Development and characterisation of a triple-negative breast cancer multicellular spheroidal model

Keith N Ncube¹, Vanessa Steenkamp¹, Duncan A Cromarty¹, Iman Van Den Bout², Werner Cordier¹

¹Department of Pharmacology, University of Pretoria, South Africa, ²Centre for Neuroendocrinology, University of Pretoria, South Africa

Background

In vitro monolayer (2D) cell culture models have limited predictive ability for the clinical outcome of potential anti-cancer therapies as these models fail to reproduce the physiological cues of the tumour micromilieu. Three-dimensional (3D) multicellular spheroids are used as an alternative, as they better resemble the *in vivo* tumour environment with regards to cellular interactions and chemical gradients. Triple-negative breast cancer (TNBC) is a sub-type of breast cancer associated with resistance, relapse and lack of target specificity. This study sought to develop and characterise a spheroidal model of TNBC.

Methods

To select a reproducible TNBC spheroidal model, BT-20 and MDA-MB 231 cell lines were compared and grown using the liquid overlay and hanging-drop assays. Growth was assessed using phase contrast microscopy and the bicinchoninic acid protein content analysis assay. Viability was assessed using the fluorescein diacetate (FDA)/propidium iodide (PI) assay. Haematoxylin and eosin staining was used for morphological evaluation. Susceptibility to doxorubicin was assessed in 2D-monolayer cultures using sulphorhodamine B staining, FDA/PI-staining, and acid phosphatase conversion. The effect of toxic concentrations of doxorubicin on spheroids' morphology and enzymatic activity was assessed and compared to 2D-monolayer cultures.

Results

Only BT-20 cells cultured using the liquid overlay assay formed reproducible dense spheroids which were subsequently used for characterisation. Spheroid diameter decreased from day four (949 µm) to day ten (787 µm), while protein content increased slightly in parallel (7.0 to 8.5 µg/spheroid), suggesting spheroidal compaction rather than cell death. The outer spheroid region had viable, well-nourished cells, while smaller, membrane-compromised cells were localized in the inner spheroid region with a clear distinction between the layers. The IC₂₅ (130 nM), IC₅₀ (320 nM) and IC₇₅ (1580 nM) of doxorubicin induced a dose-dependent reduction of monolayer cell viability and acid phosphatase activity. However, these concentrations did not alter spheroid size and acid phosphatase activity, suggesting resistance incurred by its 3D-conformation.

Conclusion

Heterogeneous cellular architecture and resistance of BT-20 spheroids resemble *in vivo* attributes of TNBC. It is essential to incorporate such a 3D model in preclinical drug screens in order to facilitate translational cancer research and potentially discover optimally effective drugs against TNBC.