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NLRP3 inflammasome in cancer and metabolic diseases

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The NLRP3 inflammasome is a multimeric cytosolic protein complex that assembles in response to cellular perturbations. This assembly leads to the activation of caspase-1, which promotes maturation and release of the inflammatory cytokines interleukin-1β (IL-1β) and IL-18, as well as inflammatory cell death (pyroptosis). The inflammatory cytokines contribute to the development of systemic low-grade inflammation, and aberrant NLRP3 activation can drive a chronic inflammatory state in the body to modulate the pathogenesis of inflammation-associated diseases. Therefore, targeting NLRP3 or other signaling molecules downstream, such as caspase-1, IL-1β or IL-18, has the potential for great therapeutic benefit. However, NLRP3 inflammasome–mediated inflammatory cytokines play dual roles in mediating human disease. While they are detrimental in the pathogenesis of inflammatory and metabolic diseases, they have a beneficial role in numerous infectious diseases and some cancers. Therefore, fine tuning of NLRP3 inflammasome activity is essential for maintaining proper cellular homeostasis and health. In this Review, we will cover the mechanisms of NLRP3 inflammasome activation and its divergent roles in the pathogenesis of inflammation-associated diseases such as cancer, atherosclerosis, diabetes and obesity, highlighting the therapeutic potential of targeting this pathway.

The innate immune system recognizes pathogenic insults as well as dead and defective cells in the body to initiate protective responses[1]. This recognition occurs through a set of germline-encoded pattern recognition receptors (PRRs) that sense pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs)[2]. The membrane-bound PRRs are classified into Toll-like receptors (TLRs) and C-type lectin receptors. The cytoplasmic PRRs include the NOD-like receptors (NLRs), the retinoic acid-inducible gene-I-like receptors (also known as RIG-I-like receptors) and absent in melanoma 2. The NLRs recognize ligands from various microbial pathogens, host cells and environmental sources. Based on their domain architecture, NLRs are subdivided into NLRPs and NLRCs. Among these, NLRP1 (mouse NLRP1b), NLRP3 and NLR family apoptosis inhibitory protein/NLRC4 are well established NLRs for their ability to assemble inflammasomes[3].

Inflammasomes are multimeric cytosolic protein complexes that assemble in response to DAMPs and PAMPs, leading to the activation of inflammatory responses. Inflammasome assembly initiates an inflammatory form of cell death known as pyroptosis, triggering the release of the proinflammatory cytokines interleukin-1β (IL-1β) and IL-18. The NLRP3 inflammasome responds to cellular perturbations and a wide variety of microbes[4–9].

The role of NLRP3 varies depending on whether it responds to sterile or microbial triggers. For example, during many infectious diseases, the NLRP3 inflammasome can be protective to the host[4–7] by modulating the release of inflammasome effector cytokines. However, NLRP3 has been directly associated with several inflammatory diseases and can play a dual role in the pathogenesis of metabolic diseases and cancer. Additionally, inflammasome-mediated cytokines produced mainly from the hematopoietic compartment also contribute by acting in autocrine and paracrine manners to form an inflammatory microenvironment[10], recruit immune cells and interfere with insulin signaling[9]—processes that promote the development of disease. Our understanding of the mechanistic connections between NLRP3 and metabolic diseases and cancer has grown tremendously in recent years, but there remain many unanswered questions with critical therapeutic implications. This Review will focus on the current understanding of the role of NLRP3 in the pathogenesis of different forms of cancer and metabolic diseases such as diabetes, obesity and atherosclerosis.

Mechanism of NLRP3 inflammasome activation

NLRP3 is a cytosolic protein with three domains: a leucine-rich repeat at the carboxy terminal, a central nucleotide-binding and oligomerization domain (NACHT), which possesses ATPase activity, and a pyrin domain (PYD) at the amino terminal[11]. The basal levels of NLRP3 expression are not typically sufficient for NLRP3 inflammasome activation. Therefore, a two-step process of priming and activation is required[12,13]. The priming step is induced by TLRs and cytokine receptors, such as the tumor necrosis factor (TNF) receptor or IL-1 receptor (IL-1R), which recognize PAMPs or DAMPs and upregulate the transcription of *NLRP3* and *IL1B*. PAMPs and DAMPs then promote NLRP3 inflammasome assembly, thereby leading to caspase-1 (CASP1)-mediated inflammatory cytokine maturation and release, and pyroptosis[14,15]. These PAMPs and DAMPs can include either microbial activators from Gram-positive and Gram-negative bacteria, viruses, fungal or protozoan pathogens or host-derived moieties such as extracellular ATP, uric acid crystals, calcium phosphate dihydrate, cholesterol crystals or glucose[4–9,16–18] (Fig. 1). However, in human monocytes, the priming step alone is sufficient to mediate CASP1 activation and IL-1β release[19,20]. In these cells, lipopolysaccharide (LPS) induces the release of endogenous ATP, which then activates the P2X7 receptor to trigger NLRP3 inflammasome activation and IL-1β maturation. An alternative NLRP3 inflammasome pathway is also activated in human monocytes in response to LPS, in which receptor-interacting serine/threonine-protein kinase 1 (RIPK1), Fas-associated death domain protein (FADD) and CASP8 are required downstream of TLR4–Toll or interleukin-1 receptor domain-containing adapter-inducing

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interferon-β (TRIF) signaling[21]. However, more studies are required to fully understand the similarities and differences between NLRP3 activation in humans and mice.

Several mechanisms have been proposed for how NLRP3 inflammasome assembly is activated, and disturbances in cellular homeostasis due to diverse triggers have been suggested to cause oligomerization of NLRP3 monomers through their NACHT domains. This leads to the recruitment of apoptosis-associated speck-like protein containing a CARD (ASC) via interaction between the PYD domain of NLRP3 and the PYD domain of ASC[22]. The CARD domain of ASC then recruits the CARD domain of pro-CASP1 to form the NLRP3–ASC–pro-CASP1 complex, or the inflammasome[23]. This facilitates pro-CASP1 cleavage to form active CASP1, which then goes on to cleave IL-1β and IL-18 into their biologically active forms. The active form of CASP1 also cleaves gasdermin D (GSDMD), which forms pores on the plasma membrane through which the biologically active forms of the inflammasome effector cytokines (IL-1β and IL-18) are released, leading to the inflammatory form of cell death known as pyroptosis[24,25]. An alternative form of NLRP3 activation occurs during influenza A virus (IAV) infection. Z-DNA-binding protein 1 (ZBP1)-mediated pyroptosis and CASP1 cleavage require the assembly of a particular form of the NLRP3 inflammasome, called the ZBP1-NLRP3 inflammasome[5,6]. ZBP1 recruits receptor-interacting serine/threonine-protein kinase 3 (RIPK3) and CASP8 to assemble the ZBP1-NLRP3 inflammasome after sensing Z-RNA during IAV infection[6]. The assembly of this inflammasome in response to IAV is crucial for host protection and is regulated by several host proteins, including molecules associated with interferon signaling and CASP6 (refs. [5,6]). Additionally, NLRP3 can be activated through non-canonical pathways by murine CASP11 or the human homologs CASP4 and CASP5. CASP11 senses intracellular LPS and cleaves GSDMD directly[26] (Fig. 1), and the pore formation provides the signal necessary for NLRP3 inflammasome activation.

