

Understanding the effects of oxidative stress on the pyroptotic pathway of programmed cell death



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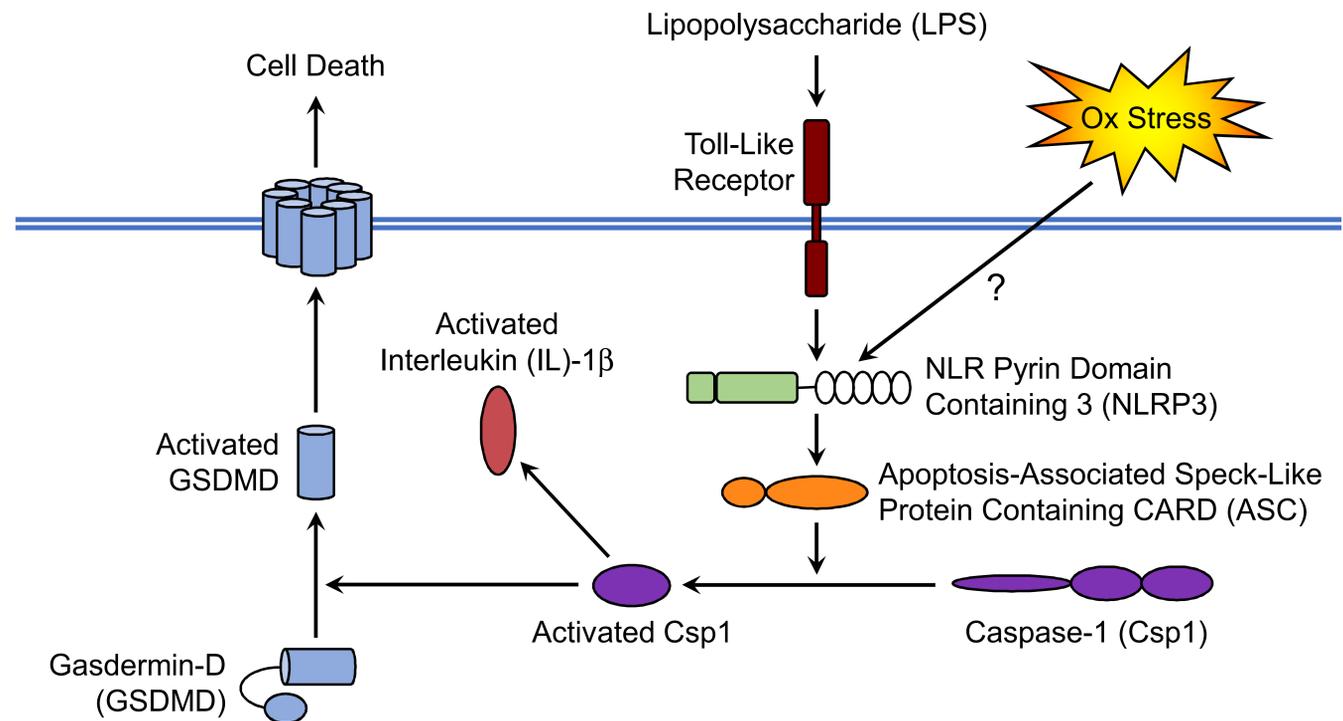
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BACKGROUND

- The development of disease is often due to programmed cell death
- Pyroptosis is a newly discovered form of cell death that is typically activated during bacterial infection
- Pyroptosis requires activation of the inflammasome leading to cleavage of Gasdermin-D by caspase-1
- A major driving component of cell death during non-infectious disease is oxidative stress
- However, whether oxidative stress can induce pyroptosis is unknown

