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Review Article

Necroptosis, The Double-Edged Sword and Its Therapeutic Implications in Cancer

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ABSTRACT

Necroptosis is a regulated cell death technique that eliminates cancer cells that are resistant to apoptosis, without requiring caspase. Necroptosis is implicated in several physiological and pathological processes. Numerous inputs can initiate the process, which is regulated by pseudokinase mixed lineage kinase domain-like protein (MLKL) and the activation of receptor-interacting serine/threonine protein kinases 1 and 3 (RIPK1, RIPK3). The well-studied executor RIPK1 affects important cellular processes and acts as a critical crossroad for several biochemical pathways through its interactions with numerous proteins. Currently, it is thought that necroptosis acts as a backup plan if apoptosis fails. Necroptosis possesses antiviral, antibacterial, and anticancer effects by getting rid of germ-filled or proliferating cells and promoting the development of a strong immune system. However, its potent anti-inflammatory and cytotoxic effects on cells can also lead to severe tissue injury, chronic sickness, and even tumor development. Not much is known about its role in the formation of tumors. In this review, we highlight recent discoveries about the biological significance of necroptosis, its conflicting functions in cancer, and its capacity to control cell destiny. As a pharmacologically controlled process, targeting necroptosis might be a valuable therapeutic intervention technique in cancer treatment.

Keywords: *Cancer; Necroptosis; MLKL; RIPK1; RIPK3.*

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Introduction

When cells are unable to sustain essential living processes, cell death results. Cell death is often divided into two categories: controlled cell death (RCD) and accidental cell death (ACD). While RCD entails a signaling cascade in which effector molecules are involved, ACD is an uncontrollable biological process. Among them, RCD is often referred to as physiologically induced programmed cell death (PCD) **[1]**. Different methods of cell death can be categorized based on their morphological appearances, immunological characteristics, or enzymatic criterion functions. Cells in organisms react to physiological or pathological

stimuli by producing different types of cell death to preserve the living body's normal function as much as possible. Autophagy typically occurs before apoptosis and initiates it. Autophagy has the greatest survival advantage and will cause programmed cell necrosis if the apoptotic cells are not removed in a timely manner **[2]**.

It is now clear that to preserve cellular homeostasis; processes for regulated cell death are required. Cell homeostasis is maintained by the physiological processes of cell division, proliferation, and death **[3]**. The biological process of cell death, which is the end of cell life, is essential for preserving the structure and functionality of healthy tissues. Necrosis and apoptosis are well-established mechanisms of cell death that have been linked to cell death, according to prior studies **[4-6]**. Genomic instability and/or inflammation cause a multi-step process that turns a normal cell into a cancerous one. The changing cells must rewire their biological processes to bypass the body's defenses against the growth of tumors as cancer progresses. One of the processes in cell transformation that encourages the emergence and spread of cancer is the inhibition of apoptosis **[7]**. Apoptosis resistance is a major component that mostly contributes to chemotherapy failure during cancer treatment **[8]**. Due to the cancer cells' strong resistance to caspase-dependent apoptosis, it was found that a unique emerging pathway is triggered in them. This indicates an alternative means of cell death in these cells and, when utilized in cancer treatment, may improve antitumor immunity. Necroptosis was the name used to describe it **[9]**.

Necroptosis was first proposed as a novel kind of programmed cell death in 2005 **[10]**. It may be identified by a phosphorylation signaling pathway that activates mixed lineage pseudokinase domain-like protein (MLKL/pMLKL) and is mediated by receptor-interacting serine/threonine protein kinase 1/3 (RIPK1/RIPK3). Death receptors cause RIPK1 and RIPK3 to become activated. RIPK3 then promotes MLKL phosphorylation. As a result, the plasma membrane is disrupted, cellular contents and damage-associated molecular patterns (DAMPs) are released, and a variety of inflammatory and immunological responses are set off, ultimately resulting in cell death **[11]**.

MLKL, which is represented by the human MLKL gene and features a protein kinase-like domain, is a member of the protein kinase superfamily **[12]** and triggers necroptosis after being phosphorylated by RIPK3. Subsequently, MLKL assembles into oligomers in the plasma membrane, where it utilizes its ability to preserve ionic homeostasis **[13]**.

