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Review

# Necroptosis and Cancer

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**Necroptosis is a programmed lytic cell death pathway, deregulation of which is linked to various inflammatory disorders. Escape from programmed cell death and inflammation play a significant role in cancer, and therefore investigating the role of necroptosis in cancer has been of great interest. Necroptosis has been shown to promote cancer metastasis and T cell death. Escape from necroptosis via loss of RIPK3 expression is a feature of some cancers. Although necroptosis is a promising novel target for cancer therapies, further investigation into its biological role in carcinogenesis is warranted. In this article we review the recently identified interplay between necroptosis and cancer, and we outline the major biological questions that require further inquiry on the road to targeting this pathway in cancer.**

## Trends

Some cancers escape necroptosis via loss of RIPK3 expression.

Necroptosis promotes cell extravasation and cancer metastasis, and is important for cell-based antitumor immunity

Necroptosis is a promising novel target for cancer therapies. However, further investigation into the biological role of necroptosis pathway in carcinogenesis and the cancer-specific role of this type of programmed cell death is warranted.

## Necroptosis – A Regulated Necrotic Cell Death Mechanism

**Necroptosis** (see Glossary) has been first discovered as a programmed cell death mode only a decade ago [1]. The importance of this pathway has driven an exponential interest in the cell death field, and as a result the pathway has been extensively studied both on the basic and translational research fronts. While much remains to be learned about the mechanism and regulation of necroptosis, the solid recognition of the pathophysiological relevance of this pathway in human diseases has already led to clinical trials of small-molecule drugs targeting necroptosis for the treatment of major diseases ranging from Crohn's disease and rheumatoid arthritis to amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (Box 1). One of the major issues with cancer therapies is drug resistance because of escape from programmed cell death, and therefore it is important to address the question of whether necroptosis plays a role in cancer resistance to therapy. Moreover, because excessive inflammation can promote cancer cell growth and metastasis, understanding the biological significance of proinflammatory cell death such as necroptosis is crucial for our understanding of cancer biology. Although much remains to be discovered and understood on this subject, with advances in our understanding of the basic biology of this pathway, recent reports have started to address the role of necroptosis in cancers. These studies have revealed that necroptosis in the tumor microenvironment may have been adapted by cancers to promote metastasis, and thus suggests the possibility of inhibiting necroptosis as an anti-metastasis strategy. On the other hand, some cancer cells may show a preferential reduction in the expression of key mediators of necroptosis, suggesting that necroptosis of cancer cells may negatively regulate tumorigenesis. In this article we discuss the emerging roles of necroptosis in the regulation of cancer cell growth and metastasis, as well as major unknowns in this field.

While the activation of **caspases** by ligands of the death receptor family, such as **tumor necrosis factor α** (TNF-α), FasL (Fas ligand), and TRAIL (TNF-related apoptosis-inducing ligand), through binding to their cognate receptors has been well established to mediate the **extrinsic apoptosis pathway** [2], the unexpected discovery that inhibition of caspases under these conditions would turn apoptotic cell death into necrosis led to investigation of the possible existence of a regulated necrotic cell pathway in mammalian cells by chemical biological approaches [1]. The activation of **tumor necrosis factor receptor 1** (TNFR1) by TNF-α leads to the formation of a transient intracellular multiprotein complex called the

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### Box 1. Necroptosis and Inflammatory Human Diseases

Necroptosis is involved in promoting inflammation under pathological conditions. Inflammation is one of the key cancer-fueling processes during tumorigenesis. Necroptosis has been implicated in several inflammatory neurodegenerative diseases including ALS [46], multiple sclerosis [9], and Niemann–Pick disease [47]. A tight connection between necroptosis and intestinal inflammation has been established in various mouse models [48,49]. In children with inflammatory bowel disease (IBD), active necroptosis was detected and this contributed to enhanced intestine inflammation [50]. In addition to IBD, necroptosis plays a key role in inflammatory skin disease [51,52]. A RIPK1 inhibitor has been advanced into in a Phase I human clinical trial for the treatment of Alzheimer disease and ALS by Denali Therapeutics. The RIPK1 inhibitor GSK2982772 developed by GlaxoSmithKline Pharmaceuticals is in Phase II clinical trials for the treatment of ulcerative colitis and psoriasis.

