Oxidative stress activates mechanisms of regulated necrosis and mitochondrial demise via CYLD

Oxidative stress is regarded as a major trigger for neuronal dysfunction and death in the ageing brain and in multiple neurodegenerative disorders. How oxidative stress mediates neuronal death and whether the associated mechanisms are accessible for therapeutic intervention strategies is not clarified. Increasing evidence suggests that oxidative stress triggers mechanisms of regulated necrosis that involve the activation of receptor interacting protein 1 (RIP1) independently of death receptor activation.

Recently, we identified a pivotal role for cylindromatosis (CYLD) driving RIP1 activation and necrosome formation in oxidative death pathways in neurons. In a model of glutamate-induced oxidative death (oxytosis) in neuronal HT-22 cells we characterized protective effects of siRNA-mediated CYLD gene silencing which involved inhibition of RIP1/RIP3 complex formation. Additionally, experiments in $CYLD^{-/-}$ mice revealed protection against traumatic brain injury demonstrating an important function of CYLD in acute neuronal injury *in vivo*. Since oxytosis and ferroptosis share common features in their signaling pathways, like GSH depletion and inhibition of GPX4, increased lipid peroxidation and mitochondrial damage, it was of great interest to investigate a potential role of CYLD in mitochondrial death pathways in paradigms of erastin-induced ferroptosis.

Here, we show in neuronal HT-22 cells that erastin-mediated formation of reactive oxygen species (ROS) triggers mechanisms of regulated necrosis independent of TNFα-signaling. In this model system of ferroptosis, erastin promotes glutathione depletion and lipid peroxidation followed by activation of RIP1 and subsequent RIP1/RIP3 necrosome formation which is regarded as a hallmark of regulated necrosis. Silencing of RIP1 by siRNA or by the RIP1 inhibitor necrostatin-1 prevented erastin-induced cell death. In contrast, the ferroptosis inhibitor ferrostatin-1 failed to protect cells against TNFα-induced classical necroptosis, a form of programmed cell death that is mediated by RIP-kinases downstream of death receptor activation. In further steps, the impact of CYLD was investigated by siRNA-mediated gene silencing or gene knockout via the CRISPR/Cas-9 system, respectively. In erastin-induced ferroptosis, CYLD depletion decreased RIP1/RIP3 complex formation and promoted neuronal survival. Furthermore, we revealed the involvement of mitochondrial fission regulating dynamin-related protein 1 (DRP-1) since genetic depletion or pharmacological inhibition of DRP-1 protected HT-22 cells against erastin toxicity. CYLD knockout prevented erastin-induced DRP-1 translocation to mitochondria as well as mitochondrial ROS production and the loss of mitochondrial membrane potential, suggesting that CYLD and DRP-1 can link mechanisms of regulated necrosis to mitochondrial pathways of cell death in paradigms of oxidative stress (Fig. 1).

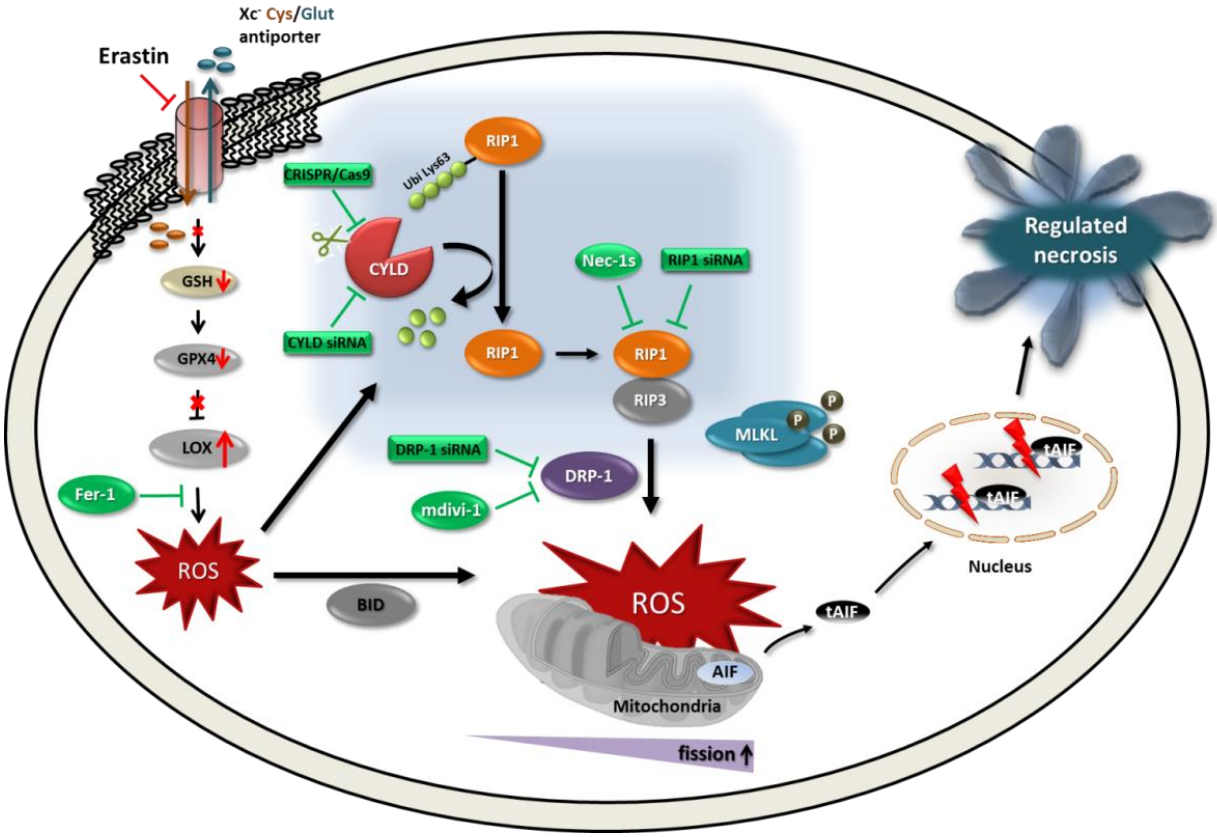

Figure 1: CYLD and DRP1 link mechanisms of regulated necrosis to mitochondrial pathways of cell death in paradigms of ferroptosis

In HT-22 cells the Xc^- transporter mediates the exchange of extracellular cystine and intracellular glutamate across the plasma membrane. Micromolar concentrations of erastin inhibit the antiporter system reducing the intracellular cystine required for the synthesis of glutathion (GSH). Depletion of GSH in turn reduces the activity of glutathion peroxidase 4 (GPX4) resulting in activation of lipoxygenases (LOX12/15), lipid peroxidation and ROS formation. This step can be blocked with the ferroptosis inhibitor ferrostatin-1, resulting in reduced lipid peroxidation and cell death. Further, ferroptosis promotes CYLD to activate RIP1 leading to RIP1/RIP3 complex formation and mitochondrial demise. Mitochondrial dysfunction and fragmentation are mediated by DRP-1, a well-known mitochondrial fission protein. The second burst of mitochondrial ROS finally leads to downstream events of ferroptosis like AIF release and subsequent necrotic cell death. All these features can be efficiently blocked by depletion of CYLD suggesting a crucial role for CYLD and DRP-1 in paradigms of neuronal ferroptosis and as a potential target for therapeutic drug approaches.