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Master of Science HES-SO in Life Sciences

Amplification and cloning of GPR81, MCT1 and HIF-1α for subsequent transfection of CHO cells

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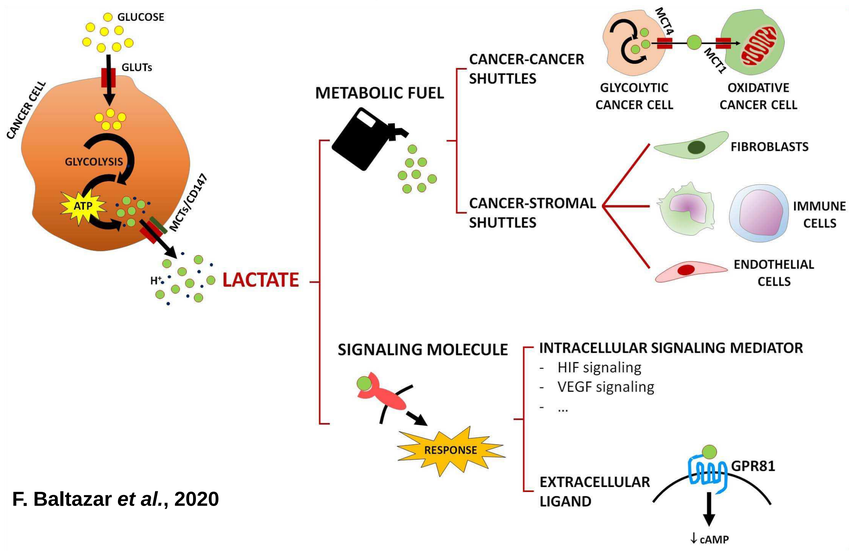
INTRODUCTION

Chinese Hamster Ovary (CHO) cells are the most widely used production system for recombinant biological compounds such as monoclonal antibodies (mAbs). While fed-batch is currently the standard of production, bottlenecks of such bioprocess include the high concentration of lactate generated over the course of cultivation, impairing growth and protein productivity. More than just a metabolic waste, recent evidence suggests new roles of lactate either as a metabolic fuel or as a signaling molecule. Lactate was found to induce several signal transduction pathways through autocrine activation of GPR81, a G-protein coupled receptor (GPCRs). The transport of lactate across membranes is achieved by proteins of the monocarboxylate transporters (MCTs) family, MCT1 being mostly associated with the uptake of lactate. Moreover, hypoxia inducible factor 1α (HIF-1α) upregulates key enzymes of the glycolytic metabolism responsible for the conversion of glucose into lactate. Those three key players in lactate metabolism of cancer cells are depicted here below.

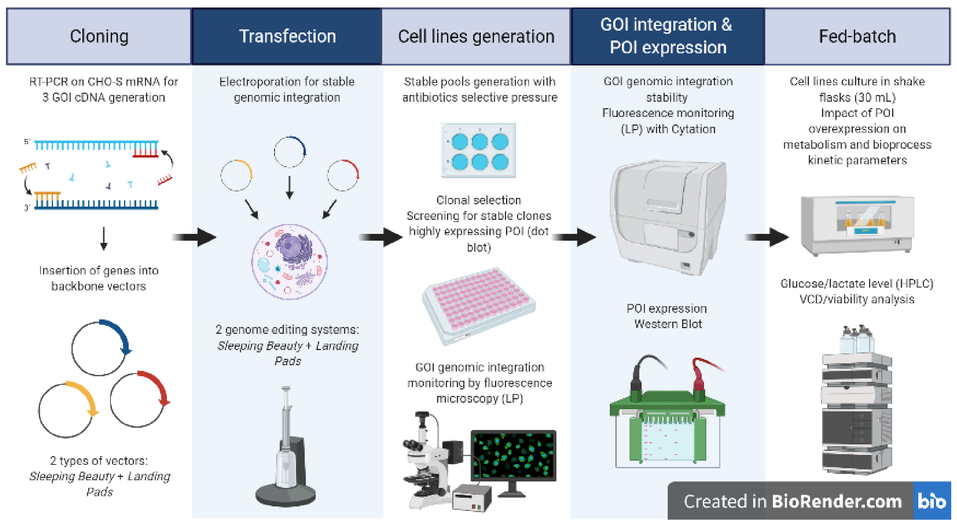


F. Baltazar *et al.*, 2020

The purpose of this master thesis was to evaluate whether overexpressing GPR81, HIF-1α and MCT1 had an impact on CHO cells cultivation in terms of viable cell density (VCD) and of cell culture duration and if a correlation can be made between overexpression of above-mentioned proteins and generation/consumption of lactic acid.