TNFR1 signaling complex – TNF-RSC (complex I) (Figure 1). TNF-RSC provides a platform to orchestrate the activation of multiple signaling pathways by mediating a complex pattern of modifications such as ubiquitination and phosphorylation to collectively determine within minutes of TNF-α stimulation if a cell may live or die. **Receptor-interacting serine/threonine protein kinase 1** (RIPK1) is recruited by the intracellular DD (death domain) motif of TNFR1, which also recruits TRADD (TNF receptor type 1 associated death domain protein), an adaptor protein. TRADD in turn recruits FADD (Fas-associated protein with death domain), a crucial adaptor for the recruitment and activation of caspase-8 to promote apoptosis. TRADD also recruits TRAF2/TRAF5 (TNF receptor-associated factor 2), redundant adaptors that mediate the recruitment of cellular inhibitor of apoptosis proteins cIAP1 and cIAP2–two important E3 ubiquitin ligases that mediate the ubiquitination of RIPK1 [3]. Notably, key regulators of RIPK1 ubiquitination, namely Cyld and XIAP (X-linked inhibitor of apoptosis protein) have been found to be mutated and overexpressed in cancer, respectively [4,5] (discussed further below).

The ubiquitination status of TNF-RSC is crucial in dictating multiple downstream events. TNF-α stimulation may promote the activation of nuclear factor κB (NF-κB), a proinflammatory pathway that mediates cell survival and the production of multiple inflammatory cytokines. On the other hand, inhibition of NF-κB pathway output by the protein synthesis inhibitor cycloheximide promotes the formation of complex-IIa – which mediates the interaction of FADD and caspase-8 to promote apoptosis in RIPK1-independent manner. Alternatively, treatment with Smac mimetics, small molecules that can promote the activation of the E3 ubiquitin ligase activity of cIAP1/2 leading to its proteasomal degradation, and consequently reducing K63 ubiquitination of RIPK1, can lead to the activation of caspase-8 and apoptosis in a RIPK1-dependent manner, termed **RIPK1-dependent apoptosis** (RDA) [6,7].

Under apoptosis-deficient conditions, however, RIPK1 is activated, as indicated by the phosphorylation of Ser166, an autophosphorylation marker for its activation [8–10]. The activated RIPK1 interacts with **receptor-interacting serine/threonine-protein kinase 3** (RIPK3) to form an alternative complex, termed complex IIb/necrosome, where these kinases interact via their RIP homotypic interaction motif (RHIM) domains [11–13]. The activated complex-IIb/necrosome in turn mediates phosphorylation of **mixed lineage kinase domain-like protein** (MLKL), a pseudokinase, and the translocation of phosphorylated MLKL to the plasma membrane to promote necrosis by disrupting the integrity of cell membrane [14–17].

Although the necroptosis pathway mediated by TNFR1 upon stimulation with TNF-α is understood best, the engagement of T cell receptors, interferon receptors, pattern recognition receptors (Toll-like receptors), and even genotoxic stimuli and anticancer drugs have been reported to activate necroptosis [18]. Because the production of TNF-α may be a common consequence of different cellular stresses, the engagement of TNFR1 by TNF-α might contribute to the activation of necroptosis in various cellular stress conditions. Given that escape from apoptosis is a hallmark of cancer, in some contexts such engagement will inevitably lead to tumor growth as a consequence of NF-κB pathway activation or of the proinflammatory

### Glossary

**Caspases:** a class of proteases that are activated by self-cleavage or cleavage by an upstream caspase and which play role in various signaling events such as apoptosis, necroptosis, and cytokine production by cleaving their target proteins. Inhibited by zVAD.fmk and Emricasan.