RIP3, a member of the receptor-interacting serine/threonine protein kinases (RIP) family, is unique from the others in the family because of its unique C-terminal domain. The encoded protein is found in the cytoplasm. It belongs to the tumor necrosis factor (TNF) receptor-I signaling complex. In some cell types, RIPK1 engages RIPK3 to start the creation of complex IIb, also called the necrosome, to start necroptosis when Casp-8 activity is inhibited, and this causes MLKL activation **[1, 14]**.

2. Mechanism of Necroptosis

The necroptotic process is initiated by activation of certain cell surface death receptors (such as Fas cell surface death receptor (FasRs), Tumor necrosis factor receptor 1 (TNFR1), interferon (IFN) receptors, and Toll-like receptors (TLRs) as well as RNA- and DNA-sensing molecules **[15]**. Three known methods activate RIPK3, which is required for the necroptotic process. First, TNFR1 ligation activates RIPK1, which subsequently attaches to RIPK3 via shared RIP homology interaction motifs (RHIM) between the two molecules. Similar to this, the contact between TLR-3 and TLR-4 draws the adapter, which possesses an RHIM that can attach to and activate RIPK3. Finally, Z-dsDNA/dsRNA-binding protein 1 (ZBP1), a cytosolic nucleic acid sensor, also contains a RIPK3-activating RHIM **[16]**. The protein oligomerizes to create an active

"necrosome" complex when RIPK3 phosphorylates MLKL, and this complex migrates to the plasma membrane. Cell death is the outcome of this process, which is characterized by the expansion of the cell, permeabilization of the plasma membrane, and loss of cellular and organelle integrity **[17]**. The leakage of potassium, chemokines, and cytokines into the circulation is the source of inflammation and immune responses **[18, 19]** as shown in **Fig. 1** which was done by Tiff software.

