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Serum amyloid A and inflammasome activation: A link to breast cancer progression?

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ABSTRACT

Breast cancer is the most frequently diagnosed cancer in women globally. Although there have been many significant advances made in the diagnosis and treatment of breast cancer, numerous unresolved challenges remain, which include prevention, early diagnosis, metastasis and recurrence. The role of inflammation in cancer development is well established and is believed to be one of the leading hallmarks of cancer progression. Recently, the role of the inflammasome, a cytosolic multiprotein complex, has received attention in different cancers. By contributing to the activation of inflammatory cytokines the inflammasome intensifies the inflammatory cascade. The inflammasome can be activated through several pathways, which include the binding of pattern associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) to toll-like receptors (TLRs). Serum amyloid A (SAA), a non-specific acute-phase protein, can function as an endogenous DAMP by binding to pattern recognition receptors like TLRs on both breast cancer cells and cancer associated fibroblasts (CAFs). SAA can thus stimulate the production of IL-1β, thereby creating a favourable inflammatory environment to support tumour growth. The aim of this review is to highlight the possible role of SAA as an endogenous DAMP in the tumour microenvironment (TME) thereby promoting breast cancer growth through the activation of the NLRP3 inflammasome.

1. Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer-related deaths among women worldwide (DeSantis et al., 2019). Although there have been many significant advances made in the diagnosis and treatment of breast cancer, numerous unresolved challenges remain, which include prevention, early diagnosis, metastasis and recurrence [1]. Breast cancer is a heterogeneous disease both clinically and at a molecular level, which contributes to the complexity of resolving these problems [2]. Carcinogenesis is the result of multiple processes which include genetic instability, proliferation abnormalities, reprogramming of the tumour microenvironment (TME), differentiation between epithelial and mesenchymal states and chronic inflammation.

It is well established that the onset and development of cancer is associated with the upregulation of pro-inflammatory cytokines and an inflammatory response. One such cytokine is interleukin-1 beta (IL-1β) which has been reported to be increased in primary breast tumours [3]. IL-1β is produced as an inactive precursor called pro-IL-1β and a multi-step inflammasome activation process is required for the processing of pro-IL-1β to active IL-1β [4]. Activation of the inflammasome has been reported in certain types of cancers such as prostate and lung cancer [5,6]. Serum amyloid A (SAA), an acute-phase protein primarily produced by hepatocytes and adipose tissues [7], plays a potential role in tumour promotion by binding to pattern recognition receptors (PRRs)

Abbreviations: CAF, cancer associated fibroblast; COX-2, cyclooxygenase-2; DAMP, damage associated molecular pattern; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; IL-1β, interleukin 1 beta; GSDMD, gasdermin D; MAPK, mitogen activated protein kinase; MMP-9, matrix metallopeptidase 9; NLRP3, nod-like receptor 3; PAMP, pathogen associated molecular pattern; PRR, pattern recognition receptor; TLR, toll like receptor; TME, tumour microenvironment; SAA, serum amyloid A.

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like toll-like receptors (TLRs) on several cell types, including immune cells like macrophages and neutrophils [8,9]. However, the role of SAA in inflammasome activation in breast cancer remains unclear. The aim of this review is therefore to highlight the possible role of SAA as an endogenous damage associated molecular pattern (DAMP) within the TME, thereby promoting breast cancer growth through activation of the inflammasome.

2. Mechanisms of inflammasome activation

The inflammasome is a multi-protein complex characterised by a nucleotide-binding and oligomerisation domain (NOD)-like receptor (NLR), an adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD domain) and procaspase-1 [10]. Surveillance of homeostatic parameters by NLRs allows the inflammasome to respond to a wide range of stimulatory PAMPs and DAMPs, including microbial DNA, RNA, cell wall components, endogenous factors and environmental contaminants. While several NLRs have been identified, inflammasomes containing the NLR family pyrin domain containing 3 (NLRP3) are the best characterised.

NLRP3 Inflammasome activation is a two-step process triggered by various PAMPs and DAMPs. The first step, referred to as the "**priming signal**", is when TLRs are auto-phosphorylated by exposure to PAMPs or DAMPs, which results in in nuclear factor kappa beta (NFκB) activation. This nuclear factor stimulates the transcription and expression of NLRP3 inflammasome components, pro-IL-1β and pro-IL-18, the level of which is otherwise relatively low in many cell types. The second step, referred to as "**activation**" is where the functional NLRP3 inflammasome is activated by initiating the assembly of the multi-protein complex in the cytosol, leading to caspase-1 activation and IL-1β and IL-18 maturation [4]. Caspase-1 also cleaves gasdermin D (GSDMD), generating a N-terminal fragment that translocates from the cytosol to the plasma membrane and forms pores through which IL-1β and IL-18 are secreted. However, excessive pore formation during infectious and sterile conditions can also result in cell rupturing (referred to as pyroptosis), resulting in cell death and the release of pro-inflammatory contents like IL-1β, IL-18 and certain DAMPs into the extracellular environment [11]. Other IL-1 family members can potentially be secreted following inflammasome activation, including IL-1α and IL-37, which is supported by the finding that a caspase-1 site also exists for IL-33 and IL-37 precursors. However, non-caspase mechanisms have shown to generate active forms of these cytokines ([12]; Chan & Schroder, 2019). In contrast to IL-1β, not much is known about the activation and secretion of IL-1α, a close homologue to IL-1β. It is suggested that IL-1α serves as a danger signal which is passively released from dying cells, but it has also been shown that NLRP3 inflammasome agonists such as uric acid crystals induces IL-1α cleavage and secretion, resulting in the co-secretion of both IL-1β and IL-1α [13]. For the purpose of this review, IL-1β will be elaborated on because of its significant association with breast cancer progression.

