Target Validation and Assay Optimisation for Anti-Viral Enzyme OAS1

Introduction and background of the project

OAS1 is a member of the 2-5A oligoadenylate synthetase family which is involved in the body's innate immune response to viral infections and has also been associated with genetic risk of several diseases. When viral dsDNA is detected, interferons induce OAS1 expression via the JAK/STAT pathway (Magusali et al., 2021), which catalyses a pathway of events leading to the degradation of cellular and viral RNA and reducing viral replication. OAS1 synthesises 2-5' oligoadenylate when viral RNA binds and dimerises to act as a second messenger and activate RNaseL. Activated RNaseL can degrade viral cytokine RNA. In addition to viral RNA degradation, OAS1 regulates cytokine signalling and aspects of cell viability (Magusali et al., 2021). Microglial OAS1 downregulation produces an increased proinflammatory response downstream of interferon stimulation and genome-wide association studies in combination with gene expression profiling have found a link between reduced OAS1 signalling and an increased risk of developing Alzheimer's disease (Magusali et al., 2021) (Salih et al., 2019). The impact of reduced OAS1 on the innate immune mechanisms, and how alterations may alter AD risk are not well understood. In this project, the effect of OAS1 knockdown in HMC3 human recombinant, immortalised microglial-like cell line on cytokine production was studied along with validation of a functional recombinant protein assay to detect changes in OAS1 enzyme activity.

Aims

-Measure the effect that knockdown of OAS1 has on cytokine production.

-Characterise, validate and optimise experimental conditions for a functional enzyme assay to quantify OAS1 enzyme activity.

Methods used and description of work

Cell splitting and plating: HMC3 cells were split every 3-4 days to maintain confluency and optimum growth conditions for cell survival. Cells were plated as required for RNA transfection and cell staining.

RNA transfection: HMC3 cells were transfected with 2.5 pmol siRNA specific to OAS1 (siRNA OAS1), a non-targeting control (siRNA Ctr) or no siRNA at all (NT). OAS1 siRNA, siRNA Ctr or lipofectamine were diluted in OptiMEM in separate vials. Three separate vials of lipofectamine were then generated, one containing OAS1 siRNA, and one containing siRNA ctr, and left at room temperature for 5 min. All cells were transfected by adding the siRNA-lipofectamine mixture directly into the media. Each SiRNA transfection condition was performed in triplicate.

Cell harvesting and RNA extraction: 48 hours after RNA transfection, cells were harvested using lysis buffer and RNA extracted using Monarch total RNA miniprep kit. Nanodrop machine was used to measure RNA concentration and A260/A280 values for each sample.

Cell fixing and immunostaining (ICC): Cells were fixed using 4%PFA and washed three times. They were then stained by immunocytochemistry (ICC) using a primary antibody for OAS1 (ab272492, concentration 1:1000) and incubated overnight at 4°C. Secondary antibodies with a conjugated fluorophore and DAPI were added to the cells to allow for visualisation under the microscope.

qPCR: A 384 well plate map was designed in excel. RNA samples were diluted to 10ng/µL and added to the wells of the plate according to the map. Three master mixes were made, each containing buffer, water and one of three primers (IL-1β cytokine primer, Beta-Actin or OAS1).

Enzyme titration optimisation: The transcreener OAS1 Assay uses a coupling enzyme that converts 2'-5'A produced by OAS1 to the products AMP and ATP. AMP is measured with a fluorescent antibody and allows quantification of enzyme product. OAS1 was serially diluted to determine the quantity of active enzyme present and determine the optimum concentration (EC50) of OAS1. Two recombinant OAS1 proteins were tested to determine the optimal one for the assay.

Data analysis and graph generation:

- qPCR data was organised in excel and GraphPad Prism used for statistical analysis and graph generation.
- Transcreener assay was performed using the PHERAstar and data analysis and graphs were done in GraphPad Prism.

Results

qPCR:

Transfected cells had significant OAS1 downregulation compared to non-transfected (NT) cells but OAS1 expression also decreased in cells treated with control siRNA, which was unexpected (Figure 1A). This may suggest siRNA affected OAS1 expression as it is involved in the recognition of dsDNA. No significant change in IL-1β expression was observed in siRNA transfected compared to NT cells but may require a larger sample size to reach significance (Figure 1B).