**Extrinsic apoptosis pathway:** caspase-dependent programmed cell death pathway initiated by binding of TNF-α, FasL, or TRAIL ligands to their cognate receptors TNFR1, Fas, and TRAIL-R1.

**Mixed lineage kinase domain-like protein (MLKL):** a pseudokinase that binds to membrane phospholipids upon phosphorylation downstream of RIPK3 activation. Upon oligomerization, forms a channel-like structure that promotes cell membrane disruption. Inhibited by the compounds necrosulfonamide and GW806742X.

**Necroptosis:** a lytic, caspase-independent, programmed cell death pathway mediated by the RIPK1/RIPK3/MLKL signaling axis and that is dependent on the kinase activity and oligomerization of RIPK1 and RIPK3 kinases, as well as on the oligomerization and membrane translocation of MLKL pseudokinase. This proinflammatory pathway has been linked to cancer and metastasis.

**Receptor-interacting serine/threonine-protein kinase 1 (RIPK1):** central kinase for cellular decision point for initiation of either apoptosis, necroptosis, or NF-κB pathway activation. Kinase activity is important for necroptosis and RIPK1-dependent apoptosis, but not for NF-κB pathway activation, where RIPK1 serves as a scaffold to recruit additional signaling players required for NF-κB pathway activation. Inhibited by the compounds Nec-1 and GSK2982772.

**Receptor-interacting serine/threonine-protein kinase 3 (RIPK3):** promotes MLKL phosphorylation to initiate the oligomerization and membrane translocation of MLKL. Has been implicated in mediating apoptosis. Expression is frequently lost in various types of cancer. Inhibited by the compounds GSK'840, GSK'843, and GSK'872.

