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Review Article

Ferroptosis, necroptosis, and pyroptosis in cancer: Crucial cell death types in radiotherapy and post-radiotherapy immune activation

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ABSTRACT

Tumor cell death and antitumor immune activation induced by radiotherapy are extensively well-studied. While radiotherapy is believed to mainly induce tumor cell necrosis and apoptosis, recent studies have shown that it can also induce ferroptosis, necroptosis, and pyroptosis in tumor cells. However, studies on the role of ferroptosis, necroptosis, and pyroptosis in radiotherapy and post-radiotherapy immune activation are limited. In this review, we summarize the comprehensive literature on the molecular mechanisms and more recent research progress related to radiotherapy-induced ferroptosis, necroptosis, and pyroptosis in tumor cells. Further, we discuss the role of tumor cells undergoing these types of cell death in immune activation after radiotherapy. In addition, we highlight some unresolved questions on the association of radiotherapy with ferroptosis, necroptosis, and pyroptosis. This review can improve our current understanding of the relationship between radiotherapy and different cell death pathways and provide a theoretical framework to improve the therapeutic effect of tumor radiotherapy in the future.

Radiotherapy is a modality that utilizes ionizing radiation to mediate tumor cell death [1]. Radiotherapy-mediated tumor cell death can be classified into accidental cell death (ACD) or regulated cell death (RCD) [2,3]. ACD is an uncontrolled passive death process, whereas RCD is a controlled death process, which involves tightly structured signaling cascades and molecularly defined effector mechanisms [4,5]. RCD

Abbreviations: ACD, accidental cell death; RCD, regulated cell death; PUFA, polyunsaturated fatty acid; PUFA-PL-OOH, PUFA-phospholipid-peroxidation; GPX4, glutathione peroxidase 4; AIFM2, apoptosis-inducing factor mitochondria-associated protein 2; $CoQH_2$, coenzyme Q; DHODH, dihydroorotate dehydrogenase; GCH1, guanosine triphosphate cyclohydrolase 1; BH_4, tetrahydrobiopterin; 4-HNE, 4-hydroxynonenal; DFO, deferoxamine; ROS, reactive oxygen species; PUFA-PLs, PUFA-phospholipids; ACSL4, acyl-CoA synthetase long-chain family member 4; ATM, ataxia-telangiectasia mutated; USP7, ubiquitin-specific protease 7; H2Bub1, histone H2B on lysine 120; HO-1, heme oxygenase-1; dsDNA, double-stranded DNA; cGAS, cyclic guanosine monophosphate-adenosine monophosphate synthase; AMPK, AMP-activated protein kinase; HMGB1, high-mobility group box-1; DAMPs, damage-associated molecular patterns; TLR4, toll-like receptor 4; DCs, dendritic cells; IFNγ, interferon gamma; ICIs, immune checkpoint inhibitors; RT-MPs, radiated tumor cell-released microparticles; ANGPTL4, angiopoietin-like 4; SOCS2, suppressor of cytokine signaling 2; CTD, C-terminus domain; NTD, N-terminal domain; DFS, disease-free survival; OS, overall survival; RIPK1, receptor-interacting protein kinase-1; RIPK3, receptor-interacting protein kinase-3; MLKL, mixed lineage kinase domain-like pseudokinase; ZBP1, Z-DNA binding protein 1; IL-8, Interleukin-8; STING, stimulator of interferon genes; cGAMP, cyclic guanosine monophosphate-adenosine monophosphate; IRF3, interferon regulatory factor 3; IFNs, interferons; TNF-α, tumor necrosis factor-α; NLRP3, NOD-like receptor family pyrin domain-containing 3; AIM2, absent in melanoma 2; NKs, natural killer cells; LPCAT3, lysophosphatidylcholine acyltransferase 3; ALOXs, lipoxygenase; POR, P450 oxidoreductase; GSH, glutathione; PUFA-PL-OH, PUFA-phospholipid-hydroperoxide; GSSG, GSH disulfide; BH_2, boron dihydride; TNFR1, tumor necrosis factor receptor 1; TRAIL-R, tumor necrosis factor-related apoptosis-inducing ligand receptor; TRADD, TNFR1-associated death domain protein; TRAF2, TNF receptor-associated factor 2; cIAP1/2, cellular inhibitor of apoptosis 1 and 2; FADD, FAS-associated death domain protein; PAMPs, pathogen-associated molecular patterns; TLR3/4, toll-like receptor 3/4; TICAM1, toll-like receptor adaptor molecule 1; NLRP1b, NOD-like receptor family pyrin domain-containing 1B; NLRC4, NOD-like receptor family CARD domain-containing protein 4; GzmB, granzyme B; GzmA, granzyme A; MOMP, mitochondrial outer membrane permeabilization; PPIF, peptidylprolyl isomerase F; PARP1, poly(ADP-ribose) polymerase 1; AIF, apoptosis-inducing factor; MIF, macrophage migration inhibitory factor; DISC, death-inducing signaling complex; ATG, Autophagy; XIAP, X-linked inhibitor of apoptosis; HSPA1A, heat shock protein family A member 1A; PTPC, permeability transition pore complex; RNF146, ring finger protein 146; ADPRHL2, ADP-ribosylhydrolase like 2; CDH1, cadherin 1; CTNNA1, catenin alpha 1; MAP1LC3β, microtubule-associated protein 1 light chain 3β; PIK3C3, phosphatidylinositol 3-kinase catalytic subunit type 3; MAP2Ks, mitogen-activated protein kinase kinases; MPO, myeloperoxidase; PADI4, peptidyl arginine deiminase 4.

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is further classified into apoptosis, ferroptosis, pyroptosis, necroptosis, autophagy-dependent cell death, immunogenic cell death, lysosome-dependent cell death, mitochondrial permeability transition-driven necrosis, mitotic death, parthanatos, entotic cell death, and NETotic cell death (Table 1) [2,4]. Previous studies have shown that apoptosis is the main RCD that is mediated by radiotherapy [6]. An in-depth understanding of the mechanism through which tumor cell death is induced by radiotherapy has revealed that radiotherapy can induce several non-apoptotic RCD types, including ferroptosis, necroptosis, and pyroptosis.

Ferroptosis is a form of iron- and lipotoxicity-dependent cell death in which the mitochondrial cristae shrink or disappear and the mitochondrial membrane ruptures [4]. Necroptosis is a cell death mode that is morphologically similar to necrosis including translucent cytoplasm and swelling organelles [4]. Pyroptosis is a form of lytic programmed cell death morphologically characterized by nuclear condensation, cell swelling, and formation of large bubbles at the plasma membrane that eventually rupture [4]. Owing to the close relationship between antitumor immunity and ferroptosis, necroptosis, and pyroptosis, these processes have recently received increasing research attention [7]. However, the roles of ferroptosis, necroptosis, and pyroptosis have not received sufficient attention in radiotherapy and post-radiotherapy immune activation.

Here, we summarize the main molecular mechanisms of ferroptosis, necroptosis, and pyroptosis, and we discuss the recent research progress of these non-apoptotic death pathways in radiotherapy and post-radiotherapy immune activation. We also highlight the unresolved issues regarding the association between radiotherapy and these cell death pathways.

Ferroptosis

Molecular mechanism underlying ferroptosis

Ferroptosis is mainly regulated by ferroptosis-executing systems and ferroptosis defense systems in the cell (Fig. 1) [8]. In ferroptosis-executing systems, intracellular polyunsaturated fatty acid (PUFA) is catalyzed to form PUFA-phospholipid-peroxidation (PUFA-PL-OOH), resulting in the accumulation of lipid peroxides in cell membranes [9,10]. Moreover, abnormally elevated intracellular iron level can result in the accumulation of lipid peroxides in cell membranes [11–14]. These excessive lipid peroxides damage membrane integrity, thereby inducing cell ferroptosis [13]. Intracellular ferroptosis defense systems include both glutathione peroxidase 4 (GPX4)-dependent and independent systems [15]. Furthermore, GPX4-independent defense systems include three signal axes: 1) the apoptosis-inducing factor mitochondria-associated protein 2 (AIFM2, also known as FSP1)-coenzyme Q (CoQH2) system; 2) the dihydroorotate dehydrogenase (DHODH)-CoQH2 system; and 3) the guanosine triphosphate cyclohydrolase 1 (GCH1)-tetrahydrobiopterin (BH4) system [8,16–19]. The ferroptosis defense systems can detoxify and maintain lipid peroxides at non-toxic levels, thus preventing cell ferroptosis [8].

