

Abstract

Necroptosis is a form of inflammatory cell death. Currently, SIGLEC8 and SIGLEC12 are two genes that have been found to play a role in necroptosis. However, there is a gap when it comes to identifying these genes as targets in the context of neurological degeneration. Utilizing cell transfection and immunofluorescence microscopy, our findings support the notion that SIGLEC8 and SIGLEC12 must be investigated further.

Introduction & Summary

Unlike apoptosis, necroptosis is messy and stems from inflammation and distress. Specifically, within the brain, it causes destruction of certain cells like microglial cells, the primary immune cells in the brain. This inflammatory response is common in neurodegenerative diseases. Elucidating the role of these genes is vital to stop the inflammation and neurodegeneration caused by various brain diseases such as Alzheimer's, Parkinsons, or MS. When SIGLEC12 was knocked out through CRISPR-Cas9, scientists found necroptosis to be defective and prevented. Further, SIGLEC8 was found to play a role in cell death in IL-5-activated eosinophils, which are white blood cells. Further research is needed to elucidate the role of these SIGLEC8 and SIGLEC12 genes in necroptosis, specifically in the neurological microglial immune cells. This study is being conducted to study how cells function when these genes are activated, and to discern how each gene plays a role in necroptosis within our cells. Current studies do not further investigate this issue within the neurological space, so this experiment will serve as a way to close this gap. In this study, it is hypothesized that SIGLEC8 and SIGLEC12 will show signs of triggering necroptosis and cell death.

References

- Noh, H., & Boms, E. (2025). SIGLEC12 mediates plasma membrane rupture during necroptotic cell death. *Nature*. <https://doi.org/10.1038/s41586-025-09741-1>
- Kano, G., Almanan, M., Bochner, B. S., & Zimmermann, N. (2013). Mechanism of Siglec-8-mediated cell death in IL-5-activated eosinophils: Role for reactive oxygen species-enhanced MEK/ERK activation. *Journal of Allergy and Clinical Immunology*, 132(2), 437-445. <https://doi.org/10.1016/j.jaci.2013.03.024>
- Gao, C., Jiang, J., Tan, Y., & Chen, S. (2023). Microglia in neurodegenerative diseases: mechanism and potential therapeutic targets. *Signal Transduction and Targeted Therapy*, 8(1), 1-37. <https://doi.org/10.1038/s41392-023-01588-0>

Methodology

To begin this experiment, we ordered the plasmids SIGLEC8 and SIGLEC12, isolating the plasmids and preparing them for cloning and PCR.

- Isolated the SIG8 plasmid
- Bacterial Transformation to insert the gene into the pEntry_Topo_Siglec8 vector



Fig 1. Plasmid isolation of SIGLEC8

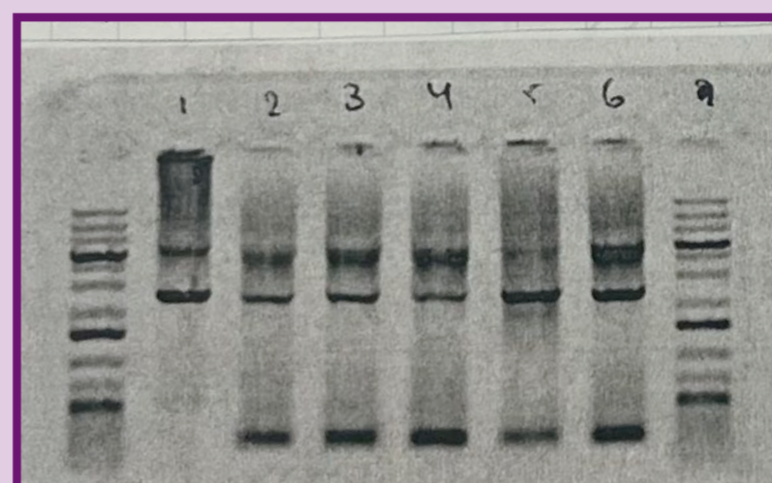


Fig 2. Gel Electrophoresis results to verify the PCR for pEntry_Topo_Siglec8

- Bacterial Transformation to insert the pEntry_Topo_Siglec8 vector into the pDest_pGama vector
- Process repeated with SIG12

We ran PCR tests and utilized Gel Electrophoresis to verify the DNA after each step to ensure the genes were ready for transfection.

