Methods Mol Biol. 2023;2701:253-259. doi: 10.1007/978-1-0716-3373-1_17.

Tumorsphere Formation Assay: A Cancer Stem-Like Cell Characterization in Pediatric Brain Cancer Medulloblastoma

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Affiliations PMID: 37574488 DOI: 10.1007/978-1-0716-3373-1_17

Abstract

Cancer is a heterogeneous disease, comprising of a mixture of different cell populations. Cancer stem cells (CSCs), also known as tumor-initiating cells (TICs), are a subpopulation of multipotent cells within the cancer that has self-renewing capability, tumor-initiating ability, multi-differentiation potential, and an inherent capacity for drug and chemoresistance. Sphere-formation assay is commonly used for enrichment and analysis of CSC properties in vitro and is typically used as a metric for testing the viability of tumor cells to anticancer agents. This model is based on the ability of CSCs to grow under ultralow-attachment conditions in serum-free medium supplemented with growth factors. In contrast to the adherent 2D culture of cancer cells, the 3D culture of tumorsphere assay exploits inherent biologic features of CSCs such as anoikis resistance and self-renewal. We describe here the detailed methodology for the generation and propagation of spheres generated from pediatric brain tumor medulloblastoma (MB) cells. As signal transducer and activator of transcription (STAT3) is known to play an important role in maintaining cancer stem cell properties, we accessed the effect of depleting or inhibiting STAT3 on MB-sphere sizes, numbers, and integrity. This may serve as a promising platform for screening potential anti-CSC agents and small-molecule inhibitors.

Keywords: Cancer stem cells; Self-renewal; Tumor-initiating cells; Tumorspheres.

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