The process of NLRP3 inflammasome activation is tightly regulated by several innate immune molecules during infection and inflammation[27–30]. Numerous positive regulators have been identified that promote NLRP3 inflammasome formation and activation. The adaptors myeloid differentiation primary response 88 and TRIF contribute to the priming of the NLRP3 inflammasome[31]. Additionally, CASP8 and FADD mediate the priming and activation of the canonical and non-canonical NLRP3 inflammasome[32]. Also, the stress granule protein DDX3X interacts with NLRP3 to promote its activation[33]. Our recent studies also found that CASP6 facilitates ZBP1-NLRP3 inflammasome activation during IAV infection[5,6]. Several other upstream regulators that are necessary for optimal NLRP3 inflammasome activation, including SHARPIN, immunity-related GTPase family member b10 and interferon regulatory factor 8, have also been identified[27,29,30]. In contrast, negative regulators of the NLRP3 inflammasome are also critical to prevent excessive activation and inflammation. The E3 ligase A20/TNF-α-induced protein 3, which has been shown to suppress inflammation in macrophages and in a mouse model of inflammatory arthritis[34], has been reported to act as a negative regulator of the NLRP3 inflammasome. In addition, the negative regulator transforming growth factor β-activated kinase 1 (TAK1) maintains NLRP3 inflammasome quiescence and preserves cellular homeostasis[28] (Fig. 1).

In addition to the role of innate immune molecules in regulation, the NLRP3 inflammasome is also controlled by cellular processes, such as ribosome stalling and translation inhibition[35], as well as post-translational modifications of NLRP3, such as phosphorylation[36] and ubiquitination[37]. Post-translational modifications are critical during the priming step to facilitate NLRP3 inflammasome activation. Additionally, recent work discovered that NEK7, a serine/threonine kinase, is also required for NLRP3 inflammasome activation[38–40]. A cryo-electron microscopy structure of human NEK7 and inactive NLRP3 showed that NEK7 binds to the leucine-rich repeat and nucleotide-binding domains of NLRP3 (ref. [40]). Future studies regarding the molecular mechanism of NLRP3 activation during various infectious and inflammatory conditions are warranted to fully understand its activation mechanism.

NLRP3 inflammasome in cancer

Inflammation is a leading hallmark for the development and progression of cancer[41]. Strong connections have been identified between inflammation and tumorigenesis, including proliferation, invasion, angiogenesis and metastasis[42]. More importantly, inflammation-mediated cytokine release orchestrated by various cells within the tumor microenvironment plays a critical role in these processes[43]. Since the NLRP3 inflammasome is a central hub of innate immunity that mediates the secretion of proinflammatory cytokines, it plays a major role in the modulation of inflammatory responses by cross-talking with other cellular compartments[44]. NLRP3 can have opposing roles in tumorigenesis. It can be tumor suppressive, which has mostly been shown in the context of colitis-associated colorectal cancer (CAC), but it can also be tumor promoting, with these effects being more evident in other forms of cancer such as gastric and skin cancer[42].

NLRP3 and human cancer

While much of our mechanistic understanding of NLRP3's roles in cancer has come from studies in mice, there is clear evidence for its physiological role in human cancers. A recent study showed that out of 24 cancers, 15 had significantly altered expression of NLRP3 inflammasome-related genes[45]. NLRP3 expression is increased in cancerous plasmacytoid dendritic cells isolated from human lung samples of patients with non-small cell lung cancer compared with its expression in healthy controls[46]. In addition, NLRP3 is constitutively expressed and activated in human melanoma cells. These cells mediate autoinflammation via CASP1 processing and biologically active IL-1β secretion without the presence of exogenous stimuli at the later stage of disease[47]. Moreover, patients with pancreatic cancer have the rs35829419-*Nlrp3* polymorphism at a greater frequency than individuals without cancer. This polymorphism may lead to excessive enzymatic cleavage of pro-IL-1β into its active form[48]. Another study found an association of the rs10733113-*Nlrp3* polymorphism with sporadic malignant melanoma in Swedish patients[49]. Likewise, the rs10754558 variant of *NLRP3* is significantly associated with gastric cancer[50]. In addition, the genetic polymorphisms *Il1b*-rs16944 and *Il18*-rs1946518 were associated with pathophysiological characteristics in patients with chronic myeloid leukemia[51]. Together, these findings strongly suggest that the NLRP3 inflammasome has a key role in cancer.

NLRP3 in tumor suppression

NLRP3 is expressed in both immune cells and colonic epithelial cells, suggesting a possible association of NLRP3 with colon cancer[52]. Several in vivo mouse models of colon cancer have shown that NLRP3-deficient mice are hypersusceptible to azoxymethane (AOM)/dextran sulfate sodium (DSS)-induced CAC[53,54]. CAC is induced by the DNA-damaging agent AOM and the colitogenic chemical DSS. The combination of AOM with DSS accelerates tumor development and is commonly used to study inflammation-mediated colorectal cancer (CRC)[55]. The protective effect of the NLRP3 inflammasome in CRC is further supported by the increased prevalence of tumors in mice lacking the adaptor ASC and CASP1, compared with wild-type mice[53,54,56] However, in contrast with these findings, another study found similar tumor burden between wild-type and NLRP3-deficient mice in response to AOM/DSS[57]. CASP1-deficient mice have increased colonic epithelial and tumor cell proliferation, which is dependent on NLRC4, but not

