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The Role of Inflammasome-Associated Innate Immune Receptors in Cancer

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
AIM2, absent-in-melanoma 2; ALR, AIM2-like receptor; AOM, azoxymethane; ASC, apoptosis-associated Speck-like protein containing a caspase activation and recruitment domain; CAC, colitis-associated colon cancer; CARD, caspase activation and recruitment domain; CRC,

ABSTRACT

Dysregulated activation of the innate immune system is a critical driver of chronic inflammation that is associated with at least 30% of all cancers. Innate immunity can also exert tumour-promoting effects (e.g. proliferation) directly on cancer cells in an intrinsic manner. Conversely, innate immunity can influence adaptive immunity-based anti-tumour immune responses via Ag-presenting dendritic cells that activate natural killer and cytotoxic T cells to eradicate tumours. While adaptive anti-tumour immunity has underpinned immunotherapy approaches with immune checkpoint inhibitors and chimeric Ag receptor-T cells, the clinical utility of innate immunity in cancer is underexplored. Innate immune responses are governed by pattern recognition receptors, which comprise several families, including Toll-like, nucleotide-binding oligomerization domain-containing (NOD)-like and absent-in-melanoma 2 (AIM2)-like receptors. Notably, a subset of NOD-like and AIM2-like receptors can form large multiprotein "inflammasome" complexes which control maturation of biologically active IL-1β and IL-18 cytokines. Over the last decade, it has emerged that inflammasomes can coordinate contrasting pro- and anti-tumour responses in cancer and non-cancer (e.g. immune, stromal) cells. Considering the importance of inflammasomes to the net output of innate immune responses, here we provide an overview and discuss recent advancements on the diverse role of inflammasomes in cancer that have underpinned their potential targeting in diverse malignancies.

Keywords: Cancer; Inflammasomes; Inflammation; Innate immunity; Pattern recognition receptors

INTRODUCTION

Dysregulated activation of the innate immune system, the first line of host defense against invading microbes and tissue injury, is a critical driver of chronic inflammation that is associated with at least 30% of all cancers, including those of the lung, stomach, pancreas, liver and colon, among others (1,2). Notably, tumour-promoting inflammation initiated and sustained by a dysregulated innate immune response is one of the hallmarks of cancer (3). In chemokines (e.g. CXCL1, CXCL8/IL-8) to sustain the activation and recruitment of inflammatory innate immune cells (1,4). This chronic inflammatory reaction in the tumour microenvironment (TME) also comprises the production of genotoxic agents (e.g. ROS),

colorectal cancer; DAMP, damage-associated molecular pattern; DC, dendritic cell; DMBA, 7,12-Dimethylbenz(a)anthracene; DSS, dextran sodium sulfate; GSDMD, gasdermin-D; HCC, human hepatocellular carcinoma; IBD, inflammatory bowel disease; MDSC, myeloid-derived suppressor cell; MEFV, Mediterranean fever; NACHT, nucleotide-binding and ATP-binding domain; NAIP, NLR family of apoptosis inhibitory protein; NLR, nucleotide-binding oligomerization domain-containing-like receptor; NLRC, NLR family CARD domain containing; NLRP, nucleotide-binding domain and leucine-rich repeat protein; NLRP1a-c, nucleotide-binding domain and leucine-rich repeat protein 1 and its three paralogs in mice; Nlrp3−/−, nucleotide-binding domain and leucine-rich repeat protein 3-deficient; NOD, nucleotide-binding oligomerization domain-containing; NSCLC, non-small cell lung cancer; PAMP, pathogen-associated molecular pattern; PDAC, pancreatic ductal adenocarcinoma; PRR, patten recognition receptor; PYD, pyrin domain; SCC, squamous cell carcinomas; SNP, single nucleotide polymorphism; TME, tumour microenvironment; TMS1, target of methylation induced silencing 1; TPA, 12-O-Tetradecanoylphorbol-13-acetate.

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angiogenic and growth factors, which collectively stimulates a pleiotropic array of tumour-promoting cellular responses (e.g. proliferation, survival, migration, epithelial-mesenchymal transition) that drive the outgrowth of transformed epithelial (tumour) cells (1).

Conversely, innate immunity can also employ several strategies to either directly or indirectly suppress tumourigenesis, the latter for example by enhancing anti-tumour adaptive immunity to eradicate tumour cells via Ag-presenting dendritic cells (DCs) which can activate NK and cytotoxic T cells (by DC-derived immunostimulatory cytokines, such as IL-27) (4-8). However, such T cell mediated anti-tumour immune responses are invariably impeded by the capacity of cancer cells to support an immunosuppressive TME via secretion of anti-inflammatory cytokines (e.g. IL-4, IL-6, IL-10, TGF β) that facilitate immune evasion and escape by cancer cells (4,5,9). This latter situation has formed the basis for the clinical implementation of immunomodulatory inhibitors (neutralizing Abs) against adaptive immune checkpoints, namely PD-1, PD-L1 and CTLA-4, in cancer patients (10). However, the clinical benefits of such immunotherapy remain controversial, especially in the context of solid cancers, with only patients from a minority of cancer types such as melanoma and subsets of non-small cell lung cancer (NSCLC) displaying robust clinical benefits (10-12). In this respect, innate immune cells and molecular regulators are only now just emerging as attractive candidates for targeted therapy in a range of cancers (7,8,13).

At the molecular level, the sensing of both invariant pathogen-associated molecular patterns (PAMPs) expressed by pathogens and commensals, and host-derived damage-associated molecular patterns (DAMPs) indicative of tissue damage, is elicited by a superfamily of evolutionarily conserved, germ-line encoded patten recognition receptors (PRRs) that are a molecular hallmark of innate immunity (14,15). PRRs comprise several distinct subfamilies that are functionally and structurally similar, namely membrane-spanning TLRs and C-lectin-like receptors that are localized to the plasma membrane or endosomes, and the cytosolic nucleotide-binding oligomerization domain-containing-like receptors (NLRs), absent-in-melanoma 2 (AIM2)-like receptors (ALRs) and retinoic acid-inducible gene 1-like receptors (16-20). PRRs are widely expressed by myeloid lineage innate immune cell subsets (e.g. macrophages, DCs, neutrophils), but are also expressed in adaptive immune cell types (B, NK, T), as well as non-immune cells (e.g. epithelial, endothelial, fibroblasts) (14,15,21).

Upon binding of PRRs to their cognate PAMP and/or DAMP ligands, a myriad of canonical signalling cascades are activated, including NF-κB and IFN regulatory factor 3 transcriptional complexes, along with PI3K/AKT and MAPKs (14,15,22). Collectively, these pathways coordinate the production of many inflammatory cytokines (e.g. IL-1β, IL-6, IL-18), IFNs and chemokines (e.g. CXCL1/KC, CXCL2/IL-8) which stimulate various inflammatory cellular processes, including immune cell recruitment, maturation, and activation (4,15-20,23). Furthermore, it has emerged that signalling from PRRs can directly affect the maturation, survival and proliferation of non-immune cells, in particular epithelial, which for example in the gastrointestinal tract can contribute to either homeostatic responses such as tissue (epithelial) remodeling, repair and barrier function, or conversely carcinogenesis (14,24-26).

A subset of cytosolic NLR and ALR family members, namely nucleotide-binding domain and leucine-rich repeat protein (NLRP)1, NLRP3, NLRP6, NLRP7, NLRP12, AIM2 and IFN-gamma inducible factor 16, along with the cytosolic PRR pyrin, form large multiprotein complexes called inflammasomes, containing the adaptor apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (CARD) (ASC) and pro-caspase-1

(**Fig. 1**) (27,28). In addition, the cytosolic NLR family of apoptosis inhibitory proteins (NAIPs) also recruit the downstream NLR family CARD domain containing (NLRC) 4 adaptor protein to assemble inflammasomes (29). These inflammasome complexes are critical orchestrators of the production of mature biologically active IL-1β and IL-18 cytokines (**Fig. 1**).

