Macromolecular Crowding (MC) potentially contributes to the Warburg effect in cancer cells

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Cancer cells simultaneously exhibit glycolysis with lactate secretion and mitochondrial respiration even in the presence of oxygen, a phenomenon known as the Warburg effect, the origin of which remains partially understood. Previous work, integrating the macromolecular crowding (MC) constraint into the Flux Balance Analysis (FBA) modeling framework (FBAwMC) strongly implied that MC represents an important bound for cellular metabolism. Here, we aim to assess the potential role of MC in affecting the energy metabolism of cancer cells. We find that cells with higher growth rates (a proxy of MC) are more glycolytic, with less mitochondrial respiration.

Keywords **— Molecular crowding (MC), glycolysis, Warburg effect**

I. PURPOSE

Cancer cells simultaneously exhibit glycolysis with lactate secretion and mitochondrial respiration even in the presence of oxygen, a phenomenon known as the Warburg effect. It is widely accepted that the Warburg effect evolves through adaptation to the hypoxic condition or oncogenic mutations. However, aerobic glycolysis is not found exclusively in cancer cells, but is also observed in rapidly dividing normal cells even under conditions of normoxia, suggesting an intrinsic characteristic of fast growing cells in constraining the cellular metabolism. Our previous studies, integrating the macromolecular crowding (MC) constraint into the Flux Balance Analysis (FBA) modeling framework (FBAwMC) successfully predicts changes in intracellular metabolic flux distribution where MC is indirectly influenced by changing the growth rate of *E. coli* cells (*1*). In addition, incorporation of MC into a yeast glycolysis kinetic model successfully predicts intracellular metabolite levels in *S. cerevisiae* (*2*).

Here, we aim to assess the potential role of MC in affecting the energy metabolism of cancer cells. We first studied a reduced flux balance model of ATP production that is constrained by the glucose uptake capacity and the MC constraint *(3)*. The model indicates that the Warburg effect is a favorable catabolic state for rapidly proliferating mammalian cells with high glucose uptake capacity. Experimentally, we examined human BJ fibroblast cell lines with serial oncogenic changes (*4*) and stepwise increase in growth rates as a proxy for MC levels to study MC effects on the energy metabolism. We find that the fastest growing cell line exhibits the highest lactate excretion rate and the lowest mitochondrial respiration rate, which are consistent with the model predictions. Besides oncogenic manipulation, we also utilized the same fibroblast cell line but with different growth rates due to the different passage numbers, again finding that faster growing fibroblasts were more glycolytic and displayed lower mitochondrial respiration. This indicates that the aerobic glycolysis may arise from the high growth rate or high MC environment. In the near future, we plan to directly manipulate intracellular MC by overexpressing an exogenous protein in HEK293 cells. The results will provide information about the correlation between the energy metabolism and MC level (intracellular density).

II. CONCLUSION

Our result indicates that even without specific oncogenic manipulation, cells with higher growth rates tend to be more glycolytic than cells with lower growth rates. It also suggests that the Warburg effect is a favorable catabolic state for *all* rapidly proliferating mammalian cells with high glucose uptake capacity due to the MC constraint in the highly crowded cytoplasm. These results may have direct relevance to chemotherapeutic strategies attempting to target cancer metabolism.

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