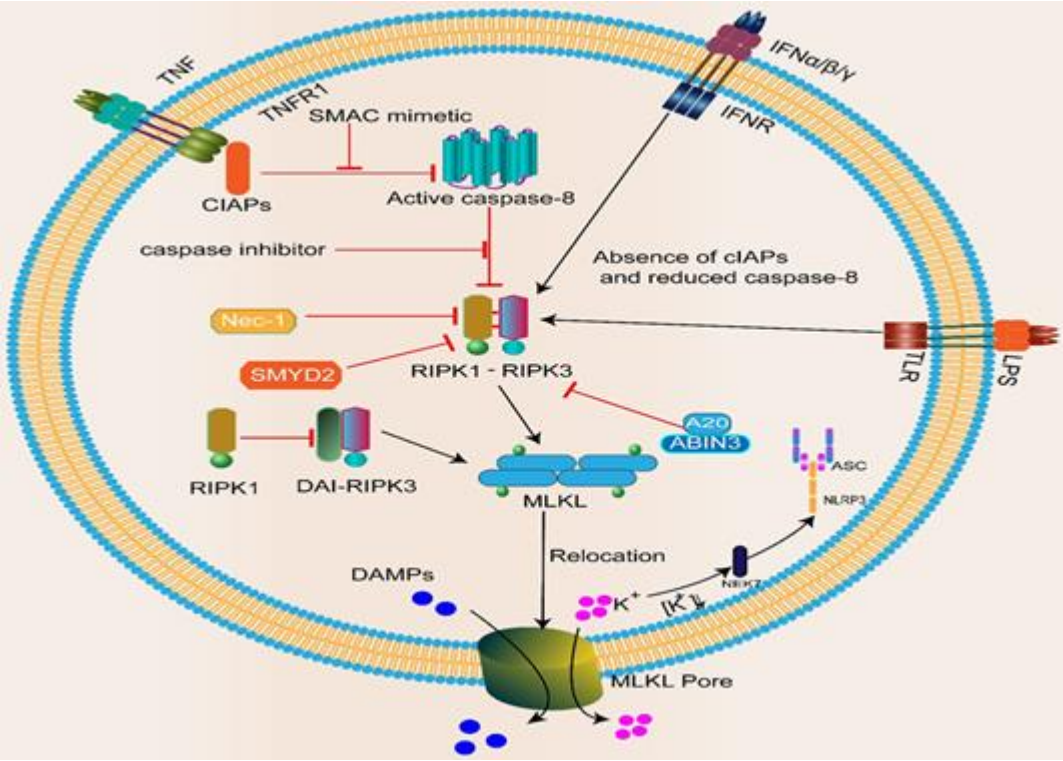

Fig. 1. Necroptosis mechanism. *Necroptosis is initiated when death receptors (TNFR, TLR, and IFNR) attach to their corresponding ligands (as shown). They encourage the formation of the RIPK1–RIPK3–MLKL signaling complex upon caspase-8 or cIAP depletion, which phosphorylates MLKL (p-MLKL). When MLKL is phosphorylated, it translocates to the plasma membrane, where it causes damage to the membrane and opens macropores. MLKL holes ultimately cause necroptosis by permitting ion inflow, cell enlargement, membrane lysis, and the consequent uncontrollable release of intracellular materials. Potassium efflux can further activate NLRP3 through NEK7 as a result of membrane disruption, which increases the release of inflammatory mediators.*

Nec-1 and SMYD2 are examples of inhibitory factors of necroptosis that have been discovered in recent investigations.

- *Receptor-interacting serine/threonine-protein kinase 1,3 (RIPK1, RIPK3), Mixed lineage kinase domain-like (MLKL), Tumor necrosis factor (TNF), Tumor necrosis factor receptor 1(TNFR1), Interferon receptor (IFNR), Cellular inhibitor of apoptosis proteins (CIAPs), Necrostatin-1 (NEC1), a lysine methyltransferase (SMYD), Lipopolysaccharides (LPS), Toll-like receptor (TLR), Damage-associated molecular patterns (DAMPs), adaptor protein apoptosis-associated speck like proteins (ASC), Tumor necrosis factor α-induced protein 3 (A2O), NLR family pyrin domain containing 3 (NLRP3).*

3. Necroptotic Mediators' Role in Cancer

MLKL, RIPK1, and RIPK3 are expected to have a critical role in regulating necroptosis in cancer. Functional modifications in the necroptotic machinery can reduce the ability of cancer cells to die and impact the prognosis due to changed interactions between RIPKs and other proteins **[38]** as seen in **(Table 1)**, key necroptotic members have been identified to be both elevated and downregulated in a range of cancer types.

Table 1. Dysregulated necroptotic mediators in different types of cancer

Model of cancer	Malfunctioning Expression	Types of sample	References
Colorectal	RIPK3 downregulation	Intestinal tissues	**[39]**
Breast	RIPK3 and MLKL downregulation	Human breast cancer cell lines	**[40, 41]**
Acute myeloid leukemia	RIPK3 downregulation	Bone marrow samples	**[42]**
Gastric	MLKL downregulation	Human gastric cancer cell lines	**[43, 44]**
Cervical Squamous Cell Carcinoma	MLKL downregulation	Tumor and adjacent epithelial tissues	**[45, 46]**
Glioblastoma	RIPK1 upregulation	Human glioma cell line	**[47, 48]**
Liver	RIPK1 downregulation	Liver cancer cells	**[49]**
Head and Neck Squamous Cell Carcinoma	RIPK1 downregulation	Head and neck carcinoma tissues	**[50, 51]**
Lung	RIPK1 upregulation	Lung cancer cell line	**[52]**
Pancreatic adenocarcinoma early-Stage	MLKL downregulation	Human pancreatic cancer tissues	**[53]**
Pancreatic ductal adenocarcinoma	RIPK1, RIPK3, and MLKL upregulation	Cell lines	**[54, 55]**
Ovarian	MLKL downregulation	Cell lines	**[56]**
Melanoma	RIPK3 downregulation	Human tissues	**[57]**

Receptor-interacting serine/threonine protein kinase 1, 3 (RIPK1, RIPK3), Mixed lineage kinase domain-like protein (MLKL).

Necroptotic mediators RIPK1 (located on chromosome 6), MLKL (located on chromosome 16), and RIPK3 (located on chromosome 14) are associated with cancer and have an effect on prognosis **[58]**. Given that the majority of cancer cells are known to express less RIPK3, RIPK3 downregulation or deletion during carcinogenesis, together with necroptosis resistance, is linked to a poor prognosis **[59]**.

