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**[Isolation and identification of brain tumor stem cells from human brain neuroepithelial tumors]**

[Article in Chinese]

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**OBJECTIVE:** To establish a simplified culture system for the isolation of brain tumor stem cells (BTSCs) from the tumors of human neuroepithelial tissue, to observe the growth and differentiation pattern of BTSCs, and to investigate their expression of the specific markers. **METHODS:** Twenty-six patients with brain neuroepithelial tumors underwent tumor resection. Two pieces of tumor tissues were taken from each tumor to be dissociated, triturated into single cells in sterile DMEM-F12 medium, and then filtered. The tumor cells were seeded at a concentration of 200,000 viable cells per mL into serum-free DMEM-F12 medium simply supplemented with B27, human basic fibroblast growth factor (20 microg/L), human epidermal growth factor (20 microg /L), insulin (4 U/L), L-glutamine, penicillin and streptomycin. After the primary brain tumor spheres (BTs) were generated, they were triturated again and passed in fresh medium. Limiting dilution assay was performed to observe the monoclonal formation. 5-bromodeoxyuridine (BrdU) incorporation test was performed to observe the proliferation of the BTs. The BTSCs were cultured in mitogen-free DMEM-F12 medium supplemented with 10% fetal bovine serum to observe their differentiation. Immunocytochemistry was used to examine the expression of CD133 and nestin, specific markers of BTSC, and the rate of CD133 positive cells. **RESULTS:** Only a minority of subsets of cells from the tumors of neuroepithelial tissue had the capacity to survive, proliferate, and generate free-floating neurosphere-like BTs in the simplified serum-free medium. These cells attached to the poly-L-lysine coated coverslips in the serum-supplemented medium and differentiated. The BTSCs were CD133 and nestin positive. The rate of CD133 positive cells in the tumor specimens was (21 +/- 6.2)% - (38 +/- 7.0)%. **CONCLUSION:** A new simplified culture system for the isolation of BTSCs is established. The tumors of human neuroepithelial tissue contain CD133 and nestin positive tumor stem cells which can be isolated, proliferate and differentiate in vitro and give rise to brain tumor spheres. This tumorigenic subset may provide both a platform for brain tumor research and a target for clinical treatment.

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