Utilization of activation-induced cytidine deaminase (AID) in mammalian cell display for therapeutic antibody discovery

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BIOTECHNOLOGY (TECH.)

INTRODUCTION

Mammalian cell display is a technology where proteins are displayed on the cell surface allowing controlled selection and screening to generate antibodies to defined targets from a population of potential binders.

Constructing large libraries in mammalian cells is difficult but the size of the library is a vital feature in increasing the probability of finding desirable antibodies against the target.

A possible solution to increasing the library size and improving its diversity could be to implement the process of antibody affinity maturation *in vitro* by utilizing activation-induced cytidine deaminase (AID). AID is an enzyme which introduces mutations to DNA by deaminating cytosine to uracil, which is then recognized as thymine.

AIM OF THE STUDY

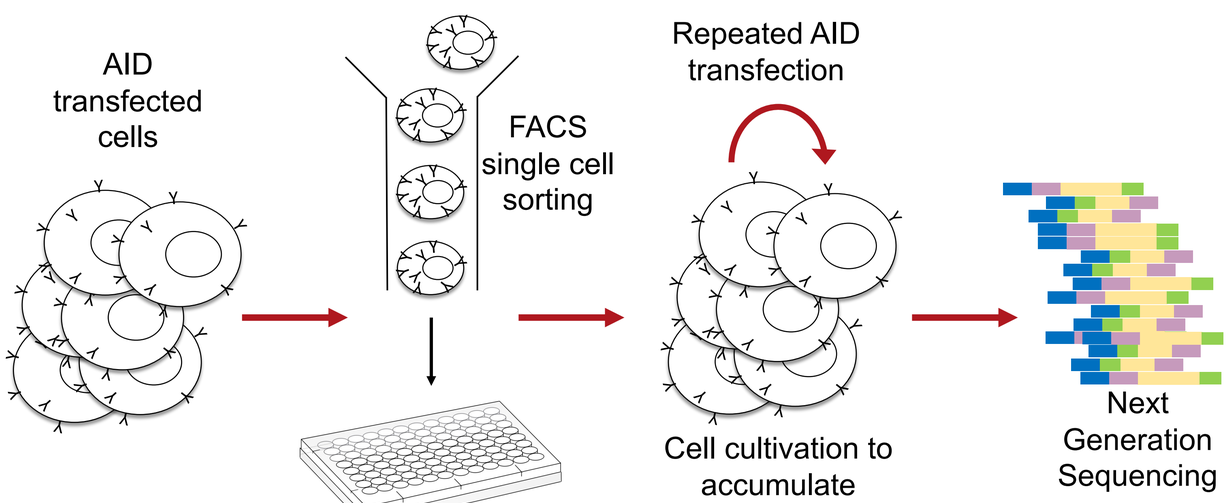
The goal of this study was to transfect Chinese hamster ovary (CHO) cells with different variations of AID and to characterize the mutations they induce on the target gene.

CHO enhancers were added to the gene constructs to study their effect on transcription efficiency and the mutation rate.

MATERIALS & METHODS

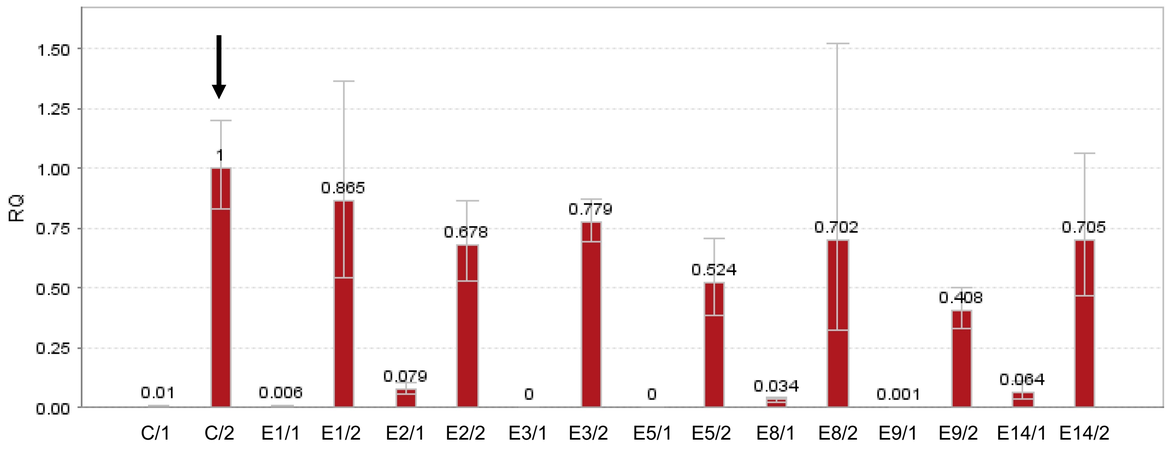
- Two versions of an scFv with human germline genes with same amino acid composition with and without quadruplex structures were compared
- 7 CHO enhancers' effect on transcription efficiency of the target genes were compared to the gene structure without enhancers with qPCR using the $\Delta\Delta C_t$ method
- Three variants of both human and mouse wild type AIDs were designed with FLAG-tag and the AID expression in the cells after transfection was analysed with western blot
- Final cell lines were composed according to the results from enhancer comparison and transfected with all the AID variants before finally extracting the gDNA and sending the samples to sequencing

