Direct Regulation of MLKL by EFhd2 to Suppress the Necrotic Signaling Pathway

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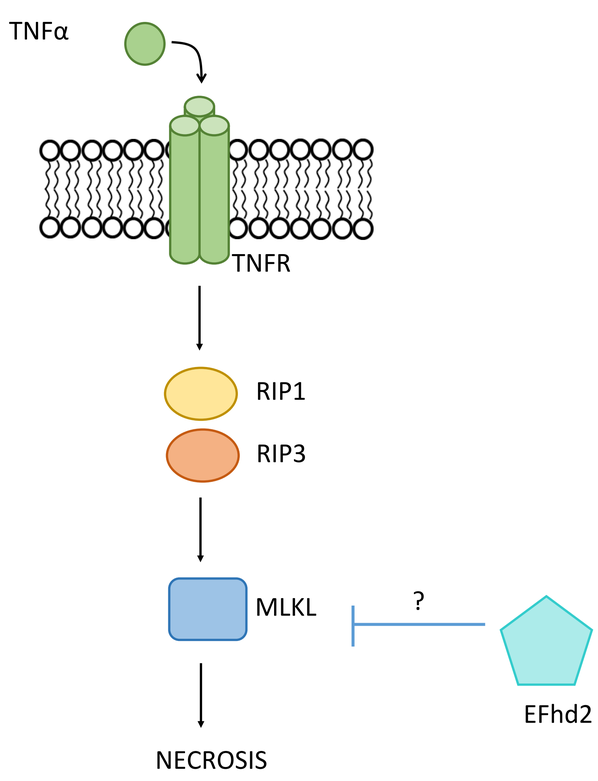
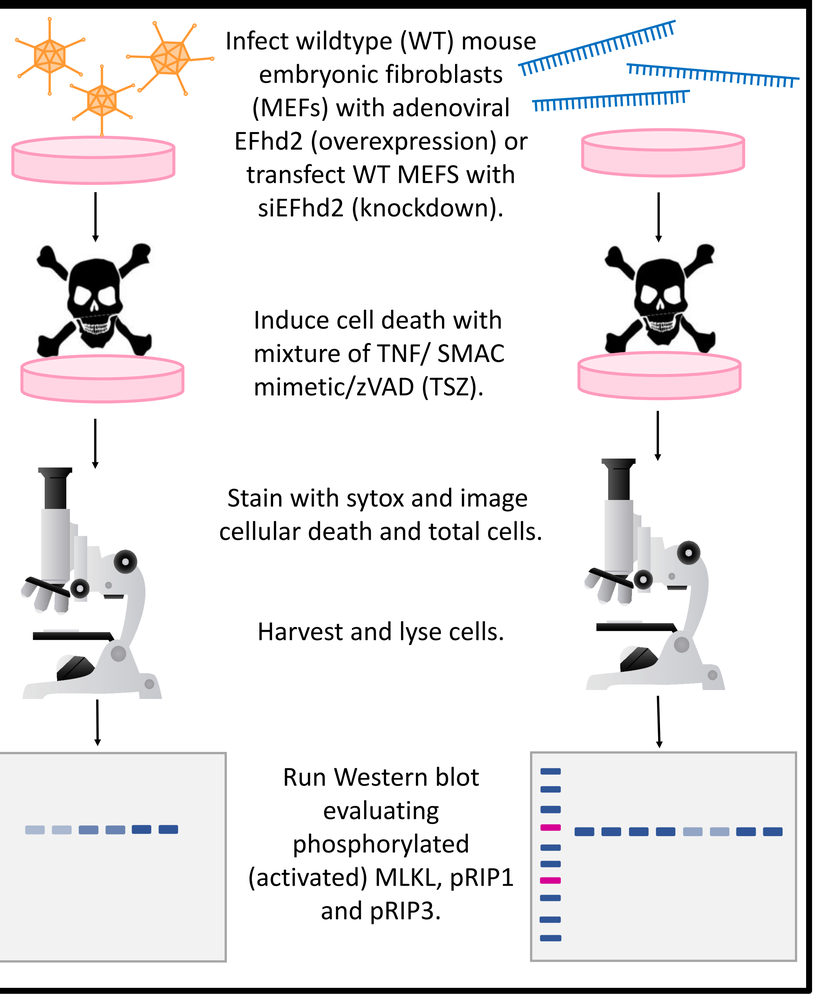
BACKGROUND