The main mechanisms that control NLRP3 inflammasome activation are as follows: the **channel model**, which is triggered by extracellular ATP that is released at the site of cellular injury. ATP activates the P2 × 7 ATP-gated ion channel, causing a rapid K^+ efflux from the cell, which is a requirement for inflammasome activation [14,15]. The **lysosome rupture model**, which is relevant for inflammasome activation by large particulate activators such as alum and silica crystals, which can often be inhaled. Inefficient clearance of such particulate matters leads to lysosome rupture, resulting in the release of cathepsin-B into the cytoplasm which will trigger the activation of the inflammasome [16]. The third model involves the **generation of ROS**. All NLRP3 inflammasome activators, including ATP and particulate matters, trigger the generation of short-lived ROS, while treatment with ROS scavengers has been shown to block the activation (Fig. 1). These mechanisms are not exclusive from each other, for example, lysosomal rupture could result in the generation of ROS and there could be other yet unknown mechanisms leading to the activation of the inflammasome.

IL-1 cytokines play important roles in regulating the inflammatory processes by binding to IL-1 receptors (IL-1R) on most cell types [17]. Upon activation, mature secreted IL-1β binds to and activates IL-1R1 on

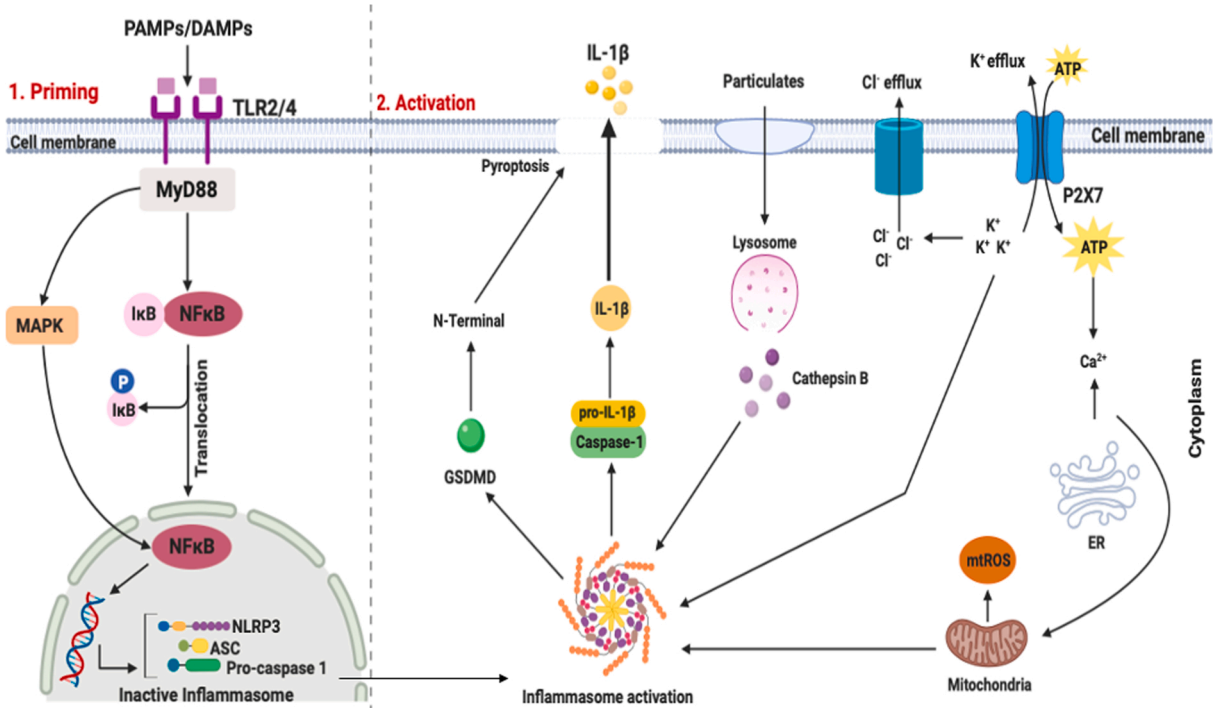

Fig. 1. Priming and activation of the NLRP3 inflammasome. During the priming step, PAMPs or DAMPs bind to TLRs on the cell membrane. Once binding occurs, it induces the downstream activation of NFκB *via* MyD88, either by phosphorylating IκB or by activating MAPKs, resulting in transcriptional upregulation of inflammasome components including NLRP3 and procaspase. During the activation step, oligomerization of the inflammasome into its active state takes place through various signaling pathways, allowing pro-IL-β to bind to caspase-1, resulting in the production and secretion of IL-1β [4].

cell surfaces (such as immune and cancer cells) in an autocrine or paracrine manner [18]. Activation of cytosolic toll- and IL-1R-like domains result in the recruitment of MyD88 and interleukin-1 receptor associated kinase-4 (IRAK4). IRAK4 undergoes autophosphorylation, which results in the phosphorylation of IRAK1 and IRAK2 and the recruitment of TNF receptor-associated factor 6 (TRAF6). IRAK1/2 and TRAF6 then dissociate from the receptor complex and activate NFκB. Once NFκB has translocated to the nucleus, it can induce the transcription of other inflammatory genes, such as *interleukin-6* (*IL-6*), *interleukin-8* (*IL-8*) and *cyclooxygenase-2* (*COX2*) [19] (Fig. 2).