Since their discovery over two decades ago, inflammasomes have been widely reported for their critical role in facilitating innate immune-mediated host defense (30-32). Moreover, it is now widely documented that dysregulated inflammasome activation is linked to many chronic inflammatory and autoimmune disorders, and cancers, the latter albeit often with contrasting tumour-promoting versus tumour-inhibiting activities (**Fig. 1**) (14,30,32-34).

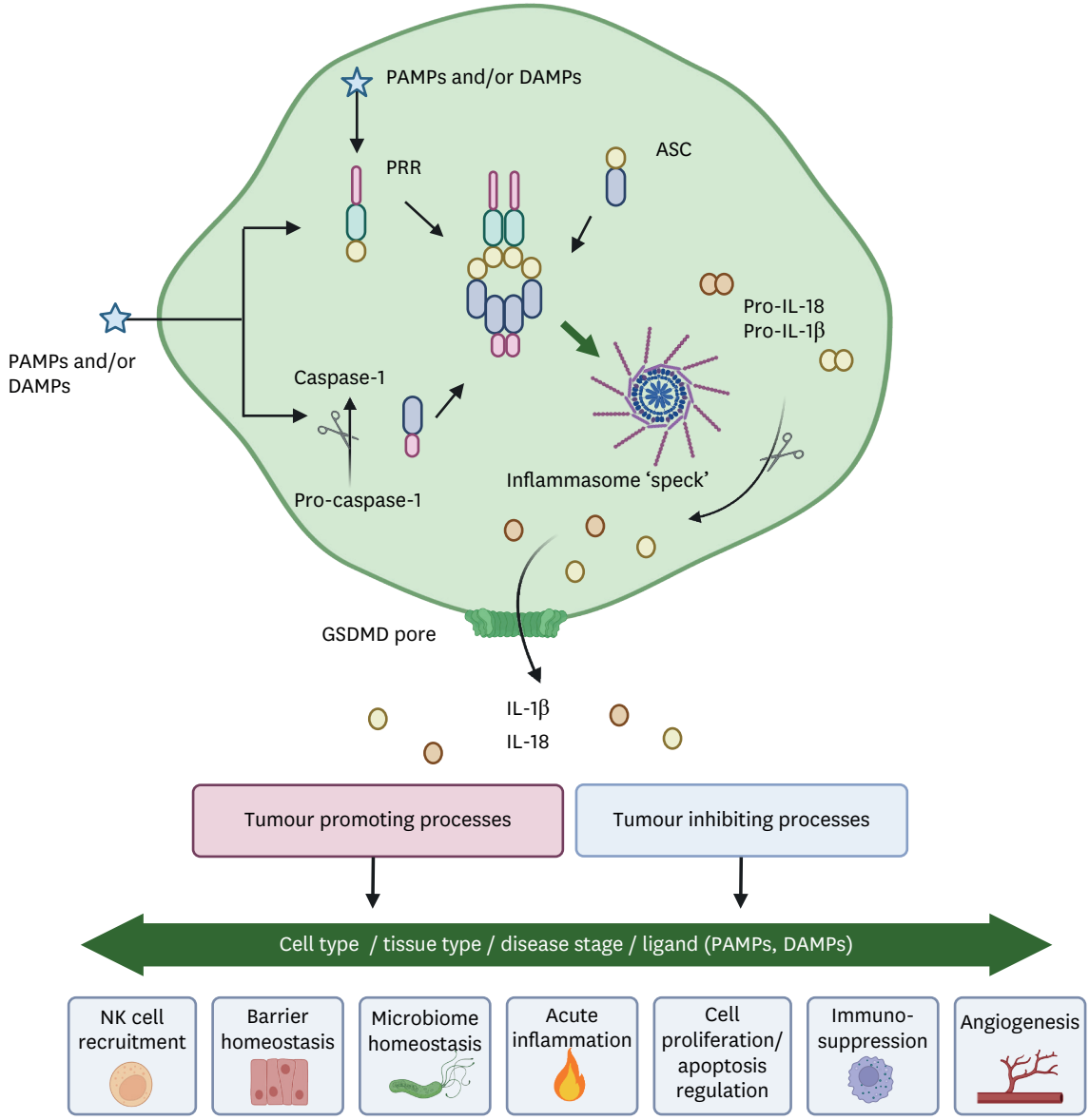

Figure 1. Schematic representation of the activation of inflammasome complexes, with an overview of diverse inflammasome-dependent downstream tumour promoting and tumour inhibiting cellular functions. The sensing of specific ligands (i.e. PAMPs and/or DAMPs) by PRRs leads to inflammasome complex assembly via recruitment of ASC and pro-caspase-1. Autocatalytic cleavage of pro-caspase-1 to bioactive (i.e. mature) caspase-1 results in the cleavage and extracellular release of bioactive IL-1β and IL-18. These inflammasome effector cytokines elicit a diverse range of intrinsic and extrinsic cellular processes that influence the development of cancer and tumour growth.

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Together with the large amount of clinical data and preclinical studies implicating excessive production of IL-1β and/or IL-18 in diverse cancer types (14,35-40), it is not surprising that there are intense research efforts globally to understand the molecular and cellular mechanisms by which inflammasomes contribute to carcinogenesis.

In this review, we will provide an overview and timely update on developments in the pathophysiology of ASC and its inflammasome-associated PRRs in cancer, including their contrasting and pleiotropic roles in specific cancer types. In addition, discussion will also incorporate the potential clinical utility of inflammasome complexes as therapeutic targets and biomarkers in cancer, as well as the emergence of inhibitors against specific inflammasome-associated PRRs and the ASC inflammasome adaptor which may serve as new anti-cancer therapeutics.

OVERVIEW OF THE COMPOSITION AND ACTIVATION OF INFLAMMASOME COMPLEXES

The canonical activation of inflammasomes is typically a two-step process, the first of which involves 'priming' via the upregulation of specific inflammasome-associated PRRs and downstream effector cytokine precursors (e.g. pro-IL-1β) by inflammatory signalling cascades, such as activation of NF-κB and JAK-STAT1/3 in response to TLRs (e.g. LPS binding to TLR4) and/or cytokines (e.g. IL-11, IFN-γ, TNFα) (24,27,41). Subsequently, the second step comprises PAMP and/or DAMP ligand activation of the core PRR(s) which triggers the recruitment and oligomerization of ASC and pro-caspase-1, the latter undergoing autocatalytic self-cleavage to become active caspase-1 and facilitating the cleavage of pro-cytokines to their bioactive forms (IL-1β, IL-18) (27). It is also notable that the canonical inflammasome-associated activities of caspase-1 involves cleavage (and activation) of the cell membrane pore-forming protein gasdermin D (GSDMD), which in high concentrations alters cell membrane permeability and facilitates release of IL-1β and IL-18 that can induce pyroptosis, a lytic and proinflammatory form of cell death (42,43). Alternatively, non-canonical activation of inflammasomes (e.g. NLRP3) leading to pyroptosis is governed by PAMP- and DAMP-sensing mouse caspase-11 or human caspases-4 and -5, whereby these inflammatory caspases promote cleavage and activation of GSDMD to induce ion efflux that subsequently leads to intrinsic NLRP3 inflammasome activation and mature IL-1β release (44,45).

The ASC inflammasome adaptor was discovered as a ≤22 kDa CARD-containing protein with pro-apoptotic activity that formed speck-like aggregates during apoptosis (46). Almost simultaneously, ASC was reported as a hypermethylated gene, termed target of methylation induced silencing 1 (*TMS1*), whose expression in human (breast) cancer cells was suppressed and associated with resistance to apoptosis, leading to the suggestion that ASC (TMS1) was a putative tumour suppressor gene (47). ASC has since been characterized as a bipartite protein comprising an N-terminal pyrin domain (PYD) and a C-terminal CARD that interact reciprocally with other PYD-containing and CARD-containing proteins, for instance inflammasome-associated PRRs such as NLRP3 (via PYD) and caspase-1 (via CARD), to form large multiprotein oligomeric filaments that condense into macromolecular ASC-containing specks (48,49).

