Abstract

Immunohistochemical Expression of Ketolytic and Glycolytic Enzymes: Impact on Personalized Therapy in Adult Astrocytic Brain Tumors

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Astrocytic tumors represent one of the most aggressive and heterogeneous forms of central nervous system (CNS) neoplasms, characterized by high invasive potential, metabolic plasticity, and poor prognosis. Despite advances in therapy and the adoption of modern molecular classifications (WHO CNS5, 2021), patient survival remains limited, particularly in glioblastoma (GBM). This thesis explores the immunohistochemical expression of key enzymes involved in glycolytic (PKM2, HK2) and ketolytic (OXCT1, BHD1) pathways, integrating biological, clinical, imaging, and survival data to identify novel prognostic biomarkers and potential targets for personalized metabolic therapies.

The study was structured around a central hypothesis: as the malignancy grade of astrocytic tumors increases, glycolytic (proliferative) enzyme expression becomes upregulated, while ketolytic (oxidative) enzyme expression declines, reflecting a strategic metabolic shift toward an aggressive glycolytic phenotype. This hypothesis was explored through an extensive literature review on tumor energy metabolism, followed by the clinical and metabolic assessment of patients diagnosed with GBM and WHO grade 4 astrocytomas, and complemented by a quantitative immunohistochemical evaluation of the four key enzymes in a cohort of 86 astrocytic tumors.

The data revealed that patients with brain tumors exhibited significantly elevated blood glucose levels compared to healthy controls (132 vs. 96 mg/dL, $p < 0.001$), and this hyperglycemia correlated negatively with survival ($r = -0.94$, $p = 0.0018$), a finding further confirmed by linear regression. Although ketone body concentrations were also higher in tumor patients, their association with survival was not statistically significant. However, patients with KB levels ≥ 0.5 mM experienced 100% mortality, suggesting that elevated ketone levels may reflect a critical metabolic imbalance. A key contributor to these metabolic changes was the use of dexamethasone, a corticosteroid commonly prescribed in neuro-oncology to reduce vasogenic edema, but which can induce hyperglycemia and potentially reduce the efficacy of radiotherapy. These findings underscore the importance of glycemic control in HGG and the need for careful monitoring of corticosteroid therapy.

Immunohistochemical analysis of metabolic markers revealed significant differences among the enzymes studied. PKM2 was highly expressed in GBM, with significantly higher H-scores compared to lower-grade astrocytomas. Although PKM2 was not an independent prognostic factor, its expression correlated with tumor grade, indicating activation of the glycolytic pathway. HK2 showed a more variable pattern, with significant differences in the proportion of positive cells but not in quantitative scores across tumor types. In GBM, HK2 expression correlated positively with ATRX, suggesting a potential interaction between epigenetic remodeling and energy metabolism.

The ketolytic enzymes BHD1 and OXCT1 exhibited globally low expression across all tumor subtypes, particularly in GBM. Although differences between groups were not statistically significant, higher expression levels were observed in grade 2 astrocytomas, potentially indicating a residual capacity for ketone body utilization in less aggressive tumors. Interestingly, OXCT1 expression was negatively correlated with p53 in astrocytomas and positively in GBM, suggesting context-dependent regulatory mechanisms. Overall, the reduced expression of these enzymes implies a metabolic rigidity in high-grade tumors, rendering them almost entirely glucose-dependent.

Direct comparison between glycolytic and ketolytic enzyme expression revealed a distinct polarization of tumor metabolism. Strong positive correlations between PKM2 and HK2, and negative correlations with OXCT1 and BHD1, support the existence of two antagonistic metabolic axes. This observation confirms that high-grade tumors favor a hyperglycolytic metabolic profile, excluding ketone utilization and thereby increasing their vulnerability to glucose restriction or glycolysis inhibitors. The findings advocate for integrating metabolic profiling into therapeutic decision-making in gliomas. For example, markers such as PKM2 and HK2 may aid in identifying aggressive tumors suitable for glycolytic inhibition strategies, while OXCT1 and BHD1 could serve as favorable prognostic markers or therapeutic targets for reactivating ketolytic metabolism. Moreover, differential expression patterns based on molecular status (IDH1, ATRX, p53) highlight the need for a personalized approach that considers both the genetic and metabolic phenotype of the tumor.

This thesis provides an original contribution through an integrative analysis of the interplay between glycolytic and ketolytic pathways in astrocytic tumors, based on a significant patient cohort. It is among the few studies to apply digital immunohistochemical quantification for the evaluation of these four enzymes and to correlate their expression with clinical, molecular, and survival data. The results confirm that GBM is a tumor type with high glucose dependency and limited capacity for ketone metabolism, supporting the rationale for exploring adjunct metabolic interventions such as ketogenic diets, glycolysis inhibitors, or ketolytic activators.

In conclusion, this research supports the concept of personalized metabolic therapy in brain tumors, demonstrating that the tumor's enzymatic profile may serve as a diagnostic and prognostic tool, as well as a guide for targeted therapeutic interventions. Integrating metabolic data into personalized clinical models could improve patient survival and reduce the toxicity associated with standard treatments for adult gliomas.