3. Inflammasome activation in cancer

Activation of NLRP3 inflammasome has been reported to occur in several malignancies including head and neck carcinoma, glioblastoma, lung, colon and breast cancer [20–22] reported NLRP3 inflammasome activation in A549 lung cancer cell lines triggered by *lipopolysaccharide* (LPS) and ATP, which significantly increased caspase-1 and IL-1β protein expression when compared to the control or LPS and ATP single-treated group. Inhibiting IL-1β activity with IL-1R antagonist (IL-1Ra) also diminished the proliferative capacity of these cancer cells. Similarly, in an *in vitro* glioblastoma model, inflammasome activation with ATP significantly increased IL-1β expression, which was significantly reduced when NLRP3 expression was suppressed [23]. In breast cancer patients, IL-1β levels positively correlated with a high rate of cancer recurrence, where it is believed to promote tumour growth, angiogenesis, invasion and metastasis [24]. In 2016, Guo et al. reported that the inflammasome as well as IL-1β play important roles in the promotion of tumour growth and metastasis in breast cancer [25]. In this study they showed that in both NLRP3-deficient and caspase-1-deficient tumour mouse models, primary tumour growth was significantly reduced when compared to the wild type controls which had high circulating IL-1β levels. Furthermore, by blocking IL-1R (with an IL-1R antagonist), tumour growth and spontaneous metastasis were reduced in caspase-1-deficient mice while wild type tumour-bearing mice treated with IL-1Ra had a reduced number of tumour nodules in the lungs. Taken together, these results indicate that inflammasome activation and IL-1β production may create favourable microenvironments for tumour metastasis.

Activation of the NLRP3 inflammasome associated with increased IL-1β production and inflammatory cell infiltration in cardiac fibroblasts has previously been reported in myocardial dysfunction, however, it has not been extensively investigated in cancer associated fibroblasts (CAFs), until recently [26]. In 2019, Ershaid and colleagues reported that the NLRP3 inflammasome is upregulated in human breast CAFs and

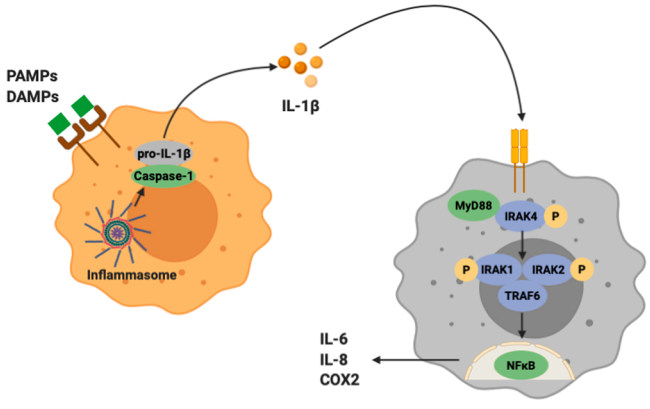

Fig. 2. IL-1β signaling in cells. IL-1β signaling is initiated by the binding of PAMPs and DAMPs to receptors on the cell membrane, resulting in the activation of the inflammasome. Following cleavage and activation, IL-1β is then released by the cell and binds to IL-1R1 on another cell, inducing NFκB-mediated transcription of pro-inflammatory cytokines.

that these fibroblasts can function as DAMP sensors. The authors also observed that multiple genes involved in the NLRP3 inflammasome pathway, including *caspase-1* and *IL-1β*, were upregulated in CAFs but not in fibroblasts isolated from normal mammary tissue. Apart from breast cancer cells, NLRP3 inflammasome components (NLRP3, caspase-1 and IL-1β) were also upregulated in breast stroma. The authors further indicated that CAF-derived inflammasome signaling promoted tumour growth in an *in vivo* mouse model [27]. Based on these observations on upregulation of IL-1β in several models and the signaling pathways associated with its secretion, it is plausible that inflammasome activation promotes breast cancer growth and development.

4. Toll-like receptor activation in breast cancer

TLRs are a family of receptors that are expressed on antigen presenting cells, like macrophages and dendritic cells, as well as fibroblasts and epithelial cells and play an important role in immune responses against infection [28]. Recent evidence indicates that TLRs are also expressed in cancer cells where it is linked to tumour proliferation and survival and the ability to alter the TME to promote tumourigenesis [29]. However, the role of TLRs in cancer development remain controversial since both anti- and pro-tumour responses are reported in literature.

Ten TLRs (TLR1−10) have been identified in humans and are classified into two subgroups, depending on their cellular localisation [30]. TLRs 1, 2, 4, 5, 6 and 10 are located on the cell surface and respond to PAMPs from micro-organisms, including lipids and bacterial proteins, or DAMPs from damaged tissues to activate innate and adaptive immune responses. TLRs 3, 7, 8 and 9 are located intracellularly in endosomes and primarily respond to nucleic acids [31]. Mammalian TLRs are comprised of an extracellular domain that contains leucine-rich repeats (for ligand binding), a transmembrane region and a cytoplasmic toll/interleukin-1 receptor (TIR) domain, which is required for intracellular signaling [32]. Receptor activation bridges two TLR molecules at the ectodomain to form a dimer with the TIR domain to activate downstream signaling. This TIR-mediated signaling involves TLR adaptor proteins, including MyD88, toll/interleukin-1 receptor domain-containing adapter protein (TIRAP), TIR-domain-containing adapter-inducing interferon-β (TRIF) and TRIF-related adaptor molecule (TRAM). TLR-ligand binding activates NFκB through the IKK complex, but various other signaling pathways capable of inducing pro-tumourigenic responses can also be activated upon ligand binding, including p38 and ERK1/2 [33,34].

