disease, can be applied to various tissues, and can show differentiated and migratory cells [12]. According to the results, the growth and migration of explants in BC02 were faster than in BC01. This was in accordance with the pathology analysis (Table 1), which showed that BC02 contained a grade III invasive carcinoma of NST value with Ki-67+30%. The cancer grew faster and had a higher spreading ability than in BC01. Grade III BC is a subtype of a tumor with characteristics similar to cancer and requires more aggressive systemic treatment, including chemotherapy [13]. Ki-67 is considered an important biomarker in BC patients. Higher levels of tumor histopathology are correlated with higher levels of Ki-67. Furthermore, the expression of the Ki-67 index significantly impacts the decision to administer adjuvant or neoadjuvant chemotherapy [14].

The morphological appearance of both specimens is shown in Fig. 1. The specimen originating from the nonmalignant tissue showed a clump not migrating in any direction but was aggregated and did not show any movement even after a few days. By contrast, BC02, which originated from malignant tissue (Fig. 1B), exhibited more separated cells moving in the opposite direction of the explant. The BC02 cells underwent a migration process involving releasing cells from the explant culture to the surrounding area; this process was observed on the second day of culture. Removing cells from primary tissue shows how tumor cells can metastasize, which involves EMT processes. The EMT is a biological process when epithelial cells polarized interact with the basal membrane to undergo biochemical changes driving the phenotype of mesenchymal cells, including increased migration capacity, invasiveness, increased resistance to apoptosis, and the potential for increased production of extracellular matrix (ECM) components [15]. Events during the EMT include decreased cytokeratin and *E-cadherin* regulation, epithelial marker expression loss, increased mesenchymal markers (such as fibronectin, N-cadherin, and vimentin), increased fibroblastic invasive phenotype, and apoptosis resistance [16–17].

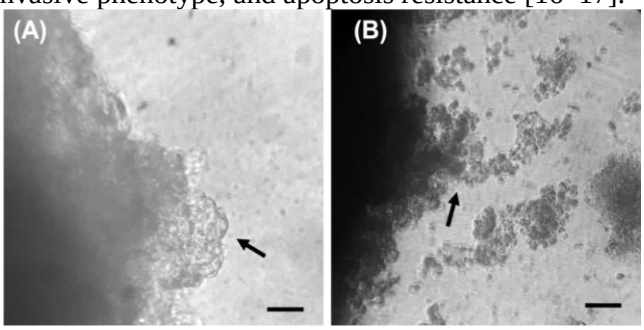

Fig. 1 Cell migrations in the BC01 and BC02 samples. The arrows show the migration process. Scale bars: 100 μm

3.2. Identification of a Mammosphere Detached from Malignant Breast Cancer Tissue

In this study, we identified a mammosphere in the explant culture of BC02 on the second day of culture (Fig. 2). The development of the mammosphere was caused by the migration and invasive potential of BC [18]. Furthermore, the mammosphere-forming cells were derived from primary BC tissue, which had cancer stem cell (CSC) characteristics. CSCs are a subpopulation of cancer cells with characteristics similar to normal stem cells and the ability to self-

repair and self-differentiate to promote tumor growth and heterogeneity. The presence of CSCs is associated with metastasis and tumor recurrence [19].

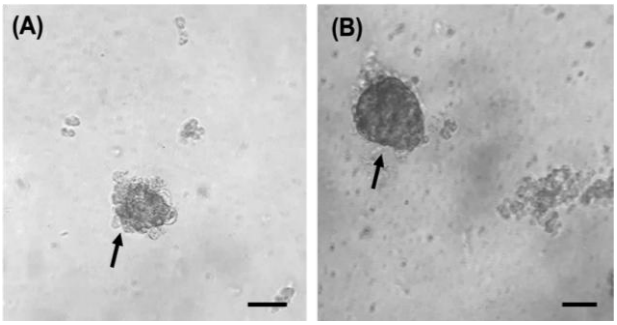

Fig. 2 The BC02 specimen showing the mammosphere on the explant culture (A) and an organoid (B). The arrows show the mammosphere and organoid formation. Scale bars: 400 μm

Molecular analysis (ER-, PR-, and HER2+ 20%) showed that the BC02 cells could be classified into HER2-enriched subtypes (Table 1). Aldehyde dehydrogenase (ALDH) has a biologically aggressive phenotype and tends to have a poor prognosis. ALDH1 cancer stem cells have ER-, Ki67, and HER2+ and are evidence of BC stem cells (BCSC) [20–21]. CSCs can acquire proliferative epithelial phenotypes and invasive mesenchyme [22], evolve rapidly, and switch between the two phenotypes, enabling them to play an important role in the EMT [23].

4. Conclusions

The findings of this study suggest that explant cultures can be used to observe the migration ability of BC cells in vitro. We show that the explant culture can maintain the tumor's microenvironment and help us understand the behavior of cancer cells and tissues. Cell migration techniques can be used to determine the anatomical pathology and immunochemical values and whether a tumor is classified as benign or malignant by estimating the time it takes for cells to separate from the primary tumor. In addition, these findings explain how the EMT process can occur and lead to metastasis via the ability of cultured cells to spread and adhere to the culture plate. This study is expected to be a reference for determining the type of drug and therapy to be administered for treating BC, especially in Indonesia. The primary challenge faced in this study was the difficulty of maintaining cells in patient-derived cell lines from tumors in vitro. This is due to the difficulty of isolating tumor tissue because the size and number of tumor specimens are limited by their natural differences from human cancers. Further research in optimizing primary cultures would provide further insight into the correlation between cell migration and cancer malignancy.

Acknowledgment

We would like to thank the Faculty of Mathematics and Natural Sciences, Universitas Indonesia, for the financial support through the FMIPA Grant No. NKB-008/UN2.F3/HKP.05.00/2021.

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