Identification of a Hypoxic Signature in Glioblastoma Cells at the Cellular and Subcellular Levels by FTIR Microspectroscopy

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Hypoxia is a frequent feature of solid tumors, and especially gliomas, and is associated with poor prognosis, resistance to radio- and chemotherapy, and tumor aggressiveness. In order to adapt therapeutic treatment to patient specific state there is a need to develop diagnostic tools suitable for widespread clinical practice to measure tumor hypoxia, its extent and its evolution. Since tumors are highly heterogeneous with normoxic, chronic or acute hypoxic, and necrotic regions, global measurements may be misleading and spatially resolved information is required. Metabolic changes such as increased glucose uptake and lipid accumulation are intrinsic markers of the energy metabolism shift occurring during hypoxia, hence, we propose that FTIR microspectroscopy could be a good tool to detect and measure the extent of hypoxic regions in tumor biopsies.

We investigated the potential of the method at the single cell level on cellular models in 2 glioblastoma cell lines in 2D culture, in HeLa cells, and in short term primary cultures derived from patient glioblastoma. We examined several hypoxic conditions mimicking those found in tumors: mild and severe hypoxia (1% and 0.1% O_2 respectively); acute (1 day), transitory (3 days) and chronic (5 days) hypoxia. The effect of the hypoxia mimetic drug dimethyloxalylglycine (DMOG) -an inhibitor of prolyl-hydroxylases, capable of upregulating HIF1 – was also studied. The most prominent and common change induced by mild hypoxia was an increase in lipid signal in all cell, and an increase in glycogen in the U87MG cell line. Each cell line presented an additional individual metabolic fingerprint. The spectral signature did not change markedly in severe hypoxia, and did not increase with the duration of the hypoxic stress, on the contrary it became more difficult to detect in the chronic hypoxia condition. The hypoxic signature was well replicated by DMOG.

Since respiration is essentially a mitochondrial event, we hypothesized that the hypoxic signature may be different in the spectra of the cytoplasm and in the spectra of the whole cell which are dominated by absorption from the nucleus. Spectra were thus recorded separately in the nucleus and in the cytoplasm of the D566MG glioblastoma cells thanks to the synchrotron source. Different hypoxic signatures were recorded in both compartments and we observed that this method improved the detection of hypoxia, particularly in the chronic hypoxia.

In conclusion, FTIR microspectroscopy allowed the detection of a spectral signature of hypoxia in all cell lines and all conditions investigated. As a follow-on experiment, we started searching this signature in tumors from an animal model of hypoxia.