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Commentary on "PANoptosis-like cell death in ischemia/reperfusion injury of retinal neurons"

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Several decades have passed since programmed-cell death (PCD) was identified. Apoptosis was first defined by Kerr in 1972, and later described by the Nobel Prices in Physiology or Medicine 2002, Sydney Brenner, John Sulston and Robert Horwitz, who defined genetic regulators of apoptosis (Diamantis et al., 2008). However, it was in 1858 when the German pathologist and biologist Rudolf Virchow identified for the first time the phenomenon of apoptosis, which he named necrobiosis, arguing that this form of cell death was completely different from the uncontrolled necrosis, suggesting the existence of two different types of cell death. Today, the knowledge in the field of cell death regulation is extensive, but still under continuous expansion.

Among all the proposed forms of PCD, pyroptosis, apoptosis and necroptosis are the most well-defined in terms of the molecular machinery responsible for the initiation, transduction, and execution of cell death. Pyroptosis is executed by gasdermin D (GSDMD) activation through the inflammatory caspases caspase-1 and caspase-11 to drive cellular swelling, plasma membrane permeabilization, and inflammation (Shi et al., 2015). Apoptosis is executed by caspase-3 and caspase-7, downstream of the initiator caspases-8, -9, and -10, leading to cell shrinkage and nuclei condensation but without membrane permeabilization (Ellis and Horvitz, 1986). Necroptosis by contrast is triggered in a caspase-independent manner through the receptor-interacting protein kinase 1 (RIPK1) and RIPK3, which activates the necroptotic executor mixed lineage kinase domain-like pseudokinase (MLKL) followed by membrane pores formation, cellular swelling, and inflammation (Holler et al., 2000). Usually, these three forms of PCD have been considered as independent pathways participating in several disorders, including nervous system diseases and degenerative conditions, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, neuroinflammation, and neuronal injury (Arrázola et al., 2019; Moujalled et al., 2021). However, increasing evidence suggests that there is an important crosstalk between pyroptosis, apoptosis, and necroptosis in a variety of pathological scenarios, thereby acting as one and integrated PCD pathway (Wang and Kanneganti, 2021). A couple of years ago, the concept of PANoptosis was introduced by Malireddi et al. (2019) establishing for the first time a possible molecular connection between pyroptosis, apoptosis, and necroptosis in the context of pathological infectious diseases with a high inflammatory component. The participation of each PCD has been established in different neurodegenerative conditions, however, the contribution of PANoptosis as an integrated PCD cascade in pathologies of the nervous system pathologies have not been addressed until now (Yan et al., 2022).

Yan et al. (2023) demonstrated that PANoptosis is triggered after nervous system injury using *in vitro* and *in vivo* models of ischemia/reperfusion (I/R) in retinal neurons. They used oxygen-glucose deprivation/recovery (OGD/R) as a model to simulate the I/R injury *in vitro* in R28 retinal cells. PANoptosis was broadly analyzed by TUNEL, ethidium homodimer III, and propidium iodide staining to detect apoptosis, pyroptosis, and necroptosis respectively after OGD/R. The detection of specific molecular markers for each PCD cascade enables the authors to determine the co-existence of the three regulated pathways in retinal neurons after OGD/R treatment. Apoptosis activation was defined by caspase-3 cleavage, increased apoptotic protein Bax and decreased Bcl-2 levels. Pyroptosis activation was confirmed by caspase-1 cleavage, increased NLRP3 levels, GSDMD cleavage, and by the presence of inflammatory interleukin-1β and interleukin-18. Finally, the increase in RIPK3-MLKL phosphorylation demonstrated the activation of necroptosis and confirmed that PANoptosis can be induced in retinal neurons by the same injury stimulus. To go further, Yan et al. (2023) defined the contribution of each pathway in the resulting neuronal death by using a serial combination of specific inhibitors. The best inhibitory condition was observed when the three PCD pathways were co-inhibited, suggesting that OGD/R-induced cell death is mainly driven by PANoptosis. However, many cellular factors must be considered to confirm this suggestion, including the fact that inhibition of one PCD pathway could lead to a compensatory effect by activating other regulated cascades of cell death. For instance, it has been shown that apoptotic triggering factors induce necroptosis activation under inhibition or deficiency of caspase-8 (Holler et al., 2000). In that sense, key components of the necroptotic pathway are also involved in the switch from apoptosis to necroptosis, mainly through RIPK3, which has versatile functions in the regulation of both cell death cascades but also participating in cell-death independent pathways, including regulation of the immune response, energy metabolism and inflammation (Zhang et al., 2009; Daniels et al., 2017). On the other hand, MLKL activation, which leads to membrane disruption, can also activate the NLRP3 inflammasome in a cell-intrinsic manner, but independently of GSDMD, the central executor of pyroptosis (Gutierrez et al., 2017). Accordingly, RIP1-dependent apoptosis has been described through flow cytometric detection of RIP3/active Caspase-3 double-positive events in live and dead populations *in vitro* (Lee et al., 2018). Therefore, crosstalk between independent PCD cascades must also be considered.

Yan et al. (2023) also determined *in vivo* whether I/R injury can induce PANoptosis-like cell death using a rat acute high intraocular pressure (aHIOP) model. Apoptosis, necroptosis, and pyroptosis were independently detected in retina sections after aHIOP and correlated with the correspondent increase of cell-death type specific markers, such as caspase-3, GSDMD, and MLKL. This evidence proposes the existence of PANoptosis-like cell death *in vivo*, indicating that retinal cells could die by executing different cell-death mechanisms after I/R injury. Further studies regarding the co-existence of individual cell-death types would better address whether pyroptosis, apoptosis, and necroptosis occur simultaneously within the same cell, or if it is cell-type dependent. In addition, the understanding of PCD pathways kinetics would allow to better define the mechanism of PANoptosis execution after acute ischemic injury. In this context, Huang et al. (2013) demonstrated that RIPK3 protein levels were rapidly elevated following aHIOP in the retina, however, some of these RIPK3 positive retinal cells colocalized with Bax and cleaved caspase-3 in the ganglionic cell layer after 12 hours of aHIOP, suggesting a differential temporal activation of individual PCD pathways.

In conclusion, the evidence provided by Yan et al. (2023) suggest that PANoptosis is likely to operate in I/R neuronal injury, providing a new research concept to study other types of neuronal injury or degenerative processes, such as many neurodegenerative diseases related to PCD. Moreover, this work arises new questions regarding the possible existence of other signaling cascades or other types of PCD that could extend the scope of PANoptosis as a therapeutic target in central nervous system diseases.

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