- Programmed cell death plays a vital role in both physiological and pathological processes from embryogenesis to disease pathogenesis in multiple organs such as liver cirrhosis and renal failure. Thus researching how necrosis is signaled is vital for advancing the treatment of multiple diseases.
- Necrosis occurs when tumor necrosis factor (TNF) receptors on cells are activated leading to the phosphorylation and activation of the kinases Receptor Interacting Protein 1 and 3 (RIP1 and RIP3). RIP3 then binds to and phosphorylates the pseudokinase Mixed Lineage Kinase domain-Like protein (MLKL). Phosphorylated MLKL then oligomerizes and translocates to the plasma membrane where it forms a non-specific pore that kills the cell.
- We have previously identified EF-hand domain family member 2 (EFhd2) as a novel MLKL-binding protein and shown that it can suppress TNF-induced necrosis. However, the molecular mechanism by which EFhd2 inhibits necrosis remains to be elucidated

MLKL Mediates TNFα-induced Necrosis but where does EFhd2 regulate MLKL?



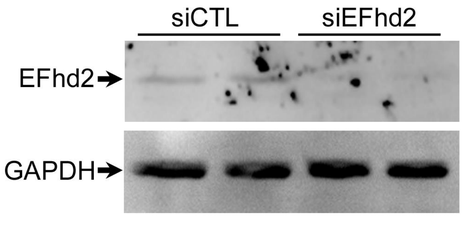
EXPERIMENT OVERVIEW & OBJECTIVES



RESULTS

Knockdown of EFhd2 increases TSZ induced cell death

Figure 1: siEFhd2 has sufficient knockdown of EFhd2 protein. *Western blot analysis of WT MEFs transfected with siRNA against EFhd2 or scrambled control (CTL).*



