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Investigating Stromal-Epithelial Interactions Using a Novel 3D Breast Cancer Organoid Model: Regulation of the Basal Epithelial Phenotype by Cancer-Associated Fibroblasts

Hanley, C.J.[1]; Thomas, G.J.[1]; Ewald, A.J.[2]

[1]University of Southampton, Southampton, United Kingdom; [2]Johns Hopkins, Baltimore, United States

Tumour organoids have provided novel insight into the intrinsic mechanisms regulating breast cancer invasion, highlighting the critical contribution of epithelial transdifferentiation to a conserved basal, keratin 14 (KRT14)-positive, phenotype. However, the mechanisms that regulate these alterations to tumour cell phenotype remain unknown.

Weighted gene correlation network analysis of RNA-Seq data from the breast cancer genome atlas revealed a highly significant positive correlation between the basal epithelial phenotype and stromal genes involved in extra-cellular matrix (ECM) organisation. To investigate this correlation experimentally we developed a novel method to co-culture autologous tumour organoids and stromal cells in 3D -directly following isolation from primary tumours. In this system, stromal cells significantly increased tumour cell transdifferentiation to a KRT14-positive basal phenotype and invasion (MMTV-PyMT and C31-Tag ductal breast cancer models).

Time-lapse and confocal microscopy showed stromal cells to be highly dynamic and motile within these 3D organotypic cultures, organising collagen fibres and interacting directly with tumour organoids. Comparison of organoids co-cultured with stromal cells or treated with stromal-conditioned media revealed that juxtacrine signalling was required for maximal impact on epithelial cell transdifferentiation and invasion. Inhibiting NOX4, an enzyme recently identified as a critical regulator of cancer associated fibroblast activation, prevented these stroma-mediated effects. Proteomic analysis of NOX4 inhibition's impact on fibroblast activation revealed a critical role of this enzyme on actin cytoskeleton remodelling, reducing stromal cell's contractility and ability to remodel the ECM.

In summary, we have developed a novel organotypic model to study tumour-stromal interactions and demonstrate the potential for targeting NOX4 to prevent stromal-mediated tumour cell invasion in ductal breast cancer.