Identification and Regulation of Heritable Biomarkers for Manufacturing Stress Tolerance in CHO to Improve Monoclonal Antibody Production Performance

Spencer Grissom
Advisor: Mark A. Blenner
Committee Members: Wilfred Chen, Kelvin H. Lee, Abhyudai Singh
Tuesday, September 30th, 2025 at 2:00 PM (EST)

Ammon Pinizzotto Biopharmaceutical Innovation Center , Room 140 |
https://udel.zoom.us/j/7826764735?omn=92785358315 | Password: Memory

---

Biologics, a class of therapeutics derived from living systems, have revolutionized modern medicine and unlocked treatment options for a wide class of diseases. For its production, Chinese hamster ovary (CHO) cells have gradually become the preferred host organism due to its compatibility with large reactor systems, high production, and patterns of human-like post-translational modifications. Any efforts to increase productivity translates to lower operating costs and increases the affordability and accessibility to therapeutics. However, large-scale bioreactors and CHO cell's inefficient overflow metabolism contribute to the accumulation of inhibitory environmental perturbations that invariably reduce cell growth and volumetric productivity. These include elevated osmolality, oxidative stress, ammonia, and lactate levels that disrupt cellular structure, cause cell-cycle dysregulation, and may induce apoptosis. Traditional cell line development (CLD) workflows often do not screen stress tolerance during clonal evaluation and therefore represents a vulnerability in isolating cell lines suited for scale-up and stress resiliency. Approaches to improve stress tolerance have largely been centered around bioreactor control strategies and knockout of metabolic or apoptotic regulating genes. These have relied on conventional heuristics for cell health, but do not consider or characterize the adaptative regulatory networks that form robust resistance. In this study, a novel approach utilizing population-based transcriptomics for the identification of unique biomarkers for bet-hedging and stress tolerance is demonstrated. Downstream genetic engineering was used to generate biomanufacturing stress-tolerant cell lines with improved growth characteristics across two different biomanufacturing-relevant environmental perturbations.

Initially, three of the most explored stress agents (ammonia, lactate, and osmolality) were used to stress shock monoclonal antibody (mAb) producing CHO cells in fed-batch to determine the phenotypic, morphological, and transcriptomic effects of perturbation. At supplemented concentrations of 10 mM ammonia and 100 mOsm/kg, cell specific growth rates, peak viable cell densities, and the integral of viable cell density (IVCD) were significantly reduced. This translated to a reduction in volumetric productivity or loss of titer. At lactate concentrations of 15 mM, inhibitory effects were not observed, possibly due to a higher baseline tolerance in the cell line or a context dependency in well-controlled environments. Disruption of lysosomal structure, hydrolase activity, and amino acid metabolism were observed in ammonia stress and an increase in surface bound transporters and translational activity were observed in osmotic stress. While this information provided insight regarding the phenotypic effects of stress, broad differential gene expression analysis highlighted confounding and ambiguous patterns of expression that convolutes the search for rational engineering targets.

While traditional transcriptomics are useful, they are often insufficient in elucidating clear targets for genetic engineering. An alternative method for identifying stress-associated biomarkers was explored using a population-based transcriptomic tool known as MemorySeq. This method utilizes RNASeq fluctuation analysis of roughly 40 single-cell derived populations after 17 generations of growth to identify highly variable genes that correlate to intermediate, transient, and heritable memory states. These unique transgenerational properties have been linked to bet-hedging and broad stress resistance mechanisms in cancer, plant, and microbial cells. Using this tool, 199 unique genes with heritable properties were identified and found to be enriched in signaling/communication, regulation of cell proliferation, and apoptosis regulation functionalities. They also significantly overlapped with the differentially expressed genes in stress shocked populations, highlighting their role in early pre-stress resistance states.

With genetic targets identified, stable and homogenous genetic engineering tools would permit replicable characterization of their effect on cell health. To streamline CHO cell line engineering efforts and regulation of native genes, a flexible and modular targeted integration toolkit was developed to accelerate vector construction and stable integration. This toolkit featured a 16 component one-pot Golden Gate (GG) reaction for plug-and-play assembly of complex mammalian expression cassettes. With efficiencies ranging from 100% in 7 element reactions to 35% in 16 element reactions, multifaceted vectors could be generated to optimize *cis*-acting regulatory elements. The toolkit also outlined a site-specific integration (SSI) workflow displaying 90-100% efficiency of complex payloads utilizing the Cre/lox recombinase system. SSI significantly reduces the transcriptional and transgene stability heterogeneity associated with random integration, therefore isolating intentional changes in vector design to phenotypic deviations.

Finally, with the MemorySeq genetic targets and the SSI toolkit, regulating native gene expression allowed for the induction of stress-tolerant phenotypes. Development and optimization of CRISPR activation/interference (CRISPRa/i) systems allowed for activation and repression of three genes with heritable properties. These included activating transcription factor 3 (*Atf3)*, immediate early response 3 (*Ier3)*, and heme oxygenase-1 (*Hmox1)*. These three genes have been indicated in other cell lines as playing a role in stress detection and response, but never in CHO. CRISPR facilitated activation of *Atf3* and *Hmox1* and repression of *Ier3* resulted in a 30-40% increase in integral viable cell density and peak viable cell density in both ammonia and osmotic stress fed-batch conditions. This translated to measurable improvements in volumetric productivity that may be further compounded in an appropriately controlled bioreactor. Concomitant with improved growth was also a reduction in broad rates of apoptosis, indicating a cytoprotective feature of some of these genes or regulatory pathways. Overall, this thesis reflects a novel approach for identifying, characterizing, and engineering stress tolerant phenotypes in CHO using heritable properties as an early-stress resistance biomarker. Continued exploration of genes displaying these properties may highlight robust rational engineering targets for the development of novel CHO host strains with improved performance in manufacturing scale-bioreactors.