

Cancer Stem Cells in Head and Neck Metastatic Malignant Melanoma

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Purpose: Malignant melanoma (MM) accounts for 60-80% of skin cancers worldwide with New Zealand and Australia having the highest incidence. Cancer stem cells (CSCs) have been identified in many cancer types including metastatic MM to the brain. This study identified and characterised CSCs in metastatic head and neck MM (HNmMM) using induced-pluripotent stem cell (iPSC) markers.

Methodology: 3,3-Diaminobenzidine (DAB) immunohistochemical (IHC) staining was performed on HNmMM tissue samples from 20 patients, for iPSC markers OCT4, NANOG, SOX2, KLF4 and c-MYC to identify CSCs. Immunofluorescence (IF) IHC staining was performed on two of these samples to localise these markers. Expression of the iPSC markers was investigated by colourimetric in-situ hybridisation (CISH) and reversetranscription quantitative polymerase chain reaction (RT-qPCR) in 6 HNmMM samples. Two HNmMM-derived primary cell lines underwent tumoursphere formation assays and Western blotting (WB) to investigate cellular functionality and protein expression of the iPSC markers, respectively.

Results: DAB IHC staining demonstrated expression of OCT4, SOX2, KLF4 and c-MYC in all 20 HNmMM tissue samples while NANOG was present in two of the samples. CISH and RT-qPCR mRNA analyses confirmed transcript expression of all five iPSC markers. IF IHC staining demonstrated an OCT4+/NANOG+/SOX2+/KLF4+/c-MYC+ CSC subpopulation within the tumour nests and the peritumoural stroma; and a further SOX2+/c-MYC+ subpopulation within the tumour nests. HNmMM-derived primary cell lines demonstrated invitro tumorsphere formation, and WB confirmed protein expression of SOX2, KLF4 and cMYC but not OCT4 and NANOG.

Conclusions: We have demonstrated three putative CSC subpopulations within HNmMM. The expression of iPSC markers by the HNmMM-derived primary cell lines and their ability to form tumourspheres in-vitro provide further evidence of the presences of CSCs which maybe a novel therapeutic target for this aggressive cancer.