Abstract

Breast cancer is a leading cause of mortality in the Western world. It is well established that the spread of breast cancer, first locally and later distally, is a major factor in patient prognosis. Experimental systems of breast cancer rely on cell lines usually derived from primary tumours or pleural effusions. Two major obstacles hinder this research: (i) some known sub-types of breast cancers (notably poor prognosis luminal B tumours) are not represented within current line collections; (ii) the influence of the tumour microenvironment is not usually taken into account.

We demonstrate a technique to culture primary breast cancer specimens of all sub-types. This is achieved by using three-dimensional (3D) culture system in which small pieces of tumour are embedded in soft rat collagen I cushions. Within 2-3 weeks, the tumour cells spread into the collagen and form various structures similar to those observed in human tumours. Viable adipocytes, epithelial cells and fibroblasts within the original core were evident on histology. Malignant epithelial cells with squamoid morphology were demonstrated invading into the surrounding collagen. Nuclear pleomorphism was evident within these cells, along with mitotic figures and apoptotic bodies.

We have employed Optical Projection Tomography (OPT), a 3D imaging technology, in order to quantify the extent of tumour spread in culture. We have used OPT to measure the bulk volume of the tumour culture, a parameter routinely measured during the neo-adjuvant treatment of breast cancer patients to assess response to drug therapy.

Here, we present an opportunity to culture human breast tumours without sub-type bias and quantify the spread of those ex vivo. This method could be used in the future to quantify drug sensitivity in original tumour. This may provide a more predictive model than currently used cell lines.

Protocol

1. Extracting Collagen from Rat Tails

   1. Place frozen fresh rat tails in 70% ethanol.
   2. Deglove the overlying skin with a scalpel exposing tendons.
   3. Strip tendons away from tail and place in 70% ethanol to sterilise.
   4. Weigh the collected tendons and transfer to acetic acid (1g tendon to 250ml 0.5M acetic acid). Mix for 48hr at 4°C.
   5. Centrifuge the tendon/acetic acid mix at 10,000g for 30min and discard the pellet.
   6. Add an equal volume of 10% (w/v) NaCl to the supernatant and allow the mix to stand overnight at 4°C.
   7. Collect the collagen-rich, insoluble bottom layer and centrifugate for a further 30min at 10,000g.
   8. Resuspend the collagen-rich material in 0.25M acetic acid at 4°C and dialyse against 1:1000 acetic acid at 4°C for 3 days, changing the dialysis buffer twice daily.
   9. Further sterilise the collagen solution by centrifugation (20,000g for 2hr) and store at 4°C.
   10. Dilute collagen as required with the addition of sterile 1:1000 acetic acid to a stock concentration of 1mg/ml.

2. 3D Assay Creation

   The 3D assay is based on a cell line assay, published previously.

   1. Multiple core biopsies are harvested from consenting patients at the time of curative surgical resection for invasive breast cancer.
   2. By eye, divide the cores using a scalpel into 1mm³ fragments. Trim and discard macroscopically distinct fat.
6. **Representative Results:**

During the development of the assay, a total of 52 breast cancer biopsy specimens with a wide range of histopathology have been used. Of these <10% (5/52) failed to provide at least one viable invasion assay. Assay failure was due to either bacterial super-infection or insufficient tumour biopsy material. Therefore application of the assay is not restricted to a specific subtype of breast cancer.

Typical assays (figure 1) fixed after 20 days in collagen culture and subsequently stained for cytokeratin. Volocity software is able to recognise both the epithelial bulk continuous with the original core and detached groups of invading cells and quantify each volume separately.
Discussion

Collagen-based assays to study the behaviour of cancer cell lines are now widely used. Nonetheless, these assays have not reflected in full the complexity of the tumour and its environment. In this study, we show that human breast cancer materials can be used in a similar manner, ex vivo. Furthermore, OPT is a useful tool to characterise the 3D expansion of breast cancer. We found that the main limitation of this technique is the availability of tumour materials (a single core biopsy is sufficient for 4-6 assays). Another limiting factor is that structural invasion occurs only in about 40% of assays. To overcome this, we regularly add any desired treatment around day 7 to all assays with visible growth.

The usage of OPT could be extended in the future to also detect bulk changes, ex vivo, resulting from drug treatment. This in turn could facilitate more individualised cancer therapy, independent of animal models. We are currently exploring the response of ER+ tumours to tamoxifen ex vivo.

Disclosures

SEW and JF are employed by MRC Technology, which is commercialising Bioptonics OPT scanners. Other authors declare no conflict of interest.

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References