Molecules and Mechanisms of MEGF10 Mediated Efferocytosis

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Abstract

Efferocytosis, the clearance of dead and dying cells, is fundamental for tissue maintenance and homeostasis (Boada-Romero et al. 2020). I performed a literature review of efferocytosis receptor Megf10 to find similarities in the mechanism of efferocytosis between Megf10 and its homologs Draper and CED-1, which my lab mates studied. Megf10 specifically is shown to be involved in tissue maintenance in the brain and mutations lead to corpse accumulation and developmental issues in the CNS (Cahoy et al. 2008)(Chen et al. 2009)(Chung et al. 2013) (Iram et al. 2016). In my literature search I organized my findings into the categories "writer, reader, eraser" framework for phosophotyrosine signaling as proposed by Lim and Pawson. (Lim and Pawson 2010). When compared, MEGF10, Draper, and CED-1 show homology in molecules as well as mechanisms, indicating conservation across phyla.

Introduction

In the Williamson lab, two other undergraduates and I studied three homologous engulfment receptors – MEGF10 in *Mus musculus*, CED-1 in *Caenorhabditis elegans*, and Draper in *Drosophila melanogaster* - to survey knowledge of the cell biology and mechanisms used in efferocytosis and to investigate our hypothesis that the process of efferocytosis is conserved. We performed systematic literature searches for our respective receptors and are working to unite our findings in a review article this fall. I will focus my poster on MEGF10.

Efferocytosis is the engulfment of dead and dying cells. Dead and dying cells left uncleared will undergo secondary necrosis, which can cause damage to surrounding tissues (Boada-Romero et al. 2020). Lack of clearance is associated with early-onset myopathy, areflexia, respiratory distress, and dysphagia (Iram et al. 2016). Phagocytes use receptors which bind to "eat me" ligands on the surface of dying cells and initiate signal transduction pathways (Boada-Romero et al. 2020). Multiple EGF-like Domains 10 (MEGF10) is a non-tyrosine kinase receptor that has Immunoreceptor tyrosine-based activation motifs (ITAMs). ITAMs are activated by the phosphorylation of the tyrosine. Once activated, ITAMs are docking sites for proteins with SH2 domains, starting the pathway of downstream signaling. (Love and Hayes 2010).

Phosophotyrosine (pTyr) signaling is fundamental to many aspects of metazoan biology. Ptyr signal transduction uses three molecules- a "writer", a "reader", and an "eraser"- to control complex signaling once activated by an external stimuli. Writers are kinases that alter SH2 domains via phosphorylation. "Readers" are proteins that recognize SH2 domain alternation. Erasers are proteins that restore SH2 domains to their original status by removing the phosphate (Lim and Pawson 2010). Utilizing these categories, I studied the molecules and mechanisms of efferocytosis via MEGF10 and can compare to its homologs.

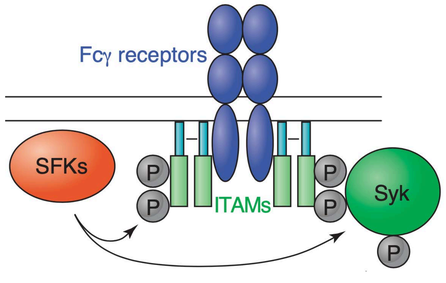

Figure1. Phosophotyrosine signaling and ITAM activation. PTyr signaling in Fcγ receptors is comparable to pTyr signaling via MEGF10. The ITAMs (light green) are phosphorylated by Src Family Kinases (SFKs). Phosphorylation of ITAM tyrosine residues enables binding of the Syk SH2 domains. SFK is a writer. Syk is a reader. Modified figure from (Berton, Mócsai, and Lowell 2005).

Methods

Performed a systematic literature search to find research articles that studied MEGF10. Literature search was performed to maintain an unbiased view. Searched through PubMed to find primary literature. Plugged relevant and promising papers into Web of Science database to find research that built off previous promising research.

Findings

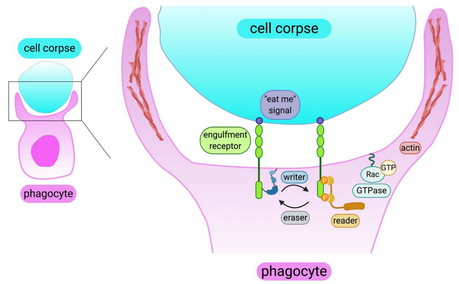

Figure 2: Engulfment signaling modules used by phagocytes across phyla. At the phagosome, the engulfment receptor recognizes an "eat me" signal displayed by the dead or dying cell. Upon recognition, the engulfment receptor is phosphorylated by the "writer'. Then the "reader" recognizes the modified ITAM via its SH2 domains. Signal transduction occurs. For example, the reader, writer, and eraser system controls the Rac pathway which controls actin at the phagosome (Mao and Finnemann, 2015).

