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Review

Molecular mechanisms of regulated necrosis

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ABSTRACT

It is now clear that apoptosis does not constitute the sole genetically encoded form of cell death. Rather, cells can spontaneously undertake or exogenously be driven into a cell death subroutine that manifests with necrotic features, yet can be inhibited by pharmacological and genetic interventions. As regulated necrosis (RN) plays a major role in both physiological scenarios (*e.g.*, embryonic development) and pathological settings (*e.g.*, ischemic disorders), consistent efforts have been made throughout the last decade toward the characterization of the molecular mechanisms that underlie this cell death modality. Contrarily to initial beliefs, RN does not invariably result from the activation of a receptor interacting protein kinase 3 (RIPK3)-dependent signaling pathway, but may be ignited by distinct molecular networks. Nowadays, various types of RN have been characterized, including (but not limited to) necroptosis, mitochondrial permeability transition (MPT)-dependent RN and parthanatos. Of note, the inhibition of only one of these modules generally exerts limited cytoprotective effects *in vivo*, underscoring the degree of interconnectivity that characterizes RN. Here, we review the signaling pathways, pathophysiological relevance and therapeutic implications of the major molecular cascades that underlie RN.

Contents

Abbreviations: AIF, apoptosis-inducing factor; ANT, adenine nucleotide translocase; CsA, cyclosporin A; CYPD, cyclophilin D; $\Delta\psi_m$, mitochondrial transmembrane potential; Fer-1, ferrostatin-1; MAMP, microbe-associated molecular pattern; MLKL, mixed lineage kinase domain-like; MPT, mitochondrial permeability transition; Nec-1, necrostastin-1; PAR, poly(ADP-ribose); PARP1, PAR polymerase 1; PPIF, peptidylprolyl isomerase F; PRR, pattern recognition receptor; PTPC, permeability transition pore complex; RIPK, receptor-interacting protein kinase; RN, regulated necrosis; ROS, reactive oxygen species; TLR, Toll-like receptor; TNFα, tumor necrosis factor α; TNFR1, TNFα receptor 1.

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1. Introduction

The Nomenclature Committee for Cell Death has recently proposed to use the adjective "programmed" to identify instances of cell death that occur in a completely physiological setting such as (post)embryonic development or the preservation of tissue homeostasis. Conversely, the term "regulated" should be employed to refer to cases of cell death that can be inhibited by specific pharmacological or genetic interventions, implying that they rely on a defined (though sometimes known to partial extents) molecular machinery. Thus, each instance of programmed cell death is by definition regulated, but not *vice versa*. Finally, the expression "accidental cell death" has been put forward to indicate cell death instances that cannot be controlled, as they generally originate from very harsh microenvironmental perturbations (Fig. 1) [1–3].

Until recently, apoptosis was considered as the only form of regulated cell death, possibly because: (1) the stereotyped morphological appearance of this cell death modality has been recognized as early as in the 1960s, mostly owing to the pioneer work of Sir Richard Lockshin [4]; and (2) the biochemical processes that regulate and execute apoptosis (including the massive activation of a class of cysteine proteases known as caspases) have emerged quite rapidly, at least in part following the milestone discoveries made by Robert Horvitz in *Caenorhabditis elegans* [5–7]. Conversely, necrosis was viewed as a merely accidental subroutine of cell death, mostly resulting from very harsh stimuli including steep changes in temperature, osmotic pressure or pH [8]. As necrosis was conceived as a (pharmacologically) incontrollable process, for a long time it generated limited interest within the scientific community. Accordingly, necrosis was mainly defined in a negative fashion, as a cell death subroutine not manifesting with apoptotic features or with an extensive vacuolization of the cytoplasm (which was considered as a sign of autophagic cell death) [9]. This begun to change only with the late 1980s, when tumor necrosis factor α (TNFα) was shown to kill cancer cells while promoting either an apoptotic or a necrotic phenotype, in a cell type-dependent fashion [10]. The possibility that – similar to apoptosis – necrosis might also occur in a regulated fashion continued to gather momentum throughout the 1990s [11–13], and was definitively confirmed in 2005, when the team of Junying Yuan discovered a groups of molecules that inhibit several instances of necrotic cell death, namely, necrostatins [14,15]. In 2008, the same authors identified receptor-interacting protein kinase 1 (RIPK1), a kinase that so far had been involved in NF-κB and apoptotic signaling, as the cellular target of necrostatin 1 [16,17]. This ignited an intense experimental effort that led to the precise characterization of the signal transduction cascade whereby TNFα can promote necrosis, at least under some circumstances [18–22].