We utilized HELA and HEK293T cells in a tray. The experimental group consisted of the cells with the SIGLEC8 and SIGLEC12 genes. Our negative control group was plain cells, our expression control group was with AAV-mCherry, and our positive control group was with the ZPB1 antibody, meant to cause a normal amount of inflammation. We wanted to observe the differences in reactions in the cells when they had the gene. We would then analyze the inflammation and cell death occurring in each group. After growing and culturing the cells and allowing them to live in our conditions, we took the slides and utilized the confocal microscope to take images. From the images, analysis of protein expression was conducted with ImageJ for the results, and expression was quantified as a percentage.



Fig 3. Confocal microscope

Results & Findings

SIGLEC8

293T Cell Line		HELA Cell Line	
Group Name	% expressed	Group Name	% expressed
Positive Control (ZPB1)	13.87%	Positive Control (ZPB1)	16.27%
Experimental Group	3.85%	Experimental Group	15.64%

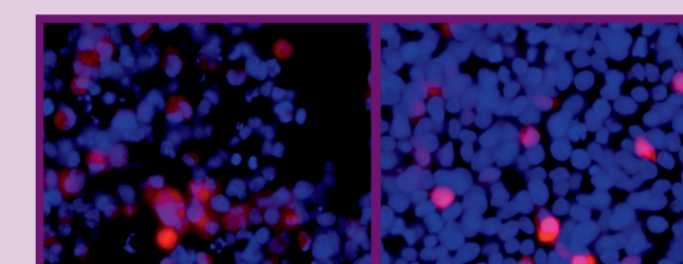


Fig 4. Images of 293T cells with SIGLEC8

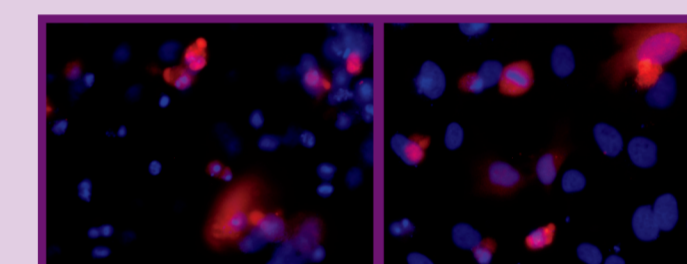


Fig 5. Images of HELA cells with SIGLEC8

SIGLEC12

293T Cell Line		HELA Cell Line	
Group Name	% expressed	Group Name	% expressed
Positive Control (ZPB1)	11.24%	Positive Control (ZPB1)	13.22%
Experimental Group	7.61%	Experimental Group	9.52%

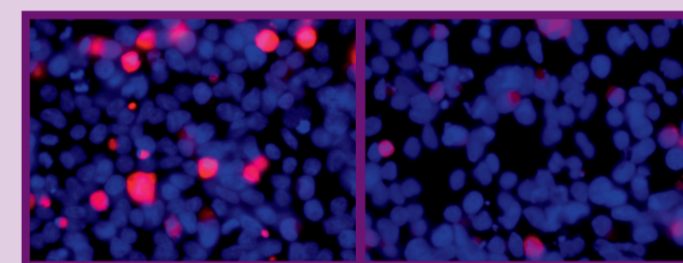


Fig 6. Images of 293T cells with SIGLEC12

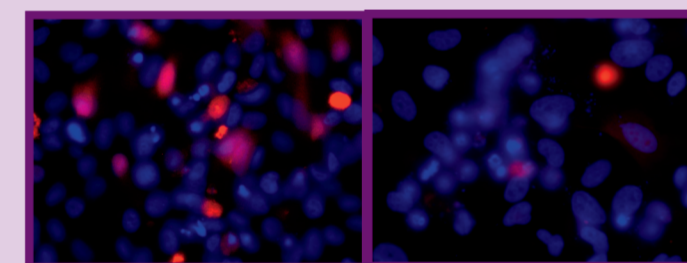


Fig 7. Images of 293T cells with SIGLEC12

Fig 4., Fig 5., Fig 6., and Fig 7. are immunofluorescence images of each cell line, demonstrating upregulated inflammatory proteins.

Discussion

When comparing cells treated with SIGLEC8 or SIGLEC12, there is a significant down regulation of inflammatory proteins. The cells showed more cell death when treated with the ZPB1 antibody from the positive control group. This disproves our initial hypothesis that these genes increase inflammation within cells. These outcomes provide evidence that SIGLEC8 and SIGLEC12 have a role in decreasing inflammation and cell death. Elucidating their roles in necroptosis will be the key for future studies, identifying them as targets and potentially contributing to neurodegeneration prevention.