EFhd2 and Drebrin-1 as Novel Regulators of Programmed Necrosis

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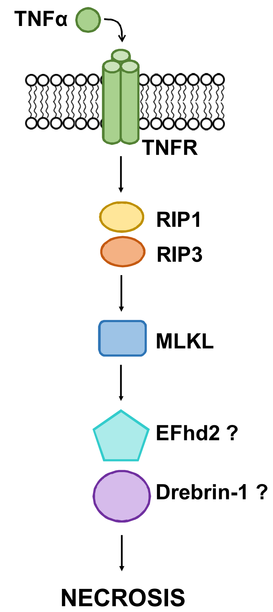
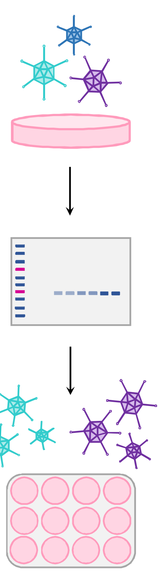
BACKGROUND

- Programmed cell death plays a vital role in both physiological and pathological processes from limb development during embryogenesis to disease pathogenesis in multiple organs. Central to both disease progression and cancer cell death is programmed necrosis.
- Programmed necrosis is initiated when TNFα binds its receptor, leading to activation of an intracellular kinase cascade.
- Despite its importance, the TNFα pathway is not fully understood and the downstream targets of the last known protein in the cascade, mixed lineage kinase domain-like (MLKL), remain unknown.
- Our previous work has identified two novel MLKL binding proteins, EFhd2 and Drebrin-1, which may act as negative regulators of necrosis.

EFhd2 and Drebrin-1 Mediate TNFα-induced Necrosis



STUDY OVERVIEW & HYPOTHESIS



Infect wild type mouse embryonic fibroblast (MEF) cells with β-galactosidase (control), murine EFhd2-Myc, or murine Drebrin-1-Myc adenoviruses

Verify degree of EFhd2 and Drebrin-1 overexpression with Western blotting and select appropriate level of infection

Overexpress EFhd2 and Drebrin-1 in wild type MEF cells and test the effects on TNFα-induced necrosis and paradoxical apoptosis

We hypothesize that overexpression of EFhd2 and Drebrin-1 will be protective for the cell, decreasing TNFα-induced necrosis

RESULTS

Knockdown of EFhd2 and Drebrin-1 Gene Expression

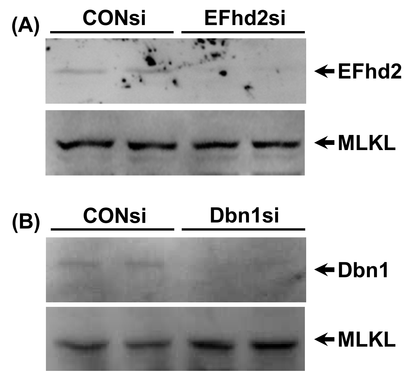


Figure 1: Successful knockdown of EFhd2 and Drebrin-1 gene expression in MEF cells. *In previous work, MEF cells were transfected for 48 hours with control (CONsi; A, B), EFhd2 (EFhd2si; A), or Drebrin-1 (Dbn1si; B) siRNAs. The cells were lysed and Western blotted for EFhd2, Drebrin-1 (Dbn1), and MLKL.*

Effects of EFhd2 and Drebrin-1 siRNA on TNFα-Induced Necrosis

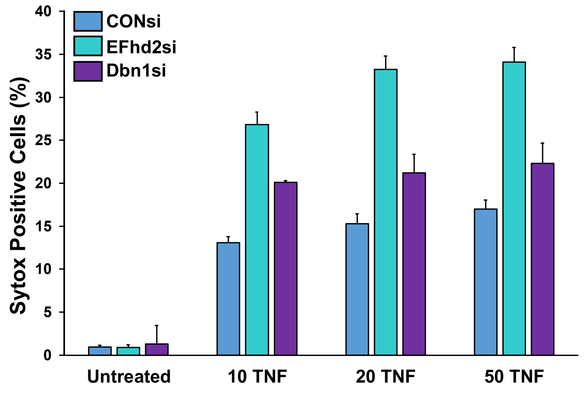


Figure 2: Effects of EFhd2 and Drebrin-1 siRNA on TNFα-induced necrosis. *In previous work, once Western blotting confirmed successful knockdown of both EFhd2 and Drebrin-1. MEFs transfected with CONsi, EFhd2si, or Dbn1si were exposed to TNFα (10, 20, or 50ng/mL) in the presence of a caspase inhibitor (zVAD-FMK, 20μM) for 4 hrs. Necrosis was then measured using Sytox Green fluorescent vital dye. Error bars = SEM, n = 3.*

