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COMMENT

The making of the glioblastoma classification

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Classification of cancer should lead to informative patients' stratification and selective therapeutic vulnerabilities. A pathway-based classification of glioblastoma uncovered a mitochondrial subtype with a unique sensitivity to inhibitors of oxidative phosphorylation. Precision targeting of cancer metabolism could provide therapeutic opportunities to a lethal neoplasm and be translated to other tumour types.

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MAIN

The history of the transcriptomic subtypes of glioblastoma

With the availability of transcriptomic, genomic and even proteomic data in the most recent years, the classification of many cancer types has evolved to coherently include molecular features that address prognostic questions and attempt to establish links between molecular subtypes, clinical parameters and therapeutic response to specific regimens.[1] The first molecular classification of glioblastoma (GBM) was reported in 2006 by Heidi Phillips and Ken Aldape.[2] It was based on differential expression of marker genes identifying three subgroups of GBM that carry mesenchymal, proneural and proliferative features, respectively. This classification was the cornerstone of subsequent subgrouping by Roel Verhaak who proposed a four-subtype classification in the context of The Cancer Genome Atlas (TCGA).[3] Later, Verhaak's group integrated information from bulk tumours and individual tumour cells and revised the classifier to three subclasses sharing mesenchymal and proneural groups with the earlier Phillips/Aldape classification.[4] The common thread between the classifications was the use of signatures of differentially expressed genes traceable as cell identity markers. The TCGA classification linked the three subclasses to distinct genetic alterations (e.g. genetic alterations of *IDH1*/*PDGFRA* and *NF1* more frequently in the proneural and mesenchymal group, respectively, whereas amplification of *EGFR* was the distinctive feature of the classical subtype).[3] These widely used GBM classifiers established that heterogeneity of molecular features is intrinsic to GBM. However, when the clinically more favourable isocitrate dehydrogenase (IDH)-mutant GBM was excluded, the transcriptomic subtyping could not convey prognostic information. Furthermore, in the era of personalised cancer therapeutics, the classifiers failed to capture vulnerabilities to selective therapeutics. The lingering question remained as to whether more informative segmentation of GBM was attainable and which molecular/biological hallmarks should be extracted to reach this goal.

A single-cell-informed and pathway-based classification of IDH wild-type GBM

As the main obstacle to accurately classify GBM is the multi-level heterogeneity, which is also embodied by the glaring diversity of normal cell infiltrates in different tumours, our group sought to build a GBM classifier based on the following pillars:

1. the classification of bulk tumours should be consistent with the biological properties of single GBM cells, thus reflecting activities intrinsic to tumour cells;
2. rather than identifying features (i.e. markers of cell identity), we should aim to extract the core functions of GBM cells, as only the identification of the main biological attributes of individual tumour cells could point to potential therapeutic targets;
3. GBM subtypes should be derived using the biological activities that not only reflect single-cell biology subclasses, but are also associated with patients' survival.

Based on the above considerations, as the first and crucial step to build a functional and clinically informative GBM classification, we devised a computational platform for the identification of functional activities in single cells selected from 5032 biological pathways. The computational platform was named as single-cell biological pathway deconvolution (scBiPaD), which integrated multiple GBM single-cell RNA-sequencing (scRNAseq) datasets while eliminating the technical variations due to different handling of scRNAseq batches.[5] Rather than clustering directly the activity values of cell sub-populations from the different datasets, we represented each sub-population with a binary vector of length 5032, with 1 indicating the enriched biological pathway. The final cluster assignment was derived from the coefficient of similarity evaluating the degree of enrichment overlap between cell populations. The typing of single cells identified four functional cellular subtypes of GBM characterised by neural development (neuronal and proliferative/progenitor) or metabolic (mitochondrial and glycolytic/plurimetabolic) attributes. Within individual tumours, cell states were distributed with distinct patterns of co-existence governed by the metabolic and neurodevelopmental axis, respectively. Interestingly, the spatial analysis of the functional states in primary GBM revealed a gradient of proliferative/progenitor to neuronal states distributed along a tumour core to periphery trajectory with enrichment of proliferative/progenitor cells in the core and progressive

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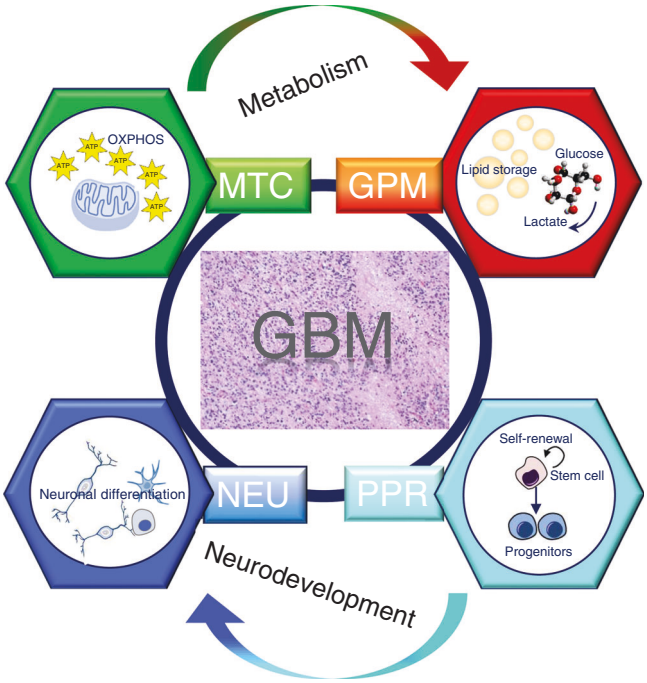

