Metabolite Imaging at the Margin: Visualizing Metabolic Tumor Gradients Using Mass Spectrometry

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Glioblastoma multiforme (GBM) tumors are highly metabolic and vascularized, yet little has been reported regarding the spatial localization of metabolic activity within these tumors. A mass spectrometry imaging (MSI) study by Randall and colleagues in this issue provides provocative observations of metabolic gradients in xenograft GBM models. The intensity of acylcarnitines is dramatically increased at tumor margins, which interface with normal tissue, but not in tumor margins at the edge of the brain. A secondary examination of drug metabolites suggests that the observed metabolic gradients are pharmacologically relevant. These findings underscore previously undescribed spatial metabolic heterogeneity in GBM biology and opportunities for MSI investigations.

See related article by Randall et al., p. 1258

They say that a picture is worth a thousand words. What is the value of thousands of pictures, each of which provides specific molecular information? This question lies at the heart of a study by Randall and colleagues (1) in this issue, in which metabolic differences between the tumor core and tumor edge are reported using intracranial mouse patient-derived xenograft models of glioblastoma multiforme (GBM) using mass spectrometry imaging (MSI). This technique allows for the multiplexed measurement of hundreds to thousands of individual molecules simultaneously using a rasterized scanning approach. Features that are unique to the biological sample under study, such as metabolites, lipids, and peptides, can be extracted to generate highly specific two-dimensional maps, decoding important spatial organization of metabolic and molecular concentrations. Using this approach, Randall and colleagues were able to describe previously undescribed metabolic heterogeneity in GBM. This is important as GBM, classified by the World Health Organization as a grade IV astrocytoma, represents the most common and aggressive malignant brain tumor affecting adults of all ages (2). Despite improvements in imaging, surgical resection, radiotherapy, and chemotherapy treatments, the prognosis for patients with GBM is poor, with a median survival of approximately 12 months and an estimated 5-year survival rate of 6.3% from diagnosis (3). This poor survival is due to the rapid proliferation, highly infiltrating growth, and invasive potential of GBM, making complete surgical resection almost impossible, resulting in tumor recurrence in most cases (2). Novel strategies and therapeutic targets are therefore imminently needed for the development of new therapeutic approaches. Although genomics, transcriptomics, and proteomics have proven to be promising prognostic tools that provide insight into the upstream signaling networks, the genetic alterations of an individual are not the only biological determinant of a tumor. Metabolite measurements not only complement genomics, transcriptomics, and proteomics information, but also encapsulate the influence of tissue biochemistry from environmental, physiologic, and pathologic factors. Therefore, metabolomics reflects the changes in phenotype that could contribute greatly to the understanding of the underlying metabolic alterations. Given the downstream position metabolites occupy in driving biology and phenotype, understanding the underlying metabolic pattern of GBM could help propose robust therapeutic targets or biomarkers for improving clinical diagnoses, prognostic characterization, and prediction of therapeutic response for patients with GBM.

GBM displays a high level of intratumoral heterogeneity as determined by single-cell genomic methods (4), characteristic of both newly detected and recurrent tumors. This intratumoral heterogeneity is likely due to the ability of GBMs to adapt to variations in the microenvironment and to therapies. The changes in cell functioning state are accompanied by metabolic variations that take place within the tumor, resulting in significant regional metabolic phenotypic heterogeneity within the same tumor. Therefore, metabolic profiling of GBM helps identify the perturbed molecular pathways and sheds light on the discovery of new diagnostic or prognostic biomarkers for GBM treatment (5). For studies of GBM metabolism, mass spectrometry of tissue extracts using LC/MS and gas chromatography–MS has been successfully employed for many years. Metabolites such as 2-hydroxyglutarate, glutamine, lipids, tricarboxylic acid cycle metabolites, and glycolytic intermediates have been reported using these techniques. In general, these approaches to metabolomics analysis require coarse sampling with relatively large tissue pieces extracted in bulk. As such, very limited spatial information can be obtained regarding differences in metabolism across a tumor, and certainly little information specific to metabolic gradients and tumor margins. In their study, Randall and colleagues addressed this gap using MSI. The specific ionization approach used in the study, matrix-assisted laser desorption/ionization (MALDI), is one of several ionization techniques (6) that can be used for MSI, with other examples including desorption ionization and secondary ion mass spectrometry.

