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1: [Ai Zheng](#). 2006 Feb;25(2):241-6.

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[Isolation and characterization of brain tumor stem cells in human medulloblastoma]

[Article in Chinese]

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BACKGROUND & OBJECTIVE: Tumor stem cells have been isolated from several kinds of solid tumors, including primary brain tumors such as glioma and medulloblastoma. This investigation was to establish a simplified culture system to isolate and passage brain tumor stem cells (BTSCs) from human medulloblastoma, observe their proliferation and differentiation, and determine the expression of normal neural stem cell antigens, CD133 and Nestin, in BTSCs. METHODS: Eleven specimens of medulloblastoma were acutely dissociated and triturated into single cells without trypsin digestion. The tumor cells were seeded into serum-free DMEM/F12 medium (200,000 viable cells/ml) containing B27 (1:50), basic fibroblast growth factor (bFGF, 20 microg/L), epidermal growth factor (EGF, 20 microg/L), insulin (4 u/L), L-glutamine, and antibiotics. After generation, the primary brain tumor spheres (BTSS) were mechanically dissociated and passaged in the above serum-free medium. The monoclonal formation experiment was performed to determine the proportion of BTSCs in medulloblastoma and to observe the formation of BTSS. The differentiation of BTSCs was induced in mitogen-free DMEM/F12 medium supplemented with 10% fetal bovine serum. The expression of CD133 and Nestin in BTSS was observed with immunofluorescence staining; the distribution of CD133-positive cells in tumor sections was assessed by immunohistochemistry. RESULTS: In each of the 11 specimens, only a minority of medulloblastoma cells showed the capacity of self-renew and proliferation. These BTSCs generated free-floating neurosphere-like BTSS in the simplified serum-free medium. The proportion of BTSCs with monoclonal formation capacity in primary tumor cells was (31.18+/-6.18)%. The BTSCs attached to poly-L-lysine-coated coverslips and differentiated when the serum-supplemented medium was added. The expression of CD133 and Nestin was detected in BTSCs. CD133-positive cells scattered or formed nest-like aggregations in tumor masses, and accounted for (33.06+/-8.57)% of all tumor cells. CONCLUSIONS: BTSCs, with the capacity of self-renew and proliferation and express CD133 and Nestin, are exist in human medulloblastoma. They could be isolated and cultured in the simplified serum-free medium, and their differentiation could be induced in serum-supplemented medium.

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