Adenoviral Overexpression of EFhd2 and Drebrin-1

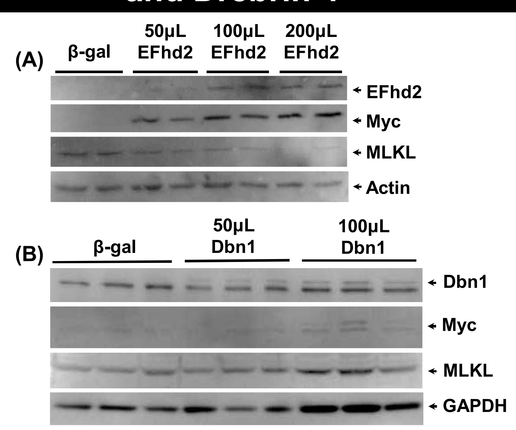


Figure 3: Adenoviral overexpression of EFhd2 and Drebrin-1 in MEF cells. *MEF cells were infected for 48 hours with adenoviruses expressing murine EFhd2-Myc (A) or murine Drebrin-1-Myc (B) at increasing concentrations (μL viral stock/mL cells). β-galactosidase was used as an infection control. Cells were lysed and subjected to Western blotting for EFhd2, Drebrin-1, Myc, and MLKL. Actin (A) and GAPDH (B) were used as loading controls. From these blots, infection levels of 50μL virus stock per mL of cells and 100μL virus stock per mL of cells were chosen for EFhd2 and Drebrin-1, respectively, to ensure adequate overexpression for necrosis and apoptosis studies.*

ACKNOWLEDGEMENTS

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Effects of EFhd2 and Drebrin-1 Overexpression on TNFα-Induced Necrosis

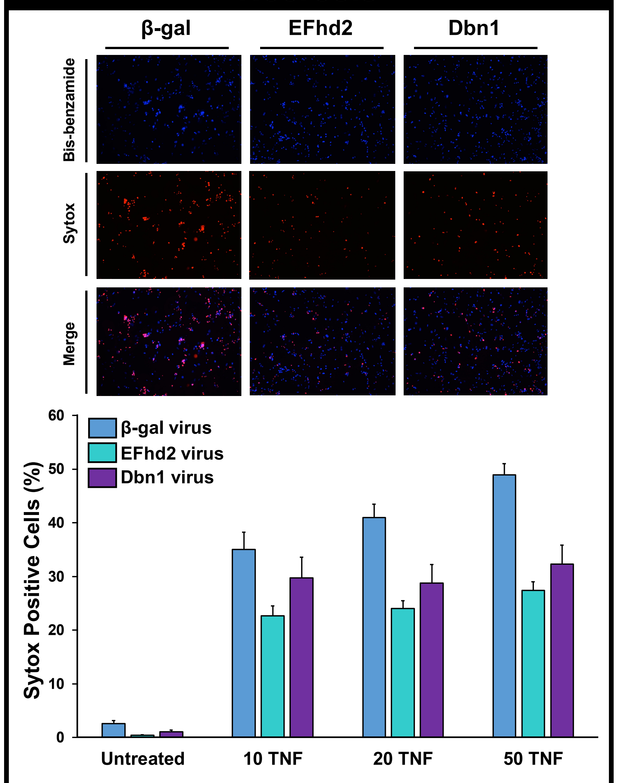

Figure 4: Effects of EFhd2 and Drebrin-1 overexpression on TNFα-induced necrosis. *MEF cells were infected for 48 hours with EFhd2 (50 μL/mL cells), Drebrin-1 (100μL/mL cells), or β-galactosidase (100μL/mL cells) adenoviruses. The infected cells were then exposed to TNFα (10, 20, or 50ng/mL) in the presence of a caspase inhibitor (zVAD-FMK, 20μM) for 4 hrs. Necrosis was then measured using Sytox Green fluorescent vital dye. Error bars = SEM, n = 3. The above series of images are representative stains of infected cells treated with 50 ng/mL TNFα.*

Effects of EFhd2 and Drebrin-1 Overexpression on TNFα-Induced Apoptosis

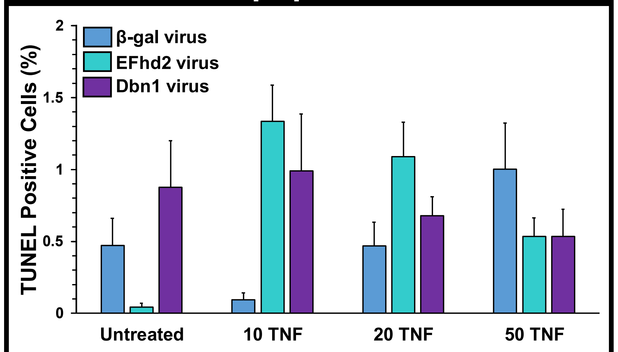

Figure 5: Effects of EFhd2 and Drebrin-1 overexpression on TNFα-induced apoptosis. *MEF cells were infected for 48 hours with EFhd2 (50 μL/mL cells), Drebrin-1 (100μL/mL cells), or β-galactosidase (100μL/mL cells) adenoviruses. The infected cells were then exposed to TNFα (10, 20, or 50ng/mL) in the presence of a caspase inhibitor (zVAD-FMK, 20μM) for 4 hrs. Apoptosis was then measured using TUNEL staining. Error bars = SEM, n = 1.*

CONCLUSIONS

- EFhd2 and Drebrin-1 appear to be negative regulators of TNFα-induced necrosis.
- Overexpression of EFhd2, and to a lesser extent Drebrin-1, appears to be protective for the cell during TNFα-induced necrosis.
- Minimal apoptosis was observed following TNFα and zVAD-FMK treatment, suggesting that the main mechanism of death taking place is necrosis.
- Future studies will further evaluate the roles of EFhd2 and Drebrin-1 in TNFα-induced necrosis.