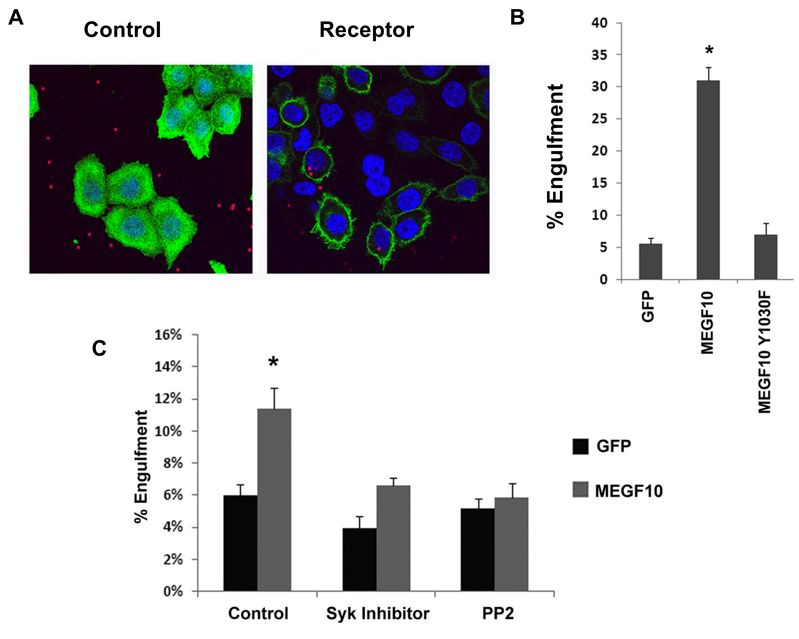

Figure 3. A. Receptor Expression Patterns. HeLa cells were transfected with GFP (left) or GFP-receptor. Microspheres (red) were added for two hours then cells were fixed. Right panel shows efferocytosis receptor localization at plasma membrane and successful engulfment of microspheres. **B. MEGF10 requires its ITAM domain for engulfment.** Percent engulfment of microspheres in HeLa cells transfected with GFP (control), MEGF10, or mutant MEGF10 (MEGF10 Y1030F) detected by confocal microscopy (MEGF10, $p = 0.0003$). **C. Syk and SFKs are required for MEGF10-mediated engulfment.** HeLa cells were transfected with GFP and MEGF10-GFP. After 48 hours cells were treated with with 1 uM Syk inhibitor (BAY 61-3606) or 1 uM PP2 for 1 h before addition of microspheres for 2 h. Cells were rinsed, fixed, and engulfment of microspheres was detected by confocal microscopy. The Syk inhibitor significantly reduced MEGF10-mediated ($p = 0.026$, $n = 3$) engulfment. Similarly, PP2, which inhibits SFKs, significantly decreased the engulfment mediated by MEGF10 ($p = 0.024$, $n = 3$). (Modified figures from Scheib, Sullivan, and Carter 2012).

Takeaways

Writers

- Fyn, Lyn, Lck, and Src are Src Family Kinases (SFKs) that phosphorylate the ITAM domains of MEGF10 and lead to successful phagocytosis. (Scheib, Sullivan and Carter 2012)

Writer	Reader	Eraser
Fyn	Gulp-1	PP2
Lyn	Syk	
Lck		
Src		

Readers

- Syk has SH2 domains and can bind phosphorylated tyrosine residues. Syk binding is increased when the ITAMs are phosphorylated by SFKs. (Scheib, Sullivan and Carter 2012).
- Gulp-1 has a phosophotyrosine binding domain. The MEGF10, GULP-1 and RAC-1 pathway is homologous to the worm pathway CED-1, CED-6, and CED-10, respectively (Morizawa et al. 2017)

Erasers

- MEGF10 does not have a specific eraser. PP2 is a nonspecific SFK inhibitor. PP2 decreased the engulfment capacity of cells by inhibiting SFKs (Scheib, Sullivan and Carter 2012).

Conclusions

Through the literature search I have identified some readers, writers, and erasers for MEGF10. When compared to my lab mates' receptors there is evidence for homology across phyla in the ITAM domains (Wu et al. 2009) as well as in the readers, writers, and erasers, and mechanisms used for efferocytosis. More research is needed to further elucidate molecules and pathways involved for efferocytosis, such as actin remolding pathways.

Future Directions

- Use knowledge from homologous receptors to guide research of MEGF10
- Identify more readers, writers, and erasers involved in MEGF10 efferocytosis signaling using biotinylation labeling and mass spectrometry
- Use research involving MerTK engineering to inform experiments with MEGF10 engineering.

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