Examining Siglec Signaling in Alzheimer's Disease

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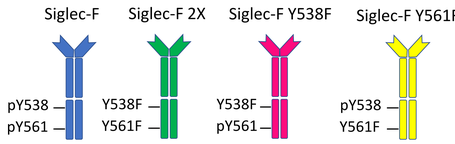
Motivation and Background

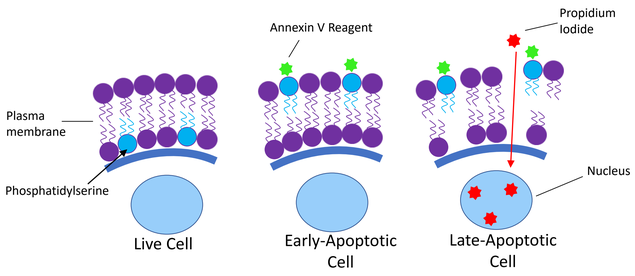
- There are few therapeutics for patients suffering from Alzheimer's disease (AD). We aim to identify possible therapeutic targets by examining inflammatory signaling pathways.
- Using Mass Spectrometry, Siglec-F was identified as an activated receptor on disease associated microglial cells of multiple mouse models of AD.
- Siglecs are cell surface receptors that bind sialic acid and are commonly found on immune cells. The cytoplasmic domains contain immunoreceptor tyrosine-based inhibitory motifs (ITIMS).
- We wanted to examine apoptotic phenotypes of BV2 cells expressing Siglec receptors to determine if altering functional tyrosine residues of these receptors will impact cell death.



Wild type and mutant receptors: functional tyrosine (Y) residues modified to inactive phenylalanine (F)

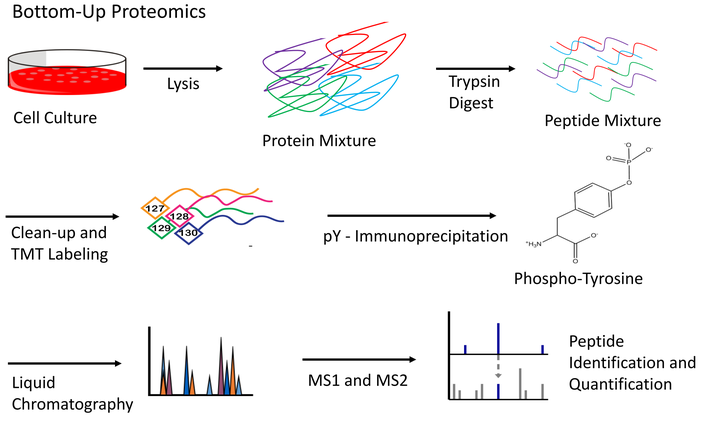
Methods: Apoptosis Assay

We used an Annexin V and Propidium Iodide to determine the occurrence of apoptosis in BV2 cell line expressing various forms of the Siglec-F receptor.

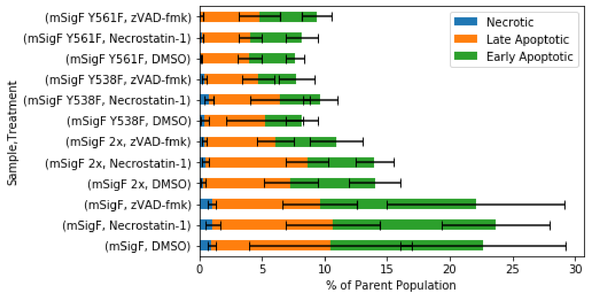


- Phosphatidylserine moves to the extracellular surface of the plasma membrane when cells begin apoptosis.
- Late-apoptotic cells have a disrupted membrane, allowing propidium iodide to enter and stain DNA in the nucleus.