As mentioned previously, diverse conditions can promote NLRP3 inflammasome assembly and a large number of receptors, including PRRs, control NLRP3 priming. Among the PRRs, TLRs, NOD2 and receptor for advanced glycation end products (RAGE) can prime NLRP3 for further activation. The overexpression of TLRs has been reported in colon, breast, prostate, lung as well as in ovarian cancer (Fukata et al., 2007; [35,36]). For the purpose of this review the focus is on TLR2 and TLR4 because of its reported association with the NLRP3 inflammasome. TLR4 is linked to prostate and ovarian cancer and was associated with a more aggressive disease outcome and increased metastatic potential [36,37]. The activation of NFκB, PI3K/Akt, COX2 and epidermal growth factor receptor signaling (EGFR) significantly contributed to the pro-tumourigenic properties of TLR4.

The TLR4 signaling pathway can be stimulated by various ligands, including PAMPs and DAMPs. It has been suggested that DAMPs play a larger role in activating TLR4. This is an important factor to consider in breast cancer development because of its highly pro-inflammatory TME, where various DAMPs are constantly secreted [38]. It was reported in previous studies that TLR4 expression is increased when compared to other TLRs in human breast cancer and that the knockdown of TLR4 inhibits proliferation and survival of MDA-MB-231 breast cancer cells [39,40]. In a different study, the authors also observed that TLR4 and

MyD88 was expressed in MCF-7 and MDA-MB-231 breast cancer cells and that the expression was increased once the cells were stimulated with LPS [41]. Apart from TLR4 expression in breast cancer, Xie et al. investigated whether TLR2 is differentially expressed in MDA-MB-231 and MCF-7 cells, to determine its role in NFκB activation and whether TLR2 promotes cancer cell invasion [42]. Their findings showed that the expression of TLR2 was present in both cell lines, but greater expression was observed in the highly metastatic and invasive MDA-MB-231 cell line. Blocking TLR2 with a neutralizing antibody decreased the number of invasive cells in the MDA-MB-231 cell line, thereby indicating that TLR2 plays an important role in triple negative breast cancers by facilitating the metastatic capabilities of these cells.

The downstream effects of TLR activation in cancer remains controversial. The signaling between tumour cells, immune cells, PAMPs and DAMPs in the TME can promote the progression of tumours or enhance anti-tumour effects through TLR signaling. In contrast to the pro-tumourigenic effects of TLRs, Lowe et al. observed that TLR2 signaling protects mice from colitis-associated colorectal cancer and showed that TLR2-deficient mice developed larger tumours than wild type mice [43]. In another study, TLR4 activation inhibited lung tumourigenesis in a mouse model which indicates its protective role [44]. A better understanding of TLRs and the TME are required to determine how these signaling mechanisms contribute to tumour development or inhibition.

DAMPs contribute to tumour development and play an important role in sterile inflammation by continuously activating PRR signaling (Gombault et al., 2013). DAMPs represent a large range of chemically unrelated mediators, such as high mobility group box 1 (HMGB1), S100 proteins, heat shock proteins and ATP. In healthy states, DAMPs are retained within a healthy cell and is released upon cellular stress or cell death, but it has been shown that DAMPs are also released from breast, lung and ovarian tumours [45]. SAA is highly upregulated in a pro-inflammatory TME [46], where it has been proposed to function as an endogenous DAMP to promote cancer growth and development because of its ability to bind to TLRs [8,9].

5. The expression and regulation of SAA in cancer

As inflammation is implicated in the etiology of many cancers, a number of inflammatory molecules are associated with cancer growth and development. One of these inflammatory molecules is SAA, an acute-phase protein (APP) primarily produced by hepatocytes and adipose tissue [7]. These acute-phase proteins are endogenous molecules that amplify innate immune responses. [7]. SAA proteins are encoded by four separate genes on chromosome 11. In humans, SAA exists in an acute-phase form, *SAA1* and *SAA2*, while human *SAA3* is a pseudogene and *SAA4* is constitutively expressed at lower levels [47]. In humans, the expression of SAA1 and SAA2 can be induced by several inflammatory signals, including IL-1β, IL-6 and LPS. In mice, SAA1 and SAA2 are predominantly produced by hepatocytes, *SAA3* encodes a functional SAA protein and is also the major form of SAA in inflammatory tissues [48]. It was initially thought that the liver was the only site of SAA synthesis, but a variety of extrahepatic tissues including breast, stomach, small and large intestine, prostate, lung, pancreas, kidney, tonsil, thyroid, pituitary, placenta, skin epidermis and brain neurons also express SAAs [49].

The expression of SAA is primarily regulated at the transcriptional level; cytokines (like TNF-α, IL-1 and IL-6) or glucocorticoids act by binding to their corresponding receptors and inducing a series of transcription factors, including NFκB (Vlasova & Moshkovskii, 2008). Purified SAA from plasma can exert cytokine-like properties by promoting the secretion of IL-1β, IL-1R antagonist and several other interleukins from lymphocytes, granulocytes, monocytes and macrophages (Patel et al., 1998; [50–52]). Other biological activities of SAA in inflammation include acting as an extracellular matrix (ECM) adhesion protein, enhancing the production of MMPs, promoting the migration and infiltration of leukocytes, angiogenesis stimulation and the production of inflammatory cytokines [53–55]. In chronic inflammation, SAA levels and pro-inflammatory molecules significantly increase, which is considered as a major regulator during tumour development [46].