Ferroptosis and radiotherapy

Since Lang et al. first reported that radiotherapy can induce ferroptosis in tumor cells, an increasing number of studies have confirmed this phenomenon [20–22]. Multiple ferroptosis-associated morphological characteristics are observed in tumor cells killed by radiotherapy, including those of lung, breast, esophageal, ovarian, renal cell carcinoma, fibrosarcoma, and melanoma [8]. Moreover, tumor cells killed by radiotherapy exhibit molecular characteristics associated with ferroptosis, such as the upregulation of tumor intracellular lipid peroxidation marker levels (such as malondialdehyde and 4-hydroxynonenal (4-HNE) and ferroptosis marker genes (such as PTGS2 and ACSL4) [21,23,24]. Likewise, ferroptosis inhibitors or the iron chelator deferoxamine (DFO) can alleviate radiation-induced damage to tumor cell lines and restore cell survival, further confirming the occurrence of ferroptosis in tumor cells induced by radiotherapy [21]. Current studies have shown that the occurrence of ferroptosis in tumor cells induced by radiotherapy is related to the abnormal accumulation of intracellular lipid peroxidation products and abnormal function of ferroptosis defense systems (Fig. 2).

Radiotherapy can upregulate intracellular reactive oxygen species (ROS) levels and promote the accumulation of lipid peroxidation. Radiation machines emit X-ray that are transported into cells in the form of waves, resulting in the production of intracellular ROS [25]. Likewise, radiotherapy can alter mitochondrial permeability, affecting mitochondrial electron transport chain activity and mitochondrial antioxidant enzyme functions, resulting in the production of large amounts of ROS in tumor cell mitochondria [21]. Intracellular ROS extract electrons from PUFA to form PUFA radicals, which then interact with oxygen molecules to produce lipid peroxyl radicals. Lipid peroxyl radicals extract H• from other molecules via the Fenton reaction to generate lipid peroxidation and trigger tumor cell ferroptosis [26]. In addition to directly increasing ROS levels in tumor cells, radiotherapy was found to indirectly promote intracellular PUFA-phospholipids (PUFA-PLs) biosynthesis to induce tumor cell ferroptosis [21]. Guang Lei et al. reported that irradiation upregulated acyl-CoA synthetase long-chain family member 4 (ACSL4) expression to promote intracellular PUFA-PLs biosynthesis, and irradiation induced lipid peroxidation was almost completely abolished in the ACSL4 knockout cells [21]. Radiotherapy can induce tumor cell ferroptosis by activating ataxia-telangiectasia mutated (ATM) kinase [20]. ATM is a serine/threonine protein kinase involved in the DNA double-strand break repair [27]. Radiotherapy can upregulate intracellular ATM expression to repress SLC7A11 expression, which downregulates cystine uptake and promotes tumor cell ferroptosis [20,28]. ATM can also decrease the nuclear translocation of metal-regulatory transcription factor 1 to increase the labile iron pool in tumor cells, resulting in ferroptosis [29]. Additionally, radiotherapy-mediated p53 activation influences tumor cell ferroptosis post radiotherapy [30]. Upregulation of the p53 protein can inhibit the GPX4-dependent system by decreasing SLC7A11 protein expression, leading to tumor cell ferroptosis [31,32]. Researchers have found that radiotherapy-activated p53 can decrease SLC7A11 expression to repress GPX4 synthesis, promoting the accumulation of intracellular lipid peroxidation products and inducing tumor cell ferroptosis. This is attributed to the binding of the p53 protein to the p53-binding sequence in the region of the SLC7A11 gene, leading to SLC7A11 transcriptional suppression [31,32]. Moreover, p53 promotes the nuclear translocation of deubiquitinase ubiquitin-specific protease 7 (USP7) to negatively regulate histone H2B on lysine 120 (H2Bub1) levels and decrease the H2Bub1 occupancy of the SLC7A11 regulatory region, thereby repressing SLC7A11 expression in tumor cells [33]. However, no study has reported that activated p53 can repress SLC7A11 expression by promoting the nuclear translocation of USP7 in tumor cells after radiotherapy. Radiotherapy also promotes iron release from heme oxygenase-1 (HO-1) or ferritin, resulting in tumor cell ferroptosis [8]. Recently, Shen et al. reported radiotherapy generated double-stranded DNA (dsDNA) to activate cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) signaling, downregulating SLC7A11 and GPX4 expression and promoting tumor cell ferroptosis [34]. Activation of the AMP-activated protein kinase (AMPK) signaling pathway is potentially linked to tumor cell ferroptosis after radiotherapy. AMPK is phosphorylated upon radiation exposure, and its activation can further

Table 1
Summary of major regulated cell death processes.

Type	Characteristics	Critical molecules	radiotherapy	immunotherapy
Intrinsic apoptosis	Type of regulated cell death (RCD) initiated by perturbations in the extracellular or intracellular microenvironment, demarcated by mitochondrial outer membrane permeabilization (MOMP), and precipitated by executioner caspases	Bcl-2, Bax, Bok, Bak, caspase protein family members	√	√
Extrinsic apoptosis	Specific variant of RCD initiated by perturbations in the extracellular microenvironment detected by plasma membrane receptors, propagated by caspase-8, and precipitated by executioner caspases	CD95, caspase protein family members, death-inducing signaling complex (DISC), Bid	√	√
Ferroptosis	A form of RCD initiated by oxidative perturbations in the intracellular microenvironment that is under constitutive control of glutathione peroxidase 4 (GPX4) and can be inhibited by iron chelators and lipophilic antioxidants	GPX4, system X_c^-, acyl-CoA synthetase long-chain family member 4 (ACSL4), arachidonate lipoxygenases (ALOXs)	√	√
Pyroptosis	A type of RCD that critically depends on the formation of plasma membrane pores by members of the gasdermin protein family, often as a consequence of inflammatory caspase activation	Caspases, GSDM protein family members	√	√
Necroptosis	A modality of RCD triggered by perturbations of extracellular or intracellular homeostasis that critically depends on mixed lineage kinase domain-like pseudokinase (MLKL), receptor-interacting serine/threonine kinase 3 (RIPK3), and (at least in some settings) the kinase activity of receptor-interacting serine/threonine kinase 1 (RIPK1)	MLKL, RIPK1, RIPK3	√	√
Autophagy-dependent cell death	A form of RCD that mechanistically depends on the autophagic machinery (or components thereof)	Autophagy (ATG)-related proteins	√	
Immunogenic cell death	A form of RCD that is sufficient to activate an adaptive immune response in immunocompetent hosts	Damage-associated molecular patterns (DAMPs)	√	√
Lysosome-dependent cell death	A type of RCD demarcated by primary lysosomal membrane permeabilization and precipitated by cathepsins, with optional involvement of MOMP and caspases	Bax, Bid, Bcl2, X-linked inhibitor of apoptosis (XIAP), heat shock protein family A member 1A (HSPA1A)		
Mitochondrial permeability transition-driven necrosis	Specific form of RCD triggered by perturbations in the intracellular microenvironment and relying on peptidylprolyl isomerase F (PPIF)	Permeability transition pore complex (PTPC), PPIF		
Parthanatos	A modality of RCD initiated by poly (ADP-ribose) polymerase 1 (PARP1) hyperactivation and precipitated by the consequent bioenergetic catastrophe coupled to apoptosis-inducing factor (AIF)-dependent and macrophage migration inhibitory factor (MIF)-dependent DNA degradation	PARP1, AIF, ring finger protein 146 (RNF146), ADP-ribosylhydrolase like 2 (ADPRHL2), MIF		
Entotic cell death	A type of RCD that originates from actomyosin-dependent cell-in-cell internalization (entosis) and is executed by lysosomes	cadherin 1 (CDH1), catenin alpha 1 (CTNNA1), microtubule-associated protein 1 light chain 3β (MAP1LC3β), ATG5, ATG7, phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3)		
Mitotic death	Specific variant of RCD (most often, intrinsic apoptosis) driven by mitotic catastrophe	p53, caspase-2, BCL2	√	
NETotic cell death	A ROS-dependent modality of RCD restricted to cells of hematopoietic derivation and associated with NET extrusion	NADPH, mitogen-activated protein kinase kinases (MAP2Ks), Raf-1 proto-oncogene, serine/threonine kinase, myeloperoxidase (MPO), peptidyl arginine deiminase 4 (PADI4), elastase, neutrophils		

activate beclin-1 (an autophagy protein) to form the beclin-1-SLC7A11 complex, subsequently inhibiting system Xc^- activity to trigger tumor cell ferroptosis [8]. However, no studies have directly demonstrated that activated AMPK, post radiotherapy, can induce ferroptosis in tumor cells.

