Role of Receptor Interacting Protein (RIP) kinases in cancer

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Abstract The Receptor Interacting Protein (RIP) kinase family consists of seven Serine/Threonine kinases, which plays a key signaling role in cell survival and cell death. Each RIP family member contains a conserved kinase domain and other domains that determine the specific kinase function through protein–protein interactions. RIP1 and RIP3 are best known for their critical roles in necroptosis, programmed necrosis and a non-apoptotic inflammatory cell death process. Dysregulation of RIP kinases contributes to a variety of pathogenic conditions such as inflammatory diseases, neurological diseases, and cancer. In cancer cells, alterations of RIP kinases at genetic, epigenetic and expression levels are frequently found, and suggested to promote tumor progression and metastasis, escape of antitumor immune response, and therapeutic resistance. However, RIP kinases can be either pro-tumor or anti-tumor depending on specific tumor types and cellular contexts. Therapeutic agents for targeting RIP kinases have been tested in clinical trials mainly for inflammatory diseases. Deregulated expression of these kinases in different types of cancer suggests that they represent attractive therapeutic targets. The focus of this review is to outline the role of RIP kinases in cancer, highlighting potential opportunities to manipulate these proteins in cancer treatment.

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Introduction

Receptor Interacting Protein (RIP) kinases are a family of Serine/Threonine kinases that play a wide variety of functional roles in cellular signaling during pathogen infection, inflammation, DNA damage, and response to extracellular stimuli. RIP1 is the founding member initially described in 1995.\(^1\) It was found to interact with the cell-surface receptor Fas/APO-1 (CD95) and therefore, named Receptor Interacting Protein (RIP).\(^1\) Since then, six additional RIP kinases have been identified, each varying in function and being classified as a RIP family member based on their homologous kinase domains (Fig. 1). Among RIP kinases, RIP1 and RIP3 are best studied as critical players in necroptosis. RIP1 was initially shown to play a role in necroptosis in the early 2000s, while RIP3's role in necroptosis was not described until 2009.\(^2\)–\(^5\)

Distinguished from other RIP kinases, RIP1 and RIP3 both contain a Respective Homotypic Interaction Motif (RHIM) domain that is involved in necroptosis through homo- or heterotypic interactions. Dysregulation of RIP kinases interferes with various signaling pathways, including cell survival and cell death pathways, which can promote oncogenic function. Because of this, it is not surprising

### Abbreviations

| AAs | amino acids |
| AML | acute myeloid leukemia |
| 5-aza-dC | 5-aza-2'-deoxycytidine |
| CARD | caspase-recruiting domain |
| cIAP | cellular Inhibitor of Apoptosis |
| CARDIAK | CARD-containing ICE associated kinase |
| CD95L | CD95 ligand |
| CLL | chronic lymphocytic leukemia |
| COR | C-terminus of Roc |
| COX-2 | cyclooxygenase 2 |
| CRC | colorectal cancer |
| CSCC | cervical squamous cell carcinoma |
| CYLD | cylindromatosis |
| DAI | DNA-dependent activator of IFN regulatory factors |
| DAMP | damage associated molecular pattern |
| DFS | disease-free survival |
| DNMT1 | DNA methyltransferase 1 |
| DIK | PKC delta-interacting protein kinase |
| DR | death receptor |
| DSS | dextran sulfate |
| EGFR | epidermal growth factor receptor |
| EMT | epithelial–mesenchymal transition |
| ESCC | esophageal squamous cell carcinoma |
| FADD | Fas-associated death domain |
| 5-FU | 5-fluorouracil |
| GEO | gene expression omnibus |
| GLUL | glutamate-ammonia ligase |
| HCC | hepatocellular carcinoma |
| HMGB1 | high mobility group box 1 |
| HNSCC | head and neck squamous cell carcinoma |
| HSP90 | heat shock protein 90 |
| ICD | immunogenic cell death |
| ICE | IL-1β converting enzyme |
| IFN | interferon |
| IL | interleukin |
| KD | knockdown |
| KIRC | kidney renal clear cell carcinoma |
| KO | knock-out |
| LRR | leucine-rich repeat |
| LRRK | leucine-rich repeat kinase |
| MDSCs | myeloid derived suppressor cells |
| MLKL | mixed lineage kinase domain like pseudokinase |
| NK | natural killer cell |
| NKT | natural killer T cell |
| NLR | nod-like receptor |
| NOD | nucleotide-binding oligomerization domain |
| NPC | nasopharyngeal carcinoma |
| OS | overall survival |
| OSCC | oral squamous cell carcinoma |
| PARP | poly-ADP ribose polymerase |
| PDA | pancreatic adenocarcinoma |
| PFS | progression free survival |
| PGAM5 | phosphoglycerate mutase 5 |
| PGE\(_2\) | prostaglandin E\(_2\) |
| PKC | protein kinase C |
| PKK | PKC-associated kinase |
| PRR | pattern recognition receptor |
| RICK | receptor-interacting serine/threonine kinase |
| RIP | receptor interacting protein |
| RIPK | receptor interacting protein kinase |
| RHIM | respective homotypic interaction motif |
| ROS | reactive oxygen species |
| Roc | Ros of complex proteins |
| Sgk288 | Sugen kinase 288 |
| SNP | single nucleotide polymorphism |
| Sp1 | specific-protein-1 |
| STS | staurosporine |
| TCGA | the cancer genome atlas |
| TAM | tumor associated macrophage |
| TLR | Toll-like receptor |
| TME | tumor microenvironment |
| TNBC | triple negative breast cancer |
| TNF | tumor necrosis factor |
| TNFR | tumor necrosis factor receptor |
| TRAIL | TNF-related apoptosis inducing ligand receptor |
| TRADD | TNF receptor-associated death domain |
| TRAF | TNF receptor-associated factor |
| TRIF | TIR-domain-containing adaptor inducing IFN-β |
| TSCC | tongue squamous cell carcinoma |
| Ub | ubiquitin |
| UHRF1 | ubiquitin-like, containing PHD and RING finger domains 1 |
| WT | wild type |
| Zbp1 | Z-DNA-binding protein 1 |
that RIP kinases are frequently upregulated or downregulated in certain types of cancer. These alterations are not only kinase-specific, but also cancer-specific. The goal of this review is to summarize the alterations of RIP kinases in cancer and discuss how these alterations impact cancer cell survival and death, metastatic potential, therapeutic response, and antitumor immune response. We will also briefly discuss various therapeutic agents known to induce necroptosis via RIP1 and/or RIP3 in cancer cells.

Structures and functions of RIP kinases

Structures of RIP kinases

RIP family kinases share 20–30% homology within a kinase domain, which is ~260–270 amino acids in length and most commonly located at the N-terminus (Fig. 1, 2). RIP1, RIP2, and RIP3 are the most similar RIP kinases based on their structures (Fig. 1, 2). RIP1 and RIP3 both contain an N-terminal kinase domain and a RHIM domain, allowing them...
to interact with each other and other RHIM domain-containing proteins to trigger necroptosis. RIP1 also contains a death domain, which enables it to interact with other death-domain-containing proteins. RIP2 contains a caspase-recruiting domain (CARD) that can interact with and activate caspase-1, an inflammatory caspase. RIP4 and RIP5 (Sugen kinase 288 [SgK288]/ANKK1) are structurally similar and both contain ankyrin repeats. Two RIP5s have been reported, with one referring to a protein related to the Dusty protein kinase and the other referring to SgK288, also known as ANKK1. RIP6 and RIP7 are most different from the other RIP family members, with each containing leucine-rich repeats (LRRs) and a Ros of complex proteins (Roc)/C-terminus of Roc (COR) domain. RIP6 and RIP7, similar to RIP4 and RIP5, contain ankyrin repeats; but these are located at the N-terminus, rather than the C-terminus. The presence of these non-kinase domains suggests protein–protein interactions are a key feature of RIP function in regulating cell death and immunity.

Functions of RIP kinases

The functions of each RIP kinase are summarized in Table 1, followed by a more detailed description below.

RIP1 and RIP3

RIP1 was initially reported to interact with Fas and Tumor Necrosis Factor (TNF) Receptor (TNFR) through its C-terminal death domain in yeast, which promoted cell death. 1 Subsequent studies showed that RIP1 can bind to other death receptors, including TNF-Related Apoptosis Inducing Ligand Receptor 1 (TRAILR1 or Death Receptor 4 [DR4]) and TRAILR2 (DR5), as well as various adaptor proteins including TNF Receptor-Associated Factor 1 (TRAF1), TRAF2, TRAF3, TNF Receptor-Associated Death Domain (TRADD), and Fas-Associated Death Domain (FADD). 7,11 In addition to mediating cell death, RIP1 has also been shown to promote cell survival, specifically through the NF-κB and MAPK pathways. 12–14 Whether RIP1 promotes cell survival or cell death is dependent on the specific cellular context and likely, its binding partners. RIP1’s kinase activity, which can be blocked by Necrostatin-1, has been shown to be critical for its role in cell death, but dispensable for RIP1-mediated cell survival. 15,16 Necrostatin-1 interferes with RIP1’s conformation, and therefore, can potentially affect its non-kinase functions. 17 The RHIM domain of RIP1 allows it to interact with other RHIM-containing proteins, such as RIP3, DNA-dependent Activator of IFN regulatory factors (DAI)/Z-DNA-binding protein 1 (Zbp1), and TIR-domain-containing adaptor Inducing Interferon (IFN) β (TRIF), which is involved in the Toll-Like Receptor (TLR) pathways. 18,19 Knock-out (KO) of RIP1 in mice leads to perinatal lethality with increased systemic inflammation and cell death. 15,16,20

Figure 2 Structure of RIP Kinases. RIP1-RIP5 (SgK288) contain N-terminal kinase domains. RIP1 contains a C-terminal death domain, while RIP2 contains a C-terminal caspase-recruiting domain (CARD). Both RIP1 and RIP3 are RHIM-containing proteins, which is located within the intermediate domain. RIP4, RIP5 (SgK288), and RIP6 contain ankyrin repeats. RIP6 and RIP7 both contain leucine-rich repeats (LRRs) and a Ros of complex proteins (Roc)/C-terminus of Roc (COR) domain. RIP7 also contains WD40 repeats. The number of amino acids (AAs) in each protein is indicated on the right.