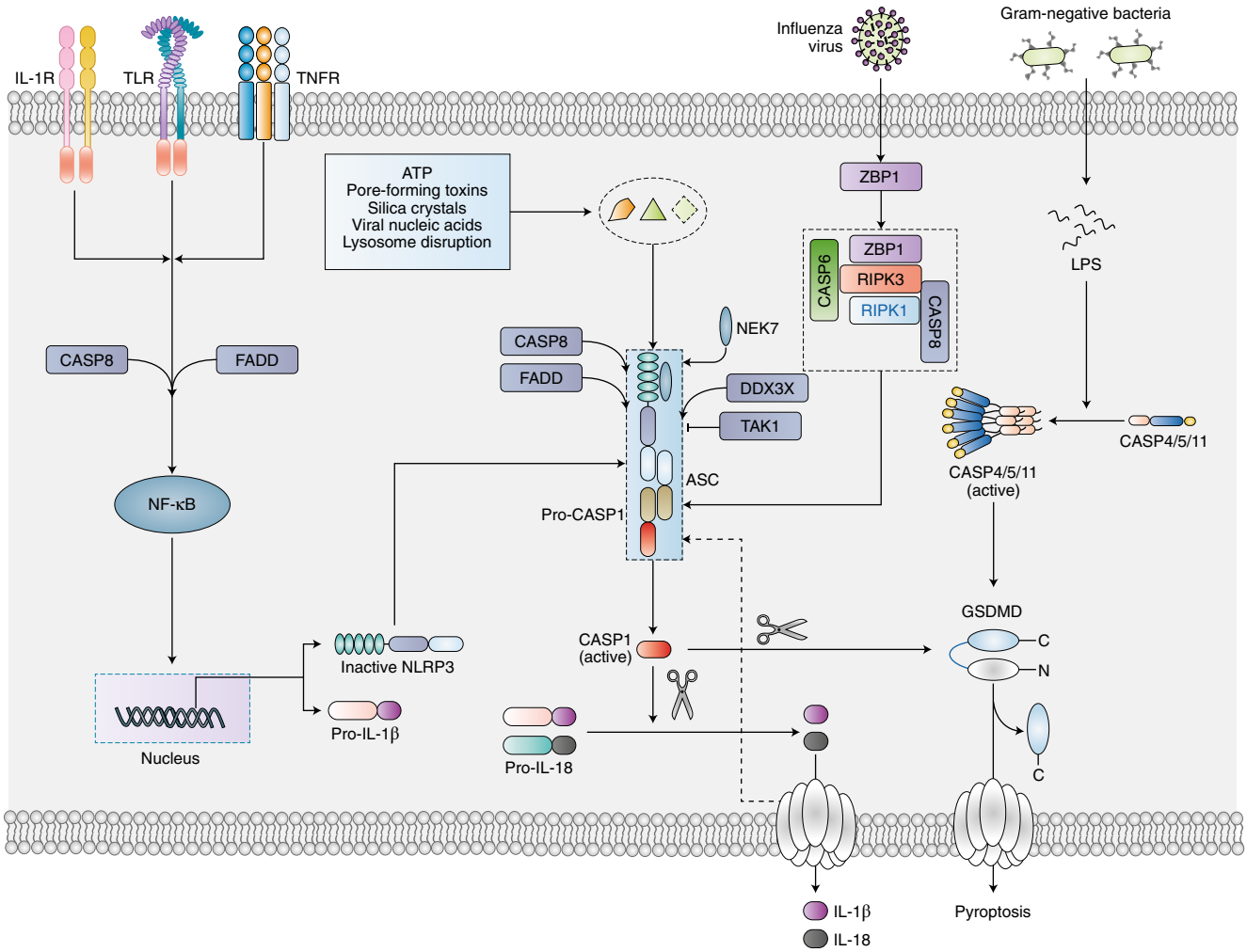

Fig. 1 | Mechanism of NLRP3 inflammasome activation. Canonical NLRP3 inflammasome activation involves two steps. The priming step occurs when inflammatory stimuli are sensed by TLRs, IL-1Rs and TNF receptors (TNFRs). The activation signal is provided by a wide range of stimuli, including ATP, pore-forming toxins, particulate matter, silica crystals and ion flux. FADD and CASP8 promote the transcription of NLRP3 and IL-1β. CASP8, FADD, transforming growth factor β–activated kinase 1 (TAK1), DDX3X and NEK7 are all reported to regulate activation of the NLRP3 inflammasome. ZBP1-dependent NLRP3 inflammasome activation occurs in response to influenza virus. Non-canonical NLRP3 inflammasome activation occurs in response to cytosolic LPS and is dependent on CASP11, which is the mouse homolog of human CASP4 and CASP5. Activated CASP1 or CASP11 cleaves GSDMD, which then forms pores in the plasma membrane, causing cell lysis (pyroptosis). GSDMD pores also facilitate the release of the mature forms of IL-1β and IL-18. NF-κB, nuclear factor-κB.

on NLRP3. Additionally, NLRP3 inhibition via mitophagy using small-molecule inhibitors prevents CAC, suggesting a detrimental role for NLRP3 in CAC[58]. The differences between studies finding positive and negative effects for NLRP3 in tumorigenesis during colon cancer could be due to differences in the gut microbiota between animal facilities, differences in the genetic background of the mouse lines and differences in experimental techniques used that affect the actual phenotype of these mice[59,60].

Besides these factors, nutrition and diet could also play a major role in modulating the outcome of CAC[61,62]. NLRP3 senses high-fat diet (HFD)-induced danger signals and promotes inflammation-induced obesity and insulin resistance[63,64]. It could be possible that NLRP3 inflammasome activation during HFD feeding creates low-grade systemic inflammation that promotes the development of CAC. Indeed, NLRP3 signaling was found to be detrimental in the presence of nutritional factors, such as high-cholesterol and HFD, which increased the inflammatory response and tumor burden in an AOM/DSS-induced mouse model of CAC[65].

