PROTEASOME-BASED SELECTION SYSTEMS FOR GENERATION OF RECOMBINANT CHOK1SV GS-KO™ CELL LINES WITH ENHANCED PRODUCTIVITY

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Chinese hamster ovary (CHO) cells are widely used industrially for the production of biotherapeutic proteins. In order to generate recombinant CHO cell lines expressing the target biotherapeutic gene(s) of interest, metabolic markers are used to select for those cells that have stably incorporated the gene(s) of interest. Such selection systems work very efficiently but do not directly select cells based upon secreted biotherapeutic recombinant protein productivity characteristics. When such biotherapeutic proteins are synthesised in eukaryotic cells, typically the polypeptide is co-translationally fed into the endoplasmic reticulum where it is folded, and if required, assembled with other polypeptides/domains, as in the case of antibodies. An overload of the capacity of the ER to fold and assemble recombinant proteins can result in upregulation of ER-associated degradation (ERAD) where unfolded or incorrectly folded or assembled material is retro-translocated out of the ER to the proteasome for degradation and recycling of amino acids. We have therefore investigated whether the susceptibility of cells to proteasome inhibitors during cell line construction when a recombinant load is placed upon the cell can be used to select for cells with a greater capacity for producing recombinant biotherapeutic proteins whilst maintaining or enhancing the quality of the material secreted. A number of proteasome inhibitors were therefore investigated, epoxomicin, MG-132 and bortezomib, at different concentrations to identify concentrations that would provide selection but not result in complete cell death. A range of concentrations was then added to CHO cells, along with MSX, during cell pool construction using Lonza's CHOK1SV GS-KO™ proprietary host cell line to investigate whether this resulted in the generation of cell pools with enhanced productivity characteristics as compared to selection using MSX alone. Using this approach, and a number of different recombinant biotherapeutic molecules, we have found that CHO pools giving enhanced product concentrations can be generated (see Figure 1 for example) and validated this in ambr15 miniature bioreactor experiments. We have thus shown that stable transfectants derived from pools that had been cultured with proteasome inhibitors were more productive than pools generated without proteasome inhibitors. Further, stable transfectants generated using proteasome inhibitors retained their higher productivity characteristics even when the proteasome inhibitors were no longer added at subculture, meaning that proteasome inhibitors are only required in the initial stages of cell line construction.
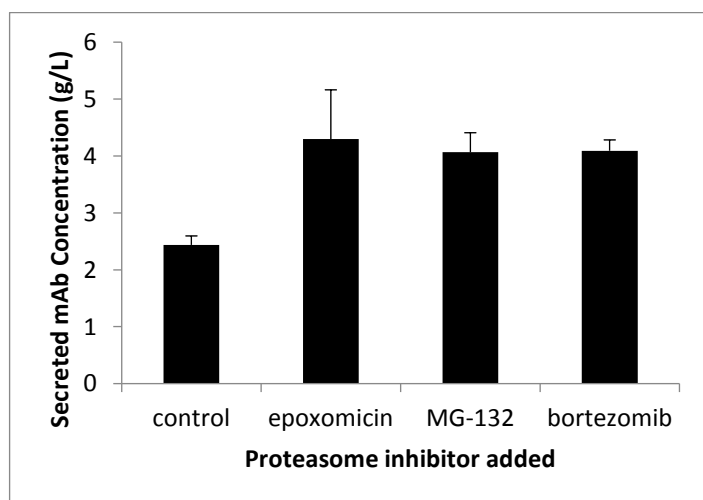
Figure 1 – Lonza CHOK1SV GS-KO™ cells were transfected with a DNA vector encoding the expression of glutamine synthetase (GS) and a monoclonal antibody, using Lonza's 4D-Nucleofector device. Stable transfectant minipools in 96-well plates were selected using glutamine-free culture medium and methionine sulphoxine (MSX), with or without the proteasome inhibitors epoxomicin, MG-132 or bortezomib. After a fed-batch overgrow of minipools in 96-well deep-well plates, product concentration was measured using FortéBio's Octet. The top 8 concentrations obtained from each plate are shown here.