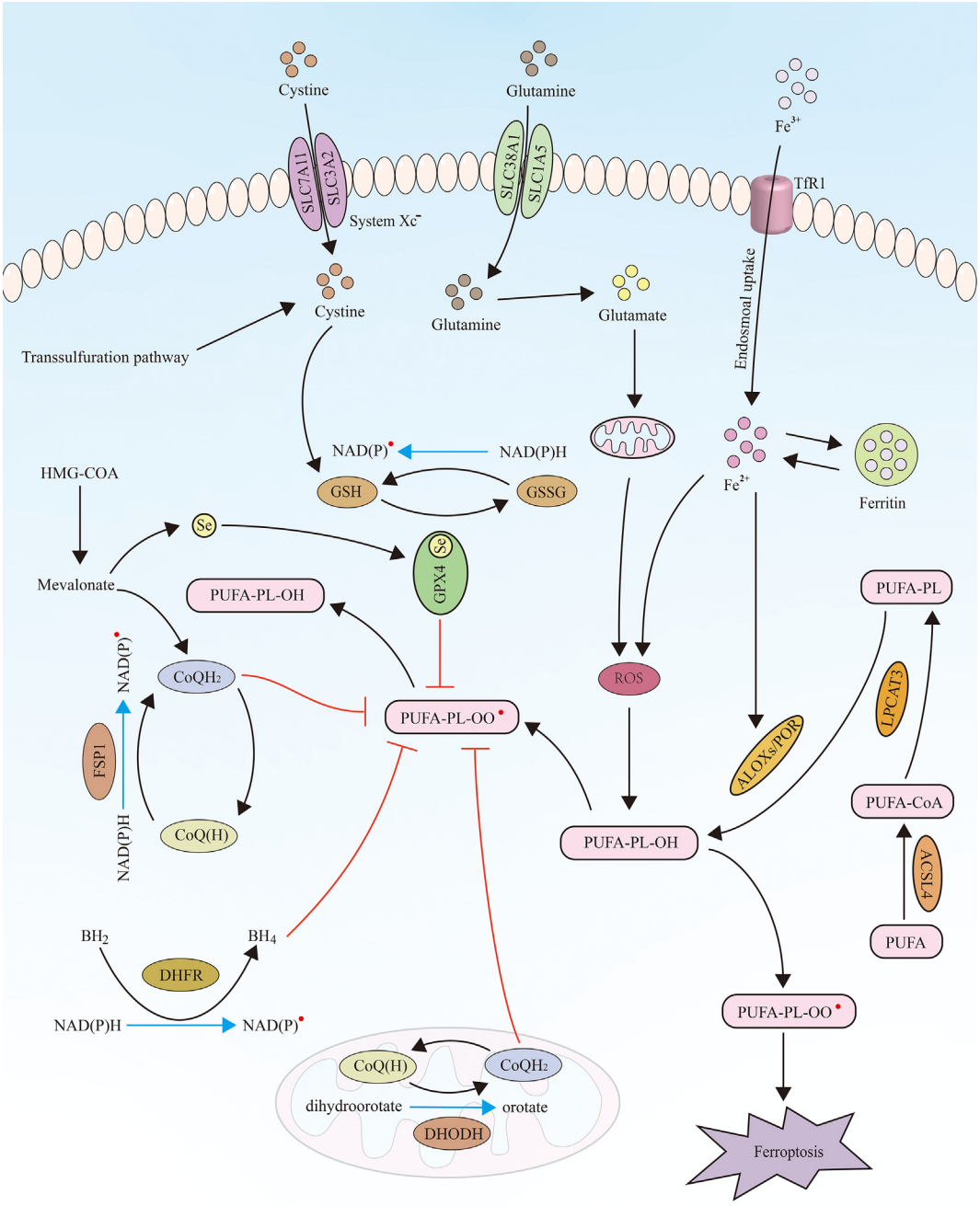
Role of ferroptosis in post-radiotherapy immune activation

Radiotherapy can inhibit tumor development by inducing ferroptosis in tumor cells [21]. However, the role of ferroptotic tumor cells in post-radiotherapy immune activation remains to be characterized. Indirect evidence suggests that ferroptotic tumor cells induced by radiotherapy can activate an antitumor immune response. High-mobility group box-1 (HMGB1), a ferroptosis-related damage-associated molecular patterns (DAMPs) released by ferroptotic tumor cells, can bind to Toll-like receptor 4 (TLR4) on dendritic cells (DCs) and enhance antitumor immunity [7]. Efimova et al. have shown that mice inoculated with ferroptotic tumor cells show the phenotypic maturation of bone marrow-derived DCs [35]. DCs is generally considered as the most impor-

tant antigen-presenting cells, which is able to uptake, process, and present antigens to activate $CD8^+$ T cell [36]. In addition, activated $CD8^+$ T cells release interferon gamma (IFNγ) to downregulate the expression of system Xc^-, promoting tumor cell lipid peroxidation and ferroptosis [34,37]. Thus, on one hand, radiotherapy can induce ferroptosis in tumor cells and activate anti-tumor immunity; on the other hand, the anti-tumor immune system activated by radiotherapy can further induce ferroptosis in tumor cells and inhibit tumor development. Some pharmacological agents capable of inducing tumor cell ferroptosis, such as immune checkpoint inhibitors (ICIs), cisplatin, and sorafenib, combined with radiotherapy, can significantly increase the number of immune cells in tumor tissues compared to that with drug treatment alone [20,38]. In addition, Wan et al. showed that injection of irradiated tumor cell-released microparticles (RT-MPs) into tumor tissue induced ferroptosis in tumor cells and increased $CD4^+$ and $CD8^+$ T cell infiltration, while combined treatment with RT-MPs and ICI delayed tumor cell growth and prolonged mouse survival [39]. In conclusion, ferroptotic tumor cells could be an important factor in immune activation following radiotherapy; however, further studies are needed to validate this aspect.