It has been proposed that a direct relationship exists between SAA concentrations and tumour grading where increased SAA levels are observed in stage IV patients with lung, breast and melanoma cancer when compared to stage I and benign cancer patients [56,57]. SAA is therefore considered as a marker to monitor tumour progression in certain cancers [58]. One theory which explains SAA's contribution to tumour pathogenesis suggests that SAA can act as an ECM adhesion protein (Malle et al., 2010). The ECM is a highly organized network of proteins, proteoglycans and glycoproteins, which form a molecular scaffold to provide binding sites for cells. However, once these factors become altered, ECM degradation can result in tumour initiation and development. SAA has binding sites for ECM components like heparin/heparan sulfate and has YIGSR-like (the Tyr-Ile-Gly-Ser-Arg peptide derived from laminin) and RGD-like (Arg-Gly-Asp) adhesion epitopes (residues 29–42) that correspond to laminin and fibronectin cell-binding domains, respectively. SAA can therefore interact with the ECM and change its affinity to different cell types [59]. Preciado-Patt and co-workers observed that the interaction between SAA and laminin coated surfaces influences TNF-α secretion from human T-lymphocytes [60]. Furthermore, Michaeli and co-authors reported that SAA enhances plasminogen activation in colon cancer (HT-29 cells) [61]. Plasminogen activation can result in ECM degradation and tissue remodeling, which is associated with inflammation and tumour metastasis. One of the main causes of ECM alterations during cancer development is its degradation by MMPs, which promotes epithelial-mesenchymal transition (EMT) and genomic instability. Increased expression of MMPs in both primary and metastatic tumours is associated with poor prognosis [62]. In 2005, Lee and colleagues observed that SAA stimulated the production of MMP-9 in THP-1 cells through NFκB as well as ERK activation [55]. It is suggested that SAA can influence tumour invasion through the ECM by stimulating the production and activation of MMPs.

With particular reference to breast cancer, SAA can be synthesized by several cell types and tissues within the TME, including mammary tissue, immune cells, adipocytes, fibroblasts, epithelial and endothelial cells. These cells may then contribute to the significant increase observed in SAA expression [63]. In an obesity model, acute-phase SAA was highly expressed in human adipocytes [64], which could also explain why SAA is significantly upregulated in breast cancer tissue. Wang and colleagues investigated the *in vitro* effect of SAA on MCF-7 breast cancer cells and observed that SAA can increase proliferation and cellular migration in these cells. SAA also promoted MCF-7 invasion by enhancing the expression of MMP-9 and MMP-2 [22]. Zhang and co-authors observed that the level of SAA in stage II, III and IV was significantly higher when compared to control, stage I and benign breast cancer patients. Furthermore, a significant increase in SAA concentrations were observed in patients with lymph node metastasis when compared to patients without metastasis [65]. SAA therefore plays an important role in cancer progression and studies have shown that SAA exerts its downstream effects by binding to receptors on several cell types [8,66].

6. Serum amyloid A as an endogenous DAMP

Inflammatory-related diseases like cancer is associated with the expression of pro-inflammatory cytokines which is enhanced by the presence of SAA. Cells within the TME can utilize inflammasome activation to promote this response (Malle et al., 2009). Several types of amyloid can activate the NLRP3 inflammasome, including SAA and amyloid-β [67,68].

There is evidence to support the binding of SAA to TLRs and its downstream signaling effects. In immune cells, Sandri and colleagues

showed that macrophage activation by SAA is TLR4-dependent [69]. In mice, macrophages from TLR4-deficient strains did not produce significant amounts of nitric oxide (NO) upon SAA stimulation when compared to wild type mice. The authors concluded that SAA acts as an endogenous TLR4 ligand [69]. Similarly, in neutrophils, SAA induced IL-1β in cell culture supernatants. However, in the presence of a caspase-1 inhibitor, SAA-induced IL-1β processing and secretion was abrogated (Migata et al., 2014). In cancer, Cheng *et al* observed that SAA stimulates TLR2 activation in HeLa (cervical cancer) cells, and this was associated with the degradation of Iκβ and an increase in NFκB activity [8]. Furthermore, relatively low concentrations of SAA induced robust phosphorylation of ERK1/2 and p38 in the Hela cells. These results suggest the involvement of TLR2 in SAA signaling. Ather and colleagues exposed peritoneal macrophages from TL2/4$^{-/-}$ and Myd88$^{-/-}$ mice to SAA and found that IL-1β secretion was primarily dependent on TLR2 and Myd88, both in the presence and absence of ATP or aluminum crystals [68].

To further support the hypothesis that SAA can function as an endogenous DAMP that activates the NLRP3 inflammasome, Shridas and colleagues observed that in J774 macrophages, SAA treatment activates caspase-1, as evidenced by cleavage of pro-caspase-1 to active caspase-1 and significantly increases IL-1β protein secretion from the cells compared to untreated controls. SAA-induced IL-1β secretion was blocked in the presence of a caspase-1 inhibitor. When bone marrow-derived macrophages (BMDMs) isolated from wild type and NLRP3$^{-/-}$ mice were treated with SAA, IL-1β secretion was decreased by more than 10-fold in BMDMs from NLRP3$^{-/-}$ mice compared to the cells from wild type mice. These results indicated that SAA stimulates IL-1β secretion in macrophages by activating NLRP3 inflammasome mediated caspase-1 activation. The authors therefore concluded that SAA acts as an "endogenous" danger signal with the ability to stimulate both essential steps (priming and activation) of NLRP3 inflammasome-mediated IL-1β secretion [70]. Yu and authors cultured keratinocytes in the presence of SAA and found that mRNA expression of caspase-1 and NLRP3 was significantly upregulated. Blocking TLR2 and TLR4 in these cells, respectively, decreased IL-1β mRNA expression by almost 75 %. Similarly, to Shridas and colleagues, the authors concluded that SAA could be one of the DAMPs in psoriasis, which induces IL-1β *via* the NLRP3 inflammasome [71].