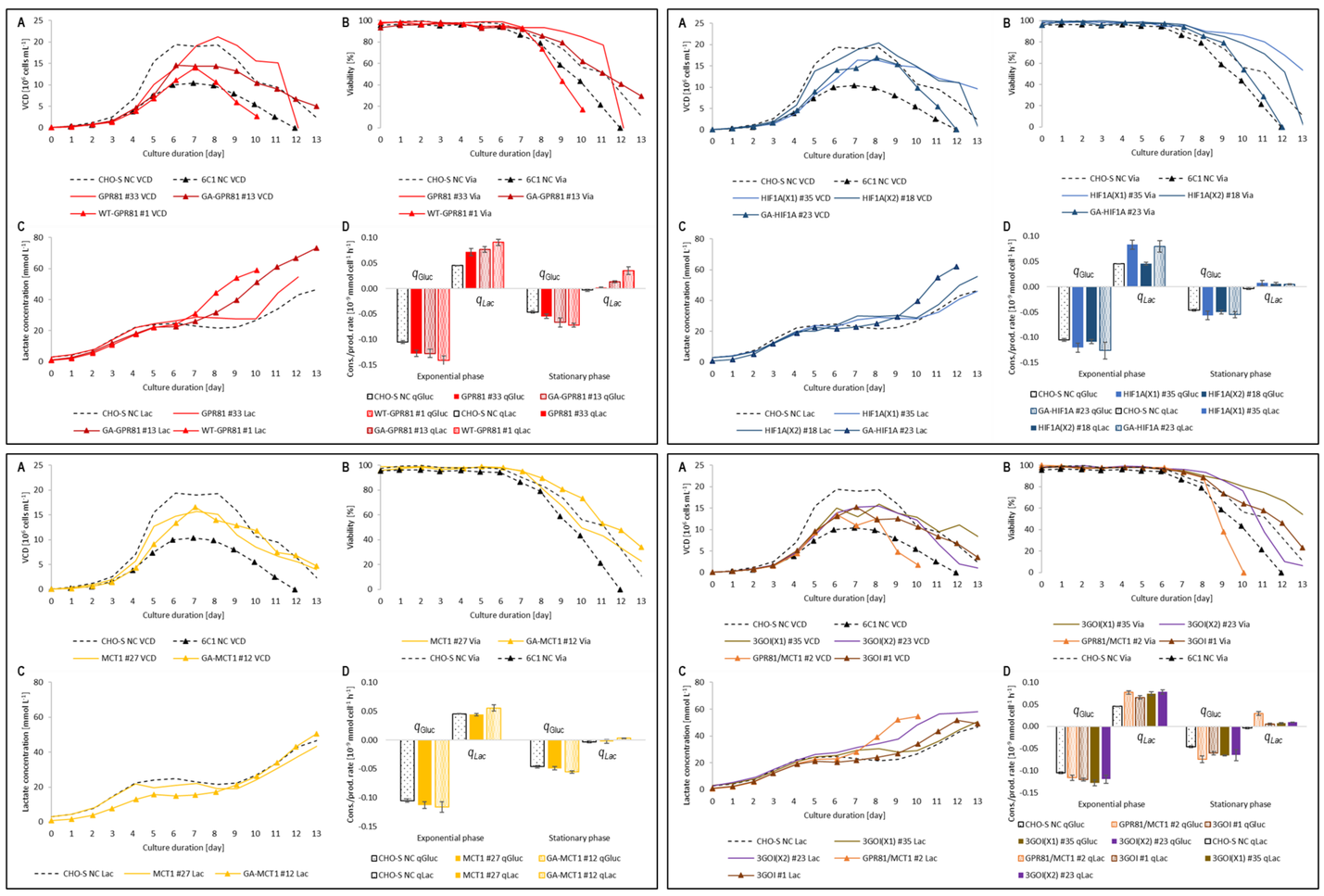
EXPERIMENTAL DESIGN

In this study, GPR81, HIF-1α and MCT1 were subcloned and stably transfected in CHO cells using Sleeping Beauty (SB) and a Landing Pad (LP) genome editing systems. Stable cell lines were then generated from selected cell pools. The impact of overexpressing these proteins in CHO cells was evaluated in fed-batch cell cultivation, focusing on viable cell density (VCD), culture duration and generation and consumption of lactate.



RESULTS

Recombinant overexpression of GPR81, HIF-1α, MCT1 or combinations of proteins thereof did not result in increased cultivation performance for a 13-days fed-batch bioprocess, in terms of maximal VCD achieved, biomass generated or generation time. However, no process development nor medium and feed optimization was performed, which could change the outcomes of this study. Indeed, the various cell lines analyzed show quite different metabolic and kinetic profiles as shown by significant differences in glucose utilization and lactate management, which could influence metabolites production and various substrates utilization during a fed-batch process. Besides, GPR81 overexpression was shown to induce a higher degree of aerobic glycolysis (Warburg effect) by increasing both glucose consumption and lactate production. Moreover, HIF-1α also demonstrated a higher production of lactic acid. However, none of the cell lines generated showed a higher uptake of lactate as additional energy source, compared to the parental CHO-S cell line, as shown below.



Growth curves (VCD), viability profiles, lactate concentration and glucose and lactate consumption/production rates for cell lines overexpressing the POI and control cell lines over fed-batch bioprocess. *Data shown are mean of triplicates. Error bars represent standard deviations calculated on triplicates. Cell lines named according to the transfected GOI. A: VCD profile, B: viability profile, C: lactate concentration, D: average glucose and lactate consumption/production rates calculated over exponential phase (day 0 to day 5) and pseudo-stationary phase (day 6 to day 8), positive values indicate production and negative values indicate consumption. Dashed black lines represent control cell lines, full lines represent SB clones, triangled-marked lines represent LP clones/control cell line. Low dotted bars represent control cell line, high dotted bars represent LP clones, full bars represent SB clones.*

CONCLUSION

Based on the design of experiments of this study, we assessed that a recombinant overexpression of GPR81, HIF-1α, MCT1 or combinations thereof did not result in increased cultivation performance for a 13-days fed-batch bioprocess, in terms of maximal VCD achieved, biomass generated, generation time or lactate uptake as additional energy source. However, payload DNA genomic integration using a Landing Pad system demonstrated very good efficiency and stability when used in combination with the process of clonal selection. Additionally, we demonstrated that GPR81 activation significantly increased the Warburg effect in CHO cells overexpressing the protein, enhancing the glucose consumption and especially lactate production. Moreover, we demonstrated that hypoxia stabilized GPR81, HIF-1α and MCT1 overexpression through a proposed regulation mechanism including lactate-mediated GPR81 signaling. Overall, to shed more light onto expression patterns of these POI and their and impact on cell metabolism, analysis of mRNA expression would be advised.

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