CONTROLLING TRYPTOPHAN OXIDATION THROUGH MEDIUM/FEED MODIFICATIONS AND POTENTIAL MOA UNVEILED BY TRANSCRIPTOMICS ANALYSIS

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Oxidation of monoclonal antibodies (mAbs) is one of the major product quality issues with potential impacts on product activity and immunogenicity. Tryptophan oxidation (Trp-ox) leads to addition of one or two oxygen atoms to the indole ring of its side chain and other subsequent degradation end products. It was reported that Trp-ox in the complementarity-determining region of a mAb led to a progressive loss of antigen binding and biological activity [1]. Trp-ox was also reported to cause color changes in near UV−visible light-irradiated and heat-stressed monoclonal antibody (mAb) drug product in liquid formulation [2]. In addition, Trp-ox was observed under real-time storage and elevated temperature conditions [3]. Recently, we observed that modifying the concentrations of copper, manganese, tryptophan, and cysteine in cell culture media/feed had a significant impact on Trp-ox levels of two mAbs in development produced in Chinese hamster ovary (CHO) cells [4]. We have demonstrated that Trp-ox level can be effectively controlled while maintaining productivity and overall suitable product quality profiles. In this presentation we will summarize those findings and the results from the systematic studies that enabled us to control the Trp-ox levels at both the shake flask and benchtop bioreactor scales. Moreover, we will describe new studies that aimed to understand the potential mechanism of action (MOA) of those components on controlling Trp-ox levels.

The advent of NGS technologies and the availability of CHO reference genomes have enabled the systematic analysis of CHO biology and its capacity for recombinant protein production [5, 6]. Here we applied transcriptomic analysis using RNA-Seq to explore the underlying mechanisms of cell culture's impact on Trp-ox. Cell samples from fed-batch bioreactors cultured with control or modified media/feed were harvested and subjected to RNA-Seq analysis. The results showed that cell culture conditions had little impact on the expression of the mAb transgenes (LC and HC), nor genes related to glycosylation, which is consistent with the previous findings on mAb productivity and glycosylation profile [4]. However, cell culture conditions did significantly alter the expression of multiple genes (fold change ≥1.5, p-value ≤0.05). Specific subsets of genes involved in control of oxidative stress and metabolism of copper, manganese, tryptophan cysteine will be discussed in detail. The analyses will focus on genes engaged in scavenging of free radicals because of their known roles in oxidation chemistry and production of reactive oxygen species (ROS). We postulate that these changes in gene expression may provide molecular means to balance the copper availability and glutathione pool, which in turn might result in the observed impact on mAb quality without changing the CHO cell growth and productivity. The work presented here provide another example of how gene expression analyses can shed additional light on potential mechanisms for observed cell culture performance and specifically in this case, changes in recombinant protein product quality attributes. Such understanding could eventually lead to a biomarker-based approach for process optimizations. To the best of our knowledge, this is the first example of using transcriptomic analysis to mechanistically understand the impact of cell culture on critical quality attributes other than glycosylation. Therefore, we believe this presentation is of great interest to general biopharmaceutical community and is relevant the themes of the Conference, especially to the section "Advances in cell culture control of product quality attributes".

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