Despite the dogma regarding the intracellular localization of ASC-containing inflammasome complexes, it has now emerged that ASC specks are also released from inflammasome-

activated cells and accumulate in the extracellular milieu of inflamed tissues (50). Although the mechanisms governing the formation, cellular release and uptake of extracellular ASC specks and their pathophysiological consequences remain to be fully elucidated, key functions of extracellular ASC include the recruitment and activation of secreted pro-caspase-1 to sustain the extracellular processing and maturation of inflammasome effector cytokines, as well as internalization by surrounding cells (e.g. phagocytic macrophages) to augment the cytosolic oligomerization of ASC-containing inflammasome complexes (49). Collectively, these biological activities of extracellular ASC are predicted to both amplify the cellular inflammatory response (incorporating pyroptosis) within inflammasome-activated cells and broaden the magnitude of cell types that are activated by inflammasome-derived signals.

NLR-containing inflammasomes

The structure and function of inflammasome-associated NLR proteins have been widely described in depth in the literature, and we refer the readers to these recent comprehensive reviews (51,52). Accordingly, this current review will briefly summarize salient features of representative NLR-containing inflammasomes. NLR family members are characterized by the presence of a central nucleotide-binding and ATP-binding domain (NACHT) that mediates NLR oligomerization, and which is commonly flanked by C-terminal leucine-rich regions—believed to modulate NLR protein activation and the sensing of PAMPs and DAMPs—and an N-terminal CARD or PYD (51).

NLRP1 was the first identified inflammasome-inducing PRR and is widely expressed in both immune and epithelial (especially keratinocytes) cells. Compared to other inflammasome-associated NLRs, human NLRP1 and its three paralogs in mice (NLRP1a-c) have unique structural features, for instance a C-terminal CARD, a function-to-find domain that facilitates constitutive NLRP1 proteolysis and inflammasome activity, and in mouse NLRP1a-c the absence of an ASC-binding N-terminal PYD (53). Collectively, these distinct features provide a rationale for the divergent mechanisms of NLRP1 inflammasome activation between species, as well as from other NLRP proteins, which in the mouse can be independent of ASC. Moreover, this functional divergence of NLRP1 between humans and mice, including in the sensing of ligands (e.g. viral dsRNA and UVB irradiation in humans; bacterial and protozoan toxins in mice), adds further complexity to the *in vivo* study of this NLR family member in mouse models and extrapolation to its role in human disease.

The best characterized inflammasome-associated PRR is NLRP3 (also known as cryopyrin), which is strongly expressed in myeloid lineage cells, as well as in specialized epithelial and endothelial cells (51). NLRP3 is activated via a large repertoire of PAMPs and DAMPs, including nucleic acids derived from microbial (viral dsRNA) and host (mitochondrial DNA) origins, mitochondrial ROS, monosodium urate and cholesterol crystals, silica, nigericin (*Streptomyces hygroscopicus*-derived antibiotic), extracellular ATP and various biotoxins (52). Notably, this scale of diversity of NLRP3 stimuli has led to the notion that NLRP3 may not physically bind to these PAMP and DAMP agonists, but rather NLRP3 is indirectly activated via sensing overlapping intracellular events triggered by these PAMPs and DAMPs, for instance mitochondrial damage and oxidative stress, ion (e.g. potassium) efflux, metabolic disturbances and disruption of lysosomal membrane integrity (52). Interestingly, the initial priming stage of NLRP3 inflammasome activation can also be modulated via post-translational modifications, such as phosphorylation (Tyr, Ser, Thr), sumoylation and ubiquitination (54). However, the upstream mechanisms by which protein modifications to NLRP3 (and potentially other NLRs) govern inflammasome activation, as well as their effect

(if any) on subcellular localization, protein stability and downstream NLRP3-dependent molecular pathways and cellular processes, remain largely unknown.

NLRC4 constitutes another subtype of inflammasome complex that is primarily activated by employing the cytosolic NAIP family of innate immune PRRs (mouse Naip1-6, human NAIP) to sense gram-negative bacterial flagellin. NLRC4-NAIP association triggers caspase-1 activation either directly by NLRC4 interacting with caspase-1, or indirectly via canonical inflammasome assembly involving ASC (55).

AIM2- and pyrin-containing inflammasomes

The AIM2 cytosolic DNA sensor was the first non-NLR family member identified that can form an inflammasome through oligomerization with ASC (56). AIM2 senses dsDNA from a wide variety of microbial (bacterial, viral) and host (nuclear dsDNA including radiation-induced dsDNA breaks, mitochondrial DNA) origins, and consistent with other inflammasome-associated PRRs is widely expressed among myeloid lineage innate immune cell subsets, as well as adaptive immune T cells and non-immune cells (e.g. gut epithelium, keratinocytes) (19,24,52,57). Interestingly, the quantity of cytosolic DNA ligand can influence AIM2 inflammasome activity and downstream cellular responses, with low concentrations of cytosolic DNA favoring AIM2 inflammasome-driven caspase-3-mediated apoptosis over caspase-1-mediated pyroptosis (58). Further highlighting the complex and diverse biological roles of AIM2 is the observation that AIM2 can also drive immune and non-immune cellular responses independent of inflammasomes, many of which curiously are linked to its cellular expression outside of the myeloid compartment (i.e. gastric and intestinal epithelial cells, T-cells) (19,24,57).

Pyrin, which is encoded by the gene Mediterranean fever (*MEFV*), is mainly expressed in myeloid lineage cells and has atypical structural features compared to other inflammasome-associated PRRs (41). For instance, mouse and human pyrin lack the central NACHT domain and C-terminal leucine-rich regions, while human pyrin contains a protein-interacting B-box zinc-finger domain, a structural coiled-coil domain and a C-terminal B30.2 domain (absent in mouse pyrin) which collectively regulate its activation, albeit by ill-defined mechanisms (59). These unique structural features of pyrin shed light on its distinct mechanism of activation which is unlikely to involve direct binding to a pathogenic ligand. In this regard, pyrin is predominantly activated by sensing perturbations in intracellular homeostasis involving bacterial effector proteins (e.g. *Clostridium difficile* toxin B) that inactive the RhoA GTPase (60).

INFLAMMASOMES IN PRECANCEROUS INFLAMMATORY AND INFECTIOUS DISEASES

Considering the diverse functions of inflammasome-associated NLRs, AIM2 and pyrin in immune and non-immune contexts, along with the plethora of inflammasome-activating DAMPs and PAMPs, it is not surprising that dysregulated expression and/or activation of these PRRs, along with ASC, are associated with the pathogenesis of a diverse range of disease states. These include autoimmune (e.g. rheumatoid arthritis, multiple sclerosis), chronic inflammatory (e.g. inflammatory bowel disease [IBD], cryopyrin-associated periodic syndrome), neurodegenerative (e.g. Alzheimer's, Parkinson's) and metabolic (e.g. diabetes, obesity, gout) diseases, as well as bacterial (e.g. *Listeria monocytogenes*, *L. pneumophila*, *C. difficile*) and viral (e.g. severe acute respiratory syndrome coronavirus 2, cytomegalovirus) infections

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(recently reviewed in (51,52)). Notably, numerous inflammasome-associated chronic inflammatory and infectious diseases are risk factors for immune-related cancers, and the following section will provide a summary of representative inflammasomes and their role in these precancerous disease settings.

