MINI-REVIEW

Connections Between Various Trigger Factors and the RIP1/RIP3 Signaling Pathway Involved in Necroptosis

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Abstract

Programmed cell death is a basic cellular process that is critical to maintaining tissue homeostasis. In contrast to apoptosis, necrosis was previously regarded as an unregulated and uncontrollable process. However, as research has progressed, necrosis, also known as necroptosis or programmed necrosis, is drawing increasing attention, not least becasu of its possible impications for cancer research. Necroptosis exhibits a unique signaling pathway that requires the involvement of receptor interaction protein kinases 1 and 3 (RIP1 and RIP3), mixed lineage kinase domain-like (MLKL), and phosphoglycerate mutase 5 (PGAM5) and can be specifically inhibited by necrostatins. Not only does necroptosis serve as a backup cell death program when apoptosis is inhibited, but it is now recognized to play a pivotal role in regulating various physiological processes and the pathogenesis of a variety of human diseases such as ischemic brain injury, immune system disorders and cancer. The control of necroptosis by various defined trigger factors and signaling pathways now offers the opportunity to target this cellular process for therapeutic purposes. The purpose of this paper is to review current findings concerning the connections between various trigger factors and the RIP1/RIP3 signaling pathway as it relates to necroptosis.

Keywords: Necroptosis - TNFR - Fas - TRAILR - RIP1-RIP3 necrosome - MLKL complex

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Introduction

In contrast to apoptosis which involves two well characterized pathways, necrosis was long considered as an uncontrolled, nonprogrammed form of cell death induced accidentally and in an unregulated fashion (Wyllie et al., 1980). However, an increasing body of evidence indicates that necrosis posses unique morphological characteristics and can also be executed via regulated mechanisms (Van Herreweghe et al., 2010).

Apoptotic cells are characterized by the rounding up of cells, pseudopod retraction, pyknosis, chromatin condensation, nuclear fragmentation, internucleosomal DNA cleavage and the appearance of apoptotic bodies (Wu et al., 2012). The dying cells initially classified as necrotic was in a negative fashion, that is, when they exhibited neither an apoptotic morphotype nor an autophagic morphotype (extensive vacuolization of the cytoplasm) (Galluzzi et al., 2011). Necrosis is characterized by cell swelling, dysfunction of mitochondria and disruption of the cell membrane, leading to the release of the cellular content, which may result in an inflammatory response (Jain et al., 2013). Not only the morphological features marks necrosis occurred, surprisingly, necrotic cell death can also be executed by its own unique signaling pathway which requires the involvement of RIP1 and RIP3, and can be specifically inhibited by necrostatins (Degterev et al., 2005; Galluzzi et al., 2011). The programmed necrosis dubbed necroptosis by Junying Yuan's laboratory (Degterev et al., 2005). Interestingly, research has demonstrated that necrosis shares identical inducing cytokines with apoptosis in certain cell lines, especially when caspases are inhibited or cannot be activated efficiently (He et al., 2009).

Binding of Ligands to Receptors

Probably the most extensively investigated model of necroptosis is that elicited by the ligation of tumor necrosis factor receptor 1 (TNFR1) (Vercammen et al., 1997). Nevertheless, TNFR1 does not constitute the sole pronecroptotic receptor described to date. Thus, other death receptors including TNFR2 (Chan et al., 2003), FS7-associated cell surface antigen (Fas, also known as CD95) (Vercammen et al., 1998; Holler et al., 2000), the TNF-related apoptosis-inducing ligand receptors 1 and 2 (TRAILR1 and TRAILR2) (Laster et al., 1988) and the Toll-like receptor (TLR) (Festjens et al., 2007) reportedly induce nonapoptotic cell death that manifests with necrotic features. It is worth noting that in a large number of experimental settings extrinsic apoptosis is triggered by death receptor ligation and proceeds through the activation of the caspase-8/-3 cascade; with or without mitochondrial involvement. Yet, in some cell types, a mechanism has been unveiled that is initiated by death receptor ligation and leads to a caspase-independent necrotic cell death.

