Physiological description of patient-derived Glioblastoma cells using fluorescence imaging

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ABSTRACT

Glioblastoma (GB) is the most malignant brain cancer and is not considered a curable disease so far. A multidisciplinary framework that integrates basic and translational research is presented attempting a better understanding of its pathophysiology. In this attempt, a carefully planned combination of experimental approaches were mobilized. Specifically, patient-specific cell cultures were established and used in experimental assays, while Light Sheet Fluorescence Microscopy (LSFM) and Confocal Microscopy (CM) were used to visualize the GB pathophysiologic factors.

Tissue from naïve-from-treatment patients was excised during brain biopsy and/or resection. After the GB case confirmation, if needed, part of the tissue was transplanted to immunodeficient mice. Followingly, the primary cell cultures were established for each GB case.

The primary cell cultures were phenotypically characterized and used in experimental assays. The well-described U87MG and T98G GB cells served as control. This work primarily focuses on proliferation and invasion, two of the most dominant GB characteristics. LSFM and CM imaging were used to monitor the growth of small-sized avascular GB spheroids using optimized imaging protocols, so that GB-specific biomarkers could be identified. In addition, preclinical drug screening was used to evaluate the efficacy of specific drugs.

Focusing on proliferation, we supported that the intratumoral heterogeneity together with the overall proliferation reflected in both the proliferation rate and the mechanical cell contact inhibition, but not the cell size, can predict the evolution of different GB cell lines (1).

We showed that the primary GB spheroids adopt a novel, cohesive pattern mimicking perivascular brain invasion, while the U87MG and the T98G adopt a typical, starburst, invasive pattern (2, 3). CM indicated alternative proliferative and adhesive characteristics of the invading cells.

We also set a preclinical drug screening tool to assess the distribution, penetration and cytotoxic potency of the Temozolomide (TMZ) and Doxorubicin (DOX) antineoplastic agents. We used LSFM in order to discriminate growth inhibition in cell division arrest from cell death. The effective doses varied over four orders of magnitude. Unlike TMZ which showed slight growth-inhibiting effects, DOX was able to accumulatively cause necrosis.

Overall, we claim that future research should be based on patient-derived GB cells and that common cell lines should only serve as landmarks to unite studies of different groups. For every primary established cell line, not only molecular, but also physiological parameters should be estimated to enable a more precise future clustering of different GB cases in order to optimize individualized therapy decisions and GB pathophysiology understanding.

REFERENCES

1. Oraiopoulou M-E, Tzamali E, Tzedakis G, Vakis A, Papamatheakis J, Sakkalis V. In Vitro/In Silico Study on the Role of Doubling Time Heterogeneity among Primary Glioblastoma Cell Lines. BioMed Research International. 2017;2017:12. doi: 10.1155/2017/8569328.
2. Oraiopoulou ME, Tzamali E, Tzedakis G, Liapis E, Zacharakis G, Vakis A, et al. Integrating in vitro experiments with in silico approaches for Glioblastoma invasion: the role of cell-to-cell adhesion heterogeneity. Scientific Reports. 2018;8(1):16200. doi: 10.1038/s41598-018-34521-5.
3. Oraiopoulou M-E, Tampakaki M, Tzamali E, Tamiolakis T, Makatounakis V, Vakis AF, et al. A 3D tumor spheroid model for the T98G Glioblastoma cell line phenotypic characterization. Tissue and Cell. 2019. doi: https://doi.org/10.1016/j.tice.2019.05.007.