Inflammasome-associated chronic inflammatory diseases that predispose to cancer are typified by IBD and its link with colorectal cancer (CRC). For example, loss-of-function mutations in NLRP3 are often found in Crohn's disease patients, and NLRP3-deficient (*Nlrp3*$^{-/-}$) mice display marked loss of epithelial barrier integrity, reduction in protective commensal bacteria, exacerbated intestinal inflammation and ultimately increased mortality in a dextran sodium sulfate (DSS) model of colitis (61,62). A general protective role for ASC-containing inflammasomes in IBD is also suggested by the exacerbated intestinal pathology (coincident with gut dysbiosis and impaired epithelial tissue repair) in mouse models for Crohn's disease and colitis coupled with genetic ablation of either ASC or other inflammasome-associated PRRs, namely NLRP6, NLRP12, or AIM2 (63-66).

Another precancerous inflammatory condition associated with dysregulated activation of ASC-containing inflammasomes is chronic gastritis triggered by *Helicobacter pylori* bacterial infection, which is linked to at least 75% of all gastric cancer cases (67). A role for inflammasomes in modulating the host response to *H. pylori* infection was first suggested by the amelioration of gastritis in ASC-deficient *H. pylori*-infected mice, including lower IL-1β production in the gastric mucosa, which was concomitant with elevated *H. pylori* colonization (68). In support of the notion that ASC-containing inflammasomes promote the pathogenesis of *H. pylori*-driven gastritis, it was subsequently reported that *Helicobacter*-infected *Nlrp3*$^{-/-}$ mice also displayed reduced gastric inflammation (69). This ameliorated gastric pathology was again accompanied by lower gastric IL-1β levels and higher bacterial colonization of the gastric mucosa, the latter likely due to impaired IL-1β-driven Th1 and Th17 responses from immune cells which are required for efficient immune clearance of *H. pylori* (69). More recently, the AIM2 inflammasome has also been implicated in *Helicobacter*-mediated immune responses, with *Helicobacter*-infected *Aim2*$^{-/-}$ mice displaying reduced gastric inflammation and hyperplasia compared to infected wild-type counterparts (70). Mechanistically, *Helicobacter*-induced gastric pathology aligned with upregulated AIM2 expression in both gastric epithelial and immune cells, and augmented AIM2 inflammasome activation was again coincident with the preferential gastric production of IL-1β, but not IL-18 (70). In addition, NLRC4 can promote gastric inflammation in response to *H. pylori* infection, as evidenced by reduced numbers of infiltrating neutrophils and DCs in the infected gastric mucosa of *Nlrc4*$^{-/-}$ mice (71). Here, bacterial clearance was increased in infected *Nlrc4*$^{-/-}$ mice, caused by the specific upregulation of IL-18 production from the gastric epithelium, which in turn led to a heightened Th17 immune response together with elevated production of antimicrobial peptides (71). Interestingly, these observations provide likely mechanistic insights into the differential cellular production and maturation of IL-1β and IL-18 predominantly in immune and gastric epithelial cells, respectively, by distinct inflammasome complexes during *H. pylori* infection.

It is also notable that the NLRP1 inflammasome has emerged as a driver of various skin inflammatory conditions, among which include the development of the inherited inflammatory and proliferative skin disorder, multiple self-healing palmoplantar carcinoma, which predisposes individuals to squamous cell carcinomas (SCCs) and is caused by germline gain-of-function mutations in *NLRP1* (exon encoding the PYD) leading to inflammasome overactivation (72).

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CLINICAL ASSOCIATIONS OF ASC AND INFLAMMASOME-ASSOCIATED PRRs IN CANCER

Over the last decade there has been a steady increase in the number of reports on the role of ASC and inflammasome-associated PRRs in cancer. A prime example of this is the emergence of clinical data indicating that genetic alterations such as single nucleotide polymorphisms (SNPs) and/or dysregulated expression of ASC (encoded by *PYCARD* gene) and numerous inflammasome-associated PRRs in tumour cells are molecular features of many types of human solid (epithelial) and blood (hematologic) cancers, and correlate with disease risk and survival outcomes (14,24,73,74). Considering that elevated production levels of the inflammasome effector cytokines IL-1β and IL-18, as well as *IL1B* and *IL18* gene mutations, are also associated with a broad range of cancers (36,40,75,76), it is not surprising that inflammasomes have emerged as innate immune targets for therapeutic strategies in cancer. However, a common trend among clinical studies is the contrasting disease associations for aberrant expression levels and/or SNPs in specific inflammasome components across cancer types. It is most likely that these discrepancies can be explained by, at least in part, the high degree of intratumoural and intertumoural genetic and molecular heterogenicity that exists in many cancers, as well as differences in study design relating to sampling, expression and genotyping (mutation detection) methodologies, and patient characteristics (e.g. geographical location, ethnicity, age, tumour stage, histological grade, anatomical location). In this regard, one must tread with caution when interpreting clinical data and disease association studies reported in the literature that are based only on single patient datasets and/or small patient numbers which do not have the statistical power to represent the heterogeneity of cancers; for this reason, in the following section only select representative studies based on clinical data for ASC and inflammasome-associated PRRs in cancer will be referred to. Nonetheless, as will be discussed in more detail later in this review, contrasting clinical data for disease associations of inflammasome components also reflect the knowledge gained from preclinical cancer models that inflammasome complexes, along with their effector cytokines, can display opposing anti- or pro-tumourigenic effects, including within the same cancer type (14).

ASC

Downregulated expression of ASC at the mRNA and/or protein level has featured in several human solid tumour types, including melanoma, SCCs, breast and lung cancers (47,77-79). Mechanistically, the silencing of *PYCARD* (i.e. ASC) gene expression in human cancers has been attributed to epigenetic pathways driving promoter hypermethylation, which leads to a subsequent blockade of cellular apoptosis in tumours, consistent with a role for ASC as a pro-apoptotic tumour suppressor (74). In support of this notion, methylation-induced suppression of ASC expression is associated with unfavourable clinical outcomes (e.g. poor prognosis, advanced disease stage, metastasis) in several human cancer types (74). Conversely, elevated ASC expression (mRNA and protein) has been reported in tumours from pancreatic ductal adenocarcinoma (PDAC) and gastric cancer patients and is predictive of poor prognosis among subsets of patients (36,80), which may suggest a different mechanism of ASC transcriptional regulation in human upper gastrointestinal cancers. It is of note that these contrasting clinical correlations for ASC among cancer types are also observed within a specific cancer (e.g. oral SCC) (74), the reasons for which, although unknown, may involve the above-mentioned variables in clinical studies.

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NLR-containing inflammasomes

While clinical investigations into the disease association of inflammasome-related PRRs in cancer are still in their relative infancy, among such NLR family members, NLRP1 and NLRP3 best exemplify studies suggesting that the altered expression and/or gene polymorphisms of these NLRs can predict cancer risk and prognosis in certain cancers (14). For example, in independent studies based on CRC patient cohorts from distinct geographical and ethical origins (Brazil, China, Sweden), patients displaying either gain-of-function mutations in *NLRP1* and/or *NLRP3* linked to augmented inflammasome activity, or elevated NLRP3 mRNA and/or protein expression, correlated with poor prognosis and/or increased risk of metastasis (81-83). Elevated *NLRP3* (and *NLRC4*) gene expression in human CRC has also been suggested from retrospective metadata analysis of several publicly-available gene expression datasets (84). However, the same meta-analysis indicated that *NLRP1* mRNA levels were consistently significantly downregulated across multiple CRC patient datasets (84). These conflicting observations in human CRC are further epitomized by a reported analysis of The Cancer Genome Atlas indicating that the expression levels of *NLRP1*, *NLRP3*, and *NLRC4* in human CRC patient tumours were significantly lower versus colon tissue biopsies from healthy controls, while in other publicly-available datasets the expression profile of these inflammasome-associated NLRs was variable (85).