Table 1 Functions of RIP kinases.

| RIP kinases (alternative names) | Functions                                                                 | References |
|-------------------------------|--------------------------------------------------------------------------|------------|
| RIP1 (RIPK1)                  | NF-κB and MAPK signaling; TLR signaling; Apoptosis and necroptosis       | 1,8,12–14, 19,38 |
| RIP2 (RIPK2; RICK; CARDIAK)   | IFN signaling; Metabolism; Necroptosis                                    | 7,8,14,50,51 |
| RIP3 (RIPK3)                  | NF-κB and JNK signaling; Wnt/β-catenin signaling; Epidermal differentiation; Cutaneous inflammation | 7–9,61,62  |
| RIP4 (RIPK4; DIK [human]; PKK [mouse]) | Dusty Protein Kinase: cell death; SgK288/ANKK1: neurodevelopment          | 7,8,14,57–60 |
| RIP5 (RIPK5)                  | Dusty Protein Kinase; SgK288; ANKK1:                                         | 7–9,61,62  |
| RIP6 (RIPK6; LRRK1)           | Endocytosis                                                              | 64,65 |
| RIP7 (RIPK7; LRRK2)           | Vesicle trafficking                                                     | 65–67 |

Abbreviations: CARDIAK: CARD-containing ICE associated kinase; DIK: PKC delta-interacting protein kinase; IFN: Interferon; LRRK: Leucine-rich repeat kinase; NOD: Nucleotide-binding oligomerization domain; PKK: PKC-associated kinase; RICK: Receptor-interacting serine/threonine kinase; RIP: Receptor interacting protein; RIPK: Receptor interacting protein kinase; SgK288: Sugen kinase 288; TLR: Toll-like receptor.
RIP3 was initially described in 1999 as an inducer of apoptosis independent of its kinase activity.\(^1\) There are conflicting reports on whether RIP3 can also activate the NF-κB pathway.\(^1\) Unlike RIP1 and RIP2, RIP3 does not contain a death domain or CARD domain (Fig. 2) and is not suspected to directly interact with death domain-containing proteins. In 2009, RIP3 was reported to be a crucial mediator of TNF-α-dependent necroptosis, which requires its kinase activity.\(^3\) Several studies showed that RIP3, when overexpressed or under certain conditions such as kinase inhibition, is able to promote apoptosis, in addition to necroptosis.\(^2\) A recent study revealed that in certain cancer cells, whether cells die by necroptosis or apoptosis is determined by the levels of heat shock protein 90 (HSP90) and CDC37.\(^3\) In some cell lines, RIP3 expression alone was sufficient to cause apoptosis via its autophosphorylation at S164/T165.\(^4\) Treatment with small-molecule RIP3 kinase inhibitors at a high concentration also results in apoptosis with blocked necroptosis.\(^2\) Similar to RIP1, RIP3 can interact with DAI/Zbp1 and TRIF through its RING domain, which are involved in necroptosis and TLR pathways, respectively.\(^5\) These interactions of RIP3 are also involved in IFN production.\(^4\) In some cases, such as during TLR stimulation, necroptosis can occur independent of RIP1 by direct activation of RIP3 through another RING domain-containing protein.\(^6\) In addition, RIP3 also regulates metabolism by interacting with various metabolic enzymes, such as Glutamate-Ammonia Ligase (GLUL), which leads to increased production of reactive oxygen species (ROS).\(^7\) Furthermore, RIP3 KO mice are viable and can rescue the embryonic lethality of caspase-8 and FADD KOs.\(^8\)

RIP1 and RIP3 functions have been elucidated largely through studies of the key steps in TNF-α-induced cell death (Fig. 3). Upon TNF-α binding to the TNFR, complex I forms, which typically consists of TRADD, TRAF2, RIP1, and E3 ubiquitin ligases cellular Inhibitor of Apoptosis 1 (cIAP1) and cIAP2.\(^9\) From this point, either cell survival or cell death can occur. The cIAPs can ubiquitinate RIP1, which leads to activation of NF-κB and MAPK pathways to promote cell survival.\(^9\) Upon cIAP degradation, RIP1 can be de-ubiquitinated by deubiquitinase cylindromatosis (CYLD).\(^10\) Upon deubiquitination, Complex IIa forms (Fig. 3), which typically includes RIP1, caspase-8, and FADD.\(^11\) Formation of this complex can trigger cell death by apoptosis. However, when caspase activity is inhibited, RIP1 can interact with RIP3 through their RING domains, forming a functional amyloid signaling complex termed “necrosome” or Complex IIb, which triggers necroptosis.\(^9,12\) Upon interaction, RIP3 becomes auto-phosphorylated, which leads to recruitment and phosphorylation of necroptotic executor Mixed Lineage Kinase domain Like pseudo-kinase (MLKL).\(^3\) Phosphorylated MLKL then translocates and oligomerizes at the plasma membrane to induce rupture, which causes release of Damage Associated Molecular Patterns (DAMPs), such as High Mobility Group Box 1 (HMGB1), stimulating an immune response.\(^3,9\) It is important to note that RIP1 and RIP3 can promote cell death independently during drug treatment or infection.\(^3\)

**Figure 3** RIP1 and RIP3 are critical mediators of necroptosis. TNF-α binds to the TNFR, promoting formation of Complex I, consisting of TRADD, TRAF2, cIAP1/2, and RIP1. cIAP1/2 ubiquitinate (Ub) RIP1, which can promote cell survival through the NF-κB and MAPK pathways. Upon de-ubiquitination by CYLD, RIP1 can interact with caspase-8 and FADD, promoting apoptosis. When caspase is inhibited, RIP1 can promote necroptosis by interacting with RIP3, leading to its autophosphorylation. Phosphorylated RIP3 recruits and phosphorylates MLKL, which translocates and oligomerizes at the plasma membrane, inducing rupture.

**Other RIP kinases**

*RIP2.* RIP2, also known as Receptor-interacting serine/threonine kinase (RICK) or CARD-containing interleukin (IL)-1β converting enzyme (ICE) associated kinase (CARDIAK), was described in 1998.\(^13\) RIP2 was shown to activate the NF-κB and JNK pathways independent of its kinase activity.\(^2,14\) RIP2 can bind to and activate inflammatory caspase-1 through their respective CARD domains.\(^5\) Caspase-1 is involved in the activation of proinflammatory cytokines, such as pro-IL-1β and pro-IL-18.\(^15\) RIP2 was also shown to interact with various adaptor proteins, such as TRAF1 and TRAF2, and with E3 ubiquitin ligases cIAP1 and cIAP2.\(^15\) Overexpression of RIP2 in some cells resulted in apoptosis.\(^9\) Furthermore, RIP2 plays a role in the innate immune response through interaction with Nucleotide-binding Oligomerization Domain 1 (NOD1) and NOD2, which are a type of pattern recognition receptor (PRR) that recognizes bacterial infection.\(^5,16\) Interaction between NOD1/2 and RIP2 can also result in activation of the NF-κB pathway.\(^5,53\)

*RIP4.* RIP4, also known as Protein Kinase C (PKC) Delta-Interacting protein Kinase (DIK) in humans and Protein Kinase C-associated Kinase (PKK) in mice, was first described in the early 2000s.\(^17,18\) RIP4 was found to activate NF-κB and JNK pathways, which require its kinase activity.\(^5,19\) The kinase domain of RIP4 alone was sufficient to stimulate these pathways.\(^5,19\) RIP4 interacts with various TRAF proteins and can be cleaved in a caspase-dependent manner during apoptosis, a common
characteristic of different RIP proteins.\textsuperscript{7} RIP4 can also phosphorylate Dishevelled proteins to stimulate Wnt/β-catenin signaling and is involved in epidermal differentiation and cutaneous inflammation.\textsuperscript{58–60} RIP4 KO in mice results in perinatally lethality and defects in the epidermis.\textsuperscript{59}

**RIP5.** In 2004, Zha et al first reported RIP5, a protein related to Dusty protein kinase.\textsuperscript{9} Overexpression of RIP5 resulted in cell death with characteristics of apoptosis, such as nuclear fragmentation.\textsuperscript{9} However, inhibition of caspases did not affect RIP5-induced death, suggesting non-apoptotic cell death.\textsuperscript{9} Due to the difference in structure of RIP5 compared to other RIP kinases (Fig. 2), Meylan et al proposed to abandon the term RIP5 for this protein kinase.\textsuperscript{8} Instead, they identified another protein commonly known as Sgk288 or ANKK1 that was fairly similar to RIP4 with approximately 35\% homology, containing both an N-terminal kinase domain and C-terminal ankyrin repeats (Fig. 2).\textsuperscript{8,10} They decided to refer to this kinase as RIP5.\textsuperscript{8} Studies suggest RIP5 plays a role in the central nervous system, specifically in neurodevelopment, and variations in this protein may be involved with psychiatric disorders, such as addiction.\textsuperscript{61,62} However, the exact physiological and biochemical functions of RIP5 remain unclear.

