METHYLATION INHIBITION FOR COPING WITH EPIGENETIC-BASED HETEROGENEITY OF CHO CELL SUBCLONES AND IMPROVING RECOMBINANT MAb PRODUCTION

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Chinese hamster ovary (CHO) cells are ideal hosts for producing recombinant proteins. However, there is a great diversity of phenotypes between cell lines and subclones. Thus, a thorough selection process that is expensive and time consuming is required to establish a production cell line. Epigenetic markers such as DNA methylation, have been proposed as responsible for regulating gene expression that leads to CHO cell heterogeneity. In the present work, four CHO cells subclones producing a monoclonal antibody (MAb) in the early stages of selection, were characterized in terms of their kinetics, cell physiology, productivity, and quality of the MAb produced. The best and worst subclones in term of MAb productivity were selected and treated with the inhibitor of DNA methyltransferase 5-aza-`2deoxycytidine (5Aza2) to determine its impact on the heterogeneity, productivity, and quality of the MAb produced. Such scarcely explored approach can be employed to accelerate the time to market of a therapeutic protein. All subclones tested showed a high glucose consumption rate and a metabolic switch from lactate production to consumption when L-glutamine was depleted. The N-glycosylation profile of the MAb produced by all subclones had a high abundance of G0, G1 and G2, fucosylated and high mannose glycans. The clones with the highest and lowest MAb productivity were selected for further studies. The high producer clone had a global cytosine methylation of 58.7 ±3.1%, whereas the lower producer had a methylation of 69.6±9.9%. After treatment with 5Aza2, the methylation extent significantly decreased to 42.1±10.7% and 45.4±7.8% in the higher producer and lower producer, respectively. A concomitant increased in specific productivity (q_p) of the lower producer of 68.2% was observed, but no change in occurred for the higher producer. Although the inhibitor slightly affected viability, no alteration in the N-glycosylation profiles was observed. In conclusion, this work shows the importance of DNA methylation on heterogeneity of a CHO cell population during generation of production cell banks.

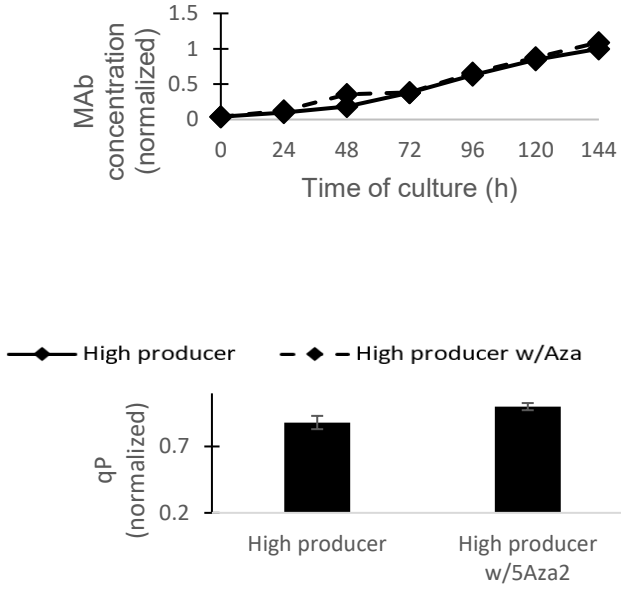

Figure 1. Kinetics of production of MAb (up) and q_p of the high producer with 5Aza2 (down).

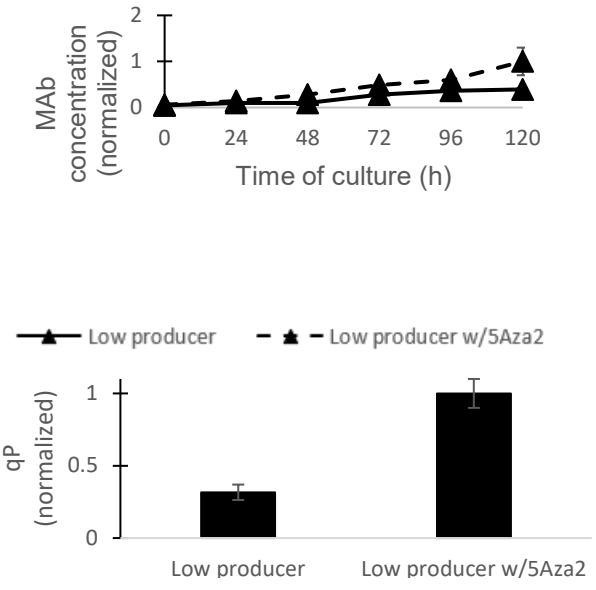

Figure 2. Kinetics of production of MAb (up) and q_p of the low producer with 5Aza2 (down).

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