Mimicking Cancer Cell Communications using Assembloids as Advanced Models of Breast Cancer

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Background

Breast cancer is the second most common cancer in Australia and accounts for approximately 20,000 new cases annually[1].

The study of breast cancer is becoming increasingly focused on cancer cells and their interactions with the surrounding microenvironment. Stromal cells, such as fibroblasts, are among the most abundant cell types found within the tumour microenvironment, known to regulate the behaviour and division of cancer cells[2]. This emphasizes the need for experimental models that can capture the complexities of cancer-stromal cell interactions in a more precise and reproducible manner.

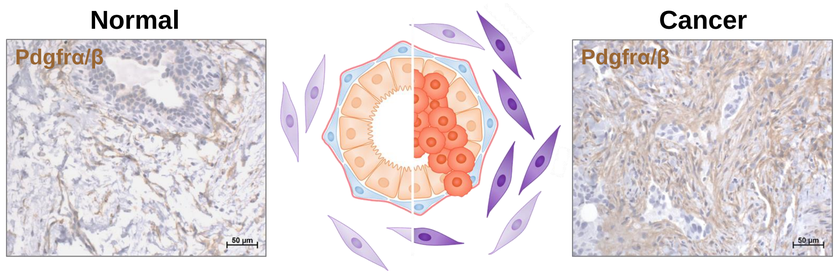


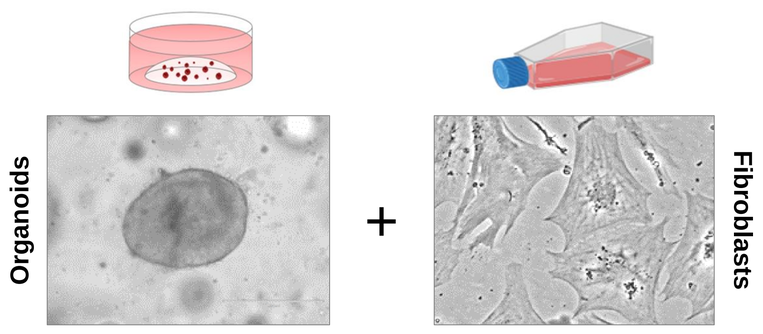
Figure 1. Expression of Pdgfrα/β positive fibroblasts in breast tissues

Assembloids are advanced multicellular culture models composed of two or more distinct cellular subtypes, such as epithelial cells and fibroblasts. They have demonstrated to better replicate the cellular diversity, architecture and physiology of tissues, scaling the dynamics of a patient's tumour externally[3]. This suggests that assembloids could serve as innovative tools for modelling breast cancer, better reflecting the cellular interactions and heterogeneity present within tissues.

Aims

- To generate normal and cancer breast assembloids and validate their accurate recapitulation of primary tissues
- To evaluate the functional capacity and drug testing potential of patient-derived breast assembloids

Methodology



1. Matching patient tissue-derived breast organoids and fibroblast cultures are collected as separate cell pellets
2. An approximate 1:10 ratio of organoid-derived epithelial cells to fibroblasts are combined in Matrigel and co-cultured as droplets
3. Incubated and set cell droplets are supplied with basic growth medium supplemented with antibiotics
4. Cells are grown in culture for 7-14 days on an orbital shaker to allow for self-organization into assembloids
5. Assembloids are fixed and collected at 14 days of culture for downstream histological analyses, such as immunofluorescence

Results

High-content imaging revealed the initial formation of normal human breast assembloids within the first 5 days of culture as cells within each Matrigel droplet become compact and self-organize into more dense and complex 3D structures, comprising of distinct epithelial and fibroblast populations.

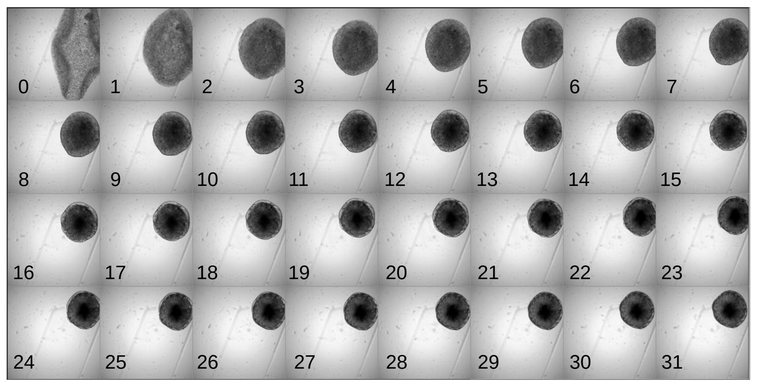


Figure 2. Generation of normal breast assembloids every 3 hours for 5 days of culture, depicting compact assembly of cells into a more advanced 3D structure

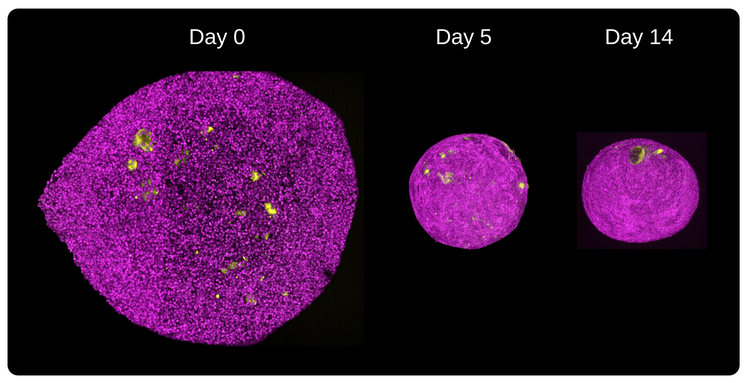


Figure 3. Live tracking of normal breast assembloids labelled with fibroblasts (magenta) and organoids (yellow) on day 0, day 5 and day 14 of culture

Co-immunofluorescence has confirmed the presence of epithelial-derived compartments in cancer (Figure 4) and normal (Figure 5) breast assembloids. These comprise of both luminal and basal epithelial cell lineages surrounded by a framework of fibroblasts, better mimicking structures found in the original tissue.