Use of ferroptosis inducers with radiation in preclinical experiments

Tumor cell radioresistance is the main cause of radiotherapy failure. Apoptotic resistance is an important cause of radioresistance in most tumors [40]. However, recent studies have shown that radiotherapy resistance in tumor cells is also associated with



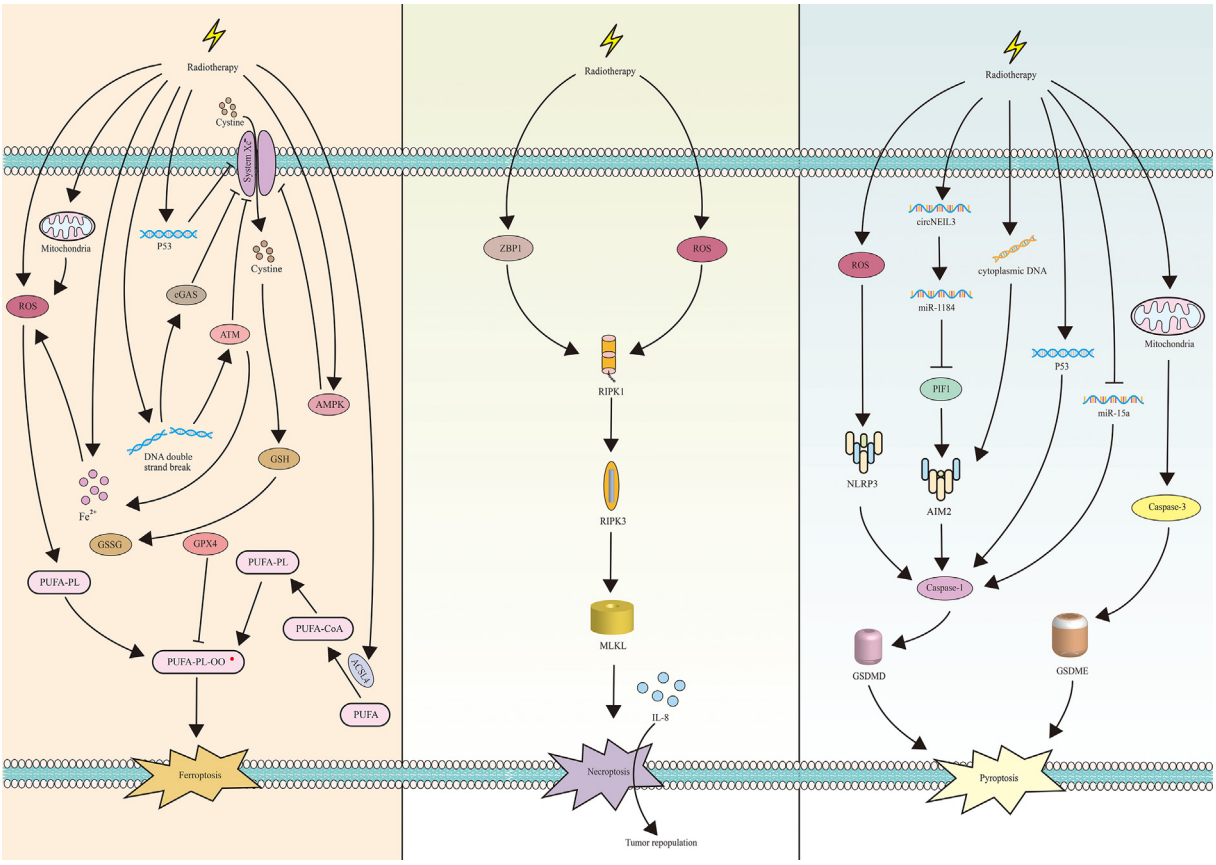


Fig. 2. Known and potential mechanisms of radiation-associated ferroptosis, necroptosis, and pyroptosis. Ferroptosis: mechanisms of ferroptosis induced by irradiation. Irradiation can promote the synthesis of PUFA, induce intracellular ROS production, increase the level of iron, and inhibit GPX4-dependent defense system to induce tumor cells ferroptosis. Necroptosis: mechanisms of necroptosis induced by irradiation. Radiotherapy can upregulate the expression of intracellular Z-DNA binding protein 1 (ZBP1) and increase intracellular mitochondrial ROS levels to induce necroptosis by activating the receptor-interacting protein kinase-1 (RIPK1)/ receptor-interacting protein kinase-3 (RIPK3) signaling pathway. Pyroptosis: mechanisms of necroptosis induced by irradiation. A) Radiotherapy can also upregulate intracellular ROS levels to activate caspase-1, which then activates GSDMD to induce pyroptosis. B) Radiation increases cytoplasmic DNA expression to activate inflammasome, leading to tumor cell pyroptosis. C) radiotherapy can upregulate the p53 protein level to activate caspase-1, inducing pyroptosis. D) High-dose radiotherapy can reduce intracellular miR-15a levels, activate caspase-1 inflammasome, and increase GSDMD, to induce tumor cell pyroptosis. E) Radiotherapy can activate the absent in melanoma 2 (AIM2) inflammasome to induce GSDMD-mediated tumor cell pyroptosis. F) Radiotherapy can activate the caspase-3-mediated pathway to induce pyroptosis.

ferroptosis resistance. Researchers reported that tumor cells can control the abnormal increase in lipid peroxides induced by radiotherapy via the upregulation of the expression of antioxidant and ferroptosis defense system-related proteins, thus suppressing ferroptosis and resulting in radiotherapy resistance [41–44]. Radiotherapy can upregulate the expression of the adiponectin receptor and further promote the protein stability of the transcription factor Nrf2, increasing the transcription and expression of system Xc^- to resist ferroptosis, thus inducing radioresistance in hepatoma [45]. Hypoxia, a prominent feature of tumor microenvi-

Fig. 1. Molecular mechanisms underlying ferroptotic pathway activation. The occurrence of ferroptosis is related to the iron accumulation and lipid peroxidation and an imbalance in the anti-oxidation system. A) Extracellular iron is recognized and taken up by TfR1 on the cell membrane, which is then transformed from Fe^{3+} to Fe^{2+} to upregulate intracellular Fe^{2+} levels. Likewise, intracellular ferritin (consisting of ferritin heavy chain, ferritin light chain, and Fe^{3+}) can be degraded by lysosomes to increase Fe^{2+} levels. The upregulation of Fe^{2+} levels can directly generate excessive reactive oxygen species (ROS) through the Fenton reaction, thereby increasing oxidative damage and inducing ferroptosis. B) Intracellular polyunsaturated fatty acid (PUFA) and CoA react with acyl-CoA synthetase long-chain family member 4 (ACSL4) to form PUFA-CoA and promote its esterification into phospholipids, whereas lysophosphatidylcholine acyltransferase 3 (LPCAT3) catalyzes the biosynthesis of PUFA-CoA and membrane phospholipid to form PUFA-PLs. The lipoxygenase (ALOXs, including ALOXE3, ALOX5, ALOX12, ALOX12B, ALOX15, and ALOX15B) family or cytochrome cytochrome P450 oxidoreductase (POR) catalyzes PUFA-containing phospholipids (PUFA-PLs) peroxidation to produce PUFA-phospholipid-peroxidation (PUFA-PL-OOH), causing ferroptosis. In addition, the upregulation of Fe^{2+} levels can increase the activity of ALOX or POR, which are enzymes responsible for lipid peroxidation and oxygen homeostasis. C) Intracellular anti-oxidation systems include two mechanisms: the glutathione peroxidase 4 (GPX4)-dependent pathway and GPX4-independent pathway. The amino acid antiporter system Xc^- (consisting of two subunits, SLC7A11 and SLC3A2) on the membrane can transport extracellular cystine into the cell to form cysteine for the further synthesis of glutathione (GSH). Under phospholipid hydroperoxidase GPX4 catalytic activity, PUFA-PL-OOH acquires electrons from GSH and forms non-toxic PUFA-phospholipid-hydroperoxide (PUFA-PL-OH), and GSH is converted to GSH disulfide (GSSG). The expression of intracellular GPX4 is controlled by selenium. As GPX4 is synthesized, the sulfur in the cysteine from the nascent GPX4 is replaced by selenium, forming a new GPX4-containing selenium, and this can increase the anti-ferroptosis activity of GPX4. The apoptosis-inducing factor mitochondria-associated protein 2 (AIFM2, also known as FSP1)-coenzyme Q ($CoQH_2$) system signaling axis is a GPX4-independent defense system. CoQ, mainly synthesized through the mevalonate pathway in the mitochondria, consumes NAD(P)H and is converted to its reduced form, $CoQH_2$, which is used as an effective lipophilic antioxidant involved in the recovery of other antioxidants. The guanosine triphosphate cyclohydrolase 1 (GCH1)-tetrahydrobiopterin (BH_4) system is another GPX4-independent defense system. Boron dihydride (BH_2) consumes NAD(P)H to generate BH_4 via dihydrofolate reductase, which is a potent radical-trapping antioxidant that can protect lipid membranes from lipid peroxidation and prevent cell ferroptosis. The dihydroorotate dehydrogenase (DHODH)-$CoQH_2$ system is also a GPX4-independent defense system. When GPX4 is inactivated, DHODH in mitochondrial membrane is significantly increased, and increased DHODH can reduce CoQ to $CoQH_2$ through consuming dihydroorotate to orotate, thereby enhancing $CoQH_2$ generation and preventing ferroptosis.