Very recently, the notion that NLRC4 is downregulated in human CRC has been further supported by the observation in multiple CRC patient cohorts that low NLRC4 mRNA and protein levels in the tumour epithelium, but not stroma, of CRC patients is associated with metastatic disease and poor overall survival (86). With respect to other cancers, recent studies have reported that low *NLRC4* gene expression was also a poor prognostic indicator in human lung cancers, and reduced *NLRP1* mRNA levels in tumours from multiple lung adenocarcinoma patient datasets representing distinct geographic regions were associated with poor survival outcomes (86,87). Interestingly, low *NLRP1* expression levels in the lungs of cancer patients coincided with *NLRP1* gene hypermethylation (87), which mimics the observed epigenetic silencing of *NLRP1* in cutaneous SCC patient biopsies (and correlation with hyper-methylation-induced suppression of ASC expression) (79).

AIM2- and pyrin-containing inflammasomes

As of writing this review article, there has been a paucity of studies investigating clinical associations between *MEFV* (i.e. pyrin) and cancer. Limited examples include increased *MEFV* gene expression in CRC patients tumours, as well as a preponderance for elevated frequencies of inherited *MEFV* gene variants in hematologic malignancies (e.g. multiple myeloma, acute lymphocytic leukemia, acute myeloid leukemia), albeit with unclear effects on pyrin expression (88,89).

By contrast, the clinical utility of AIM2 has been suggested in a range of human cancers. For instance, *AIM2* mRNA levels are upregulated and prognostic for poor survival in numerous cohorts of NSCLC patients (90,91). Similarly, AIM2 expression is upregulated and correlates with metastatic disease and/or poor prognosis in numerous other human solid tumour types, including those from patients with gastric and pancreatic cancers, and various SSCs (e.g. head and neck, cutaneous) (24,92-94). Conversely, AIM2 loss-of-function mutations and low expression levels are common in human CRC, where its reduced expression is linked to poor prognosis, suggesting a potential tumour suppressive function (85,95,96). Clinical observations for a potential tumour suppressor role of AIM2 in some cancers is also supported for human hepatocellular carcinoma (HCC), whereby reduced AIM2 mRNA

and protein expression levels are observed in liver tumour versus non-tumour tissues from independent HCC patient cohorts, and correlate with advanced stage metastatic disease and poor survival outcomes (97).

GENETIC TARGETING OF ASC AND INFLAMMASOME-ASSOCIATED PRRs IN PRECLINICAL MOUSE MODELS UNCOVERS CONTRASTING MECHANISMS OF ACTION OF INFLAMMASOMES IN CANCER

The divergent and often contrasting clinical findings for ASC and inflammasome-associated PRRs in many cancer types not only suggest a complex biological function of inflammasome complexes in the pathogenesis of cancer, but also the strong potential for opposing anti- and pro-tumourigenic activities, including within the same cancer type. Indeed, this next section will provide an overview of studies genetically targeting ASC and inflammasome-associated PRRs in preclinical mouse cancer models that have been employed to assess the broad and often contrasting mechanistic roles of inflammasomes in shaping the course of cancer over the multiple stages of tumour initiation, progression, invasion and metastasis (**Table 1**).

ASC

The contrasting roles of the ASC inflammasome adaptor in different cancer types are typified by studies investigating the effects of ASC genetic ablation in mouse models for gastrointestinal cancers. For example, in the genetically engineered *gp130*$^{F/F}$ spontaneous gastric inflammation-associated cancer mouse model, a tumour-promoting function for ASC-containing inflammasomes was reported, which interestingly was independent of gastric inflammation (36). Rather, the pro-tumourigenic function of ASC-containing inflammasomes was intrinsic to the gastric (tumour) epithelium via the selective processing and upregulated production of mature IL-18 (but not IL-1β), which in turn activated NF-κB signalling to abrogate caspase 8-mediated apoptosis in gastric epithelial cells (36). A tumour-promoting role for ASC has also been suggested in PDAC, whereby the growth of orthotopically implanted pancreatic tumour cells derived from the LSL-*Kras*$^{G12D/+}$; LSL-*Trp53*$^{R172H/+}$; Pdx-1-Cre genetic model for PDAC was reduced in mice deficient in either ASC or caspase-1 compared to wild-type recipients (98). However, since this study used established tumours in recipient mice, investigations on ASC were restricted to tumour progression only, and thus the role of ASC in pancreatic tumour initiation remains unknown. In addition, the dependence of ASC on the downstream processing of IL-1β versus IL-18 in pancreatic tumour cells or other cell types within the TME (e.g. immune cells, fibroblasts) during PDAC was not investigated, and thus remains ill-defined. In contrast to these findings in gastric and pancreatic cancers, a tumour-inhibiting role for ASC, and for that matter caspase-1, has been reported in the experimentally-induced azoxymethane (AOM) and DSS model of colitis-associated colon cancer (CAC) upon their genetic ablation in mice (99,100). Here, exacerbated inflammation-associated colon tumourigenesis upon ASC ablation was linked to the selective suppressed production of mature IL-18 (99,100). Mechanistically, the bimodal protective effect of the ASC/IL-18 inflammasome axis in the gut epithelium against CAC involved the 1) initial maintenance of epithelial barrier integrity to limit invading commensal microflora and subsequent gut inflammation, and 2) later inhibition of cellular proliferation in neoplastic regions of the colon epithelium.

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Table 1. Summary of preclinical mouse models of ASC and inflammasome-associated PRRs illustrating the diverse roles for various inflammasomes in a range of cancers

Gene	Cancer type	Model type	Function in cancer	Mechanism of action	Reference
ASC/ PYCARD	Gastric cancer	Global *Asc* knockout in genetic model of spontaneous gastric cancer	Tumour promoting	Inhibition of apoptosis of epithelial cells via IL-18	Deswaerte et al. (32)
ASC/ PYCARD	PDAC	Orthotopic xenograft of KPC cell lines in *Asc* knockout mice	Tumour promoting	Expansion of immunosuppressive macrophages in TME	Daley et al. (98)
ASC/ PYCARD	Skin cancer	Global, keratinocyte- or myeloid-specific *Asc* knockout in DMBA/TPA induced skin cancer model	Cell type dependant	Tumour promoting and pro-inflammatory in myeloid cells (via IL-1β) Tumour inhibiting in epithelial cells (via IL-18 regulated proliferation)	Drexler et al. (101)
ASC/ PYCARD	Colorectal cancer	Global *Asc* knockout in AOM/DSS induced CRC model	Protective/tumour inhibiting	Tumour suppressor via maintenance of microbiome homeostasis, epithelial barrier integrity and proliferation (via IL-18)	Allen et al. (99), Zaki et al. (100), Hu et al. (108)
NLRP3	Colorectal cancer	Global and myeloid specific knockout of *Nlrp3* AOM/DSS induced CRC model	Protective/tumour inhibiting	Tumour suppressor and anti-inflammatory function via cytokine regulation in myeloid cells	Allen et al. (99)
NLRP3	Colorectal cancer	Global *Nlrp3* knockout in AOM/DSS induced CRC model	No effect	NA	Hu et al. (104)
NLRP3	Colorectal cancer	Global *Nlrp3* knockout in AOM/DSS induced CRC model	Protective/tumour inhibiting	Maintenance of epithelial barrier integrity, anti-inflammatory, (via IL-18 activation of STAT1 pathway)	Zaki et al. (100)
NLRP3	Colorectal cancer	Gain-of-function mouse model, $Nlrp3^{R258W}$ in AOM/DSS induced CRC model	Protective/tumour inhibiting	Tumour suppressor in myeloid cells, anti-inflammatory regulation of gut microbiota (via IL-1β), Treg cell induction	Yao et al. (103)
NLRP3	Liver metastasis	MC38 transplantable CRC metastasis model and LLC lung metastasis model in *Nlrp3*-deficient mice	Protective/tumour inhibiting	Promotes NK cell maturation and activity via cancer cell production of IL-18	Dupaul-Chicoine et al. (107)
NLRP3	Skin cancer	Global *Nlrp3* knockout in DMBA and TPA-induced skin cancer model	Tumour promoting	Unclear	Chow et al. (105)
NLRP3	PDAC	Global *Nlrp3* knockout and pharmacological inhibition of NLRP3 in KPC genetic model of PDAC, and xenograft of KPC cell lines in *Nlrp3* knockout mice	Tumour promoting	Expansion of immunosuppressive macrophages and tumour promoting Th2, Th17 response in TME (via IL-1β)	Daley et al. (98)
NLRP1b	Colorectal cancer	Global *Nlrp1b* knockout mice and bone marrow reconstitution in AOM/DSS-induced CAC model	Protective/tumour inhibiting	Tumour suppressor in epithelial cells via inhibition of cell death and barrier dysfunction to maintain gut immune cell homeostasis	Williams et al. (84)
NLRP6	Colorectal cancer	Global *Nlrp6* knockout in AOM/DSS-induced CAC model	Protective/tumour inhibiting	IL-18 mediated maintenance of gut microbiome homeostasis, and inhibition of IL-6 dependant epithelial cell proliferation	Hu et al. (108)
NLRC4	Breast cancer	Orthotopic xenograft paired with high fat diet in *Nlrc4* deficient mice (Py8119, E0771 cells)	Tumour promoting	Promoting angiogenesis and tumour growth via IL-1β production of tumour associated macrophages	Kolb et al. (109)
NLRC4	CRC Liver metastasis	Orthotopic xenograft paired with high fat diet in *Nlrc4* deficient mice (MC38 cells)	Tumour promoting	Promoting angiogenesis and tumour growth via IL-1β production of tumour associated macrophages	Ohashi et al. (110)
AIM2	Hepatocellular carcinoma	DEN-induced HCC in *Aim2* deficient mice	Tumour promoting	Driving inflammation and proliferation via IL-1β production	Martínez-Cardona et al. (113)
AIM2	Hepatocellular carcinoma	Xenograft with AIM2-overexpressing HCC cells	Protective/ tumour inhibiting	Tumour suppressor via inhibition of the mTOR pathway	Ma et al. (114)
AIM2	PDAC	*Aim2* knockout in KPC model of PDAC	Tumour promoting	Unclear	Li et al. (93)
Pyrin	Colorectal cancer	Pyrin (*Mefv*) knockout in AOM/DSS-induced CAC	Protective/tumour inhibiting	Maintenance of barrier integrity, regulate microbe-induced inflammation (via IL-18)	Sharma et al. (88)