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TNFR

TNF-α is produced by activated macrophages and although currently considered an apoptotic inducer, it was first identified as an agent capable of causing tumor cell necroptosis (Oettgen et al., 1980). TNFR1 and TNFR2 are cell surface receptors that specifically bind TNF-α. While TNFR2 does not have a death domain, TNFR1 plays a principal role in TNF-α-mediated activation and triggers a series of intracellular events (Wu et al., 2012). Initially, TNF-α binds to the extracellular portion of TNFR1 to induce allosteric changes and lead to the recruitment of multiple proteins including TNFR-associated death domain (TRADD), RIP1, Fas-associated death domain (FADD), cellular inhibitor of apoptosis proteins (cIAPs), TNFR-associated factor-2 (TRAF2) and TRAF5 which together constitute the so-called complex I (Andera, 2009). At complex I, cIAPs mediate the K63-linked polyubiquitination of RIP1 triggering the canonical pathway activation of the transcription factor NF-κB. Upon TNFR1 internalization and RIP1 deubiquitination by cylindromatosis (CYLD) (or as a result of cIAP inhibition), the molecular composition of complex I changes to form complex II (also known as DISC) which is comprised of RIP1, RIP3, TRADD, FADD, and caspase-8. Normally, caspase-8 activation within the DISC initiates apoptosis coupled with RIP1 and RIP3 inactivation. When caspase-8 cannot be activated owing to a genetic condition or pharmacological intervention, RIP1 and RIP3 engage in a crosstalk entailing their phosphorylation (perhaps involving another hitherto unidentified kinase) at S161 and S199 respectively, resulting in sensitization of necrotic death induction through TNFR-1. Inhibition of CYLD via RNA interference (RNAi) robustly inhibits TNF-α-induced necroptosis (Hitomi et al., 2008), indicating that the deubiquitination of RIP1 is an important step in TNF-α-induced necroptosis. TRAF2 has been shown to be essential for TNF-α-induced necroptosis with TRAF2-/- cells exhibiting resistance to this signalling pathway (Lin et al., 2004), while ligand binding in L-M cells triggers a necrotic response (Laster et al., 1988). There is evidence suggesting that TNFR-2 signaling can potentiate necroptosis via TNFR-1 (Chan et al., 2003); however, TNF-α-induced necroptosis is normally inhibited by caspase-8 cleavage of RIP (Chan et al., 2003).

Fas

The Fas, also named CD95, APO-1, fas antigen, or tumor necrosis factor receptor superfamily member 6 (TNFRSF6) is a member of the death receptors (DR) family, a subfamily of the tumor necrosis factor receptor superfamily (Krammer, 2000). Signaling by Fas (CD95/APO1) shares similar features with TNFR signaling expect FADD is recruited directly to the death domain on Fas without the need of TRADD. Following the binding of the Fas ligand (FasL or CD95L) to its receptor, the cytoplasmic tail of Fas recruits several proteins to include cellular caspase 8, (FLICE)-like inhibitory protein (c-FLIP), FADD and pro-caspase 8, forming a membrane-bound receptor complex referred to as the CD95 death-inducing signaling complex (DISC) (Lavrik and Krammer, 2012). This leads to the reciprocal proteolytic activation of caspase-8, which in turn triggers the downstream activation of caspase 3 and eventually leads to apoptosis (Budd et al., 2006). However, cells can also undergo caspase-independent necroptotic cell death in the presence of cIAPs and caspase inhibitors. Pharmacological inhibition of cIAPs prevents RIP1 degradation leading to the formation of the ripoptosome, a signaling complex comprising RIP1, FADD, and caspase-8 (Cho et al., 2009; Vandenabeele et al., 2010; Feoktistova et al., 2011; Tenev et al., 2011). Ripoptosome-mediated apoptosis or necroptosis is dependent on the FLIP isoform used to include: c-FLIPL for Long, c-FLIPS for Short and c-FLIPR for Raji (Lavrik and Krammer, 2012). The short FLIP isoforms, c-FLIPS and c-FLIPR, block procaspase-8 activation and apoptosis (Golks et al., 2005; Fricker et al., 2010), with c-FLIPL also able to act as an anti-apoptotic molecule when present in high concentrations at the DISC (Krueger et al., 2001; Fricker et al., 2010). Previous reports suggest that c-FLIPL is involved in the regulation of necroptosis, with siRNA-mediated silencing of c-FLIPL sensitizing cells to TNF-α-induced RIP1/RIP3-dependent necroptosis (Oberst et al., 2011). Therapeutic exploitation of FasL-Fas-mediated cytotoxicity was soon an ambitious goal and during the last decade numerous strategies have been developed for its realization. Several studies have shown that Fas activation leads to necrotic cell death upon caspase inhibition in various cell lines, including L929 mouse fibroblasts, mouse embryonic fibroblasts (MEFs) and Jurkat T cells (Vercammen et al., 1998; Holler et al., 2000).