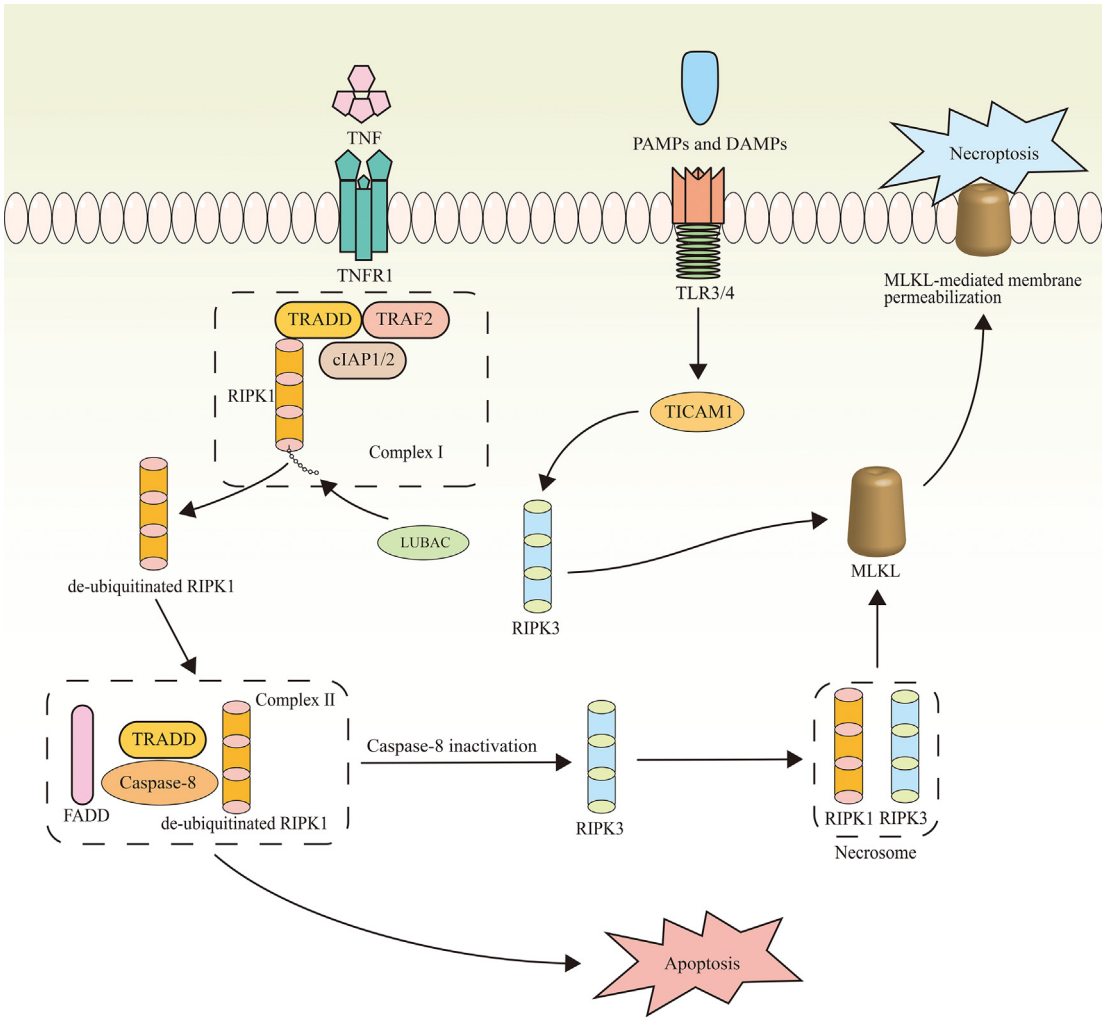


Fig. 3. Molecular mechanisms underlying necroptotic pathway activation. A) The canonical necroptosis pathway is triggered by the activation of death receptors, such as tumor necrosis factor receptor 1 (TNFR1), Fas, and TRAIL-R (tumor necrosis factor-related apoptosis-inducing ligand receptor). TNF signaling is the best characterized cell death cascade, which has been introduced here. Activated TNFR1 recruits TNFR1-associated death domain protein (TRADD), RIPK1, and TNF receptor-associated factor 2 (TRAF2), forming Complex I. Then, RIPK1 is ubiquitinated by cellular inhibitor of apoptosis 1 and 2 (cIAP1/2), and further ubiquitinated by components of the linear ubiquitin chain assembly complex (LUBAC). De-ubiquitinated RIPK1 assembles with TRADD, caspase-8 and FAS-associated death domain protein (FADD) to form Complex II. The Complex II could promote cell apoptosis when caspase-8 is activated. However, following the inhibition of caspase-8 due to pharmaceutical or genetic intervention, the Complex II promotes necroptosis. De-ubiquitinated RIPK1 associated with RIPK3 to form the necrosome, where autophosphorylation and transphosphorylation events can activate RIPK1 and RIPK3. Activated RIPK3 binds to and phosphorylates the pseudokinase mixed lineage kinase domain-like pseudokinase (MLKL), which can translocate to the plasma membrane and form pores to disrupt membrane integrity, resulting in cell death. B) Pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) binding to Toll-like receptor 3/4 (TLR3/4) leads to the subsequent activation of the Toll-like receptor adaptor molecule 1 (TICAM1; best known as TRIF). TICAM1 recruits and activates RIPK3, which subsequently promote the activation of MLKL, inducing necroptosis.

ronment, has long been considered as a critical regulator of radioresistance [46,47]. Zhang et al. recently found that hypoxia can induce the expression of angiopoietin-like 4 (ANGPTL4) protein, which is a decisive regulator of angiogenesis and an inflammatory carcinogenic mediator [46]. ANGPTL4 derived from hypoxic tumor cells was absorbed by neighboring tumor cells, resulting radioresistance of these bystander cells in a GPX4-dependent manner [46]. The suppressor of cytokine signaling 2 (SOCS2) consists of analogous structural components, a conserved C-terminus domain (CTD) including a SOCS-BOX region, a central SH2-structural domain, and an N-terminal domain (NTD) of variable length and sequence, which is a major regulator of cytokine signaling factor [48,49]. Chen et al. reported that SOCS2, as a potential prognosis predictor of radiotherapy, can promote radiosensitivity in tumor cells both *in vivo* and *in vitro* by increasing the ubiquitination degradation of SLC7A11, contributing to ferroptosis and resulting tumor cell radiosensitization [50]. Therefore, for some tumor cells that are resistant to radiotherapy, the activation of ferroptosis-executing systems or the inhibition of ferroptosis defense systems can further enhance ferroptosis and inhibit the development of radiotherapy resistance.

Ferroptosis inducers, such as erastin, FIN56, and sulfasalazine, block the activation of the ferroptosis defense system, increase total intracellular iron content, promote ROS generation, decrease the concentration of glutathione, and increase the levels of lipid peroxidation in radioresistant tumor cells, thereby enhancing tumor cell radiosensitivity by inducing ferroptosis [24,34,43,44,51–54]. Many preclinical studies have confirmed that ferroptosis inducers can enhance the efficacy of tumor radiotherapy; however, clinical trials confirming that the drugs that induce

ferroptosis can enhance the efficacy of tumor radiotherapy are limited. Only a few studies have shown that patients who possess more ferroptotic biomarkers in their tumor tissues generally have better disease-free survival (DFS) and overall survival (OS) [21,24,32,55]. Taken together, ferroptosis inducers combined with radiotherapy could enhance the effect of radiotherapy and mitigate the radioresistance of tumor cells.