The residual production of the inflammasome effector cytokine IL-18 in NLRP3-deficient mice during CAC suggests that other inflammasome sensors are probably involved in this IL-18 release[54,66]. Moreover, injection of recombinant IL-18 into NLRP3-deficient mice reduces the number of tumors formed in response to AOM and DSS, suggesting that NLRP3-mediated IL-18 has a protective role during CAC[54] (Fig. 2a). Additionally, NLRP3-mediated IL-18 signaling was found to be important in restricting CRC metastatic growth in the liver. Mice deficient in NLRP3 have increased liver CRC metastatic growth, which is mediated by impaired IL-18 signaling that negatively affects hepatic natural killer (NK) cells by interfering with their maturation, surface expression of the death ligand FasL and capacity to kill FasL-sensitive tumors[44]. Furthermore, IL-18 downregulates IL-22BP during DSS-induced damage to maintain the IL-22–IL-22BP axis, which is critically important in the regulation of intestinal tissue repair and tumorigenesis[67] (Fig. 2a). It is important to note that C57BL/6 mice with cryopyrin-associated periodic syndrome, which carry the

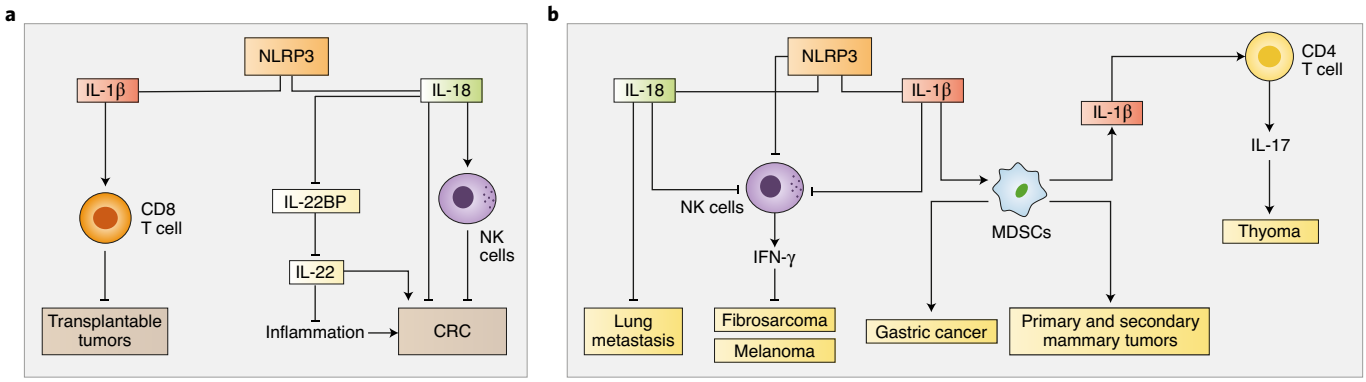

Fig. 2 | NLRP3 inflammasome in cancer. a, NLRP3 mediates the production of IL-18, which contributes to protection against CAC. IL-18 increases the tumoricidal activity of NK cells against metastasized colonic tumor cells in the mouse liver[44]. IL-18 also downregulates IL-22BP, which modulates CRC[67]. NLRP3 also drives T cell responses via IL-1β to inhibit transplantable tumor cells[134]. **b**, The NLRP3–IL-1β axis suppresses the tumoricidal activity of NK cells to promote methylcholanthrene-induced fibrosarcoma[135]. Overexpression of IL-1β mobilizes MDSCs to travel to the stomach and induces gastric cancer[75]. In primary and metastatic mammary tumors, IL-1 signaling drives the accumulation of MDSCs and promotes the tumors[74]. IL-1β also induces the secretion of IL-17 by CD4$^+$ T cells and dampens the antitumor efficacy of chemotherapeutic agents in thyoma[82]. Inflammasome-independent activity of NLRP3 suppresses NK cells and increases lung metastasis in certain models of melanoma[81,135]. IFN-γ, interferon-γ.

p.Arg258Trp substitution transcribed from a mutation in the third exon of *Nlrp3* (*Nlrp3*^{R258W})—corresponding to the p.Arg260Trp alteration frequently found in humans with Muckle–Wells syndrome[68]—maintain homeostasis in the gut and are strongly resistant to experimental colitis and CAC. Mechanistically, *Nlrp3*^{R258W} enhances IL-1β secretion in mononuclear phagocytes of the lamina propria to improve the production of local antimicrobial peptides and facilitate microbiota remodeling, leading to the induction of regulatory T cells (T_{reg} cells) that compensate for detrimental inflammation[69]. Additionally, IL-1 signaling can exhibit cell type-specific responses to set the inflammatory tone of the tumor microenvironment that determines tumorigenesis. Indeed, IL-1R signaling in T cells and epithelial cells is pro-tumorigenic, and this effect is counteracted by anti-tumorigenic IL-1R signaling in myeloid cells, particularly neutrophils. Mechanistically, IL-1R deletion in neutrophils results in bacterial invasion into tumors, leading to increased inflammation and aggressive CRC progression[70].

NLRP3 also has protective roles in hepatic cancer. The liver tumor burden from CRC metastasis is elevated in *Nlrp3*$^{-/-}$ mice[44]. Additionally, the expression of NLRP3 inflammasome components is significantly downregulated or even lost in human hepatic cancer tissues[71]. Moreover, the upregulation of NLRP3 by 17β-estradiol (E2) suppresses the growth of hepatocellular carcinoma cells[72], further suggesting a protective role of NLRP3 in hepatic cancer. Additional indirect evidence has also suggested a protective role for the NLRP3 inflammasome during tumorigenesis. Inhibiting an ion channel protein, transmembrane protein 176B, which is mainly expressed in myeloid cells, activates the inflammasome and leads to the potentiation of CD8$^+$ T cell-dependent antitumor immunity[73]. Overall, these findings suggest that NLRP3 has a tumor-suppressing role most often in CRC.

NLRP3 in tumor promotion

Despite acting as a tumor suppressor in CRC[54], the NLRP3 inflammasome promotes tumor formation in other forms of cancer. Mice deficient in CASP1 or NLRP3 have fewer lung metastases and reduced IL-1β production in an orthotopic mammary gland tumor model in which E70771 murine breast cancer cells are injected orthotopically into the fourth fat pads (murine mammary glands)[10]. Blocking IL-1R with its antagonist inhibits tumor growth and metastasis, suggesting that the activation of inflammasomes and the subsequent production of IL-1β in primary tumors creates a favorable microenvironment for tumor metastasis via the recruitment of myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages into tumor tissues[10]. It is known that IL-1 signaling drives the accumulation of MDSCs and promotes primary and metastatic mammary tumors[74]. The role for the IL-1 pathway in the recruitment of MDSCs in cancer progression is further supported by a study that found that a transgenic mouse strain overexpressing human IL-1β in the stomach is susceptible to gastric cancer development after colonization of *Helicobacter felis*, a model organism for *Helicobacter pylori* that activates nuclear factor-κB pathways in both in vitro and in vivo systems[75].