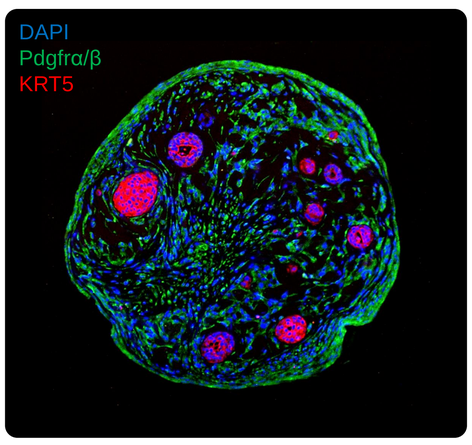


Figure 4. Presence of multiple KRT5 stained (red) basal epithelial cell compartments within a single breast cancer-derived assembloid

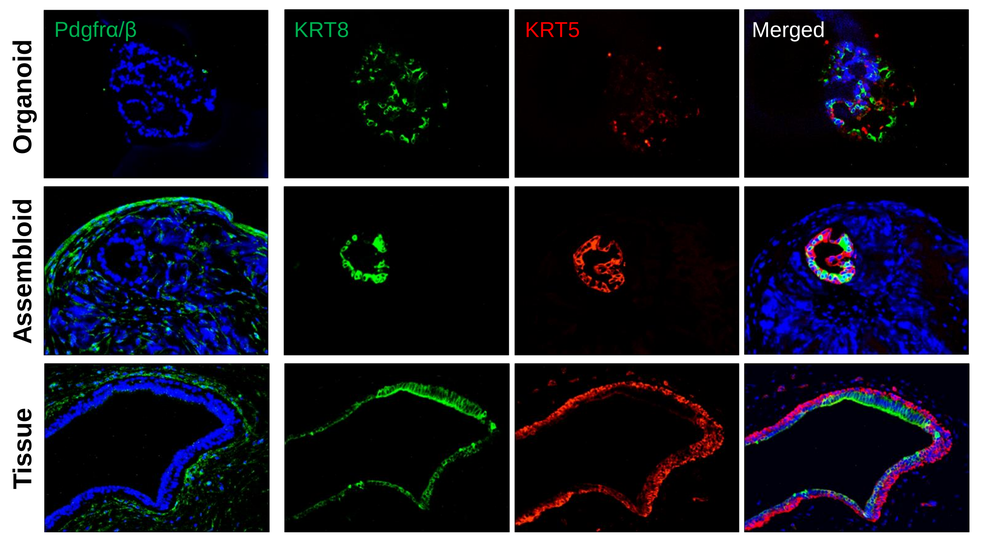


Figure 5. Positive PDGFRα/β fibroblasts (green, far left panel), KRT8 luminal epithelial cells (green, right panels) and KRT5 basal epithelial cells (red) in organoids, assembloids and primary tissue from the same healthy patient

The drug testing capacity of assembloids was assessed by first treating normal breast derived assembloids with an epidermal growth factor receptor (EGFR) inhibitor to block potential epithelial-stromal cell interactions. Treatment resulted in negative KRT8 (luminal) and KRT5 (basal) epithelial cell staining in treated assembloids compared to untreated controls.

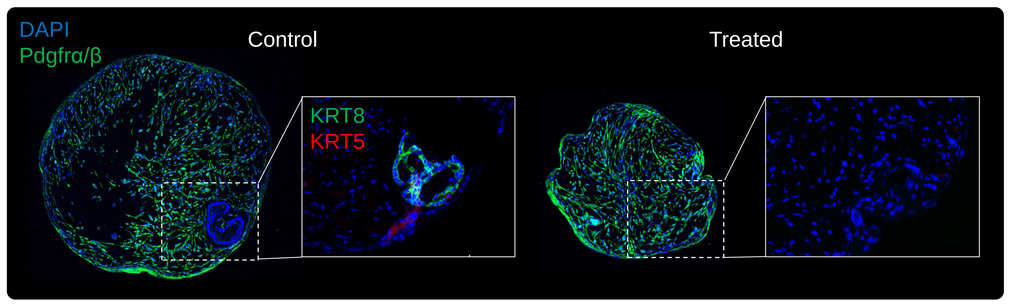


Figure 6. Normal breast assembloids untreated (left) and treated (right) with an epithelial-stromal pathway inhibitor, showing absence of luminal KRT8 (green) and basal KRT5 (red) staining in treated assembloids vs controls

Conclusion

- Our team has generated patient-derived breast assembloids by combining matching organoids and fibroblasts from healthy or tumour tissues, modelling essential direct cellular interactions
- Preliminary characterization has shown that assembloids are structurally more representative of original tissues, better reflecting breast cellular diversity compared to organoids cultured alone
- This foundational research could offer broad experimental and clinical applications of assembloids, opening up new prospects for breast cancer research

References

1. Australian Institute of Health and Welfare (AIHW) 2018. Australian Cancer Incidence and Mortality (ACIM) books. Canberra: AIHW
2. Li YR, Fang Y, Lyu Z, Zhu Y, Yang L. Exploring the dynamic interplay between cancer stem cells and the tumor microenvironment: implications for novel therapeutic strategies. J Transl Med. 2023;21(1):686.
3. Kanton S, Paşca SP. Human assembloids. Development. 2022 Oct 15;149(20):dev201120.

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