This dysregulation may be caused by oncogenes such as Tyrosine-protein kinase receptor TYRO3 (AXL/TYRO3) and Serine/threonine-protein kinase B-raf (BRAF), which may control the methylation state of the promoter and one of RIPK3's transcription factors (Sp1) **[60]**. Chemotherapy sensitivity is increased when the RIPK3 promoter is hypomethylated, restoring the kinase to its native expression and improving anticancer treatment **[61]**. Interestingly, hypoxia lowers the levels of both RIPK1 and RIPK3 mRNA expression in several colon cancer cell lines, but not promoter methylation status, which is associated with a worse prognosis **[62]**. Moreover, RIPK3 is downregulated in colorectal, breast, AML, and melanoma cancers **[63]**. Breast cancer cells lacking MLKL and RIPK3 have been found to have reduced expression of genes related to interferon-α and interferon-γ responses; however, the precise mechanisms behind this association remain unclear **[64]**. RNA-sequence research using CD34+ bone marrow cells from patients with myelodysplastic syndromes or chronic myelomonocytic leukemia showed that overexpression of MLKL was correlated with the degree of anemia **[65]**. The increased expression of RIPK1 validates its role as an inflammatory mediator and categorizes it as a predictor of a lower overall survival, even if the precise mechanism is yet unclear **[66]**. AML patients with CD34+ leukemia cells have reduced RIPK3 expression, but not RIPK1 expression, which leads to impaired apoptosis, necroptosis, and the NF-θB pathway **[67]**. In addition, a different study found that RIPK1/RIPK3 inhibition may be a helpful treatment for AML patients by reducing the leukemogenic potential of AML cells when combined with specific chimeric antigen receptor T cells (highly expressed Interferon-gamma (IFN-γ)) or other differentiation inducers **[68]**. When it comes to patients with gastric, ovarian, cervical squamous, and early-stage pancreatic adenocarcinomas, low MLKL expression is associated with tumor development and poorer survival **[69]**. The downregulated MLKL expression might hinder the necroptotic process, which would explain the biomarker's low prognostic usefulness in cancer patients **[70]**. However, human pancreatic ductal adenocarcinoma has high expression of MLKL, RIPK1, and RIPK3, which is associated with a potent chemokine manifestation that encourages tumor development **[54]**.

4. Necroptosis's Dual Sides in Cancer

More and more research has revealed the connection between necroptosis and cancer. Gaining further insight into necroptotic pathways might help create novel cancer management strategies. Cancer is characterized by its resistance to apoptosis, which is brought on by modifications in or deactivation of the caspase function **[20]**. This shows that necroptosis activation is a potential cancer treatment strategy for eliminating apoptosis-resistant cancer cells **[21]**. Necroptosis's role in cancer is well-established, though, as it may function as a tumor suppressor as well as a promoter **[22]**.

Because necroptosis can activate alternative pathways and cause inflammation under the same pathological conditions, complicating cell fate and the course of pathologies that lead to neurodegenerative diseases, inflammatory diseases, or cancer metastasis, necroptosis is a "double-edged sword" in cancer. On the one hand, its induction promotes the death of

abnormal cells, which improves prognosis **[23]**. Several hypotheses have been proposed to explain the dual role. The specific role of necroptosis in carcinogenesis cannot be determined since every kind of cancer has a different microenvironment and different mediators are involved **[24]**. On the other hand, hypoxia characteristic of solid tumors is an appropriate strategy used by cancer cells to withstand necroptosis **[25]**. In this approach, cancer cells may modify their metabolism, decreasing their susceptibility to necroptosis **[8]**.

It is believed that the DAMPs associated with inflammation, at least in part, play an odd role in initiating the necroptotic cascade in carcinogenesis **[26]**. Tumor metastasis and malignant transformation are brought on by inflammation, which is brought on by the release of DAMP (High Mobility Group Box 1; HMGB1), cytokines (Interleukin-1; IL-1), adenosine triphosphate (ATP); reactive nitrogen intermediates (RNI); reactive oxygen species (ROS); and mitochondrial DNA into the environment in response to necroptotic stimuli. However, by producing DAMP, necroptotic cells may enhance tumor suppression and activate the immune system **[2, 27]**.

Necroptotic cells supply antigens to dendritic cells (DCs), which in turn excite cytotoxic T-cells.

