Characterization of Chinese Hamster Ovary Cells Producing Coagulation Factor VIII Using Multi-omics Tools

The first public draft of a genome from Chinese hamster ovary (CHO) cells was published in 2011, an entire decade after the first draft of the human genome. This publication of a relevant CHO reference genome, in combination with the fact that the cost for DNA sequencing has dropped more than 10,000 fold over the last couple of years due to the revolution of next-generation sequencing (NGS), has dramatically accelerated CHO-omics from virtually non-existent to a vibrant growing field. The aim of this thesis was to investigate the impact of coagulation factor VIII (FVIII) production in CHO cells using omics tools. A wide range of methods were applied including whole-genome sequencing, targeted genome sequencing, mRNA sequencing, miRNA sequencing and mass spectrometry based shotgun proteomics on a number of clones in order to get a more holistic picture of the inner workings of these CHO transfectants. From the whole-genome sequencing of two CHO genomes (CHO DXB11 and the FVIII producing transfectant: F435) it was observed that roughly 20% of the genes in the genome were haploid and roughly 10% had a copy number of three or higher indicating extensive rearrangements compared to the Chinese hamster origin. The transcriptome of 14 clones producing a dynamic range of FVIII was analyzed using RNA sequencing revealing an unexpected degree of 5’ truncations of the transgene in 11 of the 14 clones. These truncations were validated using targeted genome sequencing, which also mapped the transgene insertion site in a number of clones. Furthermore, the RNA sequencing data was combined with proteomics data to investigate the impact FVIII biosynthesis exerts on CHO cells. This revealed a dose-dependent induction of the unfolded-protein response, endoplasmic reticulum stress and oxidative stress which further lead to degradation of FVIII by the endoplasmic-reticulum-associated protein degradation pathway. This is to our knowledge, the first time that such extensive omics tools have been applied to a broad panel of CHO cells producing a very complex protein. The holistic view obtained for the FVIII producing cells provide a much clearer picture of the metabolic burden associated with FVIII secretion, than could be obtained using previous indirect methods. The data and methods presented in this thesis suggest initial steps, which may be refined towards full utilization of omics technologies for analysis and engineering of industrially relevant CHO cells. Full implementation of such tools for generating specifically engineered CHO production cell lines may allow significant cost-reductions in production of complex biopharmaceuticals such as FVIII.

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