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GENETIC ENGINEERING OF MMV VIRUS RESISTANCE INTO CHO CELLS: PROBING THE ROLE OF VARIOUS CHO SIALYLTRANSFERASES IN VIRUS BINDING AND INTERNALIZATION

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Contamination by the parvovirus Mouse Minute Virus (MMV) remains a continuing challenge in CHO biopharmaceutical production processes. As part of developing a risk mitigation strategy against such events our group has evaluated the genetic engineering of Chinese Hamster Ovary (CHO) cell lines to create a new host cell line that would be resistant to MMV infection e.g by inhibiting viral attachment to a cell surface receptor. While the exact functional receptor for MMV binding to CHO cell surface is unknown, previous work in our group has validated the role of sialic acid on the cell surface as important for cell surface binding and internalization of the MMV virus.

Sialyltransferases are a group of enzymes that catalyze the transfer of sialic acid to the glycan moiety of glyoconjugates. *In-vitro* glycan-arrays have indicated that MMV binds preferentially to α-2,3 linked sialylated glycans and do not bind to α-2,6 sialylated glycans. CHO cells have six different sialyl transferases (ST3Gal 1-6), that transfer sialic acid in a α-2,3 linkage specific manner. In this work we systematically knocked out these genes and then probed for their role in MMV infectivity by challenging each cell line for their ability to resist viral entry. Results showed that St3Gal4 had a predominant effect on MMV infectivity with functional deletion resulting in a 54-88% decrease in infection compared to the Control WT cells. Additional deletion of St3Gal6 had little to no additional benefit in terms of viral resistance. Interestingly, the St3Gal4 and the St3Gal4 + 6 double mutants displayed both a decrease in viral binding to the cell surface as well as viral internalization and replication. Gene knock out of another sialyltransferase of the same family St3Gal 3 had a wide range of effect on MMV infectivity (18-84% of WT), which could be explained by a non-specific clonal effect. Based on the high resistance profile of cells with truncated O-glycosylation (COSMC knock-out clones), we hypothesized that the sialyltransferase St3Gal1 would have a significant effect on MMV infectivity, which could be further enhanced by combining it with an St3Gal4 deletion . We also sought to replace the α-2,3 sialylated phenotype with α-2,6 sialylation on the glycoproteins expressed in the viral resistant host cell lines by over-expression of the St6Gal1 gene. Such an approach would also make the therapeutic protein have more “human” like glycosylation.

Model recombinant proteins were transfected into the new host cell lines and growth, IgG productivity and product quality studied. Our data demonstrate that viral resistance against MMV virus can be incorporated into CHO production cell lines, adding another level of “defense”, against the devastating financial consequences of this virus infection, without compromising recombinant protein yield or quality.