Beyond gastric cancer, the NLRP3 inflammasome is also detrimental in a mouse model of fibrosarcoma and is dispensable for asbestos-induced mesothelioma[76] (Fig. 2b). Moreover, although the direct role of NLRP3 in head and neck squamous cell carcinoma (HNSCC) is not clearly understood[77], patients with HNSCC have an increased concentration of IL-1β in their peripheral blood. Additionally, blocking the NLRP3 inflammasome using the inhibitor MCC950 in *Tgfbr1*/*Pten* 2cKO HNSCC mice leads to inhibition of tumor growth, a decrease in immunosuppressive cell accumulation and an increase in the number of effector T cells present. Together, these data suggest that NLRP3 plays a detrimental role in HNSCC[78]. Moreover, NLRP3-mediated IL-18 production in lymphoma cells reduces dexamethasone-induced apoptosis, thereby promoting the proliferation of lymphoma cells into the S phase[79], suggesting a carcinogenic role of NLRP3-mediated IL-18 in lymphoma.

NLRP3 can also interact with the adaptive immune system to drive carcinogenesis in other tumor types. NLRP3 signaling in macrophages drives the differentiation of CD4$^+$ T cells into tumor-promoting T helper type 2 (T_H2), T_H17 and T_{reg} cell populations in pancreatic carcinoma and suppresses T_H1 cell polarization and cytotoxic CD8$^+$ T cell activation, suggesting that NLRP3 could be an attractive target for novel immunotherapies to reprogram the tumor microenvironment towards an immunogenic innate and adaptive inflammatory phenotype[80]. NLRP3 restricts antitumor T cell immunity after dendritic cell vaccination, suggesting that novel approaches are needed to improve the response to antitumor vaccines by limiting NLRP3 signaling[81]. It is possible that the expression of NLRP3 in the tumor microenvironment diminishes antitumor immunity and vaccine efficacy by promoting the

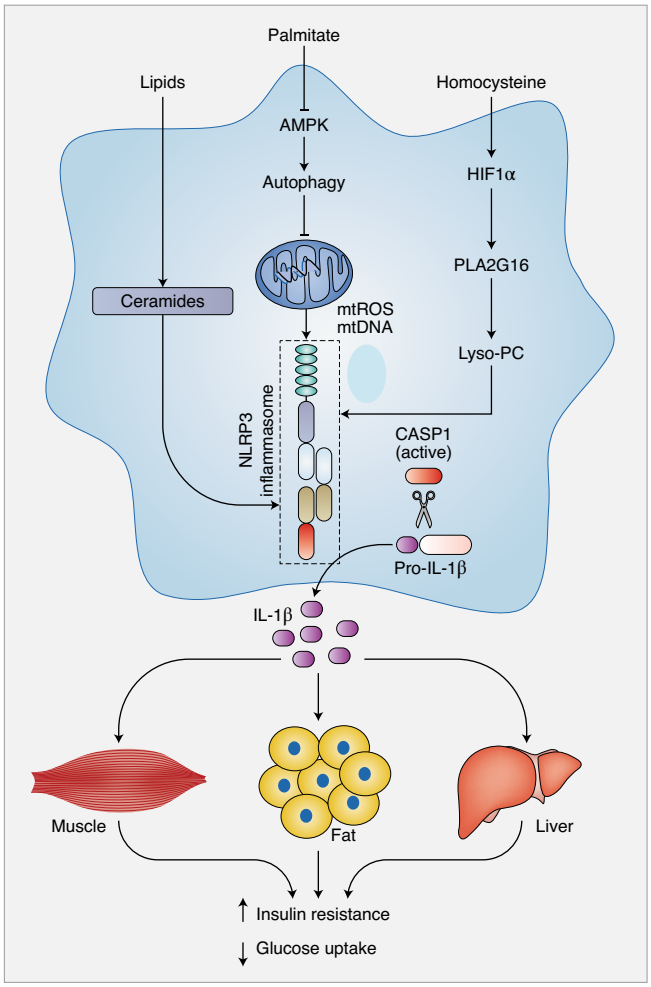

Fig. 3 | NLRP3 inflammasome in diabetes and obesity. Obesity-related danger signals, such as palmitate, lipids and ceramides, promote activation of the NLRP3 inflammasome. Ceramides are generated from the endoplasmic reticulum and/or through the conversion of sphingomyelins located in the plasma membrane. Saturated fatty acids, such as palmitate, increase mitochondrial ROS (mtROS) by reducing autophagy through the blockade of AMPK signaling. Elevated homocysteine levels can also activate the NLRP3 inflammasome through induction of the hypoxia-inducible factor 1α (HIF1α)–phospholipase A2 group 16 (PLA2G16) axis by generating adipocyte-derived lysophosphatidylcholine (lyso-PC). The release of IL-1β downstream of NLRP3 activation causes insulin resistance and reduces glucose uptake in insulin target tissues, such as muscle, liver and adipose tissues, leading to the pathogenesis of diabetes and obesity.

migration of MDSCs to the tumor site. The activation of the NLRP3 inflammasome in MDSCs by 5-fluorouracil and gemcitabine after cathepsin B release limits the antitumor efficacy of these chemotherapeutic agents[82]. IL-1β regulates CD4$^+$ T helper cell polarization in neighboring cells and drives the formation of T_H17 subsets producing IL-17, which play an active role in tumor pathogenesis and progression by enhancing the emergence of immunosuppressor MDSCs to the tumor site and reducing the tumor infiltration by cytotoxic T cells. In line with these findings, a recent study found that NLRP3-mediated IL-1β production in tumor cells creates a pro-tumorigenic pancreatic ductal adenocarcinoma microenvironment via establishing an immunosuppressive milieu mediated by M2 macrophages, MDSCs, CD45$^+$ regulatory B cells and T_H17 cells[83] (Fig. 2b).

NLRP3 may also modulate tumor responses by blocking the efficacy of immunotherapy. A recent study found that the genetic and pharmacologic inhibition of NLRP3 blocks the recruitment of granulocytic subsets of MDSCs (polymorphonuclear MDSCs) and enhances the efficacy of anti-programmed cell death protein 1 antibody immunotherapy[84]. Overall, these findings suggest that NLRP3 can have a pathogenic role in tumorigenesis by interacting with neighboring cells and other immune compartments.

