Three-Dimensional Model of Breast Tumor Stroma for Drug Testing

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ABSTRACT SUMMARY

In this work we are aiming to investigate the possibility to produce an in vitro system mimicking the tumor microenvironment by co-culturing breast cancer cells with stromal components using the Nano3D® system based on magnetic levitation. Three-dimensional (3D) tumor models for breast cancer were designed and optimized by co-culturing SUM159, triple negative breast cancer cells, and 293T fibroblasts. Three anti-cancer drugs, paclitaxel, albumin bound paclitaxel, and etoposide, were tested in the in vitro 3D system. After the addition of the anticancer drugs, flow cytometry and histological analysis for viability, structure, and proliferation demonstrated that the presence of a stromal component, specifically fibroblasts, in the in vitro 3D model significantly affects drug efficacy. Engineering an in vitro 3D tumor model that accurately represents the in vivo tumor will further benefit research efforts in anti-cancer drug and drug delivery systems testing.

INTRODUCTION

Development of cancer therapeutics is an ongoing massive effort by researchers in the academy and pharmaceutical industry. To test newly developed therapeutics and drug delivery systems, conventional two-dimensional (2D) cell cultures are utilized prior to testing on animal models. However, 2D tests frequently fail to accurately represent the performance of drugs in vivo. Several 3D models of tumor have been proposed (e.g. collagen- and Matrigel®-based models) to solve this problem.¹ These current models are usually time consuming tests which are difficult to run high throughput drug assays. The pronounced advantages of this proposed 3D in vitro system are the ability (1) to visualize tumors within 24 hours (2) to reproduce and control compositions and (3) to scale up drug assays.² The objective of this work is to assess if the structural integrity of the native tumor environment can be mimicked using this novel 3D in vitro model system for accurate anticancer drug testing.

EXPERIMENTAL METHODS

Cell Cultures:

Fibroblast (293T) (ATCC) and triple negative breast cancer (SUM159) (ATCC) cell lines were cultured with DMEM supplemented with 10% FBS and 1% P/S. Vybrant™ Cell-Labeling (Molecular Probes) was used to fluorescently label the cells. Breast cancer cells were labeled with Tracer DiO (Em: 501 nm – Green Signal) and fibroblast cells were labeled with Tracer DiD (Em: 665 nm – Red signal). 2D cultures were mixed at specific ratios of breast cancer and fibroblast cells and grown under standard cell culture conditions.

Growth of in vitro 3D tumor model:

Nanoshuttles™ were added to cells and incubated at 37 °C overnight. Using a 24-well plate, a total of 300,000 cells were added to each well at different ratios of breast cancer to fibroblast cells. Immediately after, the magnet was added on top of the well plate for the cells to levitate. (Figure 1) After a given time point, tumors were frozen in OCT, cut into 4 µm sections, and processed for either histological staining or fluorescent imaging.

Figure 1. Schematic on the development of 3D in vitro breast tumor model from a dispersed mixture of fibroblasts and breast cancer cells.

In vivo study: 3 – 4 weeks old SCID-beige female mice (Harlan) were used. Fluorescently labeled cells at a ratio of 70% fibroblasts (293T) to 30% breast cancer cells (SUM159) were injected into the mammary fat pad. After 7 days, tumors were collected and processed for either histological analysis or fluorescent imaging.

Tumor Imaging: Imaging for fluorescent and histological (H&E and Ki67) sections was performed using the NIKON® Eclipse upright microscope (brightfield and fluorescent).

Efficiency of Anti-Cancer Drugs: After the formation of 2D and 3D co-culture systems with fluorescently tagged breast cancer and fibroblast cells, paclitaxel (Taxol®), albumin-bound paclitaxel (Abraxane®), and etoposide (Vepesid®) were added. BD Fortessa flow cytometry with FITC and APC lasers was used to measure the cell viability after 30 hours treatment. The results were statistically analyzed using Student t-test (n = 3; CI 95%).

RESULTS AND DISCUSSION

Breast cancer cells (stained with Tracer DiO – green signal) were mixed with fibroblasts (stained with Tracer DiD – red signal) at different ratios and grown into 3D in vitro tumors. Figure 2A shows the fluorescent images of the different cell mixtures formed into tumors in 3 days. The heterogeneity of tumor compositions (stromal and cancer components) has been clinically observed, especially in triple negative breast cancer patients. In a
cohort study between 1985 and 1994, patients with stroma-rich tumors had a shorter relapse-free period and survival compared to patients with stroma-poor tumors. Thus, the 3D in vitro tumor system demonstrates to be an important model to produce different tumor types with controlled stromal components (i.e. fibroblasts). In Figure 2B, a clear formation of fibrotic capsule (yellow arrow) is observed in the 3D in vitro models, similarly to the in vivo tumor stroma modeled in mice. Ki67 staining for cell proliferation in Figure 2C displays that the 3D in vitro tumors cultured with a mixture of breast cancer and fibroblast cells proliferates more than 3D in vitro tumors cultured alone with breast cancer and fibroblast cells alone. The presence of fibroblasts in the 3D in vitro tumor system enhanced overall tumor proliferation. Similarly, stromal components have shown to enhance tumor growth in vivo.

To test the performance of cancer drugs on the 3D in vitro system, paclitaxel, albumin-bound paclitaxel, and etoposide were added to 2D and 3D in vitro co-cultures of breast cancer and fibroblast cells. After treating for 30 hours at a concentration of 10 µM of albumin-bound paclitaxel, free paclitaxel, and etoposide, Figure 2D shows that the cell viability in 2D co-cultures decreased by 18%, 23%, and 20%, respectively, when compared to the cell viability in the 3D in vitro system. Cell viability in 3D in vitro tumor system was significantly less affected by the anticancer drugs as compared to 2D co-cultures. One of the explanations could rely on the presence of, similar to clinically observed, transport barriers in 3D in vitro tumor system, which are absent when the cells are grown in 2D co-cultures. 2D co-cultures displayed a dispersed state of growth and attachment, forming a monolayer, whereas, 3D in vitro system produced structured tumors that created a barrier for drug penetration.

CONCLUSION
Results suggest that the new 3D in vitro cancer model mimics the structural components of in vivo tumors. The stromal components, such as fibroblasts can be controlled to form heterogeneous tumors. The presence of fibroblasts in the 3D in vitro system formed a clear fibrotic capsule and enhanced proliferation. When anti-cancer drugs were added to the 3D in vitro system, cell viability was not affected when compared to 2D in vitro co-cultures, mimicking the problems observed clinically. Engineering an in vitro 3D tumor model that accurately represents the in vivo tumor will further benefit research efforts in anti-cancer drug molecules and novel delivery systems testing.

REFERENCES


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