Necroptosis

Molecular mechanisms underlying necroptosis

The occurrence of cell necroptosis is associated with the activation of death receptors on the membrane [56]. Activated death receptors can activate the receptor-interacting protein kinase-1 (RIPK1) protein in cells, further recruiting intracellular receptor-interacting protein kinase-3 (RIPK3) to form a complex known as the necrosome, which subsequently activates RIPK3 [57]. Activated RIPK3 can phosphorylate mixed lineage kinase domain-like pseudokinase (MLKL), which is co-trafficked with tight junction proteins to the cell periphery and steadily binds at the plasma membrane to trigger cell necroptosis (Fig. 3) [58].

Necroptosis and radiotherapy

Recent studies have shown that radiotherapy can inhibit tumor development by inducing tumor cell necroptosis. The levels of various necroptosis-associated proteins, particularly MLKL, are significantly upregulated in dead tumor cells post radiotherapy [59,60]. Likewise, pretreatment of tumor cells with necroptosis inhibitors prior to radiotherapy resulted in significantly fewer dead cells compared to direct radiotherapy without pretreatment, confirming that radiotherapy can induce tumor cell necroptosis [59].

However, the mechanism underlying necroptosis induced by radiotherapy remains to be explored (Fig. 2). A recent study showed that tumor cell necroptosis induced by radiotherapy is associated with the upregulation of intracellular Z-DNA binding protein 1 (ZBP1, also known as DAI or DLM-1) expression [61]. ZBP1 is a nucleic acid sensor that mediates host-defense against certain viruses [62]. RNA produced by viruses is detected by intracellular ZBP1 to activate RIPK1 and MLKL, triggering necroptosis [63]. Additionally, Yang et al. reported that radiotherapy can upregulate ZBP1 expression in tumor cells to activate the RIPK1/RIPK3/MLKL signaling pathway, thus inducing tumor cell necroptosis [61]. Furthermore, tumor cell necroptosis induced by radiotherapy might be associated with the upregulation of intracellular mitochondrial ROS levels. Previous studies have shown that increasing mitochondrial ROS levels in tumor cells can induce necroptosis via RIPK1/RIPK3 signaling [64]. However, direct evidence of the association between radiation-induced necroptosis and increased levels of intracellular mitochondrial ROS is lacking.

The role of necroptotic tumor cells in radiotherapy also remains to be discussed. According to recent studies, tumor cell necroptosis in radiotherapy exhibits a dual role in tumor treatment. From an antitumor perspective, radiotherapy can induce tumor cell necroptosis to hinder tumor development [59,65–67]. Likewise, necroptosis inducers can enhance tumor cell radiosensitivity and tumor treatment effects. In a retrospective study, patients who are more susceptible to radiotherapy-induced tumor cell necroptosis had a better OS, whereas bioinformatics analyses showed that tumor patients with a higher necroptosis-related gene expression had a better OS [66,68]. However, studies have also shown that radiation-induced necroptosis in tumor cells can promote tumor development. Kim et al. reported that patients with more necroptotic tumor cells post adjuvant radiotherapy exhibited a shorter OS and DFS [69]. Wang et al. showed that necroptotic tumor cells, induced by radiotherapy, can release Interleukin-8 (IL-8) to promote tumor repopulation, whereas the inhibition of radiotherapy-induced tumor cell necroptosis can decrease IL-8 levels and alleviate tumor repopulation [59]. Therefore, radiation-induced necroptosis in tumor cells might have a growth-stimulating effect on surviving tumor cells [59].

Role of necroptosis in post-radiotherapy immune activation

Recently, the role of necroptotic tumor cells induced by radiotherapy in immune activation has gathered more interest. The levels of IFNγ, tumor necrosis factor-α (TNF-α), and $CD8^+$ T cells were significantly increased in the tumor tissues of mice inoculated with necroptosis-resistant tumor cells after radiotherapy, compared to those in mice inoculated with normal tumor cells [61]. Damaged tumor cell DNA induced by radiation binds to cGAS in the tumor cell cytoplasm to induce the production of cyclic guanosine monophosphate-adenosine monophosphate (cGAMP), which in turn binds immune cell-intrinsic stimulator of interferon genes (STING) to activate the transcription factors interferon regulatory factor 3 (IRF3) and NF-κB, resulting in the production of type I interferons (IFNs) and other cytokines [70,71]. The accumulation of cytoplasmic DNA in irradiated necroptotic tumor cells activates the cGAS-STING pathway to promote inflammation, thereby enhancing radiation-induced antitumor immunity [61]. Moreover, after radiotherapy, the tumor cell-intrinsic ZBP1-MLKL cascade can enhance the cross-priming of DCs to promote $CD8^+$ T cell infiltration, and the ZBP1-MLKL and cGAS-STING pathway crosstalk enhanced and prolonged antitumor immunity [61].

In addition, some studies have potentially suggested a role for necroptosis in antitumor immune activation post radiotherapy. The ectopic introduction of necroptotic tumor cells to the tumor microenvironment can promote the activation of DCs and $CD8^+$ T cells and the production of IFNγ, which can synergize and enhance the effect of immunotherapy to inhibit tumor development [72]. Overall, necroptotic tumor cells after radiotherapy can further promote the tumor therapeutic effect by enhancing antitumor immunity.

Pyroptosis

Molecular mechanisms underlying pyroptosis

The occurrence of cell pyroptosis is tightly related to the gasdermin superfamily of intracellular pore-forming effector proteins, including GSDMA, GSDMB, GSDMC, GSDMD, GSDME, and DFNB59 [73]. In addition to DFNB59, other proteins are composed of two conserved domains, the N-terminal pore-forming domain and the C-terminal repressor domain [74]. In general, intracellular gasdermin superfamily proteins are inactivated because the N-terminal pore-forming domains are masked by the C-terminal repressor domain [74]. During pyroptosis, gasdermin-N pore-forming domains are separated from the gasdermin-C repressor domains [74]. The gasdermin-N pore-forming domain then oligomerizes and forms pores in the cell membrane, disrupting membrane integrity, thereby inducing cell pyroptosis (Fig. 4) [74].

Pyroptosis and radiotherapy

Although researchers have observed that radiotherapy can induce tumor cells to undergo pyroptosis and that tumor cells can resist radiotherapy by targeting NOD-like receptor family pyrin