Taken together, these results highlight the SAA-mediated activation of several important steps in inflammasome signaling. SAA's ability to activate the inflammasome priming step (*via* NFκB pathways) by signaling through PRRs has been documented and Shridas et al. demonstrated that SAA upregulates NLRP3 mRNA expression, which is consistent with inflammasome priming [70]. In macrophages, SAA had the ability to prime and activate the NLRP3 inflammasome (supported by K^+ efflux in SAA-mediated IL-1β release) to stimulate IL-1β secretion, which distinguishes it from the numerous compounds such as ATP, pore-forming toxins, β-amyloid and crystals that are incapable of inducing IL-1β secretion in the absence of a priming stimulus. SAA therefore seems to represent as an endogenous danger signal with the unique ability of stimulating both essential steps of NLRP3 inflammasome mediated IL-1β secretion. The support for SAA-mediated inflammasome activation originates from studies using different cellular models, suggesting that this effect is not cell-type specific. This is particularly relevant in an environment such as the TME, which consists of a multitude of cell types and where SAA could potentially activate the inflammasome in several, if not all, of these cell types. It should however also be considered that the propensity of a particular cell type for inflammasome activation could affect the extent of the SAA-induced effects. Future studies comparing SAA-mediated inflammasome activation in the different cell types present in the TME would shed light on this intriguing question.

It is conceivable that in the TME of breast cancer patients, necrotic cells release various PAMPs and DAMPs into the microenvironment. These molecules can then bind to TLRs on several cell types within the TME including CAFs to activate the inflammasome through NFκB or MAPK signaling for the production of IL-1β (Fig. 3) [8,27,69]. IL-1β in the TME binds to TLRs on cancer cells to stimulate the production and secretion of SAA. SAA then binds to TLRs on cancer cells to further increase the secretion of IL-1β into the microenvironment and therefore functions as an endogenous DAMP within the TME to promote NFκB transcription to facilitate cancer growth. This positive feedback mechanism creates the desired pro-inflammatory environment necessary for cancer growth and development and ultimately, metastases and tumour recurrence in patients. Additionally, excessive pore formation following inflammasome activation could promote pyroptosis, resulting in the release of cytoplasmic components including DAMPs and pro-inflammatory cytokines such IL-1β and IL-18 [72]. This could further exacerbate a positive feedback mechanism involving IL-1β and SAA within the TME. Targeting SAA and IL-1β secretion in breast cancer patients could therefore represent a potential therapeutic approach.

7. Inflammasome inhibition and SAA as a potential therapeutic target

Excessive inflammation induced by the inflammasome is a detrimental factor in many types of cancer and inflammasome inhibitors are therefore considered a promising approach for cancer prevention and treatment by targeting upstream and downstream molecules in the inflammasome activation pathway [73]. SAA plays an important role in chronic inflammation, where it contributes to tumour initiation and progression *via* multiple signaling pathways, as well as interacting with the ECM. By inhibiting the secretion of SAA from tumour cells or by inhibiting its downstream activity, it could have beneficial therapeutic outcomes. This may be achieved by reducing the production of SAA inducers, such as IL-1, IL-6 and TNF-α. Antagonists or inhibitors for these cytokines are currently being used clinically [66].

Thalidomide, a drug that targets and inhibits the activation of caspase-1, has showed anti-tumour effects in patients with advanced myeloma [73]. In a randomised phase II trial, thalidomide combined with docetaxel (chemotherapeutic agent) resulted in the increased median survival rate in patients with metastatic androgen-independent prostate cancer. It has also been demonstrated that thalidomide reduces high levels of angiogenic factors like fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) (Figg et al., 2002). However, its antitumour activity has a moderate effect on other types of cancer and increases the risk of thrombotic events [74].

Anakinra is a recombinant form of IL-1Ra and is commonly used to treat rheumatoid arthritis and autoinflammatory diseases [75]. In a breast cancer mouse model, anakinra decreased metastatic tumour growth to bone [76]. The study also showed that anakinra failed to increase tumour cell death but suppressed cancer cell proliferation and angiogenesis. Previous studies with myeloma cells have shown that anakinra significantly reduces IL-6 levels but does not increase myeloma cell death. However, a combination therapy of anakinra and dexamethasone can induce myeloma cell death. Due to anakinras' clinical and safety record and short life, it is an ideal drug to be combined with chemotherapy. In breast cancer, Wu and authors identified an IL-1-associated inflammatory signature in primary tumours and if validated in follow-up clinical studies, could be used to stratify patients at diagnosis and justify the use of IL-1 targeting therapies. Blocking IL-1 downregulated components associated with systemic inflammation and rescued cytotoxic programs needed for anti-tumour activity [77]. Although anakinra has been approved by the US Food and Drug administration (FDA) as treatment for rheumatoid arthritis, the anti-tumour effects await further studies. Additionally, parthenolide, a sesquiterpene lactone compound found in a herb named feverfew, is used as an anti-inflammatory medicine [78]. Parthenolide is considered as a potential anti-tumour therapeutic drug that inhibits NFκB signaling and can also inhibit caspase-1 activation. In gastric and breast cancer, parthenolide inhibits tumour cell growth by downregulating NFκB