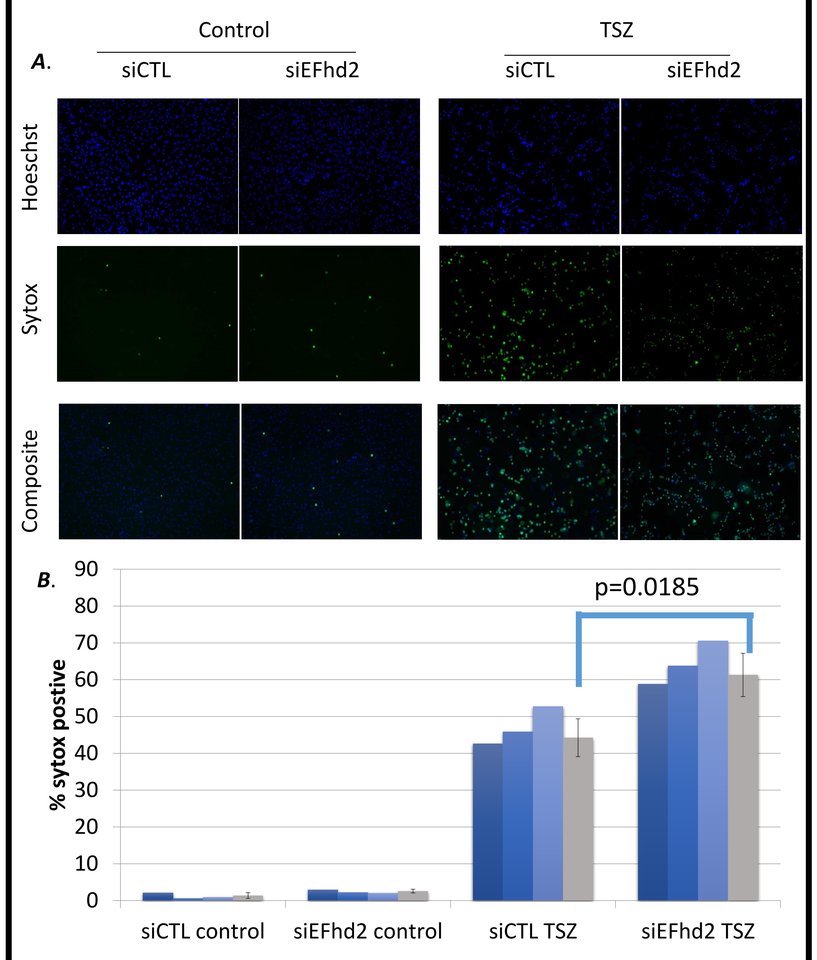


Figure 2: Knockdown of siEFhd2 increases cell death induced by TSZ. *Representative images (A) and quantification (B) of WT MEFs transfected with siRNA against EFhd2 or siCTL for 48 hours. Cells were then treated with (20µg/mLTNF-α, 1µM SMAC mimetic, 20µM ZVAD) of TSZ for 2 hours before analysis of cell death by sytox imaging. Blue bars represent individual experiments and gray bars represent mean +/- standard deviation.*

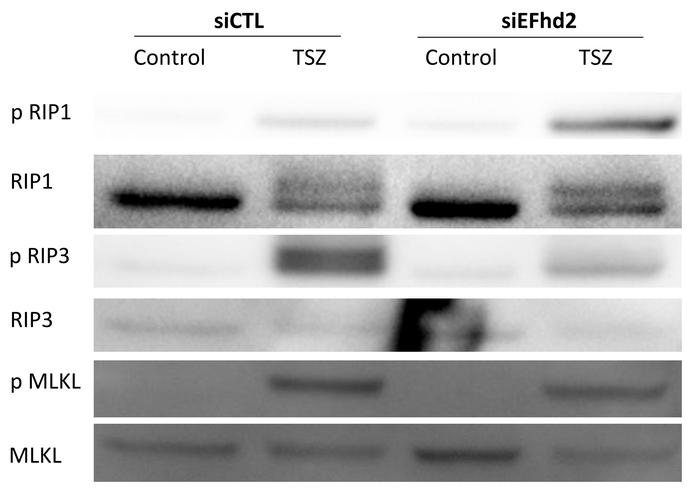
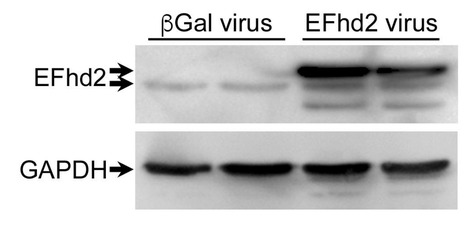


Figure 3: Knockdown of EFhd2 affects pRIP1 & pRIP3 levels. *WT MEFs were transfected with siRNA against EFhd2 or siCTL for 48 hours. The cells were collected and lysed for Western blot analysis of the RIP1/RIP3/MLKL pathway. n=1*

Overexpression of EFhd2 protects against TSZ induced cell death

Figure 4: EFhd2 virus has sufficient expression of EFhd2 protein. *Western blot analysis of WT MEF infected with EFhd2 or βgal adenovirus.*