Fig. 1 Four-group functional classification of glioblastoma. Glioblastoma was classified according to the elevation of functional activities in single cells and bulk tumours. The novel subtypes aligned with two major axes exhibiting attributes of neurodevelopment (NEU neuronal, PPR proliferative/progenitor) or divergent metabolic programmes (MTC mitochondrial, GPM glycolytic/plurimetabolic). The neurodevelopment branch recapitulates the transition from neural stem/progenitors to differentiated neurons with spatial and temporal evolution from PPR to NEU at the tumour periphery and recurrence. Within the metabolic branch, MTC cells are selectively dependent on OXPHOS for vital energy and suffer from severe loss of viability when challenged with drugs that inhibit OXPHOS. In contrast, GPM cells are highly resistant to metabolic targeting as a consequence of multiple interconnected metabolic pathways. Among the four glioblastoma subtypes, MTC tumours are associated with better clinical outcome.

acquisition of more differentiated neuronal features in tumour cells located at a higher distance from the core. The tumour periphery was populated by the most differentiated tumour cells exhibiting activation of the synaptic pathways that enable connectivity with normal neurons and drive aggressiveness of glioma.[6,7]

Next, we constructed a computational strategy in which a selected set of survival-associated biological pathways was used to classify bulk IDH wild-type GBM. The pathway-based classification of GBM returned four tumour subtypes each of which was identified by the same four biological activities uncovered in single cells. The comparative analysis of a dataset of matched primary/recurrent GBM uncovered reduction of proliferative/progenitor and gain of neuronal subtypes as the main switch associated with GBM recurrence, thus highlighting a remarkable overlap between the spatial and temporal evolution of GBM (Fig. 1).

Mitochondrial GBM exhibits vulnerability to oxidative phosphorylation inhibitors

The new pathway-based classification of GBM raises several key questions: how does the classifier compare with the "TCGA classification"? Does it deliver on prediction of clinical outcome and/or guide therapy? One key difference in the pathway-based classification was the introduction of metabolism-associated subtypes. A convincing association was found between glycolytic/plurimetabolic and mesenchymal subgroups, indicating that the glycolytic/plurimetabolic activity and mesenchymal identity are inseparable features in GBM. On the opposite spectrum of the metabolic axis, the mitochondrial group was orthogonal to the TCGA subtypes as it included similar fractions of mesenchymal, classical and proneural GBM, suggesting that oxidative phosphorylation (OXPHOS) programmes are not restricted to a specific cell identity. The proliferative/progenitor and neuronal subtypes were almost completely restricted to proneural and classical subtypes and excluded from the mesenchymal subgroup. Patients harbouring tumours classified as mitochondrial had significantly longer survival than any other group, a finding confirmed in four GBM datasets.[5] The functional traits of mitochondrial and glycolytic/plurimetabolic subgroups underpin key metabolic dependencies that have a divergent impact on metabolic vulnerability. Mitochondrial GBM relies on OXPHOS for energy production and survival, and exhibits marked sensitivity to mitochondrial inhibition. Conversely, glycolytic/plurimetabolic tumours are sustained by concurrent activation of multiple energy-producing programmes (aerobic glycolysis plus anabolism of lipids and amino acids), which manage to escape mono-targeting of metabolic pathways. We did not fully explore what could cause the better clinical outcome of mitochondrial GBM, but gliomaspheres classified as mitochondrial were more sensitive to irradiation and generally produced higher levels of reactive oxygen species than glycolytic/plurimetabolic gliomaspheres. Other mechanisms need to be investigated including the inability of mitochondrial GBM to adapt to unfavourable environments such as the inadequate energy supply triggered by limiting oxygen concentrations required for the optimal OXPHOS activity of this subtype. We have also indicated that mitochondrial complex I inhibitors delay tumour growth and prolong survival of mice bearing mitochondrial intracranial PDX when compared with mice bearing glycolytic/plurimetabolic PDX or controls receiving vehicle (A. Lasorella and A. Iavarone, unpublished data). Thus, the main actionable implication of the new pathway-based classification of GBM is that targeting OXPHOS activity is a viable therapeutic strategy in patients with the mitochondrial subtype. However, the lack of clinically applicable tests to stratify patients with mitochondrial GBM will have to be resolved to launch accurate clinical trials. We have identified transcriptomic signatures of the four GBM subtypes and they could be used to classify patients using CLIA-certified global transcriptomic profiles obtained from paraffin-embedded tissues. These assays are increasingly offered by Cancer Centres across the United States and Europe. As an alternative, we are testing selected panels of expressed genes and/or protein biomarkers to be detected by targeted assays (e.g. NanoStrings, immunohistochemistry, etc.). It will also be interesting to evaluate whether mitochondrial subtypes exist also in the context of other tumour types and whether they will have similar sensitivity to OXPHOS inhibition. A bigger targeting challenge is represented by the other GBM subtypes. Additional segregation of individual groups into even more refined and homogeneous subtypes, combined with the integration of highly informative multi-omics platforms (proteomics, phosphoproteomics, metabolomics, etc.), may offer the most promising insights to expand targeted therapeutics to larger fractions of GBM patients.

AUTHOR CONTRIBUTIONS

A.I. and A.L. conceived and wrote this Comment article.

ADDITIONAL INFORMATION

Ethics approval and consent to participate Not applicable.

Data availability Not applicable.

Competing interests A.L. and A.I. are listed as inventors on patent applications filed by Columbia University related to the diagnostic and therapeutic applications of the functional classification described here.

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