THE ROLE OF STEM CELLS MARKERS IN 3D GLIOBLASTOMA TUMOUR CELLS GROWTH

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Abstract:

Glioblastoma is the most common and aggressive type of brain tumours that is fatal with no curative treatment. Studies have shown that a subpopulation of glioma stem-like cells (GSCs) within the tumours are responsible for the poor prognosis and treatment resistance. Thus, identification and targeting of GSCs offer a promising future for the discovery of novel treatments.

This study investigated the 3D spheres generated from 2D glioma cells cultured on Matrigel where the cells structure, proliferation and average volumes in addition to the expression of Nestin, SOX2 and CD44 for stem cells detection, PIMO, HIF-1α and CAIX for hypoxia and Y-H2AX for DNA damage were determined in G7 cell line at three time points 7, 10 and 14 days. Various techniques were implemented; aseptic tissue culture, and flow cytometry for cell surface markers and cell death analysis. Furthermore, Western blotting was utilised for the detection of proteins levels. The 3D cultured G7 spheres increased in size, become more hypoxic with increasing levels of DNA damage during the process of 3D growth. The flow cytometry data revealed that the 3D spheroids become less viable with time; 93.13%, 88.35% and 78.52% of the cells were alive on day 7, 10 and 14 respectively. On the other hand, the Western blot data showed clear expression of the hypoxia markers HIF-1α and CAIX at day 14. Taken together the results from this study show GBM 3D cells become more hypoxic with the increase in size along with DNA damage, yet the tumourspheres volume does not change between day 7 and 14 which could be linked to the invasion mechanism adopted by the 3D cells.

Keywords:

Glioblastoma, Tissue Culture, Flow Cytometry, Western Blot, Glioma Stem-like cells, Nestin, HIF-1α, SOX2, CAIX, □-H2AX, PIMO, CD44.