**RIP6 and RIP7.** RIP6 and RIP7 are also known as leucine-rich repeat kinase 1 (LRRK1) and LRRK2, respectively, due to their LRR domains (Fig. 2).\textsuperscript{7,8} LRR domains are found in a variety of immune-related proteins, such as Nod-Like Receptors (NLRs), and mutations in LRR-containing proteins are associated with a variety of human inflammatory diseases.\textsuperscript{63} RIP6 plays a role in endocytosis and endosome trafficking, specifically with the epidermal growth factor receptor (EGFR).\textsuperscript{64,65} RIP7 has been shown to be involved in vesicle trafficking.\textsuperscript{65–67} These kinases are also known to play a role in neurodegenerative diseases, with most information reported being in the context of these diseases.\textsuperscript{68,69} For example, mutations in RIP6 and RIP7 are often associated with Parkinson’s disease.\textsuperscript{70,71}

**Roles of RIP kinases in cancer development**

Depending on specific cancer types, RIP kinases can be either oncogenic or tumor suppressive. The dual roles most likely reflect the functions of these kinases in cell survival, cell death, and inflammation.

**Alterations of RIP kinase expression and impacts in cancer**

RIP kinases are frequently altered in cancer mainly at their expression levels. The types and impacts of the alterations of RIP kinases in different types of cancer are outlined in Table 2 and further discussed below. RIP5 and RIP7 are not included in the discussion due to lack of their information in cancer.

### Table 2

| RIP   | Alterations and cancer types                                      | References |
|-------|-------------------------------------------------------------------|------------|
| RIP1  | • Downregulation: breast cancer, CRC, HNSCC                       | 38,72,73   |
|       | • Upregulation: gallbladder cancer, gastric cancer                  |            |
|       | • Upregulation: CRC, pancreatic adenocarcinoma, ovarian, ovarian, |            |
|       | • Upregulation: bladder cancer, CSCC, pancreatic adenocarcinoma   |            |
| RIP2  | • Downregulation: OSCC                                            | 85–91      |
|       | • Upregulation: breast cancer, CRC, gastric cancer, KIRC          |            |
| RIP3  | • Downregulation: AML, breast cancer, CRC, malignant               | 38,81,94,95,98 |
|       | • Upregulation: CRC, ovarian, prostate cancer                      | –100,103   |
| RIP4  | • Downregulation: HCC, lung cancer, TSCC                           | 58,108     |
|       | • Upregulation: bladder cancer, CSCC, ovarian, ovarian, pancreatic | –110,113–119 |
| RIP6  | • Downregulation: HCC                                             | 120        |

**Abbreviations:** AML: acute myeloid leukemia; CLL: chronic lymphocytic leukemia; CRC: colorectal cancer; HCC: hepatocellular carcinoma; HNSCC: head and neck squamous cell carcinoma; KIRC: kidney renal clear cell carcinoma; OSCC: oral squamous cell carcinoma; PDA: pancreatic adenocarcinoma; RIP: receptor interacting protein; TSCC: tongue squamous cell carcinoma.
Exposure to carcinogens led to elevated RIP1 levels in normal mouse lung tissue and human bronchial epithelial cells, and, knockdown (KD) of RIP1 resulted in increased cell death and decreased transformation upon carcinogen exposure, most likely through abrogating its function in cell survival pathways. Another study reported that expression of kinase-inactive RIP1 increased survival of mice with pancreatic tumors, suggesting the kinase activity of RIP1 is oncogenic in these tumors. Additionally, RIP1 was found to be upregulated in gastric cancers, which was correlated with worse clinical outcomes, also suggesting a pro-tumor role. Interestingly, in ovarian cancer, RIP1 appears to play dual roles, where depletion of the kinase resulted in decreased proliferation but also reduced sensitivity to cisplatin. This dual role makes sense given RIP1’s established involvement in both cell survival and cell death. It would be interesting to determine in some tumors whether RIP1 is initially upregulated to aid in cell proliferation but eventually downregulated as the cancer progresses to suppress its ability to induce cell death.

**RIP2**

RIP2 was reported to be downregulated during the progression of oral squamous cell carcinoma (OSCC), suggesting RIP2 plays a tumor suppressive role in this type of cancer. RIP2 is commonly overexpressed in cancer, with reports of high expression levels in breast cancer, kidney renal clear cell carcinoma (KIRC), CRC, and gastric cancer. RIP2 overexpression in TNBC was associated with worse PFS and, at the cellular level, resulted in activation of the NF-κB and JNK pathways, which can promote growth and survival. Pharmacological inhibition of RIP2 resulted in decreased activation of these pathways, along with decreased migratory abilities of the TNBC cells. High levels of RIP2 were also associated with worse clinical outcomes in KIRC, with high expression being associated with increased tumor grade. Higher levels of RIP2 in CRC were also associated with worse clinical outcomes. Despite overexpression of RIP2 in human CRC, RIP2 was shown to suppress colorectal tumor development in mice due to its role in regulating inflammation via NOD2. KO of NOD2 or RIP2 in mice resulted in an increased number of tumors and enhanced activation of various inflammatory genes. Furthermore, co-housing wild-type (WT) mice with RIP2 KO mice treated with dextran sodium sulfate (DSS) promoted tumorigenesis and resulted in increased tumor burden in the WT mice, compared to mice that were separated. In this study, WT mice co-housed with NOD2 KO or RIP2 KO mice had an increased incidence of colitis, which is a known risk factor for CRC, suggesting enhanced transmission of microorganisms and a role of the microbiome.

**RIP3**

RIP3 was reported to be downregulated in CRC, malignant mesothelioma, breast cancer, melanoma, lung cancer, acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and prostate cancer. Low RIP3 expression was associated with worse clinical outcomes in metastatic CRC, malignant mesothelioma, and breast cancer patients. RIP3 expression was also shown to be decreased during melanoma development. Reconstituting RIP3 expression in RIP3-silenced melanoma cells restored the ability of these cells to undergo necroptosis, suggesting a role of RIP3-mediated necroptosis in suppressing melanoma development. Furthermore, stable expression of RIP3 in lung xenograft tumors resulted in decreased tumor burden and increased immune cell infiltration. Contrarily, in some cancer cells, RIP3 appears to play an oncogenic role. Increased RIP1 and RIP3 levels were found in PDA compared to normal tissue. Treating PDA cells with chemotherapeutic agents further increased the expression of RIP3, which was associated with induction of chemokine CXCL1, increased levels of myeloid derived suppressor cells (MDSCs), and an immunosuppressive tumor microenvironment (TME). In addition to altered expression, single nucleotide polymorphisms (SNPs) of RIP3 have been reported in non-Hodgkin’s lymphoma, which again, can restrict its ability to induce cell death to inhibit onco-gensis. Furthermore, nasopharyngeal carcinoma (NPC) tumors often have loss of heterozygosity 14q11.2, where the RIP3 genomic locus resides, which could promote tumorigenesis through compromising RIP3’s ability to induce cell death.

**RIP4**

RIP4 was reported to be downregulated in lung cancer. High RIP4 expression levels in lung cancer are associated with tumor differentiation and better clinical outcomes in The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) datasets, suggesting a tumor suppressive role. This is further supported by the finding that RIP4 KD in lung cancer cells leads to enhanced STAT3 signaling and de-differentiation. Similarly, decreased RIP4 expression was correlated with poor differentiation of tongue squamous cell carcinoma (TSCC). RIP4 expression was also found to be reduced in primary human hepatocellular carcinoma (HCC) samples compared to normal tissue. Furthermore, there are conflicting reports on RIP4 expression in NPC. While one study reported RIP4 downregulation in a small number of NPC patient samples compared to normal tissue, another study described upregulation of RIP4 in NPC and an inhibitory effect of RIP4 KD on NPC cell growth. On the other hand, RIP4 expression was found to be upregulated in cervical squamous cell carcinoma (CSCC), bladder cancer, pancreatic cancer, ovarian cancer, CRC, and osteosarcoma. High RIP4 expression was associated with a shorter 5-year OS and disease-free survival (DFS) in CSCC, and also, shorter OS in bladder cancer, pancreatic cancer, ovarian cancer and osteosarcoma. Therefore, like the other RIP kinases, RIP4 has dual roles in cancer depending on the specific cancer type.

**RIP6**

RIP6 was found to be downregulated at both mRNA and protein levels in primary human HCC samples and in liver cancer cell lines compared to normal tissue. Low RIP6 expression was associated with larger tumors, suggesting an anti-tumor function of RIP6 in HCC. Stable expression of
RIP6 in liver cancer cell lines that have reduced RIP6 expression led to decreased proliferative abilities and enhanced apoptosis with an unclear mechanism. RIP6 expression in vivo also led to reduced tumor growth, suggesting RIP6 has antitumor activity against liver cancer.\textsuperscript{120}

**Genetic, epigenetic and other alterations of RIP kinases in cancer**

Few studies have described genetic alternations of the RIP family members in cancer. Analysis of COSMIC database revealed amino acid sequence changes of RIP1 and RIP3 in some cancers, resulting in missense or RHIM domain mutations that may impact their expression and functions.\textsuperscript{43,97} We analyzed cancer genomics datasets at cBioPortal (https://www.cbioportal.org/), and surprisingly, found many genetic alterations of RIP kinases in cancer, predominantly genomic amplification and mutations. Table 3 summarizes the top four tumor types with the most frequent genetic alterations of each RIP family member. The highest alteration frequencies range from 15.69% to 3.60%, corresponding to RIP7 in uterine corpus endometrial carcinoma and RIP3 in skin cutaneous melanoma, respectively. RIP2 is the most frequently amplified RIP family member in cancer, with 8.77% amplification in uterine carcinosarcoma and 8.58% amplification in breast invasive carcinoma. RIP7 is the most frequently mutated RIP family member, with 14.93% mutations in uterine corpus endometrial carcinoma and 13.86% mutations in stomach adenocarcinoma. Other genetic alterations, including structural variants, deep deletions, and multiple alterations, are rare and not included in Table 3. The functions of these genetic alterations are not well characterized. The amplification of RIP kinases likely leads to upregulation of their expression in cancer. The mutations, including silent, missense, and nonsense mutations, may result in silencing, downregulation, upregulation, or altered functions of RIP kinases in cancer.