Work flow of the final cell lines



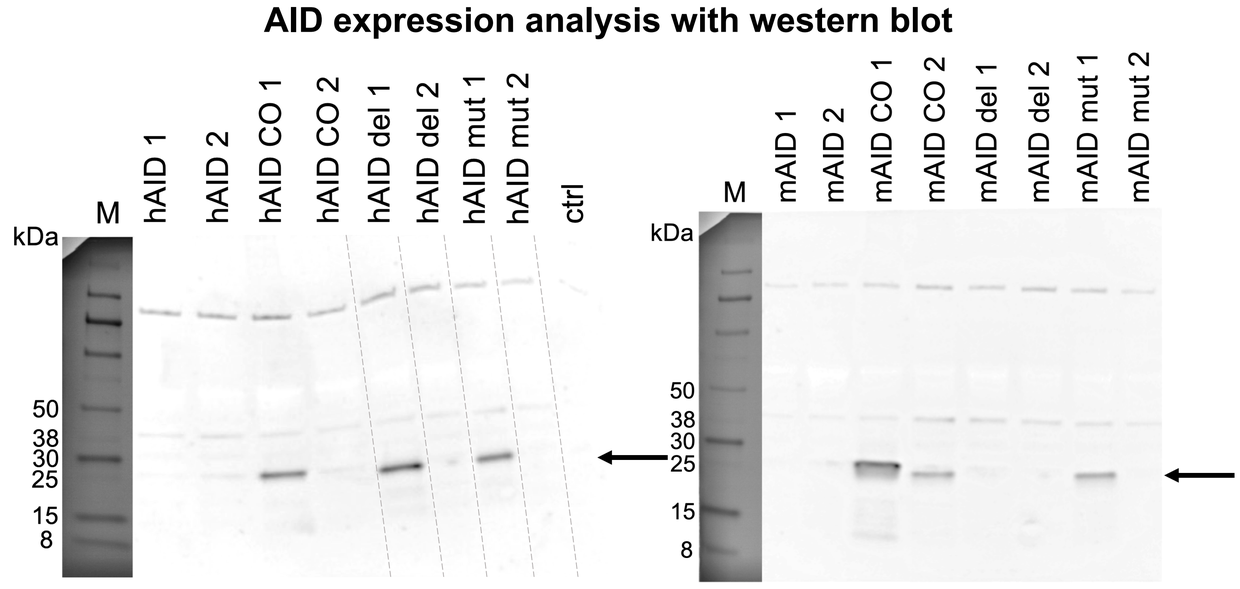
PRELIMINARY RESULTS

Relative target gene mRNA levels of enhancer containing cells



The graph presents the relative quantity value for each cell line with different enhancers named E1–14. The numbers indicate if the sample was taken during transient expression (1) or stable expression (2). The Ct values were compared to the housekeeping gene GAPDH and then to the untreated control sample (C) without enhancers (pointed with an arrow) from stable expression which value was set to 1.

AID expression analysis with western blot



Samples from cells transfected with different AIDs (codon optimized (CO), codon optimized with deletion (del), and codon optimized with deletion and point mutations (mut)). The numbers indicate if the sample was taken three days (1) or a month (2) after AID transfection. The control sample (ctrl) used was from the same cell line without any AID transfections. The samples with wild type human and mouse AIDs were also run as reference as they do not contain the detectable FLAG-tag.

DISCUSSION AND CONCLUSIONS

The chosen enhancers did not increase the transcription efficiency of the target gene. However, they could still affect how AID targets or induces mutations, which could hopefully be seen in later analysis of the mutations.

AID expression seemed to decrease significantly after transfection, which lead to repeating AID transfections to increase the possibility of acquiring mutations. This infomartion is useful in designing future experiments.

In the next step of the study, the sequencing results are analysed to characterize the mutations acquired and to see if there are any trends in how the mutations are targeted. Any differences between the different gene constructs and AID variant combinations are investigated. The results could help to improve antibody libraries and impact how we discover new antibodies for drug development in the future.

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