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COMPUTATIONAL APPROACHES FOR METABOLIC TARGET DISCOVERY IN IMMUNO-ONCOLOGY

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Background Metabolic alterations within the tumor microenvironment are thought to contribute to immunotherapy resistance. Dysregulation of metabolism in cancer cells affects the expression of surface markers and secreted metabolites, thereby impeding immune surveillance, suppressing immune infiltration, and dismantling anti-tumor immune cell function. Targeting metabolic vulnerabilities has therefore emerged as an avenue for therapeutic development.

Methods We applied genome-scale metabolic models (GEMs), contextualized with transcriptomic data, to elucidate complex metabolic networks of mammalian systems and predict selective targets. As proof-of-concept, we identified tumor-specific reactions and enzymes that may mediate resistance in B16 syngeneic tumor models but not in immunotherapy-responsive MC38 models, with and without the glutamine antagonist DON (6-diazo-5-oxo-L-norleucine), a metabolic therapy which has been shown to modulate immune cell function.¹ Flux balance analysis was performed using COBRApy on Mouse-GEM models customized with bulk RNAseq data from either B16 or MC38 lines using tINIT from the RAVEN Toolkit.²⁻³ Single-cell RNA-seq from *in vivo* B16 and MC38 tumors was analyzed using the Compass algorithm to capture differential metabolic states for *in vivo* tumor cells.⁴

Results These computational approaches revealed that B16 tumor cells have enhanced activity in glycolysis, TCA cycle, NAD metabolism, fatty acid synthesis and sphingolipid metabolism, while MC38 tumor cells show enhanced activity in fatty acid oxidation. Moreover, these findings suggest that B16 tumors have different metabolic dependencies than MC38 tumors, which may be in part responsible for altered responsiveness to immune checkpoint blockade. We experimentally validated several selective targets (MDH2, OGDH, PRODH, NAMPT, SLC35A2, and UGCG) linked to TCA cycle, NAD salvage pathway and glycosphingolipid metabolism and demonstrated that small-molecule inhibition or genetic loss of these targets in B16 tumor cells reduces *in vitro* cell proliferation and delays *in vivo* tumor growth.

Conclusions We conclude that the application of bulk- and single-cell computational methods effectively enables *in silico* exploration and identifies metabolic targets for therapeutic discovery.

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REFERENCES

1. Robert D Leone, *et al.* Glutamine blockade induces divergent metabolic programs to overcome tumor immune evasion. *Science* 2019;**366**:1013–1021. DOI:10.1126/science.aav2588
2. Ebrahim A, Lerman JA, Palsson BO, *et al.* COBRApy: CONstraints-Based Reconstruction and Analysis for Python. *BMC Syst Biol* 2013;**7**:74. <https://doi.org/10.1186/1752-0509-7-74>
3. Gustafsson J, Anton M, Roshanzamir F, Jörnsten R, Kerkhoven EJ, Robinson JL, Nielsen J. Generation and analysis of context-specific genome-scale metabolic models derived from single-cell RNA-Seq data. *Proc Natl Acad Sci* 2023;**120**(6): e2217868120. doi:10.1073/pnas.2217868120

4. Wagner, *et al.* Metabolic modeling of single Th17 cells reveals regulators of autoimmunity. *Cell*, 2021;**184**(16):4168–4185. <https://doi.org/10.1016/j.cell.2021.05.045>.

Ethics Approval All research was performed as part of Calico Life Sciences LLC AAALAC-accredited animal care and use program. All research and animal use in this study was approved by the Calico Institutional Animal Care and use Committees (IACUC).

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