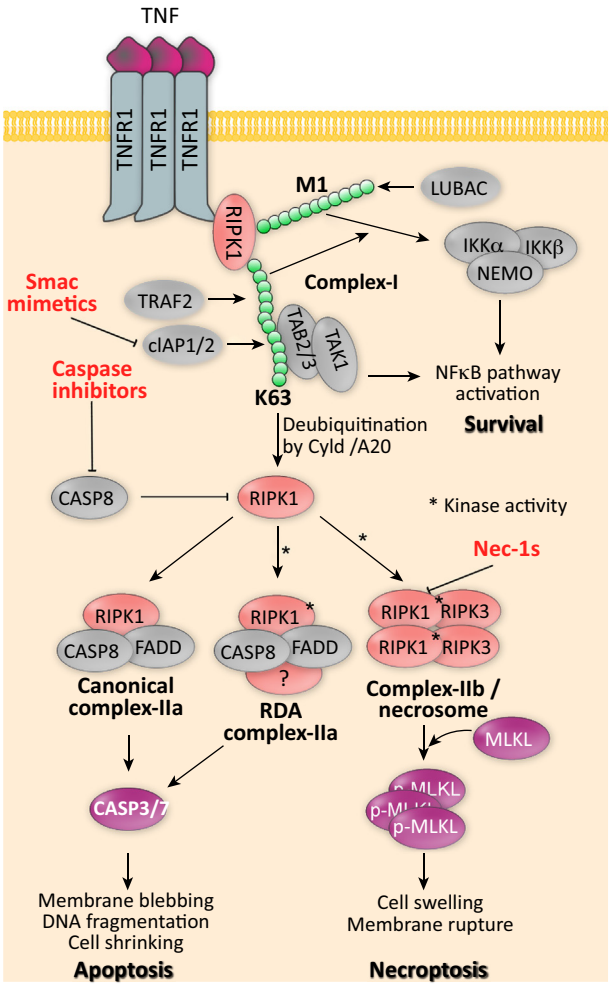


Figure 1. Canonical Apoptosis, RIPK1-Dependent Apoptosis, and Necroptosis Signaling Cascades. Tumor necrosis factor α (TNF-α) binding to TNF receptor 1 (TNFR1) results in receptor-interacting serine/threonine-protein kinase 1 (RIPK1)-mediated formation of complex-I, which is mediated by the E3 ubiquitin ligases cIAP1 and cIAP2 (cellular inhibitor of apoptosis protein 1/2) (and/or by X-linked inhibitor of apoptosis protein, XIAP) and TNF receptor-associated factor 2 (TRAF2), as well as by linear ubiquitination complex (LUBAC). Complex-I promotes survival and cytokine production via IκB-kinase (IKK)- and transforming growth factor-β-activated kinase 1 (TAK1)-mediated nuclear factor κB (NF-κB) pathway activation. Apoptosis, RIPK1-dependent apoptosis (RDA), or necroptosis pathways may be activated to promote cell death following deubiquitination of RIPK1 by Cyld and/or A20. Whereas canonical complex-IIa does not require RIPK1 kinase activity, RDA complex-IIa and complex-IIb/necrosome require RIPK1 kinase activity (*), which is inhibited by Nec-1s. The caspase-8–caspase-3 pathway is activated downstream of both canonical and RDA complex-IIa to promote apoptotic cell death, while mixed lineage kinase domain-like protein (MLKL) phosphorylation (p) and oligomerization downstream of complex-IIb/necrosome promotes necrotic cell death.

**RIPK1-dependent apoptosis (RDA):** a programmed cell death pathway that depends on caspase-8 and RIPK1 kinase activity. This pathway, unlike canonical apoptosis, can therefore be inhibited by inhibitors of RIPK1 such as Nec-1.
**Tumor necrosis factor α (TNF-α):** a cytokine that binds to the TNFR1/2 receptor. It is mainly secreted by macrophages. Can induce either cytokine production and cell proliferation, apoptosis, RIPK1-dependent apoptosis (RDA), or necroptosis, depending on the concurrent treatments and conditions. Gene symbol *TNF*.
**Tumor necrosis factor receptor 1 (TNFR1):** a single-pass type I transmembrane receptor (not a kinase) for the TNFα ligand that has docking sites for recruiting downstream signaling players such as TRADD and RIPK1 upon ligation of the receptor and its trimerization. Ligation of the receptor can induce either apoptosis, necroptosis, or NF-κB pathways depending on additional concurrent stimuli. Gene symbol *TNFRSF1*.

effect of necroptotic cell death, suggesting that inhibition of necroptosis in cancer could have beneficial therapeutic effects by reducing tumor growth-fueling inflammation levels in the tumor microenvironment.

## Necroptosis in the Tumor Microenvironment Promotes Inflammation and Cancer Metastasis

Metastasis is the leading cause of cancer-related mortality. It is increasingly recognized that metastasis involves a complex interaction of cancer cells with their microenvironment – including infiltrating immune cells and secreted cytokines, chemokines, and growth factors that collectively create an inflammatory milieu that promotes the invasive and metastatic ability of cancer cells. In this regard, as a proinflammatory form of cell death, necroptosis of non-transformed cells in the tumor microenvironment might facilitate metastasis by promoting inflammation. TNF-α has been known to play a central role in tumor progression; however, the exact downstream mechanism of this progression has not been fully elucidated. Elevated expression of TNF-α in the tumor microenvironment is a characteristic of many malignant tumors and is associated with poor prognosis [19]. Consistent with a proinflammatory nature of necroptosis and the pro-cancer role of inflammation, Nec-1 has been shown to reduce

