Shedding light on glioblastoma cellular heterogeneity

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Cellular heterogeneity is one of the key contributors to poor clinical outcome in glioblastoma (GBM). In this issue Lathia and colleagues report on the development of a novel mouse model where the GFAP-tva mouse harbors a Sox2-EGFP reporter to enable direct visualization of intratumoral cellular heterogeneity in a RCAS-PDGF-driven GBM model. Cellular heterogeneity is associated with one of the salient features of glioblastoma stem cells (GSCs). Multiple studies have elucidated that GSCs are a unique subpopulation of cells within GBM and are responsible for hierarchical cellular organization. Additionally, GSCs may potentially orchestrate multiple other aspects of GBM biology, including resistance to therapy, tumor recurrence, and cellular invasion, thus contributing to dismal clinical outcomes despite aggressive therapy. The characterizations of GSCs isolated/enriched from surgical human GBM specimens have lent support to the cancer stem cell hypothesis and have opened avenues for understanding the complex dynamics of cancer progression, recurrence, and resistance to therapy. Thus far, marker-based enrichment of GSCs has remained controversial due to the potential dissociation from their ‘stem-cell state’ once removed from their native tumor niche (e.g. perivascular).

Genetically engineered mouse (GEM) models of glioma have resulted in valuable information with respect to de novo tumor development and have recapitulated the molecular sub-grouping of human GBM. GEM models have been particularly beneficial in evaluating the oncogenic potential and targeted therapy of specific cell types in tumor development, such as simultaneously targeting of quiescent cancer stem cells along with highly proliferative non-stem cancer cells. The research community, however, urgently needs a reliable marker that will uniquely identify GSCs as well as play a key role in maintaining and regulating its properties.

The sex-determining region Y box 2 (Sox2) transcription factor is one of the four Yamanaka factors of induced pluripotency and has emerged as a marker and regulator of ‘stemness’ of cancer stem cells in multiple cancer models. Sox2, as an established stem cell marker in both normal and cancer contexts, provides a unique model system in which the reporter is also the regulator of GBM ‘stemness’.

The mouse model described by Stoltz et al. is very exciting as it merges the potentials of lineage tracing experiments with the flexibility of the RCAS-tva (as it can easily be adopted to different oncogenic stimuli) system while reporting on intratumoral cellular heterogeneity (both de novo and allografts). By undertaking a faster allograft approach (without intermediate cell culture) to evaluate cellular heterogeneity, the authors show that the relative abundance of Sox2-EGFPHigh cells appears to be specific to these allograft tumors and is in line with other GFAP-tva models. In this model the Sox2-EGFPHigh cells can give rise to Sox2-EGFPLow cells in the tumor and EGFP expression itself was tightly correlated with the state of the cell, thus recapitulating certain aspects of cancer stem cell biology. Additionally, enrichment of Sox2-EGFPHigh cells in the perivascular niche (as judged by their proximity to CD31+ blood vessels) in allograft tumors suggests that this model has great potential with respect to the characterization of the intratumoral stem cell niche. The authors further show the value of their model in identifying novel mechanisms associated with specific cellular (stem and non-stem cell) compartments of the tumor. Through kinomic analyses p-Fes, among others, was identified to have elevated phosphorylation in the Sox2-EGFPHigh fraction of sub-cutaneous allografts. Clinical correlation shows that high expression levels of Fes are correlated with poor prognosis in glioma patients. Furthermore, a significant proportion of p-Fes-positive cells were also Sox2-positive in both patient and xenograft tissues; thus, it is conceivable that these models will continue to identify novel molecules/pathways that can be evaluated as potential therapeutic targets.

Going forward, GEM models will be particularly useful for addressing multiple pressing questions in the field of GBM biology, such as the ‘seed and soil’ determinants and the plasticity status of the niche during de novo tumor formation and upon exposure to therapy. Also, these models may tell us what types of cells can populate or re-populate the niche after selective...
targeting of quiescent cells from these niches, etc. There are several cellular hierarchy and plasticity models that can help in explaining intratumoral cellular heterogeneity; depending on the relative contribution of various cell types, one may predict various scenarios for clinical response to therapy. It will be exciting to combine this GEM model described by Stoltz et al. with other immunocompetent models that specifically ablate quiescent cells and mark progenies of Sox2+ cells to address questions of cellular hierarchy and plasticity in GBM. It will also be important to connect these cellular phenotypes to responders vs. non-responders to a therapeutic regimen.

The ability to (re-) isolate these specific cell types from the tumor microenvironment is particularly exciting as it provides an opportunity to perform in-depth genomic analysis of these cells and undertake a systems biology approach to the development of pre-clinical targeted therapies. In the clinical, imaging modalities such as MRI are unique as they potentially reflect de novo tumor heterogeneity in its entirety. For a seamless translation of information from pre-clinical research to the clinical setting it is vital that some of the newer branches of research such as imaging-genomics are incorporated early on during pre-clinical research. It will be exciting to extract imaging texture features from MRI scans of tumors (with or without treatment) of GEM models and to perform texture-defined image-guided biopsies followed by genomic analyses. Once robust algorithms connecting cellular heterogeneity to radiophenotypes have been developed and validated in pre-operative MRI scans of GBM patients, it will augment prospective clinical decision-making. These are exciting times in glioblastoma research where novel genetically engineered mouse models, such as the one presented by Lathia and colleagues, shed new light on GBM heterogeneity and cancer biology.

References