Editorial

Metabolic imaging of c-Myc expression in patient-derived xenograft models of glioblastoma

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Glioblastomas form highly infiltrative tumors that have a dismal prognosis, with a median survival of just 15 months from diagnosis. This poor prognosis has been attributed to extensive inter- and intra-tumoral heterogeneity. Although glioblastomas were classified into four classes based on genomic analyses (proneural, neural, mesenchymal and classical), later studies showed that members of each class were present within individual tumors, which could explain the variable responses to treatment. As well as genetic heterogeneity, there is also microenvironmental heterogeneity, which can influence metabolism, gene expression [1] and again treatment outcomes. Standard of care treatment involves maximal surgical debulking followed by chemoradiation. However, the infiltrative behavior of glioblastomas enables them to escape this treatment, with most tumors recurring within 2–3 cm of the resection site [2]. There is, therefore, a need to develop new and better treatments.

With modern targeted drugs the challenge is to identify "actionable" mutations that allow the drug to be matched to the tumor, targeting the specific processes driving cell growth and proliferation in that specific tumor type [3]. These can be identified by DNA sequencing of tumor biopsies or, potentially, *via* analysis of circulating tumor DNA. An alternative approach is to use imaging to detect the very earliest response of tumors to treatment, which has the potential to allow rapid selection of the most effective treatment for an individual patient. Moreover, imaging may also detect differential responses within and between tumors, which arise from their genetic and microenviromental heterogeneity. An alternative and complementary imaging approach to therapy selection would be to detect underlying tumor genomic changes from pre-treatment image features and then infer prognosis and drug sensitivity. Imaging has the advantage over tissue biopsy that it can more readily detect heterogeneity (both intra- and inter-tumor) and, furthermore, by imaging tumor phenotype may be a more effective way of selecting drugs than identifying tumor mutations.

Hyperpolarization of ^{13}C-labeled cell substrates increases their sensitivity to detection in the MR experiment by more than 10,000x [4], making it possible to image the location and subsequent metabolism of a hyperpolarized ^{13}C-labelled cell substrate in the body, with spatial resolutions of 2 – 5 mm and temporal resolutions in the sub second range. The technique has now translated to the clinic [5]. In a recent study published in *Cancer Research* we showed in patient-derived xenograft (PDX) models of glioma implanted orthotopically in rats that tumor lactate labelling, following injection of hyperpolarized [1-^{13}C]pyruvate, displayed inter-tumoral heterogeneity, reflecting the intra- and inter-tumoral heterogeneity in the patients' tumors from which they were derived [6]. Labeling in some patient-derived tumors could be observed before their appearance in morphological images, suggesting that the technique could be used to detect occult disease, whereas in contrast, in other tumors it was not significantly greater than in the surrounding brain. A recent study in a glioblastoma patient also showed that tumor lactate labeling was no greater than that in the surrounding brain tissue [7] and accords with earlier work showing that gliomas oxidize much of the glucose that they consume in the TCA cycle rather than producing lactate [8, 9], which is perhaps contrary to the conventional view of tumor metabolism. Increased lactate labelling in the glioma PDXs correlated with c-Myc–driven expression of hexokinase 2 (HK2), lactate dehydrogenase A (LDHA), and plasma membrane expression of the mono-carboxylate transporters (MCTs). LDHA and the MCTs are responsible for exchange of the hyperpolarized ^{13}C label between the injected pyruvate and the endogenous lactate pool and the increased expression of HK2 may explain the increased lactate concentration in some of these tumors, which will also stimulate the exchange between pyruvate and lactate. A recent study suggested that increased lactate labeling in preclinical brain tumor models is the result of increased vascular permeability [10]. However, we found no evidence for this since we observed increased lactate labeling in PDXs that showed no signal enhancement following injection of a gadolinium-based contrast agent.

The patient-derived cells that showed the highest levels of c-Myc and lactate labeling *in vivo* were more radio-resistant *in vitro*, suggesting that the technique potentially could be used to select regions of tumors for increased radiotherapy dose ("dose painting"). However, this needs to be verified in cells in which c-Myc has been knocked down, which we showed decreased lactate labeling *in vivo*. Furthermore, it is not yet clear whether imaging with hyperpolarized [1-^{13}C]pyruvate will have sufficient spatial resolution in the clinic to make it an effective tool for targeting radiotherapy. Lactate labeling

in those PDXs that showed the highest levels of labeling was still less than that in an established cell line model (U87), suggesting the latter are not a good model of the human disease, at least in terms of their metabolism.

In summary, this study in glioma PDXs suggested that imaging with hyperpolarized [1-^{13}C]pyruvate could be used clinically to determine disease prognosis, to detect early responses to drugs that modulate c-Myc expression, and to target radiotherapy.

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REFERENCES

1. Cooper LA, et al. Am J Pathol. 2012; 180:2108-19. https://doi.org/10.1016/j.ajpath.2012.01.040.
2. Cuddapah VA, et al. Nat Rev Neurosci. 2014; 15:455-65. https://doi.org/10.1038/nrn3765.
3. Garraway LA. J Clin Oncol. 2013; 31:1806-14. https://doi.org/10.1200/jco.2012.46.8934.
4. Ardenkjaer-Larsen JH, et al. Proc Natl Acad Sci U S A. 2003; 100:10158-63.
5. Nelson SJ, et al. Science Translational Medicine. 2013; 5:198ra08. https://doi.org/10.1126/scitranslmed.3006070.
6. Mair R, et al. Cancer Res. 2018; 78: 5408-18. https://doi.org/10.1158/0008-5472.Can-18-0759.
7. Miloushev VZ, et al. Cancer Res. 2018; 78:3755-60. https://doi.org/10.1158/0008-5472.Can-18-0221.
8. Marin-Valencia I, et al. Cell Metab. 2012; 15:827-37. https://doi.org/10.1016/j.cmet.2012.05.001.
9. Maher EA, et al. NMR in Biomedicine. 2012; 25:1234-44. https://doi.org/papers3://publication/doi/10.1002/nbm.2794.
10. Miller JJ, et al. Scientific Reports. 2018; 8:15082. https://doi.org/10.1038/s41598-018-33363-5.