A major cause of downregulation of RIP kinases in cancer is promoter hypermethylation.\textsuperscript{96,121} Hypermethylation often occurs at CpG islands, which are genomic regions of 200–2000 nucleotides with at least 50% of CpG dinucleotides existing in approximately 60–70% of promoters.\textsuperscript{122} RIP3 downregulation in various cancer cell lines was found to be

| Table 3 | The most frequent genetic alterations of RIP kinases in human cancer. |
|---------|-------------------------------------------------------------------|
| RIP kinases | Tumor types | Number of cases | Frequency of genetic alterations |
| | | | Total | Amplification | Mutations |
| RIP1 | Uterine Corpus Endometrial Carcinoma | 529 | 6.99% | 5.48% | 1.51% |
| | Ovarian Serous Cystadenocarcinoma | 584 | 6.85% | 5.99% | 0.34% |
| | Skin Cutaneous Melanoma | 444 | 6.08% | 2.93% | 2.70% |
| | Liver Hepatocellular Carcinoma | 372 | 4.84% | 3.76% | 0.81% |
| RIP2 | Breast Invasive Carcinoma | 1084 | 9.04% | 8.58% | 0.28% |
| | Uterine Carcinosarcoma | 57 | 8.77% | 8.77% | 0% |
| | Prostate Adenocarcinoma | 494 | 7.89% | 7.69% | 0.20% |
| | Liver Hepatocellular Carcinoma | 372 | 7.53% | 6.99% | 0.27% |
| RIP3 | Skin Cutaneous Melanoma | 444 | 3.60% | 0.23% | 3.38% |
| | Uterine Corpus Endometrial Carcinoma | 529 | 3.59% | 0.38% | 3.21% |
| | Bladder Urothelial Carcinoma | 411 | 3.16% | 0.73% | 0.73% |
| | Lung Adenocarcinoma | 566 | 2.83% | 1.77% | 0.71% |
| RIP4 | Skin Cutaneous Melanoma | 444 | 5.86% | 0.23% | 5.41% |
| | Stomach Adenocarcinoma | 440 | 5.45% | 0.23% | 3.18% |
| | Bladder Urothelial Carcinoma | 411 | 4.62% | 0.24% | 3.89% |
| RIP5 (Dusty Protein Kinase) | Uterine Corpus Endometrial Carcinoma | 529 | 4.35% | 0.76% | 3.59% |
| | Breast Invasive Carcinoma | 1084 | 9.13% | 8.49% | 0.46% |
| | Skin Cutaneous Melanoma | 444 | 8.11% | 3.15% | 4.95% |
| | Uterine Corpus Endometrial Carcinoma | 529 | 7.56% | 2.27% | 5.29% |
| | Liver Hepatocellular Carcinoma | 372 | 6.72% | 6.45% | 0.27% |
| RIP5 (SgK288;ANKK1) | Skin Cutaneous Melanoma | 444 | 7.88% | 0.23% | 4.95% |
| | Uterine Corpus Endometrial Carcinoma | 529 | 6.05% | 0% | 5.48% |
| | Uveal Melanoma | 80 | 3.75% | 0% | 1.25% |
| | Uterine Carcinosarcoma | 57 | 3.51% | 1.75% | 0% |
| RIP6 (LRRK1) | Sarcoma | 255 | 10.20% | 7.45% | 2.35% |
| | Uterine Corpus Endometrial Carcinoma | 529 | 10.02% | 0.76% | 8.70% |
| | Skin Cutaneous Melanoma | 444 | 9.19% | 1.13% | 8.56% |
| RIP7 (LRRK2) | Uterine Corpus Endometrial Carcinoma | 529 | 15.69% | 0.76% | 14.93% |
| | Stomach Adenocarcinoma | 440 | 14.55% | 0.45% | 13.86% |
| | Lung Squamous Cell Carcinoma | 487 | 13.96% | 1.03% | 12.73% |
| | Skin Cutaneous Melanoma | 444 | 13.29% | 0.45% | 12.61% |

Data from https://www.cbioportal.org/.
caused by promoter hypermethylation, which could be restored by treatment with hypomethylating agents, such as the DNA methyltransferase 1 (DNMT1) inhibitor 5-aza-2’-deoxycytidine (5-aza-dC). \(^{96,99,100}\) Another study reported that RIP1, but not RIP2, was downregulated by promoter hypermethylation in HNSCC, which could also be reversed by 5-aza-dC treatment. \(^{73}\) Many previous studies have shown that combining 5-aza-dC, or its analogue 5-azacitidine, with other anticancer agents can enhance therapeutic sensitivity, which may involve restoration of RIP3 expression in RIP3-silenced cancer cells. However, a contradicting report showed that 5-aza-dC treatment did not restore the expression of RIP1 or RIP3 in various cancer cell lines. \(^{97}\)

Methylation of the RIP3 promoter has been shown to be maintained by Ubiquitin-like, containing PHD and RING finger domains 1 (UHRF1) by recruiting DNMT1 in CRC cell lines. \(^{123}\) KD of UHRF1 led to enhanced RIP3 expression, which is mediated by the zinc finger transcription factor specific-protein-1 (Sp1). \(^{123}\) It was also suggested that hypoxia can trigger RIP1 and RIP3 silencing, indicated by decreased RIP1 and RIP3 expression in RIP1/3-expressing CRC cells cultured under hypoxic conditions. \(^{97}\)

In addition, RIP kinase expression in cancer can be regulated by other mechanisms. One study suggests post-translational modifications and autocrine TNF-α impact upregulation, specifically for RIP1 in melanoma. \(^{79}\) Increased expression of oncoproteins may also play a role in upregulation of these kinases.

**Impact of RIP kinase alterations on cancer metastasis**

Alterations in RIP kinases can either promote or suppress the metastatic potential of cancer cells depending on the specific type of cancer and context.

**Promoting metastasis**

Several studies showed that loss or inhibition of RIP1 kinase activity reduced tumor metastasis *in vivo*. \(^{124–127}\) RIP1 inhibition led to reduced vessel sprouting, a key event in metastasis. \(^{124}\) Exposing non-small cell lung cancer cells to γ-ionizing radiation induced metastatic potential by increasing RIP1 expression and NF-κB activation. \(^{128}\) KD of RIP1 in gallbladder and gastric cancer cells resulted in reduced invasiveness *in vitro* and decreased tumor size *in vivo*. \(^{72,80}\) RIP1 was shown to promote lymph node metastasis of gallbladder cancer in an orthotopic model in nude mice. \(^{129}\) This activity of RIP1 can be explained by its role in regulating TNF-α-mediated lymphangiogenesis and lymphatic metastasis via the NF-κB–VEGF-C pathway in gallbladder cancer, further suggesting that the pro-tumor function of RIP1 is dominant in this type of cancer. \(^{130}\)

RIP2 was shown to enhance metastatic properties in TNBC, where depletion of RIP2 reduced migration and invasion of these cells *in vitro* and decreased tumor size *in vivo*. \(^{87}\) Similar *in vivo* results were observed in RIP1 or RIP3 KO TNBC cells. \(^{127,131}\) These observations are most likely due to activation of cell survival pathways by these RIP kinases. Depletion of RIP2 in KIRC also led to decreased proliferative and migratory abilities, along with decreased tumor burden, which was shown to be due to down-regulation of the NF-κB and JNK pathways. \(^{89}\)

Oncogenic roles of RIP4 have been largely attributed to NF-κB, JNK, and Wnt signaling, where KD of this kinase in various cancers reduced invasiveness both *in vitro* and *in vivo*. \(^{115–118}\) Furthermore, depletion of RIP4 in osteosarcoma cells reduced not only invasiveness, but also the epithelial–mesenchymal transition (EMT), indicated by increased E-cadherin and decreased N-cadherin expression. \(^{119}\) This effect of RIP4 is mediated by stimulation of the Wnt/β-catenin pathway, which is known to play a role in EMT.