DEN, diethylnitrosamine; KPC, $Kras^{G12D/+}$; $Trp53^{R172H/+}$; P48-Cre; LLC, Lewis lung carcinoma.

The complex and paradoxical roles of ASC in the context of inflammasomes are also evident within a specific cancer type, a key example being epithelial skin carcinogenesis. By coupling conditional cell type-specific ASC knockout mice with a 2-step chemically induced (7,12-Dimethylbenz(a)anthracene [DMBA] and 12-O-Tetradecanoylphorbol-13-acetate [TPA]) inflammation-associated skin carcinogenesis model, a tumour-promoting role was assigned for ASC-containing inflammasomes in myeloid cells, whereas a tumour-inhibiting role for ASC was observed in keratinocytes (i.e. skin epithelium) (101). The opposing functions of these ASC-containing inflammasome complexes in skin carcinogenesis were mediated by, at

least in part, their differential usage of the IL-1β (in myeloid cells) and IL-18 (in keratinocytes) effector cytokines. Indeed, these contrasting tumourigenic activities of IL-1β and IL-18 downstream of ASC-containing inflammasomes are most likely influenced by differences in their transcriptional regulation (i.e. inducible versus constitutive), expression of receptor subunits and accessory proteins, and activation of signalling pathways, in distinct immune versus non-immune cell types within an organ (40,102).

The opposing effects of ASC within a specific cancer type can also be influenced by disease stage, as has been observed in melanoma. In a study using primary human melanoma cell lines, shRNA-mediated knock-down of ASC increased cell viability and proliferation *in vitro*, and the growth of tumour xenografts following injection of ASC-silenced cell lines into nude mice was augmented (77). Conversely, in the same study ASC knock-down in metastatic human melanoma cell lines suppressed cell viability and proliferation *in vitro*, as well as impaired *in vivo* growth of tumour xenografts. Although maturation of caspase-1 (i.e. inflammasome activation) was similarly impaired upon ASC knock-down in both primary and metastatic human melanoma cell lines, the disease stage-dependent opposing roles for ASC coincided with ASC-mediated differential activation of the IL-1 receptor/NF-κB signalling cascade in primary (low activity) versus metastatic (high activity) melanoma (77). As discussed further in the next section below on NLR-containing inflammasomes, the interpretation of these findings needs to be tempered by the limitations on using immortalized cancer cell lines in which the expression of genes of interest (e.g. *PYCARD*) are artificially manipulated. Nonetheless, for ASC, it remains to be seen whether such a stage-dependent mechanism will apply to other cancer types, or whether it is restricted to melanoma.

In addition to these opposing pro- and anti-tumourigenic roles for ASC, it was recently reported that in the $Kras^{G12D}$ mouse model of NSCLC, genetic deficiency of ASC had no effect on inflammasome activation (i.e. caspase-1 and IL-1β maturation) nor tumour formation in the lungs of mice (90). It will therefore be of interest for future studies to assess whether this observation is specific for Kras-mutant lung carcinogenesis by coupling the genetic ablation of ASC with mouse models for other subtypes of lung cancer (e.g. small cell lung cancer).

NLR-containing inflammasomes

The contrasting tumour-promoting and -inhibiting activities of ASC are also a common theme among inflammasome-associated NLR family members in cancer, the best characterized being NLRP3. Indeed, at the time of writing this review, a search on PubMed® using the terms "NLRP3," "inflammasomes," and "cancer" yielded over 1,150 results. However, the caveat with many published wet-lab based studies examining the role of NLRP3 in cancer is that they have been performed exclusively on immortalized cancer cell lines in which NLRP3 expression has been artificially manipulated (knocked down or overexpressed). In addition, most studies fail to consider that the mechanistic actions of NLRP3, and for that matter other inflammasome-associated PRRs (and ASC), in cancer are influenced by cellular interactions between cancer cells and other cell types (e.g. innate and adaptive immune cells) found within the native TME. This latter point is also a limitation with many *in vivo* studies in cancer that explore the role of NLRP3 when artificially expressed outside of its native cellular and tissue environment, as is exemplified by subcutaneous xenografts derived from immortalized cancer cell lines grown in immunodeficient mice (e.g. athymic nude mice lacking T cells). For these reasons, this section will largely focus on *in vivo* studies employing *bona fide* preclinical spontaneous or experimentally-induced cancer models harbouring the genetic manipulation of endogenous NLRP3 (and other inflammasome-associated NLRs).

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The opposing, and sometimes confounding, roles of NLRP3 in cancer are best evidenced by preclinical studies on CRC using *Nlrp3*$^{-/-}$ mice. Several studies using the AOM/DSS-induced CAC model have reported that NLRP3 deficiency in mice exacerbated colonic inflammation-associated tumourigenesis—similar to observations in ASC-deficient mice—leading to the notion that the NLRP3 inflammasome (via IL-18) acts as a tumour suppressor in CRC (99,100). Furthermore, by generating reciprocal bone marrow chimeras between *Nlrp3*$^{-/-}$ and wild-type mice, it was reported that NLRP3 anti-cancer activity in myeloid-lineage hematopoietic cells coincided with the impaired production of tumour-promoting cytokines and chemokines (99). A tumour-inhibiting function for NLRP3 in CRC is also supported by the ameliorated AOM/DSS-induced CAC phenotype in mice carrying a gain-of-function *Nlrp3*^{R258W} gene mutation (103). However, while the tumour-suppressing activity of NLRP3^{R258W} was again aligned to NLRP3 expression in myeloid-lineage cells of the colon, hyper-activity of NLRP3^{R258W} inflammasomes triggered the preferential upregulation of mature IL-1β (not IL-18) to augment production of anti-microbial peptides, which in turn orchestrated an anti-inflammatory gut microbiota via immunosuppressive Treg cells (103). While these data collectively suggest that NLRP3 inflammasomes can exert multiple and diverse anti-tumour activities in CRC via their differential usage of IL-18 and IL-1β effector cytokines, some caution is warranted when interpreting these findings since the AOM/DSS-induced CAC model is known for experimental discrepancies due to variable gut microbiota present in different mouse facilities and/or differences in the genetic background of mice. Indeed, this is highlighted by another study using *Nlrp3*$^{-/-}$ mice (from a different laboratory to that of Allen et al. (99)) reporting that genetic ablation of NLRP3 in mice had no effect on AOM/DSS-induced CAC (104). On this note, the coupling of *Nlrp3*$^{-/-}$ mice with other preclinical cancer models, including those for asbestos-induced mesothelioma and gastric cancer, has suggested that the NLRP3 inflammasome is dispensable for distinct cancer types (105,106).

