Brain tumor stem cells and their diverse features

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Abstract

Objective:
Malignant brain tumors, in particular the glioblastoma mutliforme (GBM), are amongst the most lethal solid human tumors. They grow invasively, develop resistance to radiation and chemotherapy, and frequently recur. Recently, GBM tissue was classified into four distinct molecular entities, the classical, mesenchymal, neural and proneural subtype based on a combined genome, transcriptome and proteome analysis. It remains unclear whether stem-cell like tumor cells (SCLC), which represent a small subfraction of the GBM, could be grouped into similar categories and whether GBMs harbor different types of SCLC.

Material and methods:
We screened 80 human brain tumor biopsies for the presence of SCLC by immunological techniques and isolated SCLC from different glioma entities, such as glioblastoma mutliforme (GBM) and gliosarcoma (GSarc). SCLC were expanded in serum-free medium and transplanted into SCID mice. Expression of stemness factors and neural markers was investigated by immunocytochemistry, FACS, Western blot analysis and real time PCR. Single cells from SCLC cultures were seeded in 96 well plates, clonally expanded and subsequently subjected to phenotype analysis.

Results and conclusions:
We identified stem cell-like cells (SCLC) in all brain tumor biopsies analyzed so far. SCLCs that gave rise to proliferating cultures were transplanted into the brain of SCID mice to prove their tumorigenic potential. All SCLC expressed the intermediary filaments Nestin and the transcription factor Sox2. Co-expression of the intermediary filament GFAP or the marker CD133 (prominin-1), however, varied largely. The histostructural organization of Nestin+/GFAP- and Nestin+/GFAP+ cells differed between the various glioma tissues. This indicated that malignant gliomas show a high heterogeneity with respect to the presence and localization of SCLC. This also translated to SCLC cultures derived from GBM and GSarc biopsies. Several SCLC cultures exclusively contained Nestin+/GFAP- cells, whereas others encompassed only Nestin+/GFAP- cells. Mixed forms were also observed. In these cultures the ratio of Nestin+/GFAP+ to Nestin+/GFAP- was remarkably stable over many passages. The same phenomenon was observed for the presence of CD133+ and CD133- cells. In addition, we found co-expression of Nestin and CD44 in almost all SCLC subtypes, which is in contrast to data published in the novel classification of molecular GBM entities. Clonal expansion of single SCLC cells from selected cultures revealed different degrees of heterogeneity, supporting the view that SCLC subtypes may co-exist in the same culture and presumably the same tumor.

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