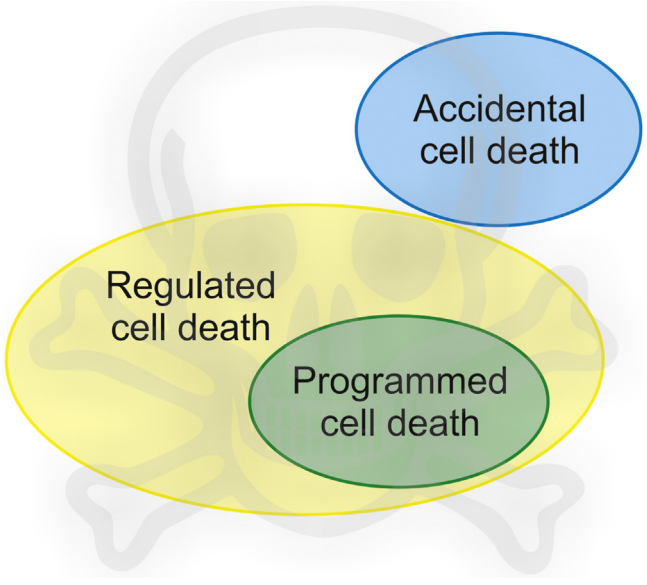

Fig. 1. Cell death nomenclature. At odds with "accidental" instances of cell death, which by definition cannot be controlled, "regulated" cell death relies upon a genetically encoded molecular machinery that can be pharmacologically modulated. Regulated cell death, be it apoptotic or necrotic, can be triggered by exogenous stimuli or occur as part of a genetically encoded physiological program, for instance (post)embryonic development or the maintenance of tissue homeostasis. Such as physiological type of regulated cell death is generally indicated as "programmed".

Since then, our knowledge on the molecular mechanisms that control and execute regulated necrosis (RN) has significantly improved [23–25]. Alongside, it has become clear that RN plays a significant role in both physiological scenarios (*e.g.*, embryonic development) and pathological settings (*e.g.*, ischemic conditions), suggesting that the pharmacological modulation of RN might provide consistent therapeutic benefits to patients affected by a large panel of disorders [23–25]. Furthermore, it rapidly turned out that RN does not occur only in caspase-incompetent cells upon the activation of the RIPK1 homolog RIPK3. Rather, there are multiple molecular circuitries that can drive RN including (but not limited to) necroptosis, mitochondrial permeability transition (MPT)-dependent RN, and parthanatos. Interestingly, the inhibition of only one of these modules generally provides limited cytoprotective effects *in vivo* [26], underscoring the elevated degree of interconnectivity of the RN signaling network. Here, we discuss the signal transduction cascades, pathophysiological relevance and therapeutic implications of the major molecular circuitries underlying RN.

2. Mechanisms of regulated necrosis

2.1. Necroptosis

The term "necroptosis" has originally been introduced in 2005 to indicate a necrostatin 1 (Nec-1)-inhibitable, and hence RIPK1-dependent, regulated form of non-apoptotic cell death triggered by TNFα receptor 1 (TNFR1) in the presence of genetic or pharmacological caspase inhibition [14]. For the next few following years, this term has been widely (but improperly) employed as a strict synonym of RN [27]. Nowadays, following the milestone discovery that RIPK1 transduces pro-necrotic signals by engaging in physical and functional interactions with its homolog RIPK3 [18–20] and the identification of multiple RIPK3-dependent but RIPK1-independent instances of RN [28–31], necroptosis is rather defined as a RIPK3-dependent molecular cascade promoting RN [32].

During necroptosis, RIPK3 gets activated in the context of a supramolecular complex that may or may not involve RIPK1, hence acquiring the ability to phosphorylate mixed lineage kinase domain-like (MLKL), a pseudokinase that binds ATP but is not catalytically active [21,33] The multiprotein complex promoting the activation of RIPK3 is commonly referred to as the necrosome [34]. Of note, when RIPK1 is not involved in the necrosome other factors that share with RIPK1 and RIPK3 a RIP homotypic interaction motif (RHIM) are [35,36]. Only two murine and human proteins other than RIPK1 and RIPK3 are known to contain a RHIM, namely, Z-DNA binding protein 1 (ZBP1, also known as DAI) and Toll-like receptor (TLR) adaptor molecule 1 (TICAM1, best known as TRIF) [35,36]. As both these proteins have already been involved in instances of necroptosis [35,36], RHIMs appear to be critical for the activation of the necrosome.

RIPK3 only engages in transient interactions with MLKL, resulting in the exposure of a positively charged N-terminal stretch [33]. This said, the precise molecular mechanisms whereby MLKL

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