NLRP3 inflammasome in metabolic diseases

The interconnection between the immune and metabolic systems is important to balance metabolic homeostasis. Imbalances between these systems leads to chronic, low-grade systemic inflammation that results in the development of metabolic disorders[85]. At the molecular level, the NLRP3 inflammasome–mediated inflammatory cytokines can act in both an autocrine and a paracrine manner in various cells within the metabolic tissues and contribute to metabolic disorders such as diabetes[63], obesity[64], atherosclerosis[17], cardiovascular disease[86], gout[16] and neurodegenerative diseases[87]. Here, we discuss the role of NLRP3 in diabetes, obesity and atherosclerosis.

NLRP3 in diabetes and obesity

Obesity is characterized by excess fat accumulation when energy intake exceeds energy expenditure, leading to pathological expansion and fundamental changes in cellular metabolism with immune modulation in the adipose tissue[88]. Ectopic fat accumulation in muscle and liver with low-grade inflammation instigates insulin resistance with the elevation of glucose in the blood[85,89]. The first study that reported the overexpression of TNF in the adipose tissue of obese mice provided a link between diabetes, obesity and the immune response[90]. Since then, several studies have reported that inflammasome components including NLRP3 are upregulated in subcutaneous adipose tissue or visceral adipose tissue (VAT) of obese patients compared with lean controls[91–95]. Likewise, calorie restriction- and weight loss-induced insulin sensitivity are associated with reduced NLRP3 and IL-1β expression in subcutaneous adipose tissue[96], further suggesting that NLRP3 is associated with diabetes and obesity.

Mice deficient in NLRP3 are protected from HFD-induced obesity and insulin resistance[96]. These mice have smaller adipocytes with reduced macrophage infiltration in the VAT compared with control mice. Moreover, HFD-fed *Nlrp3*$^{-/-}$ mice have reduced inflammasome activation in the fat and liver and have improved insulin signaling. Similarly, another study found that HFD-fed *Nlrp3*$^{-/-}$ mice have reduced weight gain and hypertrophy. Upregulation of CASP1 after HFD feeding regulates both insulin sensitivity and differentiation of adipocytes[63]. Additionally, the preadipocytes derived from *Casp1*$^{-/-}$ or *Nlrp3*$^{-/-}$ mice display higher insulin sensitivity and higher fat oxidation rates. Moreover, treatment with recombinant IL-1β and recombinant IL-1R antagonist showed that the effects of CASP1 are mediated by the processing of IL-1β[63].

The hematopoietic compartment may play a predominant role in the recognition of obesity-related DAMPs and subsequent production of IL-1β and IL-18 (refs. [9,96]). The blood glucose and insulin levels are significantly reduced in *Nlrp3*$^{-/-}$ mice fed with HFD for 3 months when compared with wild-type mice[9]. In contrast with this finding, one study has shown that *Nlrp3*$^{-/-}$ mice are not protected from weight gain, adipose tissue inflammation or glucose intolerance after 6 months of being fed with 45% fat and 1% cholesterol[97]. The discrepancies observed in these studies could be due to differences in the composition of dietary intake, the genetic background of the mice used in the study, the composition of the microbiota or the animal facilities.

A key question regarding the role of NLRP3 in diabetes and obesity is which triggers are sensed by NLRP3 to induce CASP1 activation and the release of inflammatory cytokines over the course

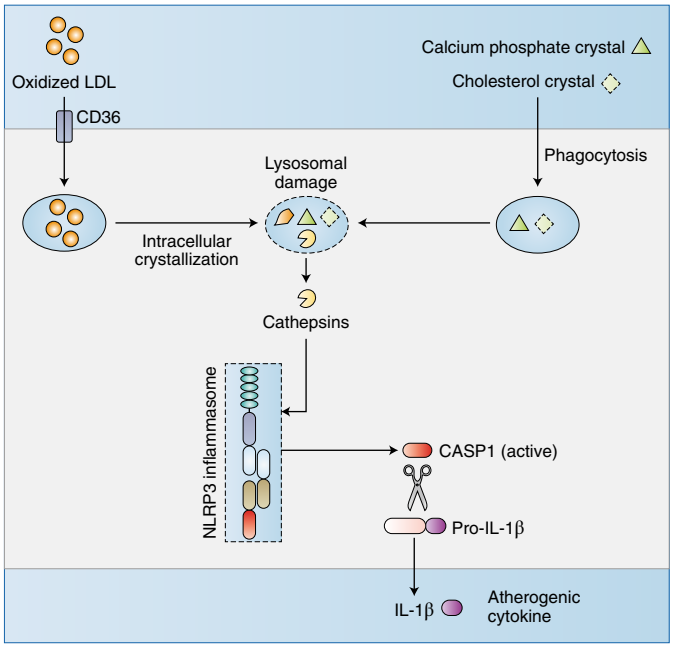

Fig. 4 | NLRP3 inflammasome in atherosclerosis. Calcium phosphate crystals and cholesterol crystals are taken into the lysosome by phagocytosis, or intracellular cholesterol crystals are formed when oxidized LDL is taken up by the scavenger receptor CD36. An excess of crystals in atherosclerosis causes lysosomal destabilization and rupture. Rupturing of lysosomes leads to leakage of the lysosomal enzyme cathepsin B, resulting in NLRP3 inflammasome activation. The release of IL-1β downstream of NLRP3 activation signals to neighboring monocytes/macrophages, endothelial cells and smooth muscle cells to promote the pathogenesis of atherosclerosis.

