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[Characteristics of morphology, differentiation related marker, and proliferation dynamics of differentiated brain tumor stem cells in vitro]

[Article in Chinese]

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OBJECTIVE: To pursue the changes of cell morphology, expression of differentiation related markers, and proliferation cycles of brain tumor stem cells (BTSCs) after differentiation in vitro. METHODS: Tumor stem cells of the line CD133(+) were obtained from two specimens from one clinical case with anaplasia ependymocytoma during operation, one specimen being obtained during the first operation and then second specimen being obtained during the second operation 6 months later on the recurrent tumor. CD133(+) cells were acquired by using magnetic sorting and then cultured to differentiate in medium containing 10% fetal bovine serum. The morphology of the cells was observed under phase contrast microscope. Cells were collected respectively before differentiation and 3, 7, 10, and 21 days after the differentiation. The cell surface markers such as CD133, nestin, glial fibrillary acidic protein (GFAP), and beta-tubulin III were detected with flow cytometry. Proliferation cycles were examined before differentiation and in the 7th day after differentiation. Normal neural stem cells (NSCs) obtained from fetal brain tissues were used as controls. RESULTS: (1) The BTSCs were round shape at the beginning, then changed to short fusiform, polygon and long fusiform. Seven days later the cells reversed to short fusiform and round shape. The cells accumulated into cell spheres and floated in the culture medium again. While the NSCs differentiated along their routine rules. (2) Both the undifferentiated BTSCs and NSCs showed high level expression of CD133 and nestin. After differentiation the BTSCs expressed CD133 and nestin, the expression levels decreased first and then increased. The expression rates of CD133 and nestin were (3.65 +/- 0.17)% and (28.99 +/- 1.26)% in the 7th day, (14.63 +/- 1.16)% and (45.46 +/- 1.27)% in the 21st day. While the positive expression rate of GFAP was higher than that of beta-tubulin III. In the 10th day the NSCs under differentiation lost the expression of CD133 and nestin. The percentage of GFAP positive cells and beta-tubulin III positive cells were (88.94 +/- 1.23)% and (11.94 +/- 0.36)% respectively. (3) All undifferentiated BTSCs were hypodiploid. After differentiation majority of the BTSCs were hypodiploid or hyperdiploid, The percentages of S phase and G(2)-M phase cells in the BTSCs were higher than that in the NSCs. The cell composition of recrudescence BTSCs was more complex than that of the primary BTSCs. All NSCs were diploid whether differentiated or not. Most of the NSCs were G(0)-G(1) phase cells. CONCLUSION: The differentiation direction of BTSCs is quietly different from that of the NSCs. There is an obvious dysdifferentiation in BTSCs.

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