**Suppressing metastasis**

It was shown that RIP1 is downregulated in metastatic-tumor-derived HNSCC cells, which increased invasiveness assessed by wound healing and trans-well migration assays. \(^{73}\) Similarly, depletion of RIP3 in AML and HCC models resulted in enhanced tumorigenesis. \(^{127,132,133}\) In CRC cells, RIP3 overexpression caused decreased metastatic potential. \(^{123}\) The effects of RIP1 and RIP3 described in these studies are most likely due to their functions in cell death, in particular necroptosis. As described above, when necroptosis is triggered, DAMPs are released, which can stimulate a local immune response and, in certain cases, can lead to activation of the adaptive immune response through immunogenic cell death (ICD). \(^{134}\) Furthermore, the effects of RIP kinases can also be mediated through the TME. \(^{127}\) For example, RIP3 expression was found to be decreased in MDSCs in the TME of CRC, which promoted tumor development via increased cyclooxygenase 2 (COX-2) and prostaglandin E₂ (PGE₂) levels, along with enhanced NF-κB activity. \(^{135}\)

RIP4 was also found to play a tumor suppressive role in lung cancer and TSCC. RIP4 overexpression in lung cancer cells reduced invasiveness *in vitro* and decreased tumor size *in vivo*, in part due to its inhibition of oncogenic STAT3 signaling. \(^{108}\) Similarly, depletion of RIP4 led to enhanced migration and invasion of TSCC cells, along with decreased caspase-8 activation during cisplatin treatment. \(^{109}\)

**Roles of RIP kinases in anticancer therapies**

**Role in determining therapeutic response**

Various anticancer agents have been shown to induce cell death mediated by RIP1 and/or RIP3. There has been a strong interest to restore cell death in cancers with RIP1 or RIP3 silencing. Variations in the expression of RIP3 was shown to impact therapeutic sensitivity in different cancer cell lines, with RIP3-silenced cancer cells having reduced sensitivity to necroptosis-inducing therapeutic agents, which could be restored through ectopic expression of RIP3. \(^{96,123}\) Reconstituting RIP3 expression in RIP3-silenced cancer cells, either ectopically or through 5-aza-dC treatment, resulted in enhanced response to various chemotherapeutic drugs, such as taxol, doxorubicin, etoposide, camptothecin, cisplatin, and 5-fluorouracil (5-FU), as shown by decreased cell viability *in vitro* and reduced
tumor burden in vivo. Similarly, RIP3 KO lung cancer and thymoma cells were also less responsive to mitoxantrone and oxaliplatin. Furthermore, caspase inhibition in some CRC cells can enhance the sensitivity to 5-FU, which is abrogated by RIP1 and/or RIP3 depletion. These studies suggest RIP kinase expression can enhance therapeutic sensitivity in certain cancer types, while loss of their expression may be a mechanism of therapeutic resistance.

Several other classes of anticancer agents can induce necroptosis in cancer cells either alone or in combination with chemotherapy. For example, Smac mimetics can stimulate the formation of the ripoptosome complex, comprised of FADD, caspase-8, and RIP1, through depletion of cIAPs, which can then trigger either apoptosis or necroptosis in various cancer cell lines. BH3 mimetics, such as obatoclax, can stimulate necroptosis in various cancer cell lines when used in combination with other anticancer agents.

A number of natural compounds can also induce necroptosis, such as shikonin and its analogues, staurosporine (STS), neoalbaconol, trichothecin, and 2-methoxy-6-acetyl-7-methyljuglone. Shikonin was shown to induce cell death through necrosis in breast cancer cells, which was not blocked by overexpression of anti-apoptotic proteins. Shikonin treatment led to increased RIP1 and RIP3 expression in pancreatic cancer cells and a synergistic effect when combined with gemcitabine. STS could induce necroptosis in lymphoma cells under caspase inhibition. Oncolytic viruses, such as vaccinia virus, have also been shown to induce necroptosis in cancer cells, such as ovarian cancer cells. Together, these data suggest inducing necroptosis can be used to overcome therapeutic resistance and enhance treatment efficacy, especially when the apoptotic pathway is inactivated.

Therapeutic targeting of RIP kinases

Various RIP kinase-targeting agents have been developed but not yet approved for cancer treatment. Some agents have advanced more in the treatment of other diseases. For example, a RIP1 inhibitor is currently under review for treating inflammatory diseases, such as rheumatoid arthritis. Other RIP1 inhibitors showed promising anticancer effects, such as reducing metastasis in mouse models and enhancing T cell activation in patient-derived tumor samples. With promising results in preclinical studies, the RIP1 inhibitor GSK3145095 was tested in Phase 1 clinical trials for certain solid tumors. FDA-approved Braf inhibitor Dabrafenib, which is currently used for treatment of Braf-mutated (V600E) melanoma, can also inhibit RIP3. These inhibitors could potentially be used in cancers where these RIP kinases play an oncogenic role. Perhaps these inhibitors could be used alone or in combination with targeted or chemotherapeutic drugs to enhance therapeutic efficacy and improve clinical outcomes for these types of cancer. Furthermore, utilizing drugs to restore expression or activate RIP kinases could also be beneficial by enhancing therapeutic sensitivity in cancers where RIP kinases play a tumor suppressive role. As noted above, cell death mediated by RIP kinases, in particular necroptosis, can increase inflammation, which may or may not be beneficial for cancer treatment. There is a fine line between the beneficial and negative effects of increased inflammation. While the antitumor immune response stimulated by inflammatory cell death is beneficial, too much inflammation can promote tumorogenesis and metastasis. Therefore, we need to better understand cancer-specific functions of RIP kinases and develop corresponding therapeutic strategies to target or restore these proteins.

Impact of RIP kinase alterations on antitumor immune response

In addition to impacting tumor intrinsic therapeutic response, emerging evidence supports that RIP1- and RIP3-dependent necroptosis influences the antitumor immune response. RIP1-dependent cell death triggered by RIP3 oligomerization has been shown to promote CD8+ T cell priming through activation of the NF-κB pathway and enhance both ICD and tumor infiltration of immune cells. RIP1-mediated cell death enhanced activation of CD8+ T cells and natural killer (NK) cells and potentiated immune checkpoint blockade against soft-tissue sarcomas. RIP3 was also reported to be involved in natural killer T (NKT) cell function, as depletion of RIP3 led to decreased NKT cell activation, cytokine production, and inflammation upon treatment with an immunostimulant in mouse melanoma and liver inflammation models. This activity of RIP3 is mediated by mitochondrial phosphoglycerate mutase 5 (PGAM5), which activates a transcription factor required for cytokine production in NKT cells. Furthermore, a recent study showed that intratumoral delivery of constitutively active RIP3 enhanced T cell response, improved survival of tumor-bearing mice, and potentiated anti-PD-1 therapy. Additionally, KO of RIP3 in mouse syngeneic lung cancer or lymphoma models resulted in reduced ICD and decreased immune cell infiltration, which is most likely due to RIP3’s critical role in necroptosis. Together, these data suggest that RIP1 and RIP3 enhance the immune response against various types of cancers.

On the other hand, necroptosis can also promote tumorogenesis due to its inflammatory effects. In a PDA model, RIP1 inhibition not only decreased tumor size, but also led to increased T cell activation and enhanced efficacy of anti-PD-1 therapy, suggesting oncogenic immunosuppressive activity of RIP1 in this model. Inhibition of RIP3 in small intestine tumors reduced levels of various inflammatory cytokines and the amount of MDSCs, suggesting that RIP3 promotes tumorogenesis through its pro-inflammatory activity in this type of tumor. Another study showed that in contrast to the in vitro growth inhibitory effects of RIP1 and RIP3 in PDA cells, KO of RIP3 or inhibition of RIP1 suppressed PDA progression in mice.
In this study, RIP3 KO tumors showed decreased tumor associated macrophages (TAMs) and MDSCs, both of which promote an immunosuppressive TME. These surprising results indicate a role of RIP1/3-mediated inflammation in PDA. Thus, alterations in RIP1 and RIP3 can both positively and negatively impact immune response depending on the cancer type due to their roles in promoting cell survival, cell death, and inflammation.

Conclusions

In conclusion, the RIP family kinases are involved in a wide variety of cellular functions and signaling pathways, with each kinase having its own unique functions. These kinases are frequently upregulated or downregulated in cancer depending on cancer types and cellular contexts, which contributes to tumor progression. A major cause of reduced expression of RIP1 and RIP3 in cancer is promoter hypermethylation, which results in gene silencing and decreased expression. Alterations in RIP kinases can lead to enhanced metastatic potential, reduced therapeutic sensitivity, and decreased antitumor immune response. Expression of RIP kinases can potentially be used as a predictive biomarker for certain therapies. However, further studies are needed to better delineate how alterations of RIP kinases influence the development and treatment response of different types of cancer. Such information is essential for the development of RIP-kinase-targeted therapies. The current knowledge suggests that RIP kinases represent attractive targets for developing improved treatments for certain types of cancer.

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K.E., J.Y., and L.Z. contributed to the conception, design and writing of this review.

Conflict of interests

The authors declare no conflict of interests.

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References

1. Stanger BZ, Leder P, Lee TH, Kim E, Seed B. RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. Cell. 1995; 81(4):513–523.

2. Holler N, Zaru R, Micheau O, et al. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol. 2000;1(6):489–495.

3. Cho YS, Challia S, Moquin D, et al. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell. 2009;137(6):1112–1123.

4. He S, Wang L, Miao L, et al. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell. 2009;137(6):1100–1111.

5. Zhang DW, Shao J, Lin J, et al. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science. 2009;325(5938):332–336.

6. Thome M, Hofmann K, Burns K, et al. Identification of CARDIAK, a RIP-like kinase that associates with caspase-1. Curr Biol. 1998;8(15):885–888.

7. Zhang D, Lin J, Han J. Receptor-interacting protein (RIP) kinase family. Cell Mol Immunol. 2010;7(4):243–249.

8. Meylan E, Tschopp J. The RIP kinases: crucial integrators of cellular stress. Trends Biochem Sci. 2005;30(3):151–159.

9. Zha J, Zhou Q, Xu LG, et al. RIP5 is a RIP-homologous inducer of cell death. Biochem Biophys Res Commun. 2004;319(2):298–303.

10. Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Hum Mutat. 2004;23(6):540–545.

11. Hu H, Huang J, Shu HB, Baichwal V, Goeddel DV. TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. Immunity. 1996;4(4):387–396.

12. Ting AT, Pimentel-Muñoz FX, Seed B. RIP mediates tumor necrosis factor receptor 1 activation of NF-kappaB but not Fas/APO-1-initiated apoptosis. EMBO J. 1996;15(22):6189–6196.

13. Kelliler MA, Grimm S, Ishida Y, Kuo F, Stanger BZ, Leder P. The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. Immunity. 1998;8(3):297–303.

14. Cuny GD, Degterev A. RIPK protein kinase family: atypical lives of typical kinases. Semin Cell Dev Biol. 2021;109:96–105.

