

# Multicellular Organotypic Models of Normal and Malignant Breast

Deborah L Holliday, Kellie T Brouillette, Anja Mueller, Linda A Gordon, J. Louise Jones.

## Introduction:

- Ductal carcinoma in situ (DCIS) accounts for 40% of screen detected breast carcinoma
- Left untreated, 25-30% of DCIS will progress to invasive carcinoma (1).
- Currently, there is no way of knowing which DCIS will progress leading to difficulties which the choice of treatment.
- Understanding biology of DCIS progression will help to predict which patients are likely to develop invasive carcinoma and potentially identify new targets for therapy.

## Modelling Breast cancer in 3D:

- Primary myoepithelial cells have been shown to inhibit tumour cell invasion (2)
- Fibroblasts play an active role in promoting tumour invasion (3)
- A model to study breast cancer progression would need to contain both of these cell types.

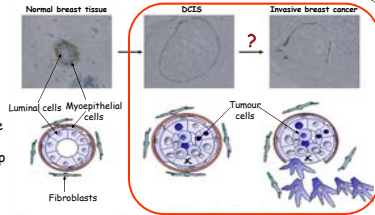


Figure 1: Structure of normal breast tissue and progression through pre-invasive breast cancer (DCIS) to invasive cancer.

## Aims:

- To develop 3 dimensional models which represent normal breast and pre-invasive breast cancer.
- To use the models to study fibroblast contribution to breast cancer invasion.

## Methods:

- Individual cell populations were isolated from normal and cancer containing breast tissue (fig 2a) and fully characterised as described (4).
- Cells pre-labelled with cell trackers were incorporated into collagen I gels with or without a fibroblast population (fig 2b).
- Gels were cultured for 7 days, fixed in paraformaldehyde and whole gel immunofluorescence performed.
- Inhibitors to the c-met receptor (100nM PHA 665752) and/or MMPs (10um GM6001) were incorporated at the time of culture set up and replenished every 48hrs.

## Quantification of co-units:

- A co-unit was defined as a luminal or MCF-7 spheroid where at least 70% was surrounded by myoepithelial cells.
- For quantification co-units were counted in 10 microscopic fields (x20) in 3 separate cultures.
- Changes in levels of specific markers were defined as the percentage of co-units expressing the markers.

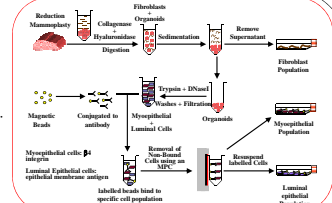


Figure 2a: Isolation of cell populations from primary breast tissue

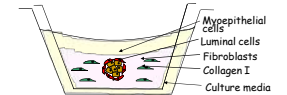


Figure 2b: Structure of 3d in vitro model

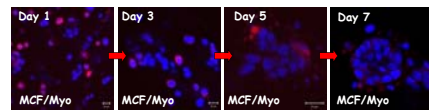


Figure 3: Time course of 3d structure formation. Blue: MCF-7 or luminal cells, Red: Myoepithelial cells

## Results 1: Development of epithelial co-unit over time in culture:

- On day 1 myoepithelial cells and MCF-7 cells are dispersed within the collagen gel.
- After 5 days in culture cells take on a more organised appearance.
- By 7 days myoepithelial cells locate around the MCF-7 cells, forming dual-cell 'co-units'.
- Co-units containing normal luminal cells with myoepithelial cells show comparable co-unit formation.

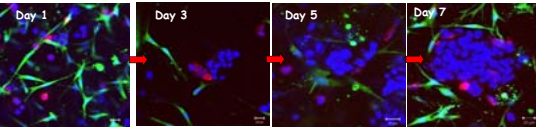


Figure 5a: Co-unit formation over time in culture. Blue cells: tumour cells, red cell: myoepithelial cells, green cells: normal fibroblasts.

## Results 3: Inclusion of fibroblast populations into the DCIS model.

- In cultures with normal fibroblasts co-units are formed after 7 days and cultures show an ordered appearance (Fig 5a).
- Inclusion of tumour-associated fibroblasts leads to disruption of co-unit formation and loss of organisation (Fig 5b).

Figure 5b: Fibroblasts (green) isolated from patients with breast cancer.

## Results 2: Characterisation of normal and DCIS models.

### Normal model

- Luminal epithelial expression of EMA and E-cadherin is observed.
- Myoepithelial cells lay down basement membrane proteins demonstrated by expression of Tenascin-C.
- Basal polarity is demonstrated by the expression of beta-4 integrin at the basal aspect of the myoepithelial cells at the interface with the basement membrane.

### DCIS model

- In keeping with the normal model MCF-7 cells express the luminal-associated EMA and E-cadherin.
- However, expression of the Tenascin-C and beta-4 integrin is decreased suggesting the presence of tumour cells interferes with normal myoepithelial cell function.

### Quantification of models

- Counting the number of co-units shows that a comparable number of co-units are formed in both cultures.
- The percentage of co-units expressing Tenascin-C and beta-4 integrin were significantly decreased in the DCIS model.

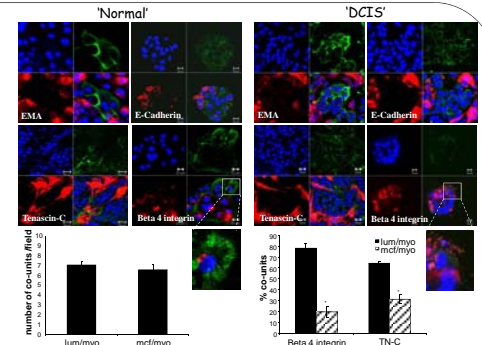
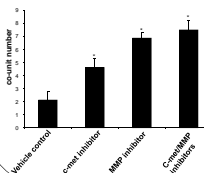
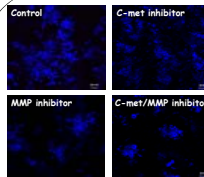


Figure 4: Immunohistochemical characterisation and quantification of normal and DCIS models



## Results 4: Inclusion of inhibitors into the DCIS model containing tumour-associated fibroblasts.

- Matrix degrading metalloproteinases (MMPs) are elevated in breast cancer and the major source is fibroblasts (5)
- Hepatocyte growth factor, expressed in high levels in TAFs, binds to the c-met on tumour cells increasing their motility (6)
- Inclusion of inhibitors to c-met or MMPs results in significantly higher formation of co-units than control cultures.

Figure 6: Effect of c-met (100nM PHA 665752) and MMP (10um GM6001) inhibitors on 'DCIS' co-unit formation

## Conclusions:

- Reproducible isolation of primary cells from normal and cancer containing breast tissue.
- Myoepithelial cells 'home' around normal and tumour luminal cells to form polarised structures.
- Beta 4-integrin expression is altered in normal myoepithelial cells cultured with tumour population.
- Epithelial co-units maintained in presence of normal fibroblasts but disrupted in the presence of tumour-associated fibroblasts.
- The fibroblast effect is at least in part mediated by MMPs and HGF.
- This is the first time that these 3 cell populations have been cultured together in an *in vitro* setting.
- This model provides an important tool enabling functional studies of stromal contribution to breast cancer invasion to be performed.

## References:

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