ELIMINATION OF THE “ESSENTIAL” WARBURG EFFECT IN MAMMALIAN CELLS THROUGH A COMBINATORIAL GENOME ENGINEERING STRATEGY

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Over the past 3 decades, mammalian cells have become the predominant production hosts for biotherapeutics, and now produce 6 of the top 10 grossing pharmaceuticals. However, the complexity of the protein-based drugs and the host cells pose major challenges that must be controlled to improve the safety, efficacy, and affordability of these pharmaceuticals. One major challenge that has plagued the industry is that immortalized mammalian cells secrete large quantities of lactic acid through the Warburg effect. This leads to premature cell death, reduced product yields, and often lower quality products. The only innovations that have mitigated lactate secretion have included enzyme inhibition, knock-down and media optimization. Indeed, numerous efforts have tried, and failed to eliminate the key enzymes involved in lactic acid secretion. This is because the enzymes have proven essential for immortalized cell growth. Here I present our work in which we discovered a panel of genes that control lactic acid secretion. Genome editing efforts targeted at individual genes or multiple genes in serial were unsuccessful, but the implementation of multiplex genome editing using CRISPR technologies in multiple mammalian cell cultures successfully eliminated lactic acid secretion and enabled the deletion of multiple “essential” genes. Surprisingly, the cells show improved metabolic and growth phenotypes, despite the elimination of this fundamental metabolic activity. To understand how immortalized mammalian cells can cope without this seemingly essential metabolic process, we conducted a comprehensive analysis of these cell lines using time-course RNASeq, metabolomics, and analysis with a genome-scale metabolic network model developed for Chinese hamster ovary cells1. We further characterize its impact on recombinant drug production yields and quality. Thus, through a detailed and multiplex metabolic engineering effort and comprehensive systems biology analysis, we have been able to engineer out a leading challenge in protein biotherapeutic development and begin to understand now a cell can survive without a seemingly essential process.