

Effect of hypoxia on the growth of glioma-initiating cells

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Introduction

Glioma-initiating cells (GICs) possess stem cell characteristics such as the ability for limitless growth, self-renewal and to differentiate into all kinds of cells found in the original tumour. GICs are held responsible for the strong radio- and chemoresistance of gliomas.

A solid tumour consists of an outer layer that is surrounded by a blood vasculature to provide both nutrition and oxygen. In the inner tumor layers, the oxygen concentration decreases with increasing distance from the blood vessels reaching numbers below 0.5 %. This hypoxic core is referred to as a stem cell niche. Studies concluded that hypoxia regulates glioblastoma stem cell properties and biomarker expression [1].

Materials and Methods

Three glioma cell lines (#10, #10-IR, and U87) were cultured as neurospheres in serum-free neurobasal A medium supplemented with B27, EGF and FGF. For the growth curves, 30.000 normoxic cells were seeded in T25 culture flasks and cultured under normoxia (21 % O₂) and hypoxia (1 % O₂). In the course of 2-3 weeks they were trypsinised and counted regularly. For the neurosphere formation assay 30.000 cells were seeded in T25 culture flasks then cultured under hypoxia and normoxia.

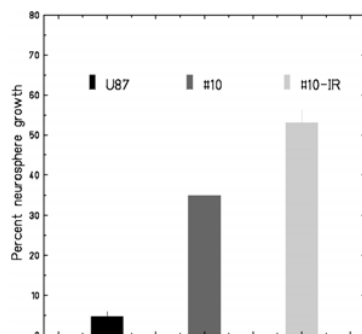


Figure 1: Percent neurosphere formation under hypoxia (1 % O₂), relative to neurosphere formation under normoxia. Error bars represent standard deviation, n=3.

Results and Discussion

The results from the neurosphere formation assay are displayed in figure1. The U87 cells showed a drastically

reduced neurosphere formation ability under hypoxic conditions (less than 5 % relative to normoxia). For the cell lines #10 and #10-IR the neurosphere formation in hypoxia was 35 % and 53 %, respectively.

Cell growth curves were compiled for cell lines #10 and #10-IR and are displayed in figure 2. Due to the reduced growth of U87 cells in hypoxic conditions it was not possible to measure growth curves for this cell line. The doubling time (td) for #10 is 54 h in normoxic conditions and 107 h under hypoxic conditions. #10-IR cells have a td of 68 h under normoxia and 79 h under hypoxia.

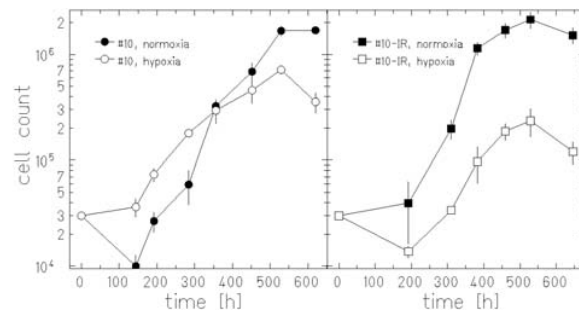


Figure 2: Growth curves of cell lines #10 (left panel) and #10-IR (right panel) under normoxic (closed symbols) and hypoxic (open symbols) culture conditions. Error bars represent standard deviation, n=3.

The cell lines # 10 and #10-IR were established as cell lines growing in serum-free self-renewal conditions [2] which promote the stem cell properties. The U87 cell line, in contrast, was established decades ago as a (bulk) glioblastoma cell line and is generally cultured in standard cell culture medium containing fetal calf serum. The ability of the cell lines # 10 and # 10-IR to grow well in a hypoxic environment, which is not shared by the U87 cells, could be a hint that the #10 and #10-IR cells are in a more stem-like state and can be used as a model for cancer stem-like cells while U87 cells are not an appropriate model for cancer stem-like cells.

References

- [1] E.E. Bar et al. "Hypoxia increases the expression of stem-cell markers and promotes clonogenicity in glioblastoma neurospheres", *Am J Pathol* 177 (2010), p. 1491–1502.
- [2] A. Barrantes-Freer et al. "Human glioma-initiating cells show a distinct immature phenotype resembling but not identical to NG2 glia", *J Neuropathol Exp Neurol* 72(4) (2013) p.307