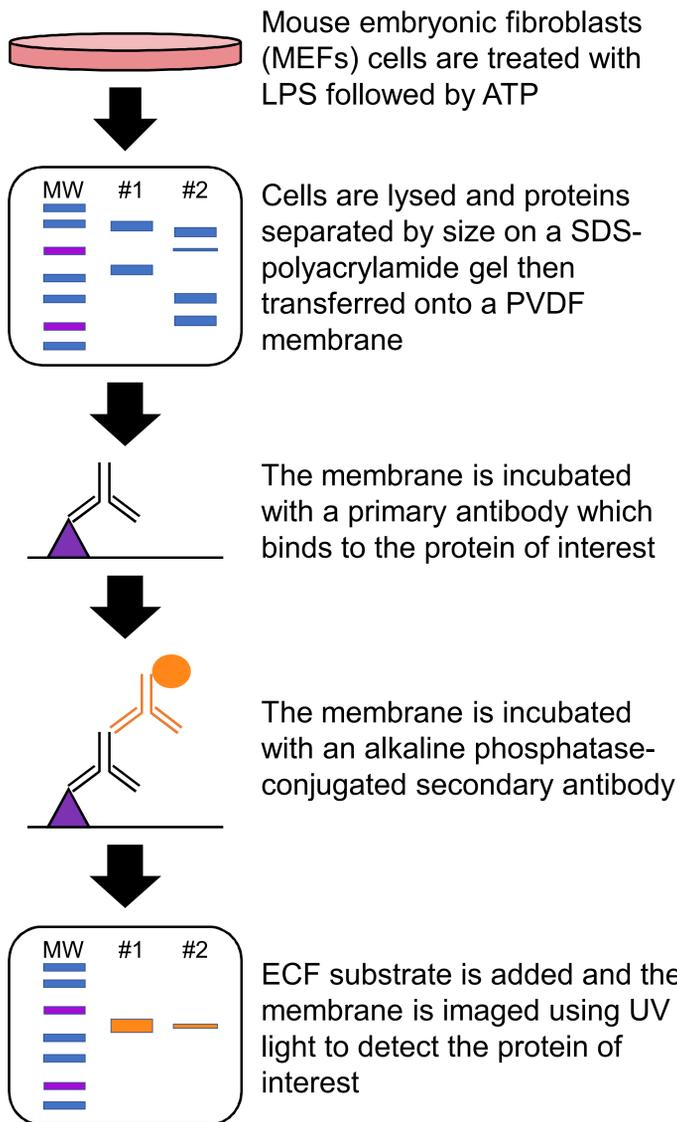
THE PYROPTOTIC PATHWAY



HYPOTHESIS

- We hypothesize that oxidative stress activates the inflammasome, leading to Gasdermin-D cleavage and cell death

METHODS



RESULTS

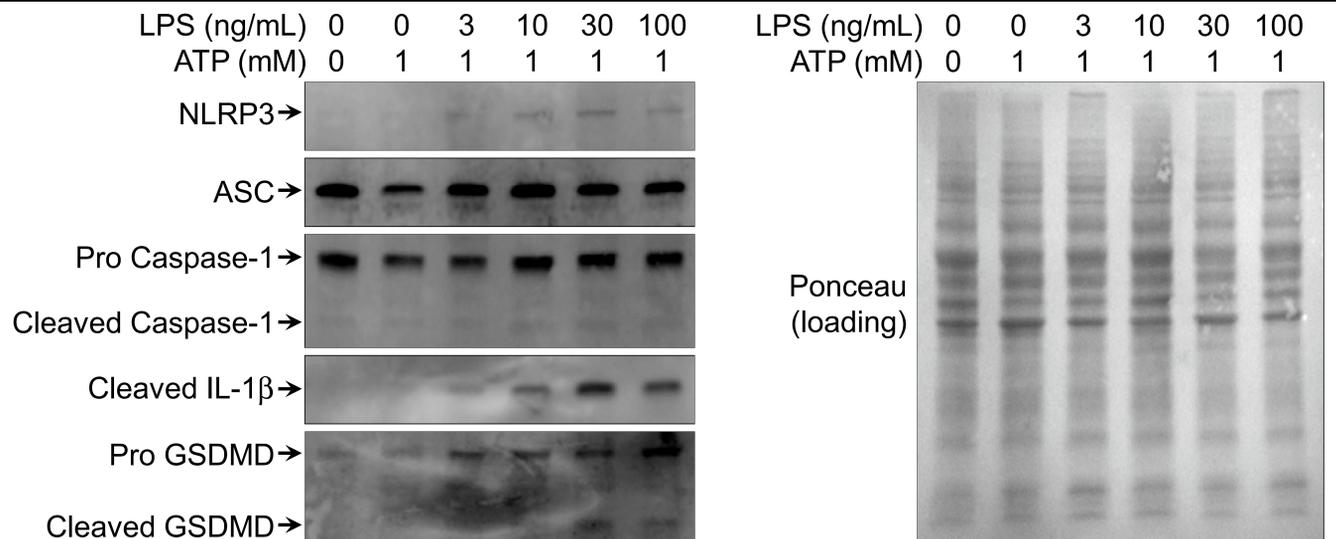


Figure 1. Induction of pyroptotic signaling in fibroblasts. MEFs were incubated with increasing amounts of LPS for 3hrs followed by incubation with 1mM ATP for 3hrs. Cells lysates were then subjected to Western blotting for NLRP3, ASC, Csp1, cleaved IL-1β, and GSDMD. Equal loading of samples was verified by Ponceau staining.

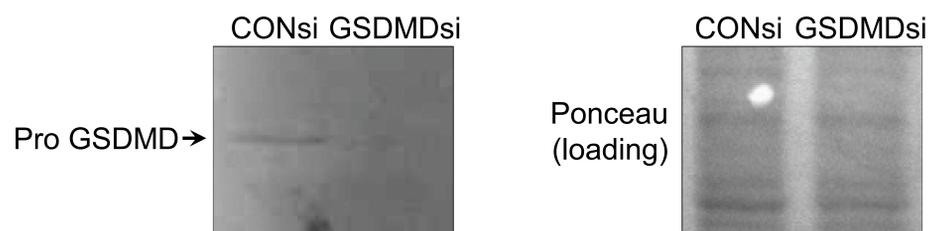


Figure 2. siRNA knockdown of GSDMD in fibroblasts. MEFs were transfected with 100nM of control or GSDMD siRNAs for 72hrs. Cells lysates were then subjected to Western blotting for GSDMD. Equal loading of samples was verified by Ponceau staining.

CONCLUSIONS AND FUTURE DIRECTIONS

- We have established that MEFs have a functional pyroptotic pathway and have successfully knocked down GSDMD in these cells
- We will expose control and GSDMD-depleted MEFs to oxidative stress and measure activation of the pyroptotic pathway and cell death