

New findings with therapeutic benefit in organ transplantation

Knowledge of the molecular mechanisms of cell death and understanding their fundamental role in a wide range of human diseases including myocardial infarction, stroke, sepsis, neurodegeneration, cancer, and organ graft ischemia-reperfusion injury, among others, is of major importance to physicians. Millions of our cells die every day, and the manner of their death conveys important information to the rest of our body. Most cell death proceeds by a pathway called apoptosis, which leads to rapid engulfment by a type of white blood cell called macrophages for degradation and no reaction from our immune systems. Alternative modes of cell death distinct from apoptosis have been identified more recently. Like apoptosis, these modes of death are highly regulated, but by nature, their role is to provoke an immune response. Two such cell death pathways are termed necroptosis and ferroptosis, which have been implicated in the pathology of delayed graft function and transplant rejection. Both of these modes of cell death differ in the underlying components from those at the heart of the better-understood apoptosis pathway, and are characterized in the ongoing process of signaling by the successive loss of membrane integrity of the affected cells associated finally with the release of highly immunogenic molecules and organelles from the dying cells that drive tissue inflammation and extensive tissue damage. Why is this so exciting? With this knowledge comes power. Knowledge of the underlying components and mechanisms in our cells that control cell death by necroptosis and ferroptosis could inform development of drugs. In the clinic, however, anticipation of which pathway might be dysregulated and the time for intervention are rarely possible, factors that may restrict therapeutic applications. However, one of the few clinically relevant situations in which cell death may be predicted and thereby therapeutically targeted within a reasonable time frame is solid organ transplantation. In this context, both necroptosis and ferroptosis contribute to the total organ damage in ischemic injury. To date, it has been assumed that drugging both necroptosis and ferroptosis pathways in combination will yield a stronger benefit compared to the inhibition of each pathway individually.

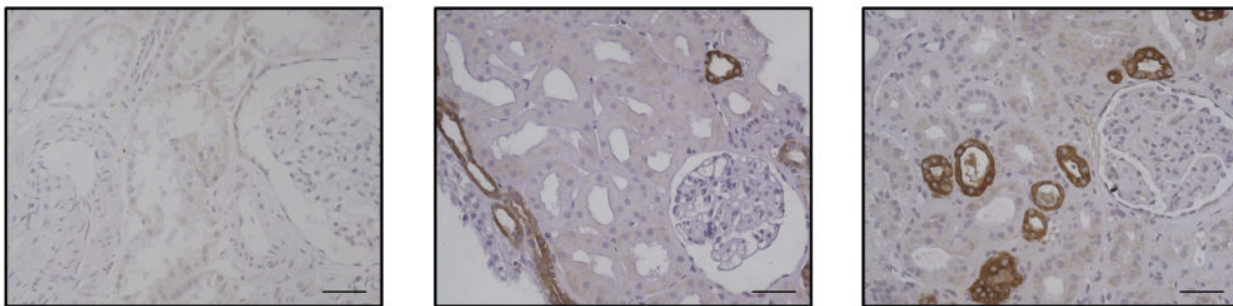


Fig. 1. Increased expression of ACSL4 in human biopsies indicates and verifies an important role for ferroptotic cell death in complex diseases like acute tubular injury. Normal renal parenchyma samples taken from a tumor nephrectomy of renal cell carcinoma served as control (left). ACSL4-positive tubuli (brown coloring) can be seen in acute tubular injury seven days post-

transplantation, showing moderate tubular injury (middle). Increased ACSL4-positivity is detected after severe acute tubular damage from a patient suffering from thrombotic microangiopathy due to atypical hemolytic-uremic syndrome (right). Scale bars = 60 μ m.

Now, our latest studies revealed that necroptosis and ferroptosis represent two alternative modes of regulated necrosis that could compensate for each another when one of them is compromised. Briefly summarized, we detected that the blocking *Acs/4*, an essential gene of the lipid metabolism, protects cells from death by ferroptosis, but simultaneously leads to sensitization to death by necroptosis. Reciprocally, we confirmed that blocking of *Mkl1*, an indispensable mediator of necroptosis, led to sensitization to death by ferroptosis, confirming that these two independent pathways talk to one another within cells. We could confirm what we saw in cultured cells holds true in an animal model of ischemia-reperfusion injury in which necroptosis as well as ferroptosis are known to underlie acute organ failure that cause tremendous tissue damage and contribute collectively to the overall organ damage. Interestingly, defective necroptotic signaling in ischemic-reperfusion injury sensitizes the cells of these animals to death by ferroptosis and *vice versa*.

Our new results exemplify the complex interconnectivity of regulated necrosis cell death pathways, and their enormous potential as drug targets in the clinic. From a clinical point of view, our current findings need to be taken into consideration in the conception and development of pharmacological intervention where therapeutic targeting of both necroptosis and ferroptosis may be necessary to negate complex diseases/processes like organ graft ischemia-reperfusion injury. Otherwise, combination therapy in patients is well established in solid organ transplantation, but has focused exclusively thus far on immunosuppression and not on cell death pathways. In this context, our data illustrate the increasing importance to identify reliable biomarkers for monitoring the exact mode of cell death operating in a wide range of human diseases. Despite its pathophysiological importance, a therapeutic-useful biomarker has not been identified so far for ferroptotic cell death. By analyzing human kidney biopsies from patients with acute tubular injury (ATI) we examined now for the first time the essential contribution of a protein, notably ACSL4, in regulating ferroptosis. As demonstrated in Figure 1, we detected robust immunostaining of ACSL4 in human ATI seven days post-transplantation, as well as in severe thrombotic microangiopathy of naive kidney. These histological samples highlight for the first time ACSL4 as a reliable biomarker of pathophysiological ferroptosis, providing a novel platform for clinical monitoring and diagnosis of ferroptosis-mediated human diseases.

James M. Murphy¹, Stefan Krautwald²

¹*The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia*

²*Department of Nephrology and Hypertension, University Hospital Schleswig-Holstein, Kiel, Germany*

Publication

[Necroptosis and ferroptosis are alternative cell death pathways that operate in acute kidney failure.](#)

Müller T, Dewitz C, Schmitz J, Schröder AS, Bräsen JH, Stockwell BR, Murphy JM, Kunzendorf U, Krautwald S

Cell Mol Life Sci. 2017 Oct