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COMMENT

Oxeiptosis—a cell death pathway to mitigate damage caused by radicals

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To live or to die is one of the most delicate decisions on a cellular level. Environmental signals are constantly integrated and can activate a multitude of pathways that either activate life-saving countermeasures or initiate cell death programs. On an organismal level, both life-saving and life-terminating mechanisms on cellular level are of central importance and play essential roles during diverse processes including growth, tissue regeneration or immune responses.

Prominent signaling messengers with multiple intracellular functions are reactive oxygen species (ROS). ROS lead to proliferation and survival of cells. However, unbalanced protein folding, energy production or fatty acid metabolism resulting from tumorigenic processes, viral infections or inflammation, trigger a pathological increase of ROS levels leading to oxidative stress [1]. While multiple enzymatic and non-enzymatic mechanisms have evolved to protect cells from such detrimental accumulation of ROS, the molecular mechanisms by which ROS sensing translates into anti-inflammatory or cytotoxic programs remain incompletely understood. It was shown that ROS can cause activation of necroptosis, the inflammasome pathway and caspase-dependent cell death, but a molecular sensor for intracellular ROS was not known. Recently, we identified "oxeiptosis", a signaling pathway that couples a ROS sensor to a non-canonical cell-death execution pathway [2].

Initial cues on the existence of such non-canonical ROS-sensitive cell death pathway arose by investigating an in vivo ozone-exposure model. Mice exposed to ozone accumulated detrimental amounts of ROS in airway epithelial cells. However, genetic deletion of numerous genes involved in canonical cell death pathways did not affect ozone-induced tissue damage. Indeed, in vitro experiments using hydrogen peroxide as ROS inducer confirmed that pharmaceutical inhibition or genetic deletion of canonical cell death components was not sufficient to significantly rescue cell death in a variety of different cell lines and primary mouse embryonic fibroblasts. Additionally, activation of autophagy or inflammasome-mediated cell-death could be excluded, suggesting the execution of a yet undescribed cell-death pathway independent of caspase-driven apoptosis, ferroptosis, pyroptosis, necroptosis and autophagy [2].

A well-known sensor for intracellular ROS is KEAP1, which gets oxidized on C-terminal cysteine residues in a ROS-dependent manner [3, 4]. This leads to a conformational change and to the release of the transcription factor NRF2, which in turn stimulates the expression of cytoprotective, ROS-scavenging genes [5]. Surprisingly, we found that KEAP1 deletion increased survival of ROS-treated cells and that simultaneous deletion of KEAP1 and NRF2 did not rescue these effects, suggesting that KEAP1—beside its cytoprotective role via NRF2 activation—additionally possesses the ability to stimulate cell death in response to high intracellular ROS levels [2].

In line with previous studies, we found that KEAP1 forms a tri-partite complex with NRF2 and the mitochondrial serine-threonine phosphatase PGAM5 [6]. Interestingly, cumulative evidence showed that PGAM5 is a key downstream effector of the oxeiptosis pathway; indeed ablation of PGAM5 rendered cells more resistant to toxic levels of ROS. Furthermore, alike the regulation of KEAP1-NRF2 affinity by intermediate levels of ROS, exposure to toxic amounts of H_2O_2 strongly reduced KEAP1-PGAM5 association. This suggests that different ROS levels specifically and finely modulate the relative composition of the KEAP1-NRF2-PGAM5 tri-partite complex [7] (Fig. 1). In turn, this results in functionally diverse cellular fates. Low levels of ROS lead to NRF2-mediated expression of cytoprotective genes and proteins. Conversely, high levels of

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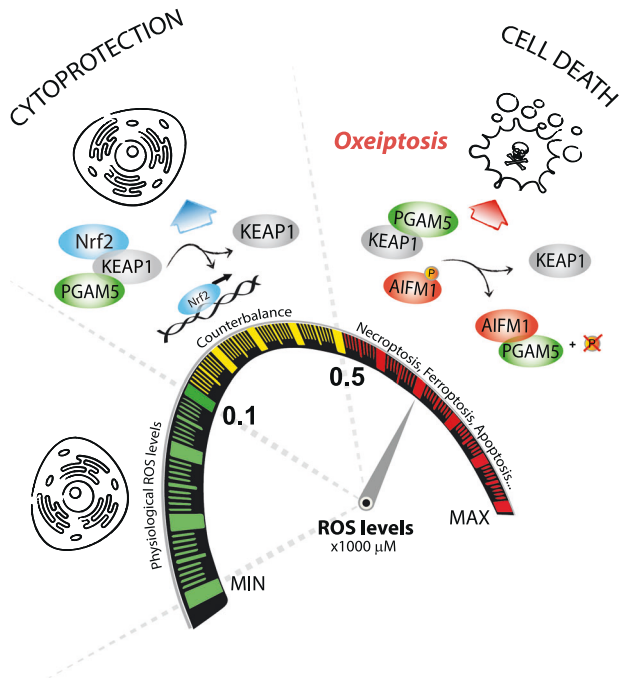