TRAILR

So far, researchers have identified 5 TNF-related apoptosis inducing ligand (TRAIL) receptors species to include 2 death receptors (DR), DR4 (TRAILR1 or TNFRSF10A) (Pan et al., 1997b) and DR5 (TRAILR2 or TNFRSF10B) (Pan et al., 1997a), and 3 decoy receptors (DcR), DcR1 (TRAILR3 or TNFRSF10C), DcR2 (TRAILR4 or TNFRSF10D) (Marsters et al., 1997) and OPG. DR4 and DR5 are widely expressed on both normal and malignant cells and contain a functional cytoplasmic death domain capable of inducing DISC formation, leading to TRAIL-induced receptor trimerization and the activation of the extrinsic apoptotic pathway (Hymowitz et al., 1999). DcR1 has no death domain and is anchored to the membrane via a glycophosphatidyl inositol (GPI) tail, whereas DcR2 was found to have a truncated, non-functional death domain. TRAIL receptor signaling is similar to CD95/Fas signaling, with the exception of decoy receptors that compete with DR4 and DR5 for binding to ultimately block cell death induction (Sheridan et al., 1997). Finally, the fifth receptor for TRAIL, OPG, exists in a dimeric, soluble, secreted form with lower affinity to TRAIL (Holen et al., 2002).

The biological effects of TRAIL are executed through its binding to its corresponding receptors to induce DR4 /DR5 trimerization, leading to an intracellular death domain alteration and resulting in DR4/DR5 activation (Mahalingam et al., 2009). Besides apoptosis induction, TRAIL induces necroptosis in both Jurkat cells (Holler et al., 2000) and murine prostate adenocarcinoma

TRAMP-C2 cells (Kemp et al., 2003). A recent study examining human HT29 colon adenocarcinomas and human HepG2 hepatocarcinoma cell lines found that an acidic pH switches TRAIL-induced apoptosis to RIP1-dependent regulated necroptosis (Meurette et al., 2005; Meurette et al., 2007) with this extracellular environmental change attributed to some pathological conditions such as cancer or inflammation. Moreover, in HT29 cells an acidic pH sensitizes only TRAIL-induced cell death but not TNF-α or FasL. Only decreased expression of DR4 or DR5 by RNA interference or use of antagonistic antibodies significantly inhibited TRAIL-induced necroptosis, showing that an acidic pH specifically alters the TRAIL death pathway.

TLR

Pattern recognition receptors (PRRs or sensors) can facilitate antigen presentation to produce an optimal adaptive immune response to protect from reinfection (Kaiser et al., 2013). Growing evidence implicates sensors in infected cell fate decisions via regulated cell death pathways. Toll-like receptors (TLRs) were the first PRRs to be identified (Kumar et al., 2011), sensing pathogen-associated peptidoglycan (TLR2), double-stranded (ds) RNA (TLR3), lipopolysaccharide (LPS) (TLR4), flagellin (TLR5), unmethylated CpG DNA motifs (TLR9), as well as other pathogen-associated molecular patterns (PAMPs) (Kumar et al., 2011). Each TLR recognizes specific ligands with its extracellular N-terminal containing leucine-rich repeats; among these, TLR3 responds to viral double-strand RNA (dsRNA) or a synthesized analog of dsRNA poly(I:C) (Alexopoulou et al., 2001), whereas TLR4 is activated by LPS (Poltorak et al., 1998; Hoshino et al., 1999), a cell-wall component of Gram-negative bacteria. TLRs activated by LPS, poly (I:C) and viral dsRNA also trigger necroptosis in various cell types such as MEF, T cells, macrophage, and L929 cells (Ma et al., 2005; Upton et al., 2010). Engagement of TLR3 or TLR4 induces necroptosis via interactions between TRIF and RIP3 through their RIP homotypic interaction motif (RHIM) domains. In the presence of the caspase inhibitor z-VAD, TRIF/RIP3 elicits downstream ROS accumulation and subsequently executes necrotic death. TLR3/TLR4 mediated necrosis further triggers the induction of inflammatory cytokines (He et al., 2011).