### Box 2. Defining a Novel Programmed Cell Death Pathway Using Chemical Biology

The discovery of the small molecule Nec-1 (methyl-thiohydantoin-Trp) and its improved analog Nec-1s (7-Cl-O-Nec-1) [5-(7-chloro-1H-indol-3-yl)methyl)-3-methylimidazolidine-2,4-dione] as highly effective and specific inhibitors of necroptosis played an important role in defining this regulated necrotic cell death mechanism and in characterizing the involvement of necroptosis in various cell and animal models of human diseases [1,8,18]. The original screening hit Nec-1 has been noted to have an off-target effect on inhibiting indoleamine 2,3-dioxygenase (IDO) with an $IC_{50}$ of ~117 μM, which is about 58-fold higher than the $IC_{50}$ for Nec-1 to inhibit necroptosis (2 μM); thus, the off-target effect of original Nec-1 in inhibiting IDO is not relevant when used at 10–20 μM [41,42]. Consistently, 1-methyl-D,L-tryptophan (1-MT), an IDO inhibitor, cannot inhibit necroptosis even when used at a very high concentration. The optimized Nec-1s has no activity towards IDO [41,42]. An oral formulation has been developed to deliver a consistent amount of Nec-1s in animal models [9]. The requirement of RIPK1 in mediating caspase-independent death of Jurkat cells was first noted by Tschopp and colleagues [43]. The ability of Nec-1s to specifically inhibit the activity of RIPK1 without affecting its scaffolding function made it possible to determine the specific role of its kinase activity in a wide range of cultured systems and animal models, and this was crucial to convincingly demonstrate the existence of necroptosis as a common cell death mechanism, rather than some oddity of a specific cell line. In this regard, the chemical biology approach beautifully accomplished the goal of traditional genetic analysis in model organisms such as the nematode *C. elegans* and fruit fly *Drosophila*, where point mutations generated by random mutagenesis are necessary to painstakingly dissect the functions mediated by different domains of multifunctional proteins. Nec-1s binds to a hydrophobic pocket between the N lobe and C lobe of the RIPK1 kinase domain and stabilizes the kinase in an inactive conformation by interacting with highly conserved amino acids in the activation loop and the surrounding structural elements [44]. Thus, Nec-1s is an excellent example of type IV kinase inhibitors which are known to show high selectivity towards their targets. Consistently, Nec-1s demonstrates exclusive selectivity towards RIPK1 [45]. Importantly, in addition to the upstream role of RIPK1 in the necroptosis pathway, the remarkable potency and selectivity of Nec-1s make it an excellent tool for translational studies on the role of necroptosis in cancer.

inflammation and colitis-associated tumorigenesis in a mouse model of the disease [20] (Box 2). Furthermore, Seifert *et al.* have shown that necroptosis promotes macrophage-induced suppression of T cell immunity in pancreatic ductal adenocarcinoma and consequently tumor cell metastasis, reinforcing the idea that targeting necroptosis in cancer can be a viable therapeutic strategy [21].

Metastasis primarily involves the spreading of individual tumor cells through the circulatory system to colonize distant organs. Extravasation of tumor cells through endothelium is an important step in metastatic spread. Tumor cells were shown to induce necroptosis of endothelial cells by activating death receptor 6 (DR6, encoded by *TNFRSF21*), another member of the death receptor family, to promote tumor cell extravasation and metastasis [22]. Treatment of mice with Nec-1, or endothelial cell-specific deletion of RIPK3 or MLKL, reduced tumor cell-induced endothelial necroptosis, tumor cell extravasation, and metastasis. Conversely, pharmacological caspase inhibition or endothelial cell-specific loss of caspase-8 promoted these processes. Thus, blocking necroptosis of endothelial cells might be a potential clinically-translatable antimetastatic therapeutic approach.

## Necroptosis and Cancer Immunity

Cross-priming, the process that stimulates naïve cytotoxic $CD8^+$ T cells, is necessary for immunity against most tumors [23]. Necroptotic cells were shown to initiate adaptive immunity by providing both antigens and inflammatory stimuli for dendritic cells (DCs), which in turn activate $CD8^+$ T cells and antitumor immunity [24]. Using an artificial system that can promote the dimerization of RIPK3 upon treatment with a dimerizer, it was shown that activation of the RIPK3/RIPK1/NF-κB pathway can provide both antigen and immune stimulation, in turn supporting DC-mediated cross-priming of $CD8^+$ T cells. Cross-priming requires the scaffolding function of RIPK1 and nuclear factor-κB (NF-κB)-mediated transcription within the dying cells. By contrast, cells undergoing passive necrosis or secondary necrosis were poor inducers of $CD8^+$ T cell responses *in vivo*. These observations suggest that the complex regulation of necroptosis mediators RIPK1 and RIPK3 in the immune response against tumors must be carefully considered during cancer therapy designs involving necroptosis-targeting agents.