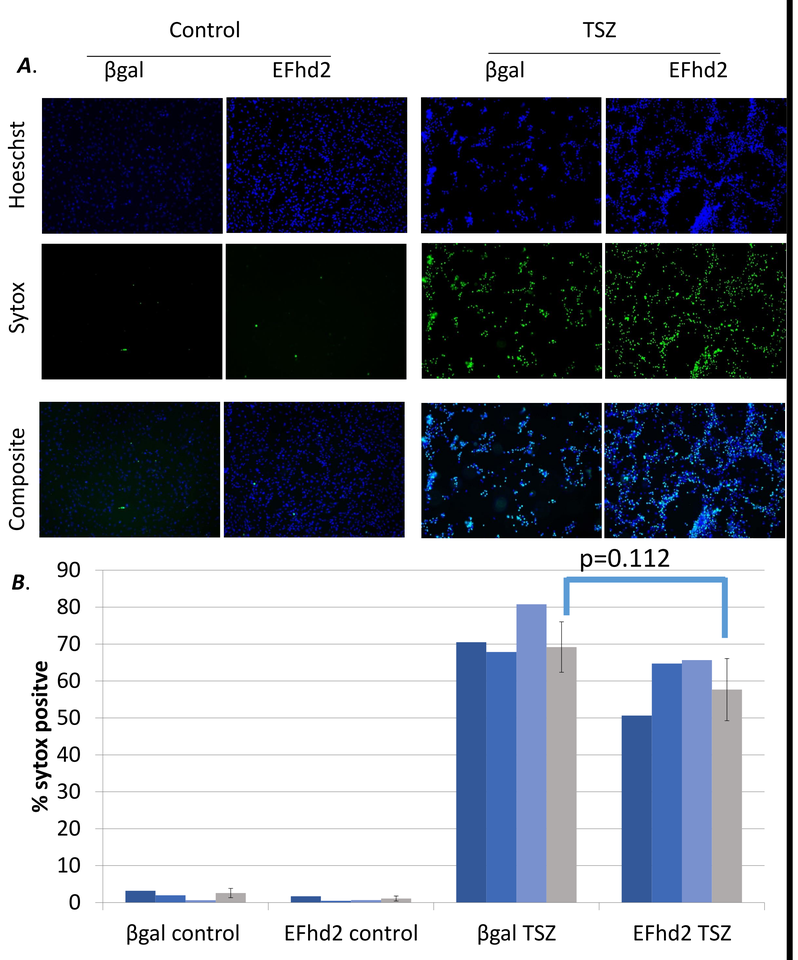


Figure 5: Overexpression of EFhd2 protects against cell death induced by TSZ. *Representative images (A) and quantification (B) of WT MEFs infected with viral EFhd2 or βgal (control virus) for 48 hours. Cells were then treated with (20µg/ml TNF-α, 1µM SMAC mimetic, 20µM ZVAD) of TSZ for 2 hours before analysis of cell death by sytox imaging. Blue bars represent individual experiments and gray bars represent mean +/- standard deviation.*

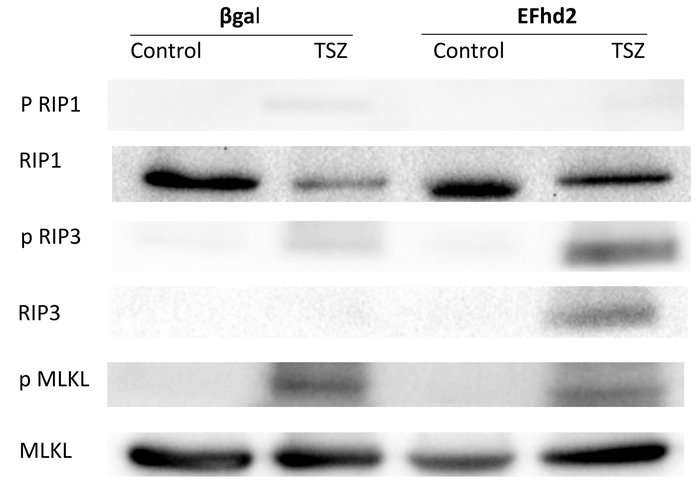


Figure 6: Overexpression of EFhd2 affects pRIP1 & pRIP3 levels. *WT MEFs were infected with EFhd2 or Bgal for 48 hours. The cells were collected and lysed for Western blot analysis of the RIP1/RIP3/MLKL pathway. n=1*

CONCLUSIONS & FUTURE STUDIES

- EFhd2 regulates TSZ induced cell death.
  - Knockdown increases cell death.
  - Overexpression decreases cell death.
- EFhd2 is an upstream regulator of MLKL activation.
  - Western blot analysis reveals a difference in pRIP1 and pRIP3 with EFhd2 augmentation.
- Western blot analysis needs to be repeated for conformation of current results.
- Further studies will be performed on direct regulation of pRIP1 and pRIP3 to conclude EFhd2 regulation of the pathway does not involve direct inhibition of MLKL.

ACKNOWLEDGEMENTS

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