Figure 1

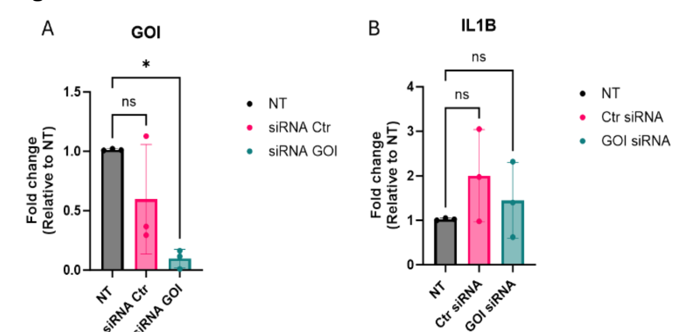

Figure 1. Gene expression of OAS1 and IL-1β measured in HMC3 cells in NT, transfected and control (scrambled siRNA) groups. Fold change relative to NT group was calculated in excel. Statistics performed using a one way-ANOVA comparing each group to the NT group, * p=0.05. n=3 biological replicates. GOI is the gene of interest (OAS1).

Immunocytochemistry:
HMC3 cells were stained to detect protein expression of OAS1 (Figure 2).

Figure 2.

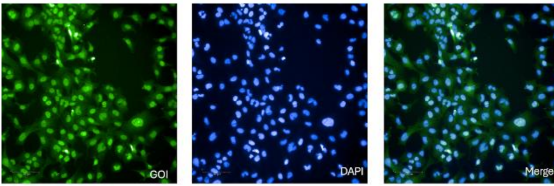

Figure 2. ICC Staining of OAS1 in HMC3. Left image shows cells stained with anti-OAS1 antibody (green), middle image is DAPI nuclear stain (in blue) and right is overlay.

Enzyme titration:
Two recombinant batches of OAS1 were compared in the enzyme assay to determine range of activity.

Figure 3.

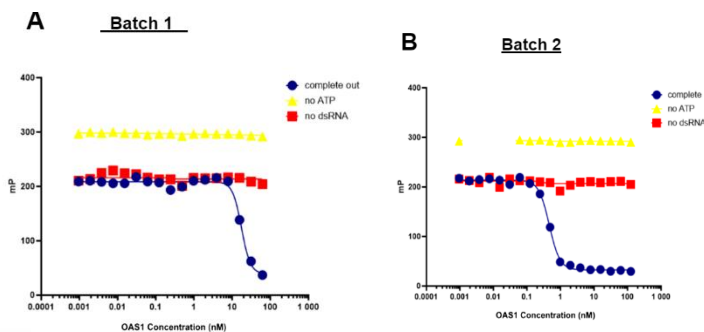

Figure 3. Batch comparison of OAS1 enzyme activity curves measured using fluorescence polarisation (mP) against increasing enzyme concentration (nM). *Assay conditions include enzyme, ATP and dsRNA (blue). Negative controls contained all the reagents minus either ATP (yellow) or dsRNA (red).*

Conclusions and future directions

OAS1 expression was reliably reduced with siRNA but larger sample sizes are required to confirm any gene expression changes in the presence of immune stimuli with reduced OAS1. In future, alternative methods to knock down OAS1, such as CRISPR gene editing, could be used in HMC3s to reduce the control background caused by siRNA. The enzyme assay reliably measures OAS1 function. Next steps will determine its utility in testing functional variants.

Departures from original plan

Due to timing and protein construct availability, my project was switched to the biochemical characterisation of an alternative genetically linked target for AD.

Value of the studentship

To the student

This studentship has increased my confidence in technical skills learned at university, e.g. PCR, RNA extraction. I have also gained new skills in cell culture, protein biochemistry and automated assays methods. I collaborated with a multidisciplinary team, gained useful skills in integrating scientific methods and following detailed protocols.

To the lab

Potential targets linked to disease by genetics require extensive biological validation before drug discovery. The cellular model will allow the lab to test functional consequences of OAS1 knock down to inform potential ways to target therapeutically, and the protein assay development work I completed validates methods to measure OAS1 enzymatic function reproducibly.

References

Magusali N, Graham AC, Piers TM, Panichnantakul P, Yaman U, Shoai M, Reynolds RH, Botia JA, Brookes KJ, Guetta-Baranes T, Bellou E, Bayram S, Sokolova D, Ryten M, Sala Frigerio C, Escott-Price V, Morgan K, Pocock JM, Hardy J, Salih DA. (2021) . A genetic link between risk for Alzheimer's disease and severe COVID-19 outcomes via the OAS1 gene. Brain. 31;144(12):3727-3741. doi: 10.1093/brain/awab337. PMID: 34619763; PMCID: PMC8500089.

Salih, D.A., Bayram, S., Guelfi, S., Reynolds, R.H., Shoai, M., Ryten, M., Brenton, J.W., Zhang, D., Matarin, M., Botia, J.A., Shah, R., Brookes, K.J., Guetta-Baranes, T., Morgan, K., Bellou, E., Cummings, D.M., Escott-Price, V. and Hardy, J. (2019). Genetic variability in response to amyloid beta deposition influences Alzheimer's disease risk. *Brain Communications*, 1(1). doi:https://doi.org/10.1093/braincomms/fcz022.