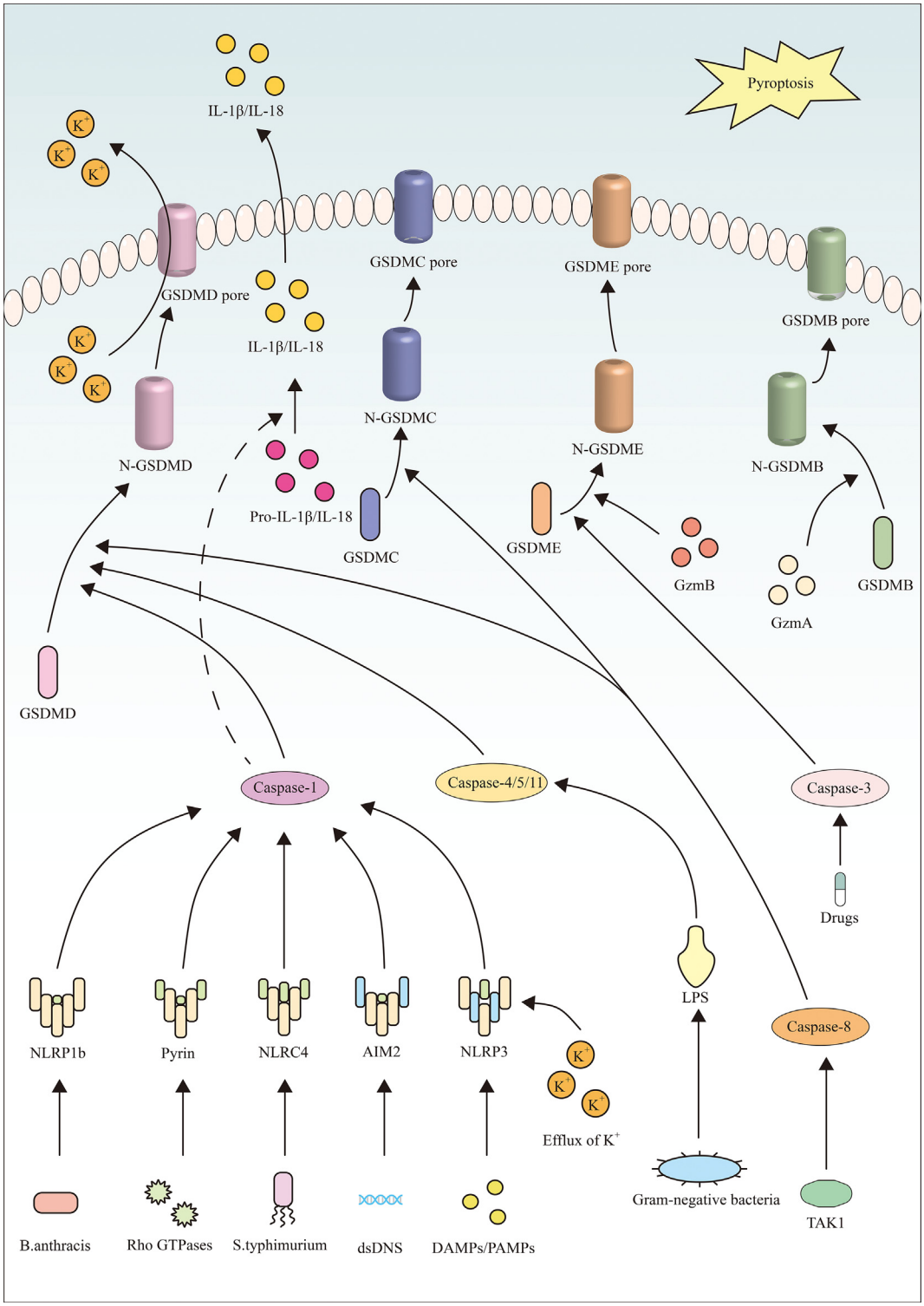


Fig. 4. Molecular mechanisms underlying pyroptotic pathway activation. A) In the canonical pathway, *Bacillus anthracis*, *Salmonella typhimurium*, double-stranded DNA (dsDNA), PAMPs, DAMPs, and toxin-induced modifications to Rho GTPases activate specific inflammasome sensors, including NOD-like receptor family pyrin domain-containing 1B (NLRP1b), NOD-like receptor family pyrin domain-containing 3 (NLRP3), NOD-like receptor family CARD domain-containing protein 4 (NLRC4), AIM2, and pyrin. Activated inflammasome sensors directly or indirectly bind and activate caspase-1, which in turn cleaves the N-terminal end of GSDMD and forms pores on the cell membrane. This results in the release of cytoplasmic content and the induction of cell pyroptosis. Activated caspase-1 also cleaves the precursor cytokines pro-IL-1β and pro-IL-18 to promote the maturation of IL-1β and IL-18. B) In the non-canonical pathway, pyroptosis does not require the activation of non-canonical inflammasomes. Gram-negative bacteria-derived lipopolysaccharide triggers the activation of caspase-4 or caspase-5 in human cells and caspase-11 in mouse cells; the caspases then cleave the N-terminal end of GSDMD to induce pyroptosis. In addition, the cleavage of GSDMD results in efflux of K^+, ultimately mediating the assembly of NLRP3 inflammasome, eventually leading to pyroptosis. C) In the caspase-3-mediated pathway, active caspase-3 cleaves GSDME to form N-GSDME, inducing pyroptosis; meanwhile, in the caspase-8-mediated pathway, inhibiting TAK1 induces the activation of caspase-8, which cleaves GSDMD, resulting in pyroptosis. In addition, caspase-8 can lyse GSDMC to produce N-GSDMC, and form pores on the cell membrane to induce pyroptosis. D) In the granzyme-mediated pathway, chimeric antigen receptor T cells rapidly activate caspase-3 in target cells by releasing granzyme B (GzmB), which activates GSDME causing extensive pyroptosis. In addition, granzyme A (GzmA), derived from natural killer cells and cytotoxic T lymphocytes, kills tumor cells via GSDMB cleavage.

domain-containing 3 (NLRP3)-mediated pyroptosis, studies on the relationship between tumor cell pyroptosis and radiotherapy, particularly those on the mechanism underlying pyroptosis induced by radiotherapy in tumor cells, are limited (Fig. 2) [75,76].

Radiotherapy can downregulate the expression of intracellular circNEIL3, which directly binds to intracellular miR-1184 to downregulate the expression of Pif1 helicase [75]. Downregulation of the expression of Pif1 helicase increases damage to DNA replication of telomeres, ribosomes, and mitochondria in tumors [77,78]. Ultimately, DNA damage can activate the absent in melanoma 2 (AIM2) inflammasome to induce GSDMD-mediated tumor cell pyroptosis [75]. Rana et al. reported that tumor cell pyroptosis-induced by radiotherapy is associated with miR-15a expression [79]. High-dose radiation can decrease intracellular miR-15a levels, activate caspase-1 inflammasome, and increase Gasdermin D, to induce tumor cell pyroptosis.

In addition, there are other potential signaling pathways underlying radiotherapy-induced tumor cell pyroptosis. Radiotherapy can upregulate intracellular p53 protein level, and the overexpression of p53 proteins in tumor cells promotes the expression of caspase-1, which activates the canonical pathway to induce tumor cell pyroptosis, suppressing tumor proliferation and progression [30,80,81]. However, whether radiotherapy can activate tumor cell pyroptosis by upregulating p53 protein remains unknown. Increased intracellular ROS levels are also associated with pyroptosis. Excess ROS cause the oxidation and oligomerization of the mitochondrial outer membrane protein Tom20, which recruits Bax to the mitochondria and facilitates cytochrome c release to the cytosol, activating the caspase-3-mediated pathway of pyroptosis [82]. Intracellular ROS also promote the activation of the NLRP3 inflammasome to induce pyroptosis, whereas a decrease in intracellular ROS levels alleviates pyroptosis by blocking the ROS/NLRP3/caspase-1 pathway [83,84]. In addition, radiation can induce DNA damage in tumor cells, leading to an increase in intracellular cytoplasmic DNA. Previous studies have shown that cytoplasmic DNA can induce cell pyroptosis by binding to AIM2 to activate caspase-1 or trigger the activation of the NLRP3 inflammasome through the cGAS-STING pathway, resulting in cell pyroptosis [85,86]. This signaling pathway has not been observed in tumor cells. Overall, the activation of p53, upregulation of ROS levels, and increase in cytoplasmic DNA post radiotherapy might be the potential activators of pyroptotic tumor cells.

Role of pyroptosis in post-radiotherapy immune activation

A large number of recent studies have reported that radiation-induced pyroptosis of tumor cells is an important participator in the activation of anti-tumor immunity. Gao et al. reported that radiotherapy-induced pyroptotic tumor cells can promote the infiltration of natural killer cells (NKs) in the immune microenvironment and activate antitumor immunity [87]. They constructed a GSDME-KO tumor cell that was resistant to radiation-induced pyroptosis and established a homograft tumor model by subcutaneously injecting mouse colon tumor cells and GSDME-KO tumor cells [87]. They found that after radiotherapy, tumor cells had significantly increased the number of NKs and $CD8^+$ T cells in the tumor microenvironment compared to GSDME-KO tumor cells [87]. They further used the corresponding antibodies to deplete NKs and reported that radiotherapy-induced pyroptotic tumor cells could inhibit tumor development by promoting NKs infiltration [87]. Cao et al. reported that GSDME-mediated pyroptosis post radiotherapy effectively promotes the infiltration of $CD8^+$ T cells by enhancing the antigen presentation of DCs [88]. In addition, pyroptotic tumor cells can leak tumor neoantigens, promote the proliferation and differentiation of immune cells, and induce antitumor immunity [7,89,90]. Pyroptotic tumor cells can increase the infiltration of $CD4^+$, $CD8^+$ T, and NKs in tumor tissue [90–92]. The expression levels of GSDME in tumor tissue are positively associated with the infiltration of macrophages and $CD4^+$ and $CD8^+$ T cells in various tumor types [93]. Conversely, when drugs are used to inhibit pyroptosis or when the expression of pyroptosis-related genes is knocked down in tumors, the levels of cytokines in the tissue post radiotherapy are significantly reduced [94]. Therefore, radiation-induced tumor cell pyroptosis might explain the immune activation post radiotherapy.