15. Degterev A, Hitomi J, Germscheid M, et al. Identification of RIP1 kinase as a specific cellular target of necrostatins. Nat Chem Biol. 2008;4(5):313–321.

16. Liu Y, Fan C, Zhang Y, et al. RIP1 kinase activity-dependent roles in embryonic development of Fadd-deficient mice. Cell Death Differ. 2017;24(8):1459–1469.

17. Xie T, Peng W, Liu Y, et al. Structural basis of RIP1 inhibition by necrostatins. Structure. 2013;21(3):493–499.

18. Rebsamen M, Heinz LX, Meylan E, et al. DAI/ZBP1 recruits RIP1 and RIP3 through RIP homotypic interaction motifs to activate NF-kappaB. EMBO Rep. 2009;10(8):916–922.

19. Cusson-Hermance N, Khurana S, Lee TH, Fitzgerald KA, Kelliler MA. RIP1 mediates the TRIF-dependent toll-like receptor 3- and 4-induced NF-kappaB activation but does not contribute to interferon regulatory factor 3 activation. J Biol Chem. 2005;280(44):36560–36566.

20. Rickard JA, O’Donnell JA, Evans JM, et al. RIPK1 regulates RIP3-MLKL-driven systemic inflammation and emergency hematopoiesis. Cell. 2014;157(5):1175–1188.

21. Pazdernik NJ, Donner DB, Goebi MG, Harrington MA. Mouse receptor interacting protein 3 does not contain a caspase-recruiting or a death domain but induces apoptosis and activates NF-kappaB. Mol Cell Biol. 1999;19(10):6500–6508.

22. Sun X, Lee J, Navas T, Baldwin DT, Stewart TA, Dixit VM. RIP3, a novel apoptosis-inducing kinase. J Biol Chem. 1999;274(24):16871–16875.

23. Yu PW, Huang BC, Shen M, et al. Identification of RIP3, a RIP-like kinase that activates apoptosis and NF-kappaB. Curr Biol. 1999;9(10):539–542.
24. Kasof GM, Prosser JC, Liu D, Lorenzi MV, Gomes BC. The RIP-like kinase, RIP3, induces apoptosis and NF-kappaB nuclear translocation and localizes to mitochondria. FEBS Lett. 2000; 473(3):285–291.

25. Khan N, Lawlor KE, Murphy JM, Vince JE. More to life than death: molecular determinants of necroptotic and non-necroptotic RIP3 kinase signaling. Curr Opin Immunol. 2014;26:76–89.

26. Mandal P, Berger SB, Pillay S, et al. RIP3 induces apoptosis independent of pro necroptotic kinase activity. Mol Cell. 2014; 56(4):481–495.

27. Moriwaki K, Bertin J, Gough PJ, Chan FK. A RIPK3-caspase 8 complex mediates atypical pro-IL-1β processing. J Immunol. 2015;194(4):1938–1944.

28. Newton K, Dugger DL, Wickliffe KE, et al. Activity of protein kinase RIP3K determines whether cells die by necroptosis or apoptosis. Science. 2014;343(6177):1357–1360.

29. Nogusa S, Thapa RJ, Dillon CP, et al. RIPK3 activates parallel pathways of MLKL-driven necroptosis and FADD-mediated apoptosis to protect against influenza A virus. Cell Host Microbe. 2016;20(1):13–24.

30. Li D, Chen J, Guo J, et al. A phosphorylation of RIPK3 kinase promotes progranulin-induced corpus luteum regression. Elife. 2021;10:e67409.

31. Kaiser WJ, Sridharan H, Huang C, et al. Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL. J Biol Chem. 2013;288(43):31268–31279.

32. Upton JW, Kaiser WJ, Mocarski ES. DAI/ZBP1/DLM-1 complexes with RIP3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vIRA. Cell Host Microbe. 2012;11(3):290–297.

33. Sarhan J, Liu BC, Muenklein HI, et al. Constitutive interferon signaling maintains critical threshold of MLKL expression to license necroptosis. Cell Death Differ. 2019;26(2):332–347.

34. Chen D, Tong J, Yang L, et al. PUMA amplifies necroptosis signaling by activating cytosolic DNA sensors. Proc Natl Acad Sci U S A. 2018;115(15):3930–3935.

35. He S, Liang Y, Shao F, Wang X. Toll-like receptors activate apoptosis to protect against influenza A virus. Cereb Cortex. 2017;27(5):2005–2009.

36. Cai Z, Liu ZG. Execution of RIPK3-regulated necrosis. Mol Cell Oncol. 2014;4(12):e960759.

37. Dillon CP, Oberst A, Weinlich R, et al. Survival function of the FADD-CASPASE-8-cFLIP(L) complex. Cell Rep. 2012;1(5):401–407.

38. Chen D, Yu J, Zhang L. Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. Immunity. 2013;38(2):209–223.

39. Inohara N, del Peso L, Koseki T, Chen S, Nunez G, RICK, a novel protein kinase containing a caspase recruitment domain, interacts with CLARP and regulates CD95-mediated apoptosis. J Biol Chem. 1998;273(20):12296–12300.

40. McCarthy JV, Ni J, Dixit VM. RIP2 is a novel NF-kappaB-activating and cell death-inducing kinase. J Biol Chem. 1998;273(27):16968–16975.

41. Sollierger G, Strittmatter GE, Garstiekwicz M, Sand J, Beer HD. Caspase-1: the inflammasome and beyond. Innate Immun. 2014;20(2):115–125.

42. Mukherjee T, Hovingh ES, Foerster EG, Abdel Nour M, Philipp DJ, Girardin SE. NOD1 and NOD2 in inflammation, immunity and disease. Arch Biochem Biophys. 2019;670:69–81.

43. Gong Q, Long Z, Zhong FL, et al. Structural basis of RIP2 activation and signaling. Nat Commun. 2018;9(1):4993.

44. He S, Wang X. RIP kinases as modulators of inflammation and immunity. Nat Immunol. 2018;19(9):912–922.

45. Inohara N, Koseki T, Chen S, et al. An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways. J Biol Chem. 2000;275(36):27823–27831.

46. Meylan E, Martinon F, Thome M, Gschwendt M, Tschopp J. DIK, a novel protein kinase that interacts with MLKL, regulates necroptosis signaling downstream of RIP3. Proc Natl Acad Sci U S A. 2011;108(50):20054–20059.

47. Bahr C, Rohwer A, Stempka L, Rincke G, Marks F, Gschwendt M. DIK, a novel protein kinase that interacts with protein kinase Cdelta. Cloning, characterization, and gene analysis. J Biol Chem. 2000;275(46):36350–36357.

48. Xu J, Wei Q, He Z. Insight into the function of RIPK4 in keratinocyte differentiation and cutaneous inflammation. Cell Death Differ. 2008;314(10):2055–2066.

49. Kaczmarek A, Vandenabeele P, Krysko DV. Necroptosis: a regulated inflammatory mode of cell death. J Neuroinflammation. 2018;15(1):199.

50. Shin N, Jeong H, Kwon J, et al. LRRK2 regulates synaptic vesicle endocytosis. Exp Cell Res. 2008;314(10):2055–2065.
Role of Receptor Interacting Protein 1591

67. Kett LR, Dauer WT. Leucine-rich repeat kinase 2 for begin-
ners: six key questions. Cold Spring Harb Perspect Med. 2012;
2(3):a009407.

68. Haugarvoll K, Toft M, Ross OA, White LR, Aasly JO, Farrer MJ.
Variants in the LRRK1 gene and susceptibility to Parkinson’s
disease in Norway. Neurosci Lett. 2007;416(3):299–301.

69. Zimprich A, Biskup S, Leitner P, et al. Mutations in LRRK2
cause autosomal-dominant parkinsonism with pleomorphic
pathology. Neuron. 2004;44(4):601–607.

70. Schulte EC, Ellwanger DC, Dihanich S, et al. Rare variants in
LRRK1 and Parkinson’s disease. Neurogenetics. 2014;15(1):
49–57.

71. Berwick DC, Heaton GR, Azeggagh S, Harvey K. LRRK2 Biology
from structure to dysfunction: research progresses, but the
themes remain the same. Mol Neurodegener. 2019;14(1):49.

72. Zhu G, Ye J, Huang Y, et al. Receptor-interacting protein-1
promotes the growth and invasion in gastric cancer. Int J Oncol.
2016;48(6):2387–2398.

73. McCormick KD, Ghosh A, Trivedi S, et al. Innate immune
signaling through differential RIP1 expression promote
tumor progression in head and neck squamous cell carcinoma.
Carcinogenesis. 2016;37(5):522–529.

74. Ying Z, Chen W, Yin J, et al. RIPK1 is a negative mediator in
Aquaporin 1-driven triple-negative breast cancer progression
and metastasis. NPJ Breast Cancer. 2021;7(1):53.

75. Zhang Y, Du J, Duan X, et al. RIPK1 contributes to cisplatin-
induced apoptosis of esophageal squamous cell carcinoma cells
via activation of JNK pathway. Life Sci. 2021;269:119064.

76. Brown MF, Leibowitz BJ, Chen D, et al. Loss of caspase-3
sensitizes colon cancer cells to genotoxic stress via RIP1-
dependent necrosis. Cell Death Dis. 2015;6(6):e1729.

77. Park S, Hatanpaaj KJ, Xie Y, et al. The receptor interacting
protein 1 inhibits p53 induction through NF-κappaB activation
and confers a worse prognosis in glioblastoma. Cancer Res.
2009;69(7):2809–2816.