Methods: Mass Spectrometry for Cell Signaling



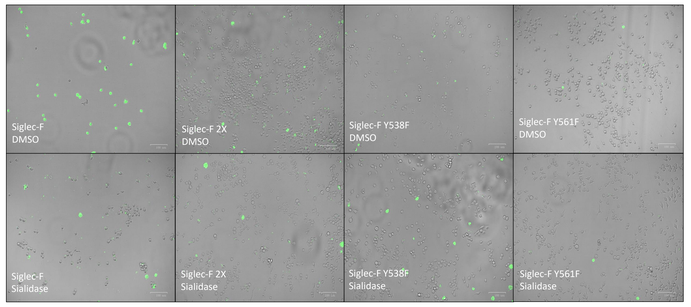
Results: Wild Type Siglec-F increases apoptosis compared to Y->F mutations



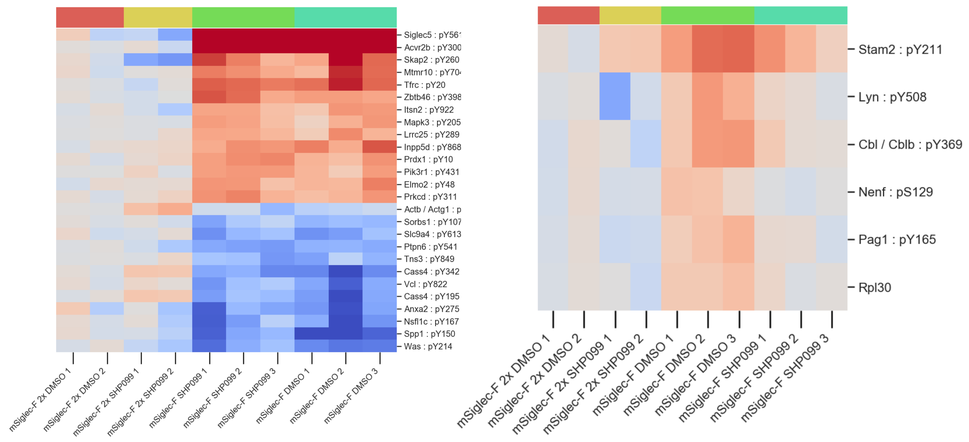
- zVAD-fmk is a pan-caspase inhibitor used to block apoptosis.
- Necrostatin-1 is a RIPK1 inhibitor used to block necroptosis.
- DMSO is added at an equivalent volume to wells not being treated with inhibitor
- Neither inhibitor was able to reduce the detected programmed cell death (apoptosis/necrosis) and in some cases, the apoptotic population was larger for those treated with the inhibitors.

Annexin V green channel merged with Brightfield images

- Sialidase is an enzyme known to cleave sialic acid off of cell surface proteins.
- This removes potential ligands for Siglec-F to bind.
- The amount of apoptotic cells expressing wild type Siglec-F decreases with treatment of sialidase.



Results: Phospho-Tyrosine Peptide Analysis of BV2 Cells Treated with SHP099



- SHP099 is a small molecule inhibitor of SHP-2 signaling protein. It prevents SHP-2 from binding to Siglec-F exposed ITIM.
- Left: The difference between the wild type Siglec-F and the double mutant, Siglec-F 2X, is expected because the tyrosine residuals make up the functional ITIM.
- Right: Use of SHP099 identified several SHP-2 dependent proteins. String network predicts co-expression and interactions between SHP099 regulated sites as well as interactions with proteins present in endocytosis pathways.

Conclusions

- Expression of Siglec-F receptor increases apoptosis in BV2 cells compared to the two phenylalanine mutants.
- zVAD-fmk and Necrostatin-1 failed to block apoptosis, suggesting an alternate pathway leads to apoptosis when Siglec-F is activated.
- Sialidase was able to reduce the number of apoptotic cells, especially in the wildtype Siglec-F receptor. This shows that prevention of Siglec-F activation can reduce cell death.
- Tyrosine analysis identified SHP-2 dependent and independent interactions.

A known and unknown signaling pathway of Siglec-F

- Siglec –F is activated by sialic acids and binds SHP-2.
- If cells are treated with sialidase, there are minimal ligands to bind Siglec-F.
- SHP-2 activation can lead to inflammation.
- SHP099 inhibits SHP-2 binding.
- Treatment with SHP099 did not prevent apoptosis in BV2 cells.
- This suggests there must be an alternate signaling pathway interacting with Siglec-F that leads to apoptosis.

Future Work

- Translate these finding into iPS-derived microglial cells.
- Potential inhibitors of other Siglec pathways will be tested with BV2 cells to evaluate if there is a change in apoptosis.
- SHP099 will be tested in CK-p25 mouse model to observe changes in microglial signaling.

References

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