ELIMINATION OF THE WARBURG EFFECT IN CHINESE HAMSTER OVARY (CHO) CELLS IMPROVES CELL PHENOTYPE AS A PROTEIN PRODUCTION PLATFORM

Hooman Hefzi, Novo Nordisk Foundation Center for Biosustainability at the University of California, San Diego
hhefzi@ucsd.edu
Soo Min Noh, KAIST
Iván Martínez Monge, Autonomous University of Barcelona
Nusa Pristovsek, Novo Nordisk Foundation Center for Biosustainability, DTU
Marianne Decker, Novo Nordisk Foundation Center for Biosustainability, DTU
Maria Javidi, Novo Nordisk Foundation Center for Biosustainability, DTU
Kristian Jensen, Novo Nordisk Foundation Center for Biosustainability, DTU
Anders Holmgaard Hansen, Novo Nordisk Foundation Center for Biosustainability, DTU
Stefan Kol, Novo Nordisk Foundation Center for Biosustainability, DTU
Johnny Arnsdorf, Novo Nordisk Foundation Center for Biosustainability, DTU
Zulfiya Sukhova, Novo Nordisk Foundation Center for Biosustainability, DTU
Karen Kathrine Brondum, Novo Nordisk Foundation Center for Biosustainability, DTU
Patrice Ménard, Novo Nordisk Foundation Center for Biosustainability, DTU
Sara Bjorn, Novo Nordisk Foundation Center for Biosustainability, DTU
Helle Petersen, Novo Nordisk Foundation Center for Biosustainability, DTU
Lars Nielsen, Novo Nordisk Foundation Center for Biosustainability, DTU
Helene Faustrup Kildegaard, Novo Nordisk Foundation Center for Biosustainability, DTU
Gyun Min Lee, Department of Biological Sciences, KAIST
Bjorn Voldborg, Novo Nordisk Foundation Center for Biosustainability, DTU
Nathan E. Lewis, Novo Nordisk Foundation Center for Biosustainability, UCSD

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Lactate is a common metabolite and is central to many important processes. One of its more prominent roles is in the Warburg effect, in which cancer cells exhibit high rates of glycolytic flux followed by secretion of lactate, even in the presence of oxygen. This fermentation of pyruvate to lactate via lactate dehydrogenase (Ldh) accompanies increased proliferation of cancer cells and several other types of rapidly proliferating cell types in immune cell activation and embryonic development. Aerobic glycolysis is also prominent in biotherapeutic protein production, where mammalian production cells often secrete high levels of lactate. The accumulation of lactate is deleterious for cell growth, viability, product formation, and quality, both directly via acidification of the media and indirectly through base addition to control culture pH.

Despite a clear genetic target, efforts to eliminate lactate secretion via knockout of Ldh(s) in mammalian cells have been unsuccessful, pointing to the essentiality of Ldh mediated NAD regeneration. A wide variety of approaches have been utilized to limit lactate accumulation in culture, including knockdown or inhibition of Ldh, replacement of glucose with alternate sugars, controlled feeding strategies, and many others, however none have proven successful in eliminating the Warburg effect.

We report the elimination of the Warburg effect in a CHO cell line by using CRISPR/Cas9-based engineering to simultaneously knockout enzymes responsible for lactate production and ancillary regulators. The resulting cell lines remain proliferative while consuming significantly less glucose and can be used to generate protein producing lines using standard industrial processes. In a pH-controlled fedbatch process, the Warburg null cells require minimal base addition to maintain a stable pH, allowing an extended growth phase. The knockout strategy was also successfully applied to a CHO cell line producing Rituximab, again resulting in a prolonged growth phase. Additionally, protein production was maintained, while product quality was improved with increased glycan galactosylation. Thus, CHO cells without the capacity of Warburg metabolism may be useful for engineering production cell lines with enhanced bioproduction traits.