78. Wang Q, Chen W, Xu X, et al. RIP1 potentiates BPDE-induced
transformation in human bronchial epithelial cells through
catalase-mediated suppression of excessive reactive oxygen
species. Carcinogenesis. 2013;34(9):2119–2128.

79. Liu XY, Lai F, Yan XG, et al. RIP1 kinase is an oncogenic driver
in melanoma. Cancer Res. 2015;75(8):1736–1748.

80. Zhu G, Chen X, Wang X, et al. Expression of the RIP1 gene and
its role in growth and invasion of human gallbladder carci-
noma. Cell Physiol Biochem. 2014;34(4):1152–1165.

81. Seifert L, Werba G, Tiwari S, et al. The necrosome promotes
pancreatic oncogenesis via CXCL1 and Mincle-induced immu-
ne suppression. Nature. 2016;532(7598):245–249.

82. Park S, Zhao D, Hatanpaaj KJ, et al. RIP1 activates PI3K-Akt via
a dual mechanism involving NF-kappaB-mediated inhibition of
the mTOR-S6K-IRS1 negative feedback loop and down-
regulation of PTEN. Cancer Res. 2009;69(10):4107–4111.

83. Patel S, Webster JD, Varfolomeev E, et al. RIP1 inhibition
blocks inflammatory diseases but not tumor growth or me-
tastases. Cell Death Differ. 2020;27(1):161–175.

84. Zheng XL, Yang JJ, Wang YY, et al. RIP1 promotes prolifera-
tion through G2/M checkpoint progression and mediates
cisplatin-induced apoptosis and necroptosis in human ovarian
cancer cells. Acta Pharmacol Sin. 2020;41(9):1223–1233.

85. Wang X, Jiang W, Duan N, et al. NOD1, RIP2 and Caspase12 are
potentially novel biomarkers for oral squamous cell carcino-
moma development and progression. Int J Clin Exp Pathol.
2014;7(4):1677–1686.

86. Zhang H, Chin AI. Role of Rip2 in development of tumor-
infiltrating MDSCs and bladder cancer metastasis. PloS One.
2014;9(4):e94793.

87. Singel SM, Batten K, Cornelius C, et al. Receptor-interacting
protein kinase 2 promotes triple-negative breast cancer cell
migration and invasion via activation of nuclear factor-kappaB
and c-Jun N-terminal kinase pathways. Breast Cancer Res.
2014;16(2):R28.

88. Jaafar RF, Ibrahim Z, Ataya K, Hassanieh J, Ard N, Faraj W.
Receptor-interacting serine/threonine-protein kinase-2 as a
potential prognostic factor in colorectal cancer. Medicina.
2021;57(7):709.

89. Li D, Tang L, Liu B, Xu S, Jin M, Bo W. RIPK2 is an unfavorable
prognosis marker and a potential therapeutic target in human
kidney renal clear cell carcinoma. Aging. 2021;13(7):
10450–10467.

90. Chen Y, Chen Y, Zhang J, et al. Fusobacterium nucleatum
promotes metastasis in colorectal cancer by activating auto-
phagy signaling via the upregulation of CARD3 expression.
Theranostics. 2020;10(11):323–339.

91. Yang Q, Tian S, Liu Z, Dong W. Knockdown of RIPK2 inhibits
proliferation and migration, and induces apoptosis via the NF-
κB signaling pathway in gastric cancer. Front Genet. 2021;12:
627464.

92. Udden SM, Peng L, Gan JL, et al. NOD2 suppresses colorectal
tumorigenesis via downregulation of the TLR pathways. Cell
Rep. 2017;19(13):2756–2770.

93. Couturier-Maillard A, Secher T, Rehanan A, et al. NOD2-
d Mediated dysbiosis predisposes mice to transmissible colitis
and colorectal cancer. J Clin Invest. 2013;123(2):700–711.

94. Seifert L, Miller G. Molecular pathways: the necroso-A target
for cancer therapy. Clin Cancer Res. 2017;23(5):1132–1136.

95. Wang KJ, Wang KY, Zhang HZ, et al. Up-regulation of RIP3
alleviates prostate cancer progression by activation of
RIP3/MLKL signaling pathway and induction of necroptosis.
Front Oncol. 2020;10:1720.

96. Koo GB, Morgan MJ, Lee DG, et al. Methylation-dependent
loss of RIP3 expression in cancer represses programmed necro-
osis in response to chemotherapeutics. Cell Res. 2015;
25(6):707–725.

97. Mariwaki K, Bertin J, Gough PJ, Orlowski GM, Chan FK.
Different roles of RIPK1 and RIPK3 in TNF-induced necroptosis
and chemotherapeutic agent-induced cell death. Cell Death
Dis. 2015;6(2):e1636.

98. Nguies AL, El Bouazati H, Metuin D, et al. RIP3 is down-
regulated in human myeloid leukemia cells and modulates
apoptosis and caspase-mediated p65/RelA cleavage. Cell
Death Dis. 2014;5(8):e1384.

99. Tan Y, Sementino E, Cheung M, et al. Somatic epigenetic
silencing of RIPK3 inactivates necroptosis and contributes to
chemo-resistance in malignant mesothelioma. Clin Epigenetic
Res. 2021;27(4):1200–1213.

100. Wang Q, Wang P, Zhang L, et al. Epigenetic regulation of RIP3
suppresses necroptosis and increases resistance to chemo-
therapy in nonsmall cell lung cancer. Transl Oncol. 2020;
13(2):372–382.

101. Won KY, Min SY, Song JY, Lim SJ, Han SA. Clinical significance
of receptor-interacting protein 3 and Parkin, essential mole-

cules for necroptosis, in breast cancer. J Breast Cancer.
2015;6(4):e1729.

102. Conev NV, Dimitrova EG, Bogdanova MK, et al. RIPK3 Death
Dis. 2013;6(9):e1884.

103. Geserick P, Wang J, Schilling R, et al. Absence of RIPK3 pre-
dicts necroptosis resistance in malignant melanoma. Cell
Death Dis. 2015;6(9):e1864.

104. Feng X, Song Q, Yu A, Tang H, Peng Z, Wang X. Receptor-
interacting protein kinase 3 is a predictor of survival and plays
a tumor suppressive role in colorectal cancer. Neoplasma.
2015;62(4):592–601.

105. Cerhan JR, Ansell SM, Frederiksen ZS, et al. Genetic varia-
tion in 1253 immune and inflammation genes and risk of non-
Hodgkin lymphoma. Blood. 2007;110(13):4455–4463.
106. Meng MB, Wang HH, Cui YL, et al. Necroptosis in tumorigenesis, activation of anti-tumor immunity, and cancer therapy. Oncotarget. 2016;7(35):57391–57413.

107. Mutirangura A, Pornthanakasem W, Siruranpong V, Supiyaphun P, Voravud N. Loss of heterozygosity on chromosome 14 in nasopharyngeal carcinoma. Int J Cancer. 1998;78(2):153–156.

108. Kopparam J, Chiffelle J, Angelino P, et al. RIP4 inhibits STAT3 signaling to sustain lung adenocarcinoma differentiation. Cell Death Differ. 2017;24(10):1761–1771.

109. Wang X, Zhu W, Zhou Y, Xu W, Wang H. RIPK4 is downregulated in poorly differentiated tongue cancer and is associated with migration/invasion and cisplatin-induced apoptosis. Int J Biol Markers. 2014;29(2):e150–e159.

110. Heim D, Cornils K, Schulze K, et al. Retroviral insertional mutagenesis in telomerase-immortalized hepatocytes identifies RIPK4 as novel tumor suppressor in human hepatocarcinogenesis. Oncogene. 2015;34(3):364–372.

111. Ge Y, He Z, Xiang Y, et al. The identification of key genes in nasopharyngeal carcinoma by bioinformatics analysis of high-throughput data. Mol Biol Rep. 2019;46(3):2829–2840.

112. Gong Y, Luo X, Yang J, Jiang Q, Liu Z. RIPK4 promoted the tumorigenicity of nasopharyngeal carcinoma cells. Biomed Pharmacother. 2018;108:1–6.

113. Azizmohammadi S, Azizmohammadi S, Safari A, et al. High-level expression of RIPK4 and EZH2 contributes to lymph node metastasis and predicts favorable prognosis in patients with cervical cancer. Oncol Rep. 2017;25(4):495–501.

114. Liu JY, Zeng QH, Cao PG, et al. RIPK4 promotes bladder urothelial carcinoma cell aggressiveness by upregulating VEGF-A through the NF-κB pathway. Br J Cancer. 2018;118(12):1617–1627.

115. Qi ZH, Xu HX, Zhang SR, et al. RIPK4/PEBP1 axis promotes pancreatic cancer cell migration and invasion by activating RAF1/MEK/ERK signaling. Int J Oncol. 2018;52(4):1105–1116.

116. Yi H, Su YZ, Lin R, et al. Downregulation of RIPK4 expression inhibits epithelial-mesenchymal transition in ovarian cancer through IL-6. J Immunol Res. 2021;2021:875450.

117. Liu DQ, Li FF, Zhang JB, et al. Increased RIPK4 expression is associated with progression and poor prognosis in cervical squamous cell carcinoma patients. Sci Rep. 2015;5:11955.

118. Kim SW, Oleksyn DW, Rossi RM, et al. Protein kinase C-associated kinase is required for NF-κB signaling and survival in diffuse large B-cell lymphoma cells. Blood. 2008;111(3):1644–1653.

119. Yi Z, Pu Y, Gou R, et al. Silencing of RIPK4 inhibits epithelial-mesenchymal transition by inactivating the Wnt/β-catenin signaling pathway in osteosarcoma. Mol Med Rep. 2020;21(3):1154–1162.

