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3-Dimensional Reconstruction of the Breast Tumour Microenvironment: Mediation of Tumour Progression by T_{REG} Lymphocytes and NK Cells



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A thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

FACULTY OF HEALTH SCIENCES
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Declaration

I, Tanya Nadine Augustine, declare that this thesis entitled "3-Dimensional Reconstruction of the Breast Tumour Microenvironment: Mediation of Tumour Progression by T_{REG} Lymphocytes and NK Cells" is my own unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



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Tanya Nadine Augustine

__23rd__ day of ___October___ 2014 at ___Parktown___

Abstract

Breast tumour progression involves complex interactions between malignant cells and the tumour microenvironment. It is increasingly apparent that immunity is a critical determinant for tumour progression. T regulatory (T_{REG}) lymphocytes, which dominate tumour infiltrating lymphocyte populations, are implicated in facilitating tumour immunoediting processes and suppressing Natural Killer (NK) cell anti-tumour function. To investigate such cellular interaction, experimentation traditionally involves using reductionist 2-dimensional culture systems that do not recapitulate the spatial dimensions of the *in vivo* microenvironment. Three-dimensional (3D) culture systems, conversely, recreate these dimensions, allowing tumour cells to assume a phenotype more representative of the tumour microenvironment.

Given that immunity is a critical factor in determining tumour progression, a novel 3D culture system was established to investigate the interactions between T_{REG} lymphocytes, NK cells and hormone-dependent (MCF-7) or hormone-independent (MDA-MB-231) breast cancer cells. Lymphocyte subpopulations were magnetically isolated, with the efficacy of the sorting procedure verified using flow cytometry. To generate 3D cultures, cell populations were resuspended in growth factor-reduced Matrigel and cultured for 72 hours. This culture system proved effective for RNA extraction for downstream applications; for immunolocalisation of selected tumour biomarkers (ER-α, TGF-β, MUC-1 and EGFR) for qualitative analysis; and for acquisition of cytokine data (IL-1β, IL-2, IL-6, TNF-α, IFN-γ, CCL2, CCL4 and CXCL8) for quantitative multivariate statistical analysis.

Immune mediation was shown to induce the disruption of cell-cell associations, altering the expression of biomarkers and secreted cytokine profiles. Collectively, these results reflect tumour cell subversion of NK cell and/or T_{REG} lymphocyte function to promote tumour progression by generating an inflammatory microenvironment. While hormone-dependent and hormone-independent breast cancer cells differed in their specific response to immune mediation, the mechanisms by which they elicited responses resulted in similar

outcomes – that of enhanced evasive and invasive capacity. It is necessary to further elucidate the relationship between the investigated cytokines, biomarkers and immune cells, to understand their interactions and potentially provide more information for therapeutic intervention, given that these factors may contribute to tumours not responding favourably to combined modalities of therapy.