Fig. 1 Intracellular exposure to intermediate ROS levels triggers conformational changes in KEAP1 via oxidation of its C-terminal cysteines, resulting in dissociation of the KEAP1-NRF2 complex and subsequent nuclear translocation of NRF2. In the nucleus, Nrf2 stimulates expression of cytoprotective genes that scavenge ROS. Under high intracellular ROS levels KEAP1 releases PGAM5, which binds and dephosphorylates AIFM1 at Ser^{116} executing a cell death program. This new signaling pathway involving KEAP1-PGAM5-AIFM1 is named *oxeiptosis.*

ROS activate a PGAM5-dependent signaling pathway that culminates in the execution of a cytotoxic program (Fig. 1). KEAP1 therefore functions as a critical switch that quantitatively monitors intracellular ROS levels. This may be regulated by some of the numerous cysteine residues in its c-terminus. Indeed, oxidation of a subset of these cysteines (C151, C273 and C288) by ROS has been shown to regulate NRF2 release [3, 4]. Similarly, PGAM5 activation may be regulated by oxidation of an alternative set of KEAP1 cysteine residues, thereby allowing the protein to integrate quantitative information on ROS levels and to initiate appropriate signaling pathways.

Mass spectrometric analysis of PGAM5 precipitates identified AIFM1 (Apoptosis-Inducing Factor 1 Mitochondrial)—a protein that has been shown to induce caspase-independent cell death [8]—among its cellular interaction partners. Notably, knockdown experiments of AIFM1 alone or in combination with PGAM5 and KEAP1 similarly reduced ROS-mediated cell-death, further pointing towards the existence of a linear signal transduction pathway along the KEAP1-PGAM5-AIFM1 axis. We could show that PGAM5 binds and dephosphorylates the evolutionary highly conserved Serine116 of AIFM1 and we pinpointed $AIFM1^{Ser116}$ phosphorylation status as read-out of oxeiptosis activation. Furthermore, in our experimental system AIFM1 does not translocate to other cellular compartments [8], indicating a role of activated AIFM1 in mitochondria.

To address the physiological relevance of oxeiptosis, we employed an ozone-exposure model in vivo. Notably, $Pgam5^{-/-}$ mice exposed to ozone showed increased inflammatory parameters, as compared to control animals, indicating that oxeiptosis negatively regulates inflammatory responses upon detrimental ROS levels. Influenza A virus infection leads to ROS accumulation in infected cells [9]. Notably, influenza A virus mediated inflammation and morbidity were increased in $PGAM5^{-/-}$ mice, further suggesting a central role of oxeiptosis to prevent overshooting immune reactions during viral infection. Notably, data from previous studies in our group showed that KEAP1, PGAM5 and AIFM1 are targeted by evolutionary diverse viruses [10]. It will be important to clarify whether oxeiptosis is positively or negatively regulated by different viruses, and how each viral family would benefit from its differential perturbation.

Altogether, these findings show that oxeiptosis serves as additional cell death pathway with anti-inflammatory apoptosis-like phenotype that operates in parallel to other cell death pathways. This model is supported by influenza A virus infection experiments in *Ripk3*-deficient mice [11], which show decreased signs of inflammation while deletion of *Pgam5* in the same in vivo model has the exact opposite phenotype [2]. It will be important to establish the mutual interconnections between oxeiptosis and the different cell death pathways and to identify the molecular mechanisms underlying their preferential activation. Different cell types may respond differentially to ROS accumulation. While oxeiptosis clearly plays an important role in fibroblasts and epithelial cells, lymphocytes appear to be more resistant and may rely on alternative pathways [12].

Increased ROS accumulation has been linked to various physiological and pathological conditions, including aging, virus infection and cancer [1]. It is therefore likely that oxeiptosis contributes to progression of various diseases. For instance, mutations in KEAP1 have been linked to progression of lung [13] and prostate cancer [14] and both, Pgam5 and Aifm1 have been associated with neuropathological symptoms in vivo [15]. Further studies will determine the involvement of oxeiptosis in diverse diseases and the potential clinical benefit of therapeutically targeting this pathway.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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