Common Necroptotic Signals are Elicited by Various Factors

TNF-α, FasL, TRAIL and TLR related ligands have been shown to be able to induce necrotic death under certain conditions. Their respective receptors stand at the tip of a very complex signaling hub, yet these different cytokines appears to employ the same molecular machinery as seen in apoptosis.

RIP1–RIP3 necrosome

Upon binding with their agonists, these death receptors induce cells towards either survival or death depending on the circumstances. Complex I situated at the crossroads of cell survival and death, switching between various signaling pathways in response to an array of stimuli and microenvironments. CYLD is essential to the formation of complex II (O'Donnell et al., 2007; Cho et al., 2009; He et al., 2009), acting as a deubiquitinating enzyme causing RIP1 release form complex I to enable its recruitment to complex II (Micheau and Tschopp, 2003; Cho et al., 2009; He et al., 2009). Complex II may activate two downstream signaling pathways: apoptosis and necroptosis. When caspase-8 is activated, it prevents RIP1 activity thru cleavage, driving complex II into a pro-apoptosis mode. Cleavage of RIP1 by caspase-8 not only abrogates the stimulatory role of RIP1 in the activation of the NF-κB pathway (Kim et al., 2000), but also exhibits a negative effect on necroptosis due to the required RIP1 kinase activity (Wang et al., 2008). However, when caspase-8 is blocked, phosphorylated RIP1 and RIP3 form a necrosome and initiate necroptosis (Holler et al., 2000). Among initiators, the TNF-α/TNFR-induced pathway has received the most intensive scrutiny. RIP1 and RIP3 play a central role in TNF-α/TNFR-induced necroptosis (He et al., 2009). Like the formation of complex II during an apoptotic process, necrosome formation appears to be facilitated by the deubiquitination of RIP1 (O'Donnell et al., 2011; Vanlangenakker et al., 2011). While it is clear that the RIP1/RIP3 complex is the core of the necrosome, the understanding of the molecular regulation of necroptosis remains limited. Unlike RIP1, RIP3 does not participate in NF-κB or apoptosis signaling (Moquin and Chan, 2010). RIP3 accelerates recruitment of RIP1 to the necrosome and the kinase activity of both proteins is required for this process (Cho et al., 2009). However, one researcher showed that shikonin, a naturally occurring naphthoquinone, can induced necroptosis in MCF-7 cell (Han et al., 2007), yet Wang's group reported that MCF-7 cells do not expression RIP3 (He et al., 2009). So does this mean there are different biomarkers for necrosis depending on the stimuli? Recent studies have shown that RIP1 and RIP3 form an amyloid structure through their RHIMs and that this heterodimeric structure could be the backbone of the large necrosome complex (Li et al., 2012). The scaffolds of amyloids may function as a crucial platform for recruiting other components, such as MLKL, and stimulating the downstream execution mechanisms of necroptosis.

MLKL Complex

MLKL and PGAM5 are two key signaling molecules downstream of RIP1 and RIP3 (Chan and Baehrecke, 2012; Sun et al., 2012; Wang et al., 2012). When treating human cells with the MLKL chemical inhibitor necrosulfonamide ((E)-N-(4-(N-(3-methoxypyrazin-2-yl) sulfamoyl) phenyl)-3-(5-nitrothiophene-2-yl) acrylamide), also referred to as NSA, TNF-α-induced necroptosis is effectively block to support that MLKL is required for this signaling pathway (Sun et al., 2012). MLKL was also identified in the immunoprecipitation of RIP3, demonstrating a direct interacts with RIP3, with a reduction in MLKL levels protecting cells against TNF-α-induced necroptosis. It has also been shown that upon necroptosis induction, MLKL is strongly recruited to phosphorylated RIP3 and is subsequently phosphorylated