In addition to the role of NLRP3-cotaining inflammasome complexes in suppressing the development of primary tumours in the colon, NLRP3 can also suppress the metastatic outgrowth of CRC tumours to the liver. Using a transplantable CRC model (MC38 murine primary colon carcinoma cells) of liver metastasis on a syngeneic C57BL/6 background, *Nlrp3*$^{-/-}$ mice (but not *Aim2*$^{-/-}$ or *Nlrc4*$^{-/-}$ mice) displayed a markedly higher metastatic tumour burden in the livers compared to wild-type counterparts (107). In addition to the anti-cancer activity of the NLRP3 inflammasome/IL-1β axis in immune cells in primary CRC, the NLRP3 inflammasome protected against CRC liver metastasis in a cancer cell intrinsic manner dependent on IL-18. Specifically, tumour cell-derived IL-18 promoted the maturation and tumouricidal activity of NK cells by upregulating FasL surface expression to facilitate NK cytotoxicity against metastatic liver growth, and this NLRP3 inflammasome-mediated immunosurveillance was also functional in the metastatic Lewis lung carcinoma model (107). These observations provide further evidence, along with those previously discussed for ASC in models of skin cancer, of how inflammasomes can have diverse roles within a specific cancer type which can be modulated by differences in cell type and disease stage.

Intriguingly, the NLRP3 inflammasome can also act as a tumour promoter in distinct cancers. For example, in the DMBA/TPA-induced inflammation-associated skin carcinogenesis model, *Nlrp3*$^{-/-}$ mice displayed a heightened resistance to the development of skin lesions (105). This protection from skin carcinogenesis upon NLRP3 deficiency mirrors the elevated resistance of myeloid-specific ASC knockout mice to the same chemically-induced skin cancer model (101), suggesting that myeloid-derived NLRP3 is a major tumour-promoting inflammasome-associated PRR in skin carcinogenesis. In a similar vein, the impaired

pancreatic tumour progression observed upon orthotopic implantation of PDAC tumour cells into ASC-deficient recipient mice was also reported for orthotopically implanted PDAC cells in *Nlrp3*$^{-/-}$ recipients, thus implicating NLRP3-containing inflammasomes in supporting PDAC progression (98). Here, NLRP3 activation in myeloid-lineage cells (i.e. macrophages) supported an immunosuppressive TME and modulated the balance of T cell polarization towards tumour-promoting Th2, Th17 and Treg cells, which highlights the potential of innate immunity (via inflammasomes) to shape adaptive immune-mediated anti-tumour responses. In PDAC, the NLRP3 inflammasome/IL-1β axis has also been shown to promote tumourigenesis in a cancer cell intrinsic manner, whereby tumour-derived IL-1β production shaped an immunosuppressive TME in the pancreas characterized by the expansion of both innate (e.g. tumour-associated macrophages, myeloid-derived suppressor cells [MDSCs]) and adaptive (e.g. Th17) immune cell types (35).

With respect to other inflammasome-associated NLR family members, independent studies have reported that mice deficient in either NLRP1b, NLRP6 or NLRC4 are more susceptible to AOM/DSS-induced CAC, consistent with these NLRs also playing a tumour suppressor role in CRC, albeit by distinct mechanisms which remain to be fully elucidated (84,104,108). In this regard, using reciprocal bone marrow chimeras between *Nlrp1b*$^{-/-}$ and wild-type mice, it was proposed that the anti-tumour activities of NLRP1b are intrinsic to the colonic epithelium where NLRP1b-containing inflammasomes promote immune homeostasis in the gut by protecting against cell death and barrier dysfunction of the mucosal epithelium (84). The exacerbated CAC phenotype of *Nlrp6*$^{-/-}$ mice was associated with impaired IL-18 production, leading to gut dysbiosis and a subsequent chronic inflammatory response driven by CCL5 which in turn augmented IL-6 signalling, the latter driving proliferation of the colonic epithelium to promote tumourigenesis (108). These observations suggest that NLRP6, like NLRP1b, also maintains gut immune homeostasis by preventing dysbiosis of the intestinal microbiome, yet via a molecular circuit involving crosstalk between the NLRP6 inflammasome/IL-18 axis, chemokine and cytokine networks. By contrast, the anti-tumourigenic function of NLRC4 in CRC was independent of changes in inflammation, but rather was attributed to impairing proliferation and augmenting apoptosis of colonic epithelial cells - in response to gut commensal microbes - in a tumour (epithelial) cell intrinsic manner (104).

By contrast, the coupling of *Nlrc4*$^{-/-}$ mice with syngeneic orthotopic transplant models for high-fat diet-induced primary breast cancer and CRC liver metastasis revealed that NLRC4 deficiency impaired the progression of tumours (109,110). In both scenarios, the tumour-promoting activity of the NLRC4 inflammasome was localized to myeloid-derived tumour-associated macrophages and facilitated by the preferential production and release of IL-1β, which in turn upregulated the expression of angiogenic factors (e.g. vascular endothelial growth factor) to drive angiogenesis and support tumour growth. These observations suggest that the NLRC4 inflammasome/IL-1β axis can also promote tumourigenesis in certain cancers, and potentially those with a link to obesity.

AIM2- and pyrin-containing inflammasomes

AIM2 has also been reported to display diverse and contrasting roles in various cancer types, albeit with the caveat that many of the tumour-related activities of AIM2 reported thus far have been independent of inflammasomes. In this respect, we refer readers to the following articles which cover in depth the inflammasome-independent mechanisms of action—tumour-promoting and tumour-inhibiting—of AIM2 in numerous *in vivo* cancer models (e.g. CRC, gastric cancer, NSCLC) (14,24,90,111,112).

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The role of AIM2-containing inflammasomes in their native cellular and tissue contexts in cancer is ill-defined, largely due to a paucity of preclinical *in vivo* studies using *bona fide* spontaneous or experimentally induced cancer models coupled to genetic modulation of endogenous AIM2. Among the few such studies, in the diethylnitrosamine-induced experimental model for HCC, the formation of liver tumours in *Aim2*$^{-/-}$ (as well as *Casp1*$^{-/-}$) mice was significantly lower compared to wild-type mice, and coincided with reductions in hepatic damage, inflammation and cellular proliferation, as well as caspase-1 activation and IL-1β production (113). Furthermore, it was reported that the primary cellular source of elevated AIM2 inflammasome activation (i.e. upregulated AIM2 expression, caspase-1 activation and IL-1β production) in the liver during early stages of HCC was Kupffer cells (resident liver macrophages). While these observations are consistent with a role for AIM2 inflammasomes in promoting the onset of inflammation-associated liver carcinogenesis, we note that this contrasts clinical data supporting a potential tumour suppressor role for AIM2 in human HCC (97). In this respect, the artificial over-expression of AIM2 in established xenografted tumours derived from immortalised human HCC cell lines has been shown to suppress tumour growth, and the anti-tumour effects of AIM2 inflammasome activity aligned with blockade of the mTOR pathway which is implicated in many cancers (114). These contrasting roles for AIM2 inflammasomes in HCC are reminiscent of those for ASC in various cancer models and may also be attributed to disease stage-specific (i.e. established versus onset) and cell type-specific (i.e. cancer cells versus Kupffer immune cells) differences in AIM2 inflammasome activation in HCC.