$CD8^+$ T cells are key mediators of anticancer immunity. A subset of $CD8^+$ T cells with $CD62L^{lo}$ phenotype that are unable to survive for a long period *in vivo* may explain their inability to control tumor growth [25]. Therapeutically activating and maintaining the survival of tumor-infiltrating T lymphocytes are now regarded as crucial in controlling the durability of cancer immunotherapy [26]. In addition, in cancer immunotherapy such as adoptive cell transfer therapy (ACT), which involves the engineering of T cells with tumor-specific T cell receptors extracted from the patient, expanding them *in vitro*, and then returning to the same patient to kill the cancer cells, a key limiting factor is the difficulty in obtaining a sufficient quantity of T cells with tumor-specific T cell receptors. It has been shown that tumor-reactive T cells might express elevated levels of RIPK1 and RIPK3 and undergo necroptosis upon T cell receptor (TCR) restimulation with cognate antigen, which can be inhibited by RIPK1 inhibition [27]. In particular, the increased susceptibility of $CD62L^{lo}$ to TCR restimulation-induced necroptosis might be primarily responsible for decreased $CD62L^{lo}$ T cell subset persistence. Inhibition of necroptosis of splenic T cells derived from Pmel mice – a mouse model that carries a rearranged T cell receptor transgene specific for the mouse homolog (*pmel*$^{Si}$ or *pmel-17*) of human premelanosome protein PMEL (also called gp100) and the T lymphocyte-specific *Thy1*$^{a}$ (Thy1.1) allele, and that is commonly used as a system for studies in immunotherapy – enhanced the survival of $CD8^+$ T cells and reduced tumor burden. Thus, blocking necroptosis by inhibiting RIPK1 in activated tumor-reactive T cells used in immunotherapy may improve the outcome of ACT as well as promoting the survival of tumor-infiltrating T cells to enhance the efficacy and application of cancer immunotherapies.

## Downregulation of Key Necroptosis Mediators in Cancer

Downregulated expression of multiple key mediators of necroptosis has been reported in cancers. CYLD is a deubiquitinating enzyme that promotes necroptosis [28]. Loss of the deubiquitinating activity of CYLD is involved in mediating familial cylindromatosis, an autosomal dominant predisposition to tumors of skin appendages termed cylindromas. Downregulation of CYLD was also found in chronic lymphocytic leukemia, which led to resistance to TNF-α-induced necroptosis [5]. Transcriptional repression by lymphoid enhancer-binding factor 1 (LEF1), a downstream effector of the Wnt/β-catenin pathway, was involved in mediating downregulation of CYLD. Transcription of CYLD was also found to be negatively regulated by Snail, which promotes tumor progression in malignant melanoma [29]. However, the consequence of downregulating CYLD in cancers might not be limited to resistance of necroptosis *per se* because CYLD also serves as a negative regulator of NF-κB, an important mediator of inflammation and tumor growth [30,31].

Reduced expression of RIPK3 has been reported in primary colorectal cancers relative to paired normal colorectal mucosa cells. In a cohort of 112 patients, low RIPK3 expression constituted an independent prognostic factor for overall survival and disease-free survival [32]. In addition, in an expression profiling study of RIPK1 and RIPK3 in $CD34^+$ leukemia cells from a cohort of patients with acute myeloid leukemia and $CD34^+$ cells from healthy donors, the expression of RIPK3, but not of RIPK1, was significantly reduced in most cancer samples [33]. Moreover, downregulated RIPK3 expression was found in malignant tissues from 75 breast carcinoma patients, regardless of tumor subtype [34]. Furthermore, the patients with greater than median RIPK3 mRNA expression exhibited improved metastatic relapse-free survival over a 10 year period.