by RIP3 (Sun et al., 2012). Among necrosomes, MLKL serves as an adaptor to bring the RIP1-RIP3 necrosome complex into proximity with other RIP3 substrates. PGAM5 has two splice variants, PGAM5L and PGAM5S (Chan and Baehrecke, 2012). In the presence of NSA, MLKL and PGAM5L binding to RIP3 is enhanced and the phosphorylation of PGAM5L by RIP3 is unaffected. Interestingly, the phosphorylation of PGAM5S is abolished in the presence of NSA, suggesting that the inhibitor likely stabilizes the RIP3 complex preventing it from engaging the downstream effector PGAM5S. Thus, it appears that on the mitochondrial membrane PGAM5L tethers the RIP1-RIP3-MLKL necrosome (complex II) to PGAM5S. RIP1-RIP3-MLKL-PGAM5L form a dynamic and transient complex that is recruited to PGAM5S on the mitochondrial membrane and then activates PGAM5S-Drp-1 (Chan and Baehrecke, 2012). In short, the extrinsic death receptor mediated signaling (e.g., TNF-α like death cytokines) and intrinsic death receptor-independent signaling (e.g., oxidative stress) converges upon the PGAM5-Drp-1 axis to induce necroptosis (Chan and Baehrecke, 2012).

Chemical Stress-induced Necroptosis

Wang's group demonstrated that six cell lines including human colon cancer HT-29 cells, human T cell leukemia Jurkat, CCRF-CEM cells, human monocytic leukemia U937 cells, mouse fibrosarcoma L929 cells, and mouse embryonic fibroblasts (MEFs) underwent necroptosis following the treatment of Smac mimetic in conjunction with caspase inhibitors (He et al., 2009). Unlike other cell lines, all these cells express RIP3 which acts as a key switch point in the TNF-α-induced cellular apoptotic and necrotic pathways (He et al., 2009). Sitosterol-induced death in macrophages is caspase-independent and involves neither the unfolded protein response nor JNK (Bao et al., 2006). Treatment of MCF-7 and HEK293 cells with shikonin, a naphthoquinone, induces cell death that could be prevented by necrostatin-1 (Nec-1). This small molecular compound induces a dominant necroptosis to circumvent cancer drug resistance and is mediated through P-glycoprotein, Bcl-2 and Bcl-xL (Han et al., 2007). Cadmium (Cd) induces necrotic death in Chinese hamster ovary (CHO) K1 cells, Nec-1 rescues cells from necrotic death through the death domain receptor (DR) signaling pathway (Hsu et al., 2009). Glutamate, a major excitatory neurotransmitter in the CNS, induced oxytosis in HT-22 cells which can be inhibited by Nec-1 (Xu et al., 2007). Hemin, a hemoglobin oxidation by-product, induces necroptotic cell death in cortical astrocytes in a dosage dependent manner within 5 h of treatment and is preceded by increased inflammatory gene expression (COX-2, IL-1beta, TNF-alpha, and iNOS) and a rapid depletion of intracellular glutathione (GSH). Astrocytic inflammation may contribute to the pathogenesis of intracerebral hemorrhage (ICH) making therapeutic targeting of GSH or other related genes beneficial in an effort to reduce the inflammation response (Laird et al., 2008). Necroptosis contributes to NMDA-induced excitotoxicity in cultured rat cortical neurons (Li et al., 2008). Aluminum (Al) which has been considered a risk factor in this etiology can induce necroptosis in SH-SY5Y neuroblastoma cells (Zhang et al., 2008). Arachidonic acid induces oxidative death in oligodendrocyte precursors with RIP1 kinase playing a critical role in this process (Kim et al., 2010).

Perspectives

Necrotic cell death has long been neglected as a process lacking physiological relevance. Times have changed, and with broad physiological implications a blueprint for necroptosis is now emerging. Despite these gains, much remains to be learned about cell specific death regulation, how these signal moleculars may influence each other, and whether the core features of necroptosis (such as RIP-RIP3 activation axis) are built in a single interdependent circuit or several independent, mutually stimulatory (self-amplifying) circuits exists. It is hope that we can augment necroptosis therapeutically to compensate for mutations in cell death pathways.

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