of weight gain. Several endogenous ligands have been reported to be required for activation of the NLRP3 inflammasome and IL-1β maturation. One key example is islet amyloid polypeptide (IAPP), which is co-secreted with insulin to form an amyloid deposit in the pancreas. IAPP leads to NLRP3 inflammasome activation and IL-1β maturation via lysosome destabilization in mouse macrophages[98]. Additionally, transgenic expression of human IAPP in mice leads to spontaneous development of diabetes, further highlighting the pathogenic role of IAPP[98]. During HFD feeding, changes in the gut microbial composition or reduced gut permeability lead to increased circulating levels of LPS, which can provide the primary signal for the upregulation of NLRP3 and IL-1β[99]. Additionally, ceramides, saturated fatty acids, reactive oxygen species (ROS), mitochondrial dysfunction and ATP released from necrotic adipocytes can act as the secondary signal for NLRP3 inflammasome activation via an autocrine or paracrine signaling mechanism[100]. For example, palmitic acid regulates 5′ AMP-activated protein kinase (AMPK) activation, which diminishes autophagy and promotes the accumulation of mitochondrial ROS, leading to inflammasome activation as well as IL-1β–mediated insulin resistance[9]. Similarly, another study found that obesity-related lipotoxic ceramides induce CASP1 activation in an NLRP3-dependent manner[96,101]. Palmitic acid induces the activation of a stress response element, inositol-requiring transmembrane kinase endoribonuclease-1, which enhances chronic low-grade inflammation and NLRP3 inflammasome activation during HFD feeding[102]. However, oleic acid, which is an unsaturated fatty acid, negatively regulates NLRP3 via AMPK activation, and mice fed with oleic acid exhibit reduced adipose IL-1β and improved insulin resistance[103]. Additionally, the amino acid homocysteine can act as a DAMP, and lysophosphatidylcholine provides the secondary signal to activate the inflammasome in both adipocytes and adipose tissue macrophages, to induce insulin resistance[104]. One of the inflammasome-dependent cytokines, IL-1β, reduces glucose uptake in adipocytes by reducing the messenger RNA expression of insulin receptor substrate-1 and blocking its downstream events, which are essential for glucose mobilization[105] (Fig. 3). The detrimental role of IL-1β in inducing adipose tissue inflammation and insulin resistance is further supported by a study in which the lack of IL-1R protected mice from HFD-induced adipose tissue inflammation, coincident with improved glucose homeostasis[106]. By contrast, the acute physiological elevation of IL-1β after feeding stimulates glucose disposal and reduces adipose tissue inflammation[107]. Overall, these data suggest that NLRP3 is a molecular sensor of obesity-associated danger signals, and that NLRP3-mediated, CASP1-dependent IL-1β secretion and inflammation in key metabolic tissues, including liver, fat and muscle, promote obesity-induced inflammation and insulin resistance (Fig. 3).

The NLRP3 inflammasome has also been associated with age-related metabolic complications. Mechanistically, NLRP3 mediates age-related chronic inflammation and controls catecholamine-induced lipolysis in adipose tissue macrophages, resulting in increased visceral obesity and defective free fatty acid mobilization[108]. Furthermore, NLRP3-mediated IL-1β and IL-18 contribute to the expansion of adipose B cells in VAT, which impairs metabolic homeostasis[109]. Hence, inhibiting NLRP3-dependent B cell accumulation could be targeted to reverse metabolic impairment in aging.

NLRP3 in atherosclerosis

Atherosclerosis is a chronic inflammatory disease in which extensive immune cell infiltration, lipid deposition and vascular smooth muscle cell proliferation lead to the formation of atherosclerotic plaques in the arterial wall. In particular, higher expression of NLRP3 inflammasome components such as NLRP3, ASC, CASP1, IL-1β and IL-18 in carotid atherosclerosis plaques suggests an association of NLRP3 with the pathogenesis of atherosclerosis[110]. Additionally, a high level of NLRP3 expression is strongly correlated with the severity of coronary artery stenosis[111].

The direct function of NLRP3 in the pathogenesis of atherosclerosis has been investigated using different in vivo mouse models. The detrimental role of NLRP3 in atherosclerosis is mostly dependent on its effector cytokine, IL-1β[112,113]. Bone marrow transplantation of *Ldlr*$^{-/-}$ mice with bone marrow derived from *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$, *Il1a*$^{-/-}$ or *Il1b*$^{-/-}$ mice followed by feeding with a Western-type high-cholesterol diet (Teklad Adjusted Calories 88137; 21% fat (wt/wt), 0.15% cholesterol (wt/wt) and 19.5% casein (wt/wt); no sodium cholate) for 8 weeks results in significantly reduced atherosclerosis and inflammasome-dependent IL-18 levels compared with *Ldlr*$^{-/-}$ mice transplanted with wild-type bone marrow[17], providing genetic evidence for the role of the NLRP3 inflammasome in atherosclerosis. In line with this, a more recent study reported that bone marrow transplantation from *Casp1/11*$^{-/-}$ mice to *Ldlr*$^{-/-}$ mice leads to the development of significantly smaller atherosclerotic plaques than *Ldlr*$^{-/-}$ mice transplanted with wild-type bone marrow[114]. However, another study demonstrated that there was no reduction in the progression of atherosclerosis in *Apoe*$^{-/-}$*Nlrp3*$^{-/-}$, *Apoe*$^{-/-}$*Asc*$^{-/-}$ or *Apoe*$^{-/-}$*Casp1*$^{-/-}$ mice with respect to *Apoe*$^{-/-}$, after feeding with a HFD (KlibaNafag 3200 supplemented with 1.25% wt/wt cholesterol and 15% wt/wt cacao butter) for 11 weeks, suggesting that atherosclerosis can progress independent of the NLRP3 inflammasome[115]. The discrepancy observed in these studies could be due to differences in the mouse models, the time of diet feeding and the type of atherogenic diet used. It is possible that an overabundance of dietary cholesterol in combination with the longer feeding might bypass the NLRP3 inflammasome and activate other inflammatory pathways. The inflammasome-independent cytokines IL-1α and

high-mobility group box protein 1 also promote atherosclerosis and are able to induce strong inflammatory immune responses[116]. *Ldlr*$^{-/-}$ mice transplanted with bone marrow from *Il1a*$^{-/-}$ mice showed significantly reduced atherosclerosis compared with *Ldlr*$^{-/-}$ mice transplanted with bone marrow from *Il1b*$^{-/-}$ mice after 16 weeks of atherogenic diet feeding[117]. These findings suggest that IL-1α has a predominant role in the progression of vascular inflammation and atherosclerosis during prolonged atherogenic diet feeding.

Mechanistically, low-density lipoprotein (LDL), cholesterol crystals and calcium phosphate crystals are the major drivers of NLRP3 inflammasome activation in atherosclerosis (Fig. 4). Cholesterol crystals activate the NLRP3 inflammasome in macrophages in vitro in a process that involves phagolysosomal damage. The phagocytosed crystals cannot be engulfed or digested, resulting in leakage of the lysosomal enzyme cathepsin B, which activates the NLRP3 inflammasome[17]. Intraperitoneal injection of cholesterol crystals induces acute inflammation in wild-type mice that is absent in mice deficient in the components of the NLRP3 inflammasome, cathepsin B, cathepsin L or IL-1 (ref. [17]). Altogether, these in vitro and in vivo data suggest that crystalline cholesterol acts as an endogenous danger signal, and its deposition is an early cause of inflammation, not the consequence of inflammation. Additionally, oxidized LDL leads to cholesterol crystallization and activation of priming signals to induce NLRP3 and pro-IL-1β expression[118]. Similar findings describe that oxidized LDL mediates the endocytic pathway via the PRR CD36 and forms crystals or fibrils, which result in lysosomal disruption and NLRP3 activation[118]. Macrophages that lack CD36 fail to release IL-1β, which is dependent on the NLRP3 inflammasome[118]. In summary, endogenous danger signals produced during atherosclerosis provide both the priming signal to upregulate the expression of inflammasome components and the activation signal to activate the NLRP3 inflammasome, which provokes the pathogenesis of atherosclerosis.