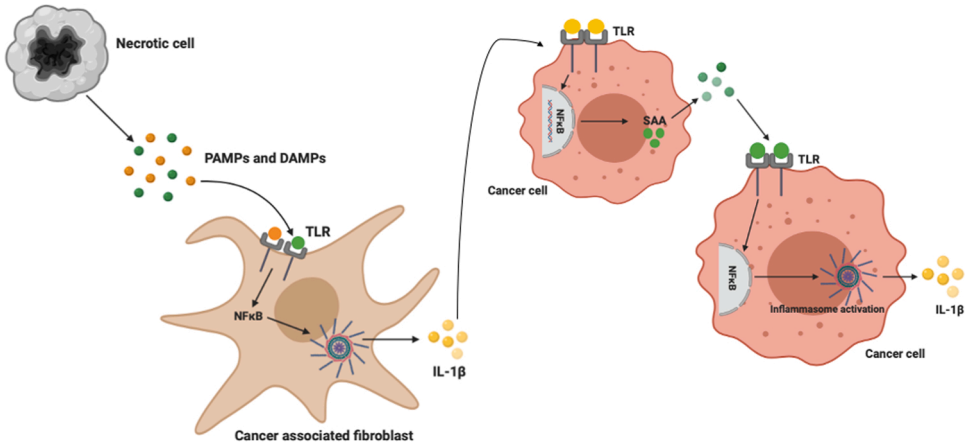

Fig. 3. Positive feedback mechanism for IL-1β and SAA secretion in the TME.

phosphorylation [79]. However, its anti-cancer potential is limited by poor solubility and bioavailability [80].

In contrast to IL-1β, the development of IL-18 inhibitors is less advanced. One agent that targets IL-18 is the GSK-1,070,806 antibody, however it has not been evaluated in a cancer setting. Kang and authors observed that IL-18 is secreted by B16 murine melanoma cells and enhanced IL-18 expression was positively correlated with the pathogenesis and metastasis of malignant skin tumours. These effects were reduced in the presence of IL-18-binding protein (IL-18BP), which inhibits IL-18 receptor binding [81].

Blocking the binding of SAA to its receptors will prevent the resulting downstream pro-inflammatory processes. When focusing on TLR4, TAK242 is an effective and specific TLR4 inhibitor that binds to the Cys-747 intracellular domain and blocks interactions with downstream effectors [82]. Zandi and authors demonstrated that TLR4 inhibition by TAK242 significantly decreased the viability of ovarian and breast cancer cells [83]. TAK242 also inhibited the activity of MMP-2 and MMP-9 in ovarian and breast cancer cells. Another TLR4 antagonist, C34, inhibits TLR4 *in vivo* and *in vitro* [84]. C34 inhibits TLR4 signaling *in vivo* in the presence of LPS in a NFκB-luciferase transgenic mouse strain. To determine the *in vitro* effect of C34, the authors treated macrophages and enterocytes with LPS. In these cells C34 significantly reduced the LPS-induced NFκB translocation from the cytoplasm to the nucleus.

Developing inhibitors for TLRs remain challenging due to the large number of cell surface TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10,) and the significant homology shared with the IL-1R-family. Furthermore, TLRs may also require dimerisation either as homo- or heterodimers for functional activity. Heterodimerisation appears to substantially influence the potency of ligand binding (eg. TLR1/2 and TLR2/6) and should be taken into consideration. Cheng and colleagues tested CU-CPT22, a TLR2 antagonist against a panel of homologous TLRs and found that CU-CPT22 only inhibits TLR1/2 signalling without affecting other TLRs [85]. CU-CPT22 also inhibited downstream signalling transduction, NO production was supressed as well as the release of IL-1β. Additionally, MMG-11, a potent TLR1/2 and TLR2/6 antagonist, inhibits NFκB activation induced by TLR2 ligands in mouse macrophages and ultimately prevents the secretion of pro-inflammatory cytokines, including TNF and IL-1β [86]. Thus SAA, being an activator of both TLR2 and 4, could potentially serve as a therapeutic target for chronic inflammatory diseases including cancer.

Neutralizing monoclonal antibodies that target SAA could be utilized to prevent its inflammatory effects. Similar products to target pro-inflammatory cytokines like IL-1 and IL-6 has been developed. To date, canakinumab, an IL-1β neutralizing human monoclonal antibody is used to treat cryopyrin-associated periodic syndromes (CAPs) in children and adults [87]. During a randomized, double-blinded, placebo-controlled trial of patients with lung cancers and atherosclerosis, researchers found that canakinumab significantly decreased lung cancer mortality by targeting the IL-1β innate immunity pathway [88]. Currently, canakinumab is being applied in clinical trials that focus on non-small cell lung cancer (NSCLC), triple negative breast cancer (TNBC), metastatic melanoma and colorectal cancer. Another phase III clinical trial Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) demonstrated that canakinumab significantly reduces the incidence and mortality of lung cancer. Goh and authors investigated a similar product, P2D7, which blocks IL-1β from binding to IL-1R (Goh et al., 2014). The engineered antibody had a higher affinity for IL-1β than canakinumab and potently neutralized human and mouse IL-1β.

Anti-IL-6 therapies have also been developed to treat cancer. Current IL-6 inhibitors include siltuximab, a humanized monoclonal antibody which is used in clinical trials for myeloma and prostate cancer [89]. It binds to and neutralizes human IL-6 directly and decreases the levels of unbound IL-6 and also prevents receptor binding and thereby exerts anti-cancer effects [90]. Blocking SAA could also limit metastasis. Double-knockout $Saa1^{-/-}$ $Saa2^{-/-}$ mice implanted with pancreatic adenocarcinoma cells failed to show features of a pro-metastatic niche in the liver [91]. Similarly, mice inoculated with lung Lewis carcinoma cells transfected with SAA1 or SAA2, resulted in metastasis and colonization to the lung [92]. This indicates that in *in vivo* models, SAA1 and SAA2 contributes to cancer metastasis. In breast cancer, conditioned media from VMR cells transduced with SAA1 and SAA3, respectively, enhanced invasiveness of MDA-MB-231 cells *in vitro* when compared with controls [93].