These MSI techniques provide both spatial and molecular information directly from tissue sections, without labeling and without a priori knowledge, and thereby provide comprehensive and unbiased tissue characterization. A major advantage of MSI is the ability to correlate molecular detail to the tumor histopathology, allowing the demarcation of the tumor to be established and thus enhancing the ability to identify spatially differentiated features. MALDI-MSI has been applied in various metabolomic and lipidomic studies of

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cancerous tissues (7), as well as direct distribution monitoring of drug compounds, their metabolites, and target engagement in pharmaceutical research (7). To date, MALDI MSI has been mostly utilized to screen for new biomarkers of disease, particularly in the field of cancer research. Studies have suggested that MALDI MSI holds great promise in evaluating tumor margins and acquiring information on tumor typing, grading, prognosis, and prediction. In practice, this information has been slow to emerge. For example, Clark and colleagues and Dilillo and colleagues (8, 9) utilized this high resolution MALDI MSI method to identify biomarkers capable of distinguishing human pediatric brain tumors and to resolve isobaric lipids, metabolites, and tryptic peptides in a GBM mouse model. While some metabolites associated with GBM were successfully identified, no initial observation on the intratumoral metabolic heterogeneity using MSI was reported. Challenges include limited access to the technology, relatively low sample throughput, difficult sample preparation, and informatic challenges in analyzing the very large datasets produced.

In this article by Randall and colleagues, MALDI ionization was combined with a Fourier transform ion cyclotron resonance mass analyzer, which allows the measurement of mass signals with very high resolution and precision. Mass-to-charge ratios with a mass accuracy below 1 part per million can be determined using this setup (9). Other mass analyzers with lower resolution can also be successfully employed for mass analysis. For example, the only FDA-approved routine clinical use MALDI MS uses a time-of-flight analyzer. In this study, the authors observed an increased level of acylcarnitines at the edge of the tumor compared with the tumor core, as well as at the interface between tumor and normal tissue and not at the tumor edge adjacent to the outer edge of the brain, suggesting that GBM cells in the core and at the infiltration/proliferating tumor edge undergo different relative levels of fatty acid metabolism, resulting in intratumoral metabolic heterogeneity. This result was extended to three-dimensions for a representative tumor by examination of incremental sections 160 μm apart, demonstrating consistency of the acylcarnitine signature throughout the tumor. mRNA sequencing provided further preliminary confirmation that fatty acid metabolism transcripts were indeed altered as expected in samples with higher acylcarnitine content.

In terms of treatment, there are numerous challenges to chemotherapy for GBM tumors. One of the main challenges is the limited and heterogeneous drug delivery across the blood–brain barrier (BBB). The physiologic purpose of this BBB is to maintain normal brain function by protecting the central nervous system from toxins and pathogens (10). However, this same selective permeability restricts the delivery of potentially useful drugs from reaching the tumor cells. During tumor progression, the BBB is disrupted and is then referred to as the blood–tumor barrier (BTB). Although the BTB is more permeable than the BBB, its heterogeneous permeability to small and large molecules, as well as heterogeneous perfusion contributes to heterogeneous drug distribution in brain tumors. Hence, the BBB is still one of the critical factors limiting the efficacy of therapy in GBM. In a correlative analysis of the dataset, Randall and colleagues examined a number of other small molecules including heme and ATP. Heme visualization acts as a proxy for vasculature, providing an estimate of blood vessel size and tumoral vascularization. Worth noting is that the visualization may be confounded by previous bleeds where residual heme is present. ATP levels were observed to be inversely correlated with acylcarnitines, suggesting a role for vasculature in the distribution of acylcarnitines to the tumor.

To understand the association between tumor metabolism and drug efficacy, the elucidation of spatial relationships between drugs and biomolecules using different imaging techniques, both within the tumor as well as in the surrounding microenvironment, may provide important prognostic information. A common genetic feature of GBM is the overexpression/amplification of EGFR, with gene amplification noted in 40%–60% of patients with GBM and therefore, it is an attractive therapeutic target. In this study, the authors investigated the relationship between the distribution of erlotinib, a small-molecule EGFR tyrosine kinase inhibitor and its response. Interestingly, they found that higher intensity of erlotinib was detected in the tumor core compared with the tumor edge, demonstrating an inverse distribution of acylcarnitines. This potentially groundbreaking finding directly defines a metabolic gradient within the tumor correlated to heterogeneous drug distribution, suggesting a single drug might be ineffective in treating this disease due to the observed intratumoral heterogeneity. GBM development is driven by genetic and molecular alterations that target different signaling pathways, including the PI3K, p53, retinoblastoma, EGRF, VEGF, and PTEN pathways. Attempts to target these pathways with a single drug have given negative results in the clinic, possibly due to this type of metabolic gradient and the physiochemical properties of individual drugs. Overall, this study represents an important step in the spatial metabolomic profiling of GBM using MSI techniques. Further work will be required to understand whether these results extend to human tumors and to establish whether there is a relationship between metabolic gradients and GBM subtypes. It is clear, however, that MSI offers a previously undescribed window into the intratumoral metabolic heterogeneity, providing useful information for the design of therapeutic approaches through metabolic modulation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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