Summary and future perspectives

The association of NLRP3 with cancer and metabolic diseases has been reported from both animal and human studies. NLRP3 has contrasting roles in tumorigenesis, demonstrating both detrimental and beneficial effects. The beneficial effect is well reported only in the context of CAC[53,54,56] and some forms of liver cancer[44]. However, detrimental effects are observed in other cancers and metabolic diseases, including diabetes, obesity and atherosclerosis. The inflammasome effector cytokines IL-1β and IL-18, which are produced as a result of NLRP3 activation, are major effector molecules aggravating these diseases. These cytokines generate and maintain an inflammatory microenvironment that supports the pathogenesis of inflammatory diseases. The inflammatory microenvironment surrounding cancer cells inhibits the effect of NK- and T cell-mediated immune surveillance, aggravating the pathogenesis of cancer. Specifically, the detrimental role of IL-1β for the initiation and progression of inflammation-associated metabolic disease and cancer has recently been reported in patients enrolled in the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS)[119]. In this study, patients who received canakinumab, an anti-IL-1β antibody, had reduced levels of high-sensitivity C-reactive protein compared with the placebo group, suggesting that the inhibition of IL-1β reduces cardiovascular burden[120]. These clinical data are further supported by a mouse study in which inhibition of NLRP3 or IL-1β was shown to reduce the incidence of atherosclerosis[17]. Additionally, CANTOS found a 77% reduction in lung cancer fatality and a 67% reduction in lung cancer incidence, although the reduction was not statistically significant for non-lung cancers[119]. Overall, the findings from CANTOS suggest that IL-1β has a detrimental role in lung cancer, where inflammation is the major driver of tumorigenesis. The pathogenic role of IL-1β has been further observed during NLRP3 sensing of metabolic perturbations, where IL-1β acts in both an autocrine and paracrine manner to interfere with insulin signaling, leading to the development of metabolic diseases[63,64]. Overall, modulating NLRP3 or its downstream inflammatory cytokines could have promising therapeutic effects in the development of metabolic diseases or cancer.

Inflammasome-mediated inflammatory cytokine release depends on gasdermin family proteins. GSDMD has been identified as an executioner of pyroptosis that forms pores on the membrane[24,25]. Pyroptosis in gasdermin E-positive cancer cells has also been observed due to CASP3 activation in response to chemotherapeutic drugs[121,122]. Additionally, programmed death ligand 1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumor necrosis[123], suggesting crosstalk between major cell death pathways in cancer. Indeed, recent studies have shown crosstalk between pyroptosis, apoptosis and necroptosis, defining PANoptosis as a unique, physiologically relevant, inflammatory programmed cell death pathway activated by specific triggers and regulated by the PANoptosome complex, which provides a molecular scaffold for contemporaneous engagement of key molecules from pyroptosis, apoptosis and necroptosis[124–127]. PANoptosis has been implicated in infectious and autoinflammatory diseases, cancer and beyond[6,124–129], and PANoptosis prevents tumorigenesis in both spontaneous and inflammation-induced mouse models of CAC[125]. In the context of metabolic disease, proinflammatory cytokines and DAMPs released from both immune and non-immune cells activate inflammatory cell death in various metabolic tissues, including the liver and pancreas, leading to insulin resistance[130,131]. The combination of TNF and interferon-γ specifically has been reported to induce pancreatic β cell death[132], which may be linked to the ability of these cytokines to drive PANoptosis[129]. It would be interesting to investigate whether NLRP3 can form unique PANoptosome complexes to regulate PANoptosis in the context of metabolic and inflammatory diseases and cancer.

The divergent roles of NLRP3 in the pathogenesis of cancer raise new opportunities and challenges to fully understand how it carries out both the pro-tumorigenic as well as anti-tumorigenic functions. These diverse functions could be affected by the tumor microenvironment influencing the activation of NLRP3. Additionally, the differential expression of NLRP3 in various cells and tissues suggests it has distinct roles in the pathogenesis of tumors originating in specific tissues. For example, NLRP3 expression is upregulated in lung adenocarcinoma and small cell lung cancer, where the role of NLRP3 is pathogenic[133]. Likewise, the expression of NLRP3 is downregulated in hepatic cancer cells, where the role of NLRP3 appears to be beneficial[71]. The function of NLRP3 in particular cancers could also depend on the effects of other mutations on its expression, the tumor type, the stage of tumor development and the effector molecules downstream of NLRP3. Therefore, NLRP3 signaling has complex effects on tumor initiation and progression through its modulation of antitumor immunity, cell death, proliferation, angiogenesis and metastasis. Given that the outcomes of inflammasome signaling are diverse across tumor types, gaining insight on how to manage this diversity will also be an important area for future investigations.

Overall, it is critical to continue to improve our mechanistic understanding of NLRP3 and its divergent functions across diseases. It is possible that NLRP3 may undergo different forms of activation in response to DAMPs versus PAMPs. Hence, examining the highest-resolution structure of NLRP3 in response to inflammatory and infectious triggers could improve our understanding of the specific functions of NLRP3 in the context of these diseases. Moreover, one possible mechanism driving the divergent roles of NLRP3 in cancers could be cell- and tissue-specific functions of NLRP3 in different stages of tumorigenesis. Thus, the use of cell- and tissue-specific NLRP3 deletions, the use of mice with similar genetic backgrounds and the use of littermates will provide valuable

insight to understand the differential functions of NLRP3 in inflammatory and infectious diseases. Identifying the specific functions of the NLRP3 inflammasome, along with the pathways and regulatory mechanisms that control its activation, in disease-specific contexts will support evidence-based therapeutic design and drastically improve our ability to balance the beneficial and detrimental functions of this critical innate immune molecule to benefit patients.

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