Taken together, several possible therapeutic approaches to prevent SAA binding and downstream activity like receptor inhibitors and neutralizing antibodies exist, however these effects have mostly only been observed in immune cells and evidence for the inhibition of signaling transduction in cancer cells remains limited. By targeting SAA through the development of neutralizing monoclonal antibodies and limiting its function as an endogenous DAMP could potentially be used as a therapeutic strategy for treating chronic inflammatory diseases. Targeting inflammasome activation in cancer could be a promising approach for cancer therapy, but the contrasting roles of inflammasomes suggests that due to the heterogeneity of cancer, specific treatment approaches depending on the type of cancer should be considered. A useful approach to consider may be to target TLRs, SAA, IL-1β and components of the inflammasome simultaneously. By neutralizing SAA, several downstream effects might be observed, which includes NLRP3 inflammasome inhibition, but it is still possible that other DAMPs in the TME could bind to TLRs and result in inflammasome activation. Therefore, blocking TLRs might prevent this from occurring and could also limit the production of other pro-inflammatory cytokines in the TME such as IL-6 and TNF-α. Inhibiting components of the NLRP3 inflammasome like

caspase-1 will result in the decreased production and secretion of IL-1β into the TME. Taking the above-mentioned into consideration, a recommended therapeutic approach could be to neutralize SAA and inhibit TLRs simultaneously or to neutralize SAA along with inhibiting components of the inflammasome.

8. Conclusion

Inflammation affects all stages of tumour development and IL-1β, a pro-inflammatory cytokine, plays an important role in inflammation induced tumourigenesis (Voronov et al., 2003). The NLRP3 inflammasome is an intracellular signaling complex that functions to regulate innate immune activity by modulating the production of pro-inflammatory cytokines, such as IL-1β. SAA, an acute-phase protein, can bind to pattern recognition receptors like TLRs on several cell types within the TME to stimulate the production of IL-1β, thereby creating a favourable inflammatory environment that supports tumour growth. SAA is a molecule capable of triggering the generation of pro-inflammatory cytokines as well as activating NLRP3 inflammasome, two mechanisms known to promote tumour development and metastasis. Reports indicate that SAA triggers growth and metastasis of tumours in several experimental model systems. SAA can therefore be a potential therapeutic target for cancer treatment.

Author contribution

All authors contributed to the writing and editing of this review.

Declaration of Competing Interest

The authors state no conflict of interest.

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Ms Carla Fourie Department of Physiological Sciences, Stellenbosch University, South Africa. I am part of the Cancer Research Group in the Physiological Sciences department at the University of Stellenbosch. I am currently busy with my PhD, with Professor Anna-mart Engelbrecht as my supervisor. I am mostly interested in signaling mechanisms and pathways that influence breast cancer growth and development. The aim for my PhD is to determine the role of Serum amyloid A (SAA) and inflammasome activation in breast cancer progression as well as understanding the interaction between cancer cells and other cell types in the tumour microenvironment, such as cancer associated fibroblasts.

Dr Preetha Shridas Department of Internal Medicine, University of Kentucky, University of Kentucky, Kentucky 40,536, USA. Education: Ph.D (1999) Biochemistry, Bharathiar University, India. I am a biochemist with extensive experience in cellular and molecular biochemistry. For the past couple of years, my research focus has been on the role of Serum Amyloid A (SAA), an acute phase protein, whose level in plasma increases more than a 1000-fold during inflammation. I have published several peer-reviewed research papers on SAA. SAA is predominantly expressed by the liver and released into the circulation, where it is transported by high density lipoprotein (HDL). My research has indicated that lipid-

free SAA is a pro-inflammatory molecule, capable of activating NLRP3-mediated inflammasome. Lipid free-SAA and HDL-bound SAA have differences in inflammatory properties, HDL masks inflammatory properties of SAA. I am currently on investigating the mechanisms by which SAA is liberated from HDL to exert its pro-inflammatory properties in chronic inflammatory diseases like atherosclerosis and abdominal aortic aneurysm.

Dr Tanja Davis Department of Physiological Sciences, Stellenbosch University, Stellenbosch. I am a cancer researcher with a keen interest in understanding how cancer thrives in its environment. Currently, I am a postdoctoral fellow with Prof Willem de Villiers at Stellenbosch University. I completed my PhD also at Stellenbosch University, in the research group of Prof Anna-Mart Engelbrecht. My current research bridges several interests of mine, including cancer, inflammation and innate immunity. More specifically, I'm working to understand the role of Serum Amyloid A (SAA) in colitis-associated colon cancer, as well as the interaction between SAA and macrophages in this environment.

Professor Willem JS de Villiers Rector and Vice-Chancellor of South Africa's Stellenbosch University, Professor Willem de Villiers is committed to supporting students to achieve their potential and has worked in the UK, America and South Africa to support them. In the 1990′s Wim travelled to the UK and completed a DPhil in Immunology at Oxford University in 1995. He became Head of Gastroenterology at the University of Kentucky and Administrative Head of the Good Samaritan Hospital in Lexington. Working as a gastroenterologist, he became a respected medical researcher and was featured in the Best Doctors in America publication. He has shown the importance of macrophages (and their pattern recognition receptors) in many murine models of gut inflammation. He then contributed significantly to the clinical development and wide-spread application of targeted monoclonal antibody therapies in numerous patients with complicated inflammatory bowel disease. This work was published in top medical journals such as the New England Journal of Medicine and Gastroenterology. Moving back to South Africa in 2013, he became the Dean of Health Sciences at the University of Cape Town, before moving to his current position at Stellenbosch in December 2014.

Professor Anna-Mart Engelbrecht PhD, Medical Physiology. Department of Physiological Sciences, Stellenbosch University