AIM2 inflammasomes may also play a role in promoting PDAC, as suggested by genetic ablation of AIM2 in an oncogenic Kras-driven genetic model for PDAC which ameliorated pancreatic tumourigenesis and was associated with lower serum levels of IL-1β (93). However, these results need to be treated with some caution because the authors did not report caspase-1 activation levels in pancreatic tumours, nor did they assess other indicators of inflammasome activity, such as ASC speck formation. Furthermore, the treatment of this PDAC mouse model with neutralizing Abs against either IL-18 or IL-1β had no effect on pancreatic tumourigenesis (93), thus leading to uncertainty as to whether AIM2 via these inflammasome effector cytokines is truly implicated in this model of pancreatic tumourigenesis.

With respect to pyrin in cancer, despite again there being limited *in vivo* preclinical studies, pyrin-containing inflammasomes have been reported to protect against CAC (88). Specifically, exacerbated AOM/DSS-induced CAC in *Mefv*$^{-/-}$ mice was associated with impaired inflammasome-driven mature IL-18 production, leading to compromised integrity of the colon epithelial barrier and the increased likelihood of microbial-driven, tumour-promoting intestinal inflammation (88).

INFLAMMASOME THERAPEUTIC TARGETING STRATEGIES, AND CONCLUSIONS

The knowledge gained over the last decade relating to the diverse and contrasting pro- and anti-tumourigenic activities of inflammasomes highlights the need for caution when considering the clinical implementation of therapeutic targeting of inflammasome complexes in specific cancers. Although the direct targeting of ASC or inflammasome-associated PRRs in cancer has yet to successfully progress to the clinic, preclinical studies using the first-in-class small molecule inhibitor MCC950 have provided proof-of-concept

that the tumour-promoting activity of NLRP3 may be pharmacologically targeted in some cancer types (e.g. PDAC, head and neck SCC (14,115). In one study on head and neck cancer, MCC950 treatment reduced tumour burden and circulating IL-1β levels which coincided with lower numbers of MDSCs, tumour-associated macrophages and Treg cells in tumours, suggesting that the pharmacological blockade of NLRP3 can modulate the cellular milieu of the TME (116). The *in vivo* anti-cancer activity of MCC950 also extends to its use in combination with anti-PD-1 immune checkpoint inhibitors in several preclinical tumour models. Specifically, Ab-mediated PD-1 blockade activated the NLRP3 inflammasome which promoted MDSC recruitment into tumours and dampened the efficacy of immunotherapy. However, MCC950 significantly boosted the response to anti-PD-1 immunotherapy which coincided with abrogated MDSC tumour infiltration and elevated levels of tumour-infiltrating $CD8^+$ T cells, leading to marked reduction in tumour growth (117).

More recently, next-generation NLRP3-specific small molecule inhibitors with enhanced bioavailability, pharmacokinetics and pharmacodynamics have also been successfully deployed as anti-cancer agents in preclinical mouse models. For example, oral administration of the OLT1177® NLRP3 inhibitor can suppress tumour growth in mouse models for melanoma, metastatic breast cancer and PDAC by activating anti-tumour immunity (e.g. expansion of cytotoxic $CD8^+$ T cells, reduction in MDSCs) to overcome an immunosuppressive TME (118-120). Moreover, combination of OLT1177® with either immune checkpoint inhibitors (e.g. anti-PD-1 in metastatic breast cancer and melanoma) or standard-of-care chemotherapy (e.g. gemcitabine in PDAC) enhanced the anti-tumour efficacy of these monotherapies, indicating the potential for OLT1177® as a therapeutic adjuvant in specific cancers (118-120). In the context of chemotherapy, these observations for OLT1177® build on an earlier report that in various syngeneic mouse cancer models (e.g. thymoma, lung cancer, melanoma), the administration of gemcitabine and 5-fluorouracil activated NLRP3-dependent IL-1β production in MDSCs which abrogated the anti-cancer efficacy of these chemotherapeutics by supporting an immunosuppressive TME (121). Moreover, the potent anti-cancer effects of these chemotherapeutics were restored in either in *Nlrp3*$^{-/-}$ (or *Casp1*$^{-/-}$) mice. Interestingly, targeting inflammasomes may also assist in preventing side-effects associated with chemotherapeutics. For example, doxorubicin-driven bone loss and leukopenia in tumour-free wild-type mice is associated with activation of myeloid-derived NLRP3 (and AIM2) inflammasomes resulting in marked pyroptotic immune cell death, and these doxorubicin-induced pathologies are ameliorated in *Aim2*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice (122). Collectively, these findings highlight the potential utility of targeting inflammasomes to augment the efficacy and safety of chemotherapeutics.

It is also worth noting other strategies to therapeutically target inflammasome complexes. In the absence of specific inhibitors against other inflammasome-associated PRRs, a potential therapeutic avenue to directly block ASC itself in the context of inflammasomes has been realized by the advent of anti-ASC camelid-derived nanobodies. Specifically, intracellular inflammasome activation leads to the cellular release and accumulation of inflammation-inducing extracellular ASC-containing aggregates (i.e. specks) which are observed in inflamed tissues and body fluids of both infectious and inflammatory mouse disease models, as well as in patients presenting with autoimmune pathologies (50). Recently, anti-ASC nanobodies were shown to target and neutralize the pro-inflammatory activity of extracellular ASC specks. Furthermore, the prophylactic and/or therapeutic systemic administration of anti-ASC nanobodies to mouse disease models of gout and Ag-induced arthritis ameliorated the onset and/or progression of these inflammatory pathologies, thus providing the first

preclinical evidence that disease-associated activities of ASC-containing inflammasomes in chronic inflammatory and autoimmune diseases can be therapeutically blocked (123). This seminal finding now paves the way to explore whether extracellular ASC specks are detectable in cancer, and if so, can the administration of anti-ASC nanobodies possess anti-cancer activity in preclinical cancer models.

We also note that therapeutic agents against IL-1β and IL-18 present as indirect approaches to block the effects of dysregulated inflammasome activation in disease, including in cancer, which have been covered in detail elsewhere (14,124,125). In this respect, targeting IL-1β or IL-18 in cancer is likely to face the same challenges as strategies to target ASC and specific inflammasome-associated PRRs. These include navigating whether these cytokines display anti- or pro-tumourigenic activities in specific cancer types, and if altered production of these cytokines in cancer is aligned to dysregulated inflammasome activity or inflammasome-independent mechanisms (e.g. cathepsins).

In summary, despite the promise of these early preclinical findings in *in vivo* cancer models, it remains to be seen whether therapeutic approaches to block the activity of NLRP3, and for that matter ASC and other inflammasome-associated PRRs, will translate to the clinic. Indeed, considering the likelihood from clinical data and preclinical models that inflammasomes also exert anti-cancer activity in certain cancer settings (e.g. CRC), it will be prudent to consider the development of inflammasome-related PRR agonists - based on disease-associated PAMPs and/or DAMPs - as immune adjuvants to bolster the efficacy of anti-cancer therapeutics in specific cancer types. The knowledge that opposing inflammasome activities in cancer are governed by several factors, including cell type-specificity (e.g. immune versus epithelial cell), will also require future therapeutic approaches to incorporate emerging cell- and organ-specific delivery systems (e.g. nanoparticles). As our collective understanding builds on the clinical importance of specific inflammasomes and their downstream effectors and molecular pathways in cancer, this promises to enable identification of biomarkers for individual inflammasomes to advance a new area of innate immune-based precision medicine in oncology.

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