120. Zhong F, Wu Q, Xia G, Liu L, Yu T. RIP6 suppresses tumor cell growth in hepatocellular carcinoma. Clin Lab. 2020;66(9):1851–1858.

121. Fukasawa M, Kimura M, Morita S, et al. Microarray analysis of promoter methylation in lung cancers. J Hum Genet. 2006;51(4):368–374.

122. Jung G, Hernández-Illán E, Moreira L, Balaguer F, Goel A. Epigenetics of colorectal cancer: biomarker and therapeutic potential. Nat Rev Gastroenterol Hepatol. 2020;17(2):111–130.

123. Yang C, Li J, Yu L, et al. Regulation of RIP3 by the transcription factor Sp1 and the epigenetic regulator UHRF1 modulates cancer cell necroptosis. Cell Death Dis. 2017;8(10):e3084.

124. Angel C, Vasiliou K, Valsis AF, et al. RIPK1/RIPK3 promotes vascular permeability to allow tumor cell extravasation independent of its necrototic function. Cell Death Dis. 2017;8(2):e2588.

125. Li Y, Xiong Y, Zhang G, et al. Identification of 5-(2,3-dihydro-1H-indol-5-yl)-7-hydroxy[2,3-d]pyrimidin-4-amine derivatives as a new class of receptor-interacting protein kinase 1 (RIPK1) inhibitors, which showed potent activity in a tumor metastasis model. J Med Chem. 2018;61(24):11398–11414.

126. Strilic B, Yang L, Albarrán-Juárez J, et al. Tumour-cell-induced endothelial cell necroptosis via death receptor 6 promotes metastasis. Nature. 2016;536(7615):215–218.

127. Zhu F, Zhang W, Yang T, He SD. Complex roles of necroptosis in cancer. J Zhejiang Univ Sci B. 2019;20(5):399–413.

128. Kang AR, Cho JH, Lee NG, et al. RIP1 is a novel component of γ-irradiation radiation-induced invasion of non-small cell lung cancer cells. Int J Mol Sci. 2020;21(13):4584.

129. Zhu G, Du Q, Chen X, et al. Receptor-interacting serine/threonine-protein kinase 1 promotes the progression and lymph metastasis of gallbladder cancer. Oncol Rep. 2019;42(6):2435–2449.

130. Li CZ, Jiang XJ, Lin B, et al. RIP1 regulates TNF-α-mediated lymphangiogenesis and lymphatic metastasis in gallbladder cancer by modulating the NF-κB-VEGF-C pathway. Oncotarget. 2018;11:2875–2890.

131. Liu X, Zhou M, Mei L, et al. Key roles of necroptotic factors in promoting tumor growth. Oncotarget. 2016;7(16):22219–22233.

132. Vucur M, Reisinger F, Gautheron J, et al. RIP3 inhibits inflammatory hepatocarcinogenesis but promotes cholestasis by controlling caspase-8- and JNK-dependent compensatory cell proliferation. Cell Rep. 2013;4(4):776–790.

133. Höckendorf U, Yabal M, Herold T, et al. RIPK3 restricts myeloid leukemogenesis by promoting cell death and differentiation of leukemia initiating cells. Cancer Cell. 2016;30(1):75–91.

134. Ruan H, Leibowitz BJ, Zhang L, Yu J. Immunogenic cell death in colon cancer prevention and therapy. Mol Carcinog. 2020;59(7):783–793.

135. Yang H, Zhao H, Zhang Q, et al. A RIPK3-PEC(2) circuit mediates myeloid-derived suppressor cell-potentiated colorectal carcinogenesis. Cancer Res. 2018;78(19):5586–5599.

136. Jing L, Zhai ME, Cui J, et al. CNOT3 contributes to cisplatin resistance in lung cancer through inhibiting RIPK3 expression. Apoptosis. 2019;24(7–8):673–685.

137. Yang H, Ma Y, Chen G, et al. Contribution of RIP3 and MLKL to immunogenic cell death signaling in cancer chemotherapy. Oncoimmunology. 2016;5(6):e1149673.

138. Oliver Metzig M, Fuchs D, Tagscherer KE, Grüne HJ, Schirmacher P, Roth W. Inhibition of caspase primes colon cancer cells for 5-fluorouracil-induced TNF-α-dependent necroptosis driven by RIP1 kinase and NF-κB. Oncogene. 2016;35(26):3399–3409.

139. Tenev T, Bianchi K, Darding M, et al. The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. Mol Cell. 2011;43(3):432–448.

140. Su Z, Yang Z, Xie L, DeWitt JP, Chen Y. Cancer therapy in the necroptosis era. Cell Death Differ. 2016;23(5):748–756.

141. Pietkiewicz S, Eils R, Kramer PH, Giese N, Lavrik IN. Combinatorial treatment of CD95L and ganciclovir in pancreatic cancer cells induces apoptotic and RIP1-mediated necroptotic cell death network. Exp Cell Res. 2015;339(1):1–9.

142. Wang Y, Zheng Y, Hao Y. Rucaparib (Rubraca(®)) induces necrosis via upregulating the expression of RIP1 and RIP3 in ovarian cancer cells. Pharmacie. 2020;75(6):242–245.

143. Zhang Z, Ju F, Chen F, et al. GDC-0326 enhances the effects of 5-Fu in colorectal cancer cells by inducing necrototic death. Onco Targets Ther. 2017;10:2519–2530.

144. Dunai ZA, Imre G, Barna G, et al. Staurosporine induces necrototic cell death under caspase-compromised conditions in U937 cells. PLoS One. 2012;7(7):e41945.
145. Han W, Li L, Qiu S, et al. Shikonin circumvents cancer drug resistance by induction of a necroptotic death. *Mol Cancer Ther.* 2007;6(5):1641–1649.
146. Yu X, Deng Q, Li W, et al. Neoalbaconol induces cell death through necroptosis by regulating RIPK-dependent autocrine TNFα and ROS production. *Oncotarget.* 2015;6(4):1995–2008.
147. Gong Y, Fan Z, Luo G, et al. The role of necroptosis in cancer biology and therapy. *Mol Cancer.* 2019;18(1):100.
148. Sun W, Yu J, Gao H, et al. Inhibition of lung cancer by 2-methoxy-6-acetyl-7-methyljuglone through induction of necroptosis by targeting receptor-interacting protein 1. *Antioxid Redox Signal.* 2019;31(2):93–108.
149. Liu T, Sun X, Cao Z, Sun X. Shikonin-induced necroptosis in nasopharyngeal carcinoma cells via ROS overproduction and upregulation of RIPK1/RIPK3/MLKL expression. *Oncotarget.* 2019;12:2605–2614.
150. Zhao X, Quan J, Tan Y, et al. RIP3 mediates TCN-induced necroptosis through activating mitochondrial metabolism and ROS production in chemotherapy-resistant cancers. *Am J Cancer Res.* 2021;11(3):729–745.
151. Chen C, Xiao W, Huang L, et al. Shikonin induces apoptosis and necroptosis in pancreatic cancer via regulating the expression of RIP1/RIP3 and synergizes the activity of gemcitabine. *Am J Transl Res.* 2017;9(12):5507–5517.
152. Xuan Y, Hu X. Naturally-occurring shikonin analogues-a class of necroptotic inducers that circumvent cancer drug resistance. *Cancer Lett.* 2009;274(2):233–242.
153. Whilding LM, Archibald KM, Kulbe H, Balkwill FR, Öberg D, McNeish IA. Vaccinia virus induces programmed necrosis in ovarian cancer cells. *Mol Ther.* 2013;21(11):2074–2086.
154. Wu Y, Dong G, Sheng C. Targeting necroptosis in anticancer therapy: mechanisms and modulators. *Acta Pharm Sin B.* 2020;10(9):1601–1618.
155. Harris PA, Marinis JM, Lich JD, et al. Identification of a RIP1 kinase inhibitor clinical candidate (GSK3145095) for the treatment of pancreatic cancer. *ACS Med Chem Lett.* 2019;10(6):857–862.
156. Hou J, Ju J, Zhang Z, et al. Discovery of potent necroptosis inhibitors targeting RIPK1 kinase activity for the treatment of inflammatory disorder and cancer metastasis. *Cell Death Dis.* 2019;10(7):493.
157. Li JX, Feng JM, Wang Y, et al. The B-Raf(V600E) inhibitor dabrafenib selectively inhibits RIP3 and alleviates acetaminophen-induced liver injury. *Cell Death Dis.* 2014;5(6):e1278.
158. Yatim N, Jusforgues-Saklani H, Orozco S, et al. RIPK1 and NF-κB signaling in dying cells determines cross-priming of CD8+ T cells. *Science.* 2015;350(6258):328–334.
159. Smith HG, Jamal K, Dayal JH, et al. RIPK1-mediated immunogenic cell death promotes anti-tumour immunity against soft-tissue sarcoma. *EMBO Mol Med.* 2020;12(6):e10979.
160. Kang YJ, Bang BR, Han KH, et al. Regulation of NKT cell-mediated immune responses to tumours and liver inflammation by mitochondrial PGAM5-Drp1 signalling. *Nat Commun.* 2015;6:8371.
161. Snyder AG, Hubbard NW, Messmer MN, et al. Intratumoral activation of the necroptotic pathway components RIPK1 and RIPK3 potentiates antitumor immunity. *Sci Immunol.* 2019;4(36):eaaw2004.
162. Wang W, Marinis JM, Beal AM, et al. RIPK1 kinase drives macrophage-mediated adaptive immune tolerance in pancreatic cancer. *Cancer Cell.* 2018;34(5):757–774.
163. Jayakumar A, Bothwell ALM. RIPK3-induced inflammation by I-MDSCs promotes intestinal tumors. *Cancer Res.* 2019;79(7):1587–1599.