RIPK3 protein was found to be absent in about two-thirds of the >60 cancer cell lines tested [34]. While only 20% of the hematopoietic cell lines examined lack RIPK3 expression, 80% of other cell lines have no detectable RIPK3. Treatment with the hypomethylating agent decitabine restored RIPK3 expression in multiple cell lines, suggesting that DNA methylation plays a role in silencing RIPK3 expression. Consistently, knockdown of maintenance methyltransferase

DNMT1 led to RIPK3 expression. Thus, reactivation of RIPK3 expression in cancer cells by modulators of DNA methylation might provide an opportunity for developing new anticancer treatments targeting necroptosis activation in cancer.

In addition, the expression of MLKL in cervical squamous carcinoma was found to be negatively correlated with histological grade and lymphatic metastasis, and low expression of MLKL indicated poor prognosis [35]. Similarly, reduced expression of MLKL in pancreatic adenocarcinoma and in resectable primary ovarian cancers was also found to correlate with decreased overall survival and decreased disease-free survival [36,37].

Taken together, downregulation of the expression of key mediators of necroptosis, including CYLD, RIPK3, and MLKL, appears to occur commonly in some types of cancers. However, the mechanisms that promote such downregulation remain unclear. Because necroptosis may be a common consequence of different cellular stress responses including chemotherapeutics, which may be directly or indirectly mediated by TNF-α simulation allowing cancer cells to survive and grow in an intrinsically inhospitable environment, this may have selected for low-expressers of CYLD, RIPK3, and MLKL. On the other hand, it is possible that the expression of dominant oncogenes may also provide a selection pressure for cancer cells with resistance to necroptosis. In this regard, we still know very little.

RIPK3 may also mediate necroptosis-independent roles in cancers, such as inhibition of the inflammasome allowing malignant progenitor differentiation – which is inhibited in many acute myeloid leukemia (AML) patients [38]. RIPK3 knockout mice transplanted with bone marrow cells transduced with AML-driving mutant gene *FLT3-ITD* had a poorer survival than wild-type mice. Therefore, in some cancers targeting RIPK3 directly may lead to adverse effects.

## RIPK1-Dependent Apoptosis and Cancers

Although loss of CYLD, RIPK3, and MLKL expression may have the potential to increase the fitness of cancer cells, there is no evidence for common loss of RIPK1 expression in cancers, which is not surprising because loss of RIPK1 expression would result in sensitization to apoptosis and loss of cell fitness [39]. Thus, because most tumor cells maintain normal levels of RIPK1 expression, the activation of RIPK1-dependent apoptosis (RDA) might provide an opportunity for cancer treatment.

Overexpression of IAP family proteins, including cIAP1, cIAP2, and XIAP, is often found in a variety of human tumors as a result of genomic amplification and has been linked to poor prognosis [4]. While it was originally thought that aberrant activation of IAPs might affect apoptosis in cancer cells by inhibiting caspases and promoting pro-survival NF-κB signaling, it is now clear that a major role of cIAP1/2 is to mediate lysine (K) 63 ubiquitination of RIPK1. Downregulation of cIAP1/2 by Smac mimetics promotes the activation of RIPK1, which in turn mediates RIPK1-dependent apoptosis under apoptosis-compatible conditions and necroptosis under apoptosis-deficient conditions. Thus, the retention of RIPK1 expression and downregulation of key mediators of necroptosis in cancer cells suggests the possibility of activating RIPK1-dependent apoptosis as an anticancer therapeutic strategy [40].

## Concluding Remarks

The importance of necroptosis in promoting metastasis and cross-priming, and its proinflammatory nature, reinforce the notion of targeting this process in cancer. However, downregulation of necroptosis mediators such as RIPK3 and MLKL in tumors suggests an escape mechanism from necroptosis in cancer (Figure 2). Therefore, in some cancers, induction of necroptosis to shrink tumors may require further therapeutic intervention to regain the

### Outstanding Questions

What is the contribution of necroptosis to the development of human cancers in general?

What is the mechanism that promotes the loss of RIPK3 in specific cancers?

What is the contribution of necroptosis to the tumor microenvironment?

Can necroptosis be modulated as a treatment for cancers?

Can necroptosis of T cells be modulated to enhance cancer immunotherapy?

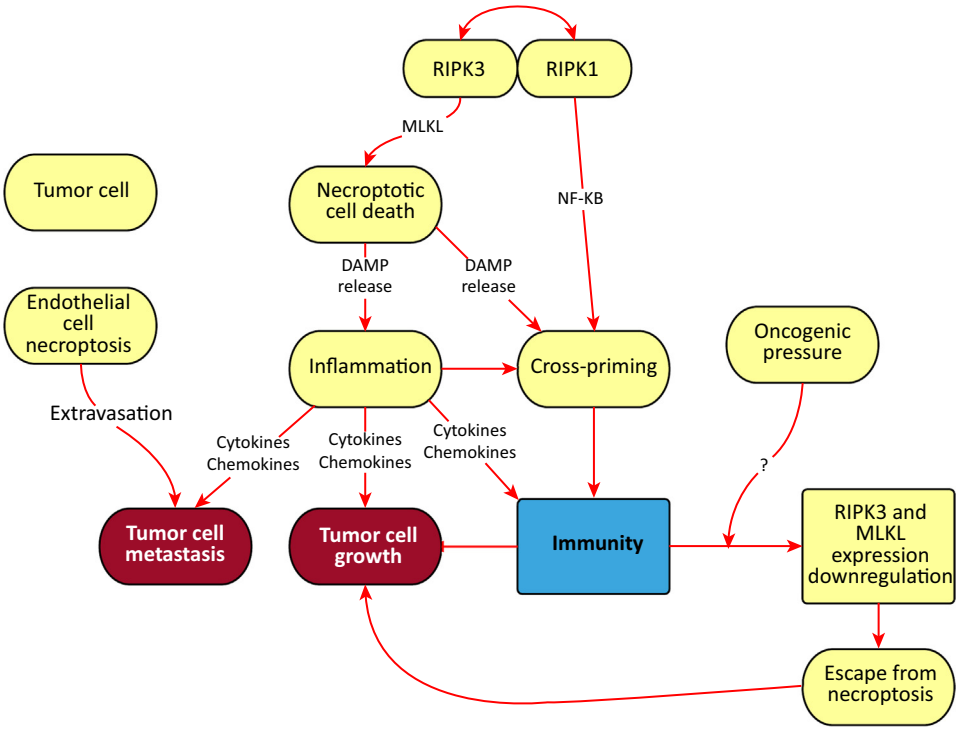


Figure 2. Role of Necroptosis in Cancer. Necroptotic cell death promotes inflammation, via the release of damage-associated molecular patterns (DAMPS), fueling cancer growth and metastasis while also promoting stimulation of naïve $CD8^+$ T cells via an event, called cross-priming, which is required for immune defense against cancer. Moreover, by killing endothelial cells via necroptosis, tumor cells achieve extravasation and metastasis, suggesting that inhibition of necroptosis in endothelial cells would restrict metastasis. While loss of RIPK3 an MLKL expression in many cancers results in escape from necroptosis, it is not known whether oncogenic forces play role in this process leading to escape from necroptosis.

expression of necroptosis mediators. Owing to its essentiality for cellular fitness, RIPK1 expression, on the other hand, is not downregulated in cancer, opening doors for RDA-mediated cancer therapy development. Currently, there is insufficient evidence for a conclusive verdict about whether necroptosis promotes or restricts cancer cell growth and/or metastasis in general. Further research in various cancer types will be necessary to determine whether induction versus inhibition of necroptosis/RDA has a significant therapeutic window. Moreover, important biological issues remain (see Outstanding Questions), such as (i) whether necroptosis contributes to carcinogenesis, (ii) how some cancers might lose RIPK3 expression and escape necroptosis, (iii) what might be the contribution of necroptosis to tumor microenvironment, and (iv) how necroptosis may be manipulated to increase the efficacy of cancer immunotherapies.

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