Introduction

The influenza A virus (IAV) is a highly contagious virus that kills 250,000-500,000 people per year worldwide[1] [2]. This season is especially bad, with the flu reaching epidemic levels and killing 84 children in the US so far[3]. Every year, scientists predict which flu strains are most likely to circulate and grow a vaccine for these strains inside chicken eggs[4]. This is an expensive 6-12 month process with several key limitations[5]. If the flu strains are wrongly predicted, it will take 6-12 months to develop a better vaccine. Also, some viral strains mutate so quickly that they are challenging to grow in eggs. This is true of this seasons H3N2 strain, with a vaccine only 10-30% effective[6] [7].

A potential alternative to the egg-based vaccine is an engineered system in which influenza genes are inserted into a mammalian cell, and the cell itself produces the virus necessary for a vaccine. This would enable vaccines to be produced more quickly and prevent mutations during production such as those occurring for the H3N2 strain. However, if the cell produces too much virus too quickly, it will kill itself. To prevent this while still maximizing production, different virus genes need to be produced at different amounts and rates. To do so, production could be controlled by an inducible promoter system.

Promoters are essential DNA regions which enable a gene to be transcribed and protein to be expressed. Whereas some promoters are continuously active, inducible promoters are regulated by factors called inducers, which bind to the promoter and either activate or inhibit transcription. Extensive research has been done to engineer inducible promoter systems which can be inserted in cells and used to turn genes on and off at will, like a light switch [8] [9] [10]. Of these, the Tet-On system and cumate-inducible system have demonstrated tight control in mammalian cells, defined as producing little to no protein before induction, but producing significant protein after induction.

The regulatory possibilities of these systems remains to be explored. The Tet-On and cumate system can turn genes on and off with switch-like behavior, but if 'dimmer switch' regulation was possible, the inducible promoter systems could be used to produce a small, medium, or large amount of protein based upon the amount of inducer added. In an engineered system for vaccine production, this could be used to maximize vaccine production without killing the cell. Better vaccines could be produced more quickly.

Hypothesis

We aim to determine the regulatory effects of several inducible promoters on the gene expression of several common mammalian cell lines. We hypothesize that some combination of inducible promoter and mammalian cell line will provide tight and regulatable control of gene expression, such that different inducer concentrations will consistently and predictably provide 'dimmer switch' control of gene expression.

Methods and Analysis

Two inducible promoter systems, the Tet-On system[11] and the cumate-inducible system[12], will be inserted into three cell lines, CHO-K1, HEK 293, and MDCK, and induced at six different concentrations[13]. These systems have already been designed to promote transcription of a red fluorescent protein (RFP), as this makes it easy to quantify gene expression in terms of fluorescence. In all experiments below, RFP expression will be quantified at the RNA level using qRT-PCR, and on the protein level using fluorescence imaging and flow cytometry.

For insertion, the inducible promoters have already been packaged into lentiviral vectors. Such vectors contain viral machinery which 'infect' a cell and insert many copies of a desired sequence into the host cell's genome. This will be used to insert each inducible promoter system into the genome of each cell line. However, the number of inducible promoters successfully inserted will vary based upon the amount of viral vector added. This will cause RFP expression

to also vary, making further results unreliable. To prevent this, a calibration curve will be run to normalize future data to the amount of viral vector added. All three cell lines will be infected by six different concentrations of viral vector, and induced to promote RFP production. RNA and protein expression will be determined and plotted against the amount of viral vector. This plot will be used to normalize all further results.

Tight control will then be assessed in these infected cell lines with non-infected cells as a negative control. Half the cells will be induced and half will not. RFP production on the RNA and protein level will be plotted and compared between the two groups. In a tight system, uninduced and induced expression would vary significantly, with little non-induced expression.

Finally, the regulatory capability of both inducible promoters will be tested by inducing all infected cell lines at six different inducer concentrations, with non-infected cells as a negative control and a fluorescent cell line as a positive control. RFP production on the RNA and protein level will be determined for all induction concentrations of all promoter systems and all cell lines, and the results will be plotted and compared. If different induction concentrations lead to significant differences in RNA and protein expression, this would indicate 'dimmer switch' control of gene expression and protein production.

Outcome

This project will reveal the applicability of the Tet-On system and the cumate-inducible system for gene regulation in CHO-K1, HEK 293, and MDCK cells. If the hypothesis is disproven and the inducible promoter systems lack tight, 'dimmer switch' control of RNA and protein expression, other inducible promoter systems such as VAC, LacI, PEACE, and TraR could be tested[14 15]. If our hypothesis is verified and the gene expression systems tested demonstrate tight, regulatory control of virus gene expression, we can conclude the system may be ideal for regulated IAV vaccine production, providing a better vaccine and saving lives.

References

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