(CD8+ T lymphocytes) via a procedure known as antigenic cross-priming. It has been shown that in addition to DAMP release, T cell activation requires RIPK1-mediated signaling and nuclear factor kappa light chain enhancer of activated B cells (NF-θB) triggered transcription to organize adaptive immunity. The release of DAMPs during necroptosis may be crucial to understanding the immune system's seemingly contradictory functions in immune surveillance and tumor promotion in cancer **[28]**.

Recent research indicates that RIPK1 may be a unique immunomodulatory target for the creation of innovative anticancer drugs. It has been shown that via changing tumor-associated macrophages, RIPK1 kinase activity inhibition enhances anticancer immunity **[29]**. Therefore, blocking RIPK1 kinase enhances the anticancer effect by opposing the immunosuppressive necroptotic tumor microenvironment **[30]**.

RIPK3 is also necessary for the anti-tumor immune response. Previous studies have shown that RIPK3 is not involved in the activation of B cells, T cells, or macrophages[3, 4]; however, recent data suggests that RIPK3 regulates the activation of Natural Killer T cells (NKT), which sets off the immune response and induces the lysis of cancer cells **[31, 32]**.

The two roles of necroptosis and the metastatic process have been connected. Metastasis, or the ability of cancer cells to move to other places in the body, is the primary cause of mortality for cancer patients **[33]**. Necrotization of metastatic cells is still in its early stages, which is triggered by many adverse circumstances, such as immune system activation, hypoxia, DNA mutations, and excessive production of reactive oxygen species (ROS) inside cells **[5, 6]**. Contradictory evidence, however, suggests that necroptosis acts as a trigger for metastasis **[34]**. The binding of death receptor 6 (DR6) on the surface of these cells to its ligand, amyloid precursor protein, is shown to enhance endothelial cell death and tumor cell extravasation **[30]**. Furthermore, RIPK1/RIPK3 promotes the phosphorylation of heat shock protein 27 in lung endothelial cells with permeability factor treatment (VEGF-A, VEGF-B, and basic fibroblast growth factor (FGF-b)), which facilitates the extravasation of tumor cells without necroptosis **[35]**.

According to this evidence, necroptosis can have a controversial function in the growth of

malignancies. It will be interesting to develop new methods that might target necroptosis for cancer treatment while still making use of well-established therapeutic approaches like immunotherapy, radiation therapy, or the delivery of chemotherapy medicines. In this case, RIPK1 activity modulation may be a helpful therapeutic approach for state-of-the-art regimens **[36]**. RIPK1 inhibitors may be a useful alternative treatment option for people who do not react to anti-TNF therapy. Many broad-spectrum multitargeting tyrosine kinase inhibitors have been approved by the Food and Drug Administration (FDA) for treatment against solid and hematological cancers. Nowadays, phase I and II clinical studies are investigating a small number of drugs that have been found to directly target RIPK1 to cure degenerative and inflammatory diseases. In clinical trials, RIPK1 inhibitors have not shown to be a successful cancer treatment. For the treatment of pancreatic cancer, only one RIPK1-targeting drug is presently being investigated in stages I and II studies **[37]**. Thus, it will be essential to keep studying the precise role of RIPK1 activity in cancer models to develop innovative therapies **[30]**.

Conclusions and Future Perspectives

A significant barrier to the therapeutic application of this phenomenon is that necroptosis is a rather conservative defense mechanism that can be evaded due to the adaptability of pathogens and tumor cells. The primary mechanisms of necroptosis under different clinical conditions remain unknown. Moreover, how cells assess cellular stress events to initiate cell death pathways and control the transition between different cell death modes remains unknown. Despite the fact that necroptosis has been extensively studied in both animal and cell models, further investigation is required to ascertain whether controlling necroptosis might have therapeutic benefits. Future research on necroptosis should include multidisciplinary techniques, such as the construction of artificial organoids that may closely resemble the complex structure of the human body. To sum up, the results that are now available suggest that treating necroptosis may offer promising therapeutic opportunities and emphasize the significance of considering all aspects of necroptosis's complexity when creating novel treatment approaches.

Declaration

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing interests

The authors declare that no competing interest exists.

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Authors' contributions

All authors have read and approved the final manuscript.

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