Application prospects of ferroptosis-, necroptosis-, and pyroptosis inducers in radiotherapy

Although radiotherapy can limit tumor development and improve the OS of patients, radiotherapy alone usually fails to achieve ideal treatment outcomes [1,95]. Thus, it is often combined with other tumor treatment strategies, such as chemotherapy, immunotherapy, and targeted therapy, to enhance the tumor treatment efficacy [96–98]. Recently, cell death inducers have been used as adjunctive drugs to enhance the efficacy of radiotherapy; of them, ferroptosis inducers are among the most promising [21,24]. Multiple iron metabolism-associated proteins participate in tumor initiation, proliferation, and metastasis; thus, tumor cells exhibit an increased iron demand compared to normal cells and are more likely to undergo ferroptosis [99]. Ferroptosis inducers, combined with radiotherapy, can more easily induce the accumulation of intracellular ROS to overcome the threshold of the antioxidation system and induce tumor cell ferroptosis, and combination therapy is associated with lower toxicity to normal tissues [9,21,24]. In material and pharmaceutical sciences, emerging ferroptotic drugs have been reported, and antitumor experiments using the combination of ferroptosis inducers and radiotherapy have reported encouraging results [100,101]. However, studies have been primarily based on animal models, and there is limited clinical data on the toxicity of ferroptosis inducers. Whether ferroptosis inducers can be applied to clinical settings remains to be explored [9]. In addition, several aspects regarding the use of ferroptosis inducers remain to be addressed, including their long-term effects on normal tissues and organs, effect of radiation and ferroptosis inducer combination therapy on normal tissues, appropriate radiation dose and dose of ferroptosis inducers for combination treatment, and methods to enhance tumor treatment effects in clinical settings by combining immunotherapy, radiotherapy, and ferroptosis inducers.

Unlike those of ferroptosis, the roles of pyroptosis and necroptosis in cancer therapy have only recently received attention, although these processes have long been understood. Multiple antitumor agents, such as 5-FU and lobaplatin, can induce tumor cell necroptosis or pyroptosis and usually have better tumor treatment outcomes when combined with radiotherapy [102,103]. However, necroptotic or pyroptotic tumor cells can also release inflammatory factors to promote tumor cell repopulation and development, while pyroptotic tumor cells can release multiple inflammasomes to injure normal tissues [59,104,105]. Therefore, the effect of enhancing necroptosis or pyroptosis in tumor cells during radiotherapy on the therapeutic outcome remains to be explored.

Table 2
Clinical trials on radiotherapy may related to the ferroptosis, necroptosis, and pyroptosis of tumor.

Disease	Cell death type	Trial number	Intervention/treatment	Associated biomarkers
Non-small cell lung cancer, small cell lung cancer, thymoma, thymus neoplasms	Ferroptosis	NCT00921739	Radiation: esophageal sparing IMRT	Glutathione (GSH)
Breast cancer	Ferroptosis	NCT00266331	Drug: reduced glutathione	GSH
Biopsy confirmed head and neck cancer and tumors of the chest	Ferroptosis	NCT05054517	Dietary supplement: glutamine	GSH
Radiated-induced injuries in patients with rectal cancer	Ferroptosis	NCT04713332	Dietary supplement: vitamin E Dietary supplement: hydrogen rich water Dietary supplement: placebo	GSH
Prostate cancer, radiation therapy	Ferroptosis	NCT01917890	Dietary supplement: curcumin Dietary supplement: placebo	GSH
Anal cancer, squamous cell carcinoma, radiation exposure	Ferroptosis	NCT03386500	Drug: BMX-001	4-hydroxynonenal (4-HNE)
Locally recurrent head and neck squamous cell carcinoma, nasopharyngeal squamous cell carcinoma, sinonasal squamous cell carcinoma	Necroptosis	NCT03803774	Drug: birinapant Radiation: intensity-modulated radiation therapy	Caspase 3, MLKL
Cutaneous T cell lymphoma, graft vs host disease, graft rejection	Pyroptosis	NCT05333367	Other: samples collection	GSDMD, caspase-3
Adenocarcinoma of the pancreas, recurrent pancreatic cancer, stage II pancreatic cancer, stage III pancreatic cancer, stage IV pancreatic cancer	Pyroptosis	NCT00047307	Drug: alvocidib Drug: gemcitabine hydrochloride Radiation: 3-dimensional conformal radiation therapy Other: laboratory biomarker analysis	Caspase-3
Mucinous adenocarcinoma of the rectum, stage IIA rectal cancer, stage IIB rectal cancer, stage IIC rectal cancer, stage IIIB rectal cancer, stage IIIC rectal cancer	Pyroptosis	NCT01197664	Drug: paricalcitol Radiation: radiation therapy Other: laboratory biomarker analysis Drug: fluorouracil	Caspase-3
High-risk cancer, locally advanced breast cancer	Pyroptosis	NCT03978663	Radiation: neoadjuvant radiotherapy	Caspase-3
Early-stage breast carcinoma	Pyroptosis	NCT02212860	Radiation: stereotactic body radiation then surgery	Caspase-3
Locally recurrent head and neck squamous cell carcinoma, nasopharyngeal squamous cell carcinoma sinonasal squamous cell carcinoma	Pyroptosis	NCT03803774	Drug: birinapant Radiation: intensity-modulated radiation therapy	Caspase-3
Glioblastoma, recurrent glioblastoma	Pyroptosis	NCT01849146	Drug: adavosertib Radiation: radiation therapy Drug: temozolomide	Caspase-3

Conclusions and perspectives

Recent studies have established a beneficial role of ferroptosis, necroptosis, and pyroptosis in radiotherapy. Radiotherapy can induce tumor cell ferroptosis, necroptosis, and pyroptosis, thus hindering tumor development. However, ferroptotic, necroptotic, and pyroptotic tumor cells can further activate antitumor immunity post radiotherapy. Some clinical trials have investigated the relationship of radiotherapy with ferroptosis, necroptosis, and pyroptosis (Table 2). Additionally, radiotherapy-induced ferroptosis, necroptosis, and pyroptosis have attracted significant attention. However, some questions remain to be answered, such as: what is the role of necroptosis and pyroptosis in tumor development post radiotherapy?

Although radiotherapy can inhibit tumor proliferation by inducing necroptosis and pyroptosis in tumor cells, the role of necroptotic and pyroptotic tumor cells in the subsequent tumor microenvironment remains to be explored. Necroptotic and pyroptotic tumor cells can activate antitumor immunity and inhibit tumor development [7]. However, necroptosis and pyroptosis, which are forms of inflammation-related RCD, initiate an inflammatory response, resulting in tumor development. For example, necroptotic tumor cells can release IL-1β and IL-18 and promote the infiltration of inflammatory cells to trigger inflammation, resulting in angiogenesis in the tumor microenvironment and tumor cell proliferation and metastasis [59]; moreover, during pyroptosis, the activation of inflammasomes can promote the maturation and release of inflammatory factors, such as IL-18, IL-1β, IL-10, which cause an inflammatory cascade response, thus promoting tumor development [106]. Therefore, the role of necroptotic and pyroptotic tumor cells in tumor cell recurrence and metastasis after radiotherapy remains a matter of concern.

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Authors' contributions

Jianxin Xue provided the idea. Youke Wang wrote the review. Yali Wang and Jin Pan revised this review. Jianxin Xue and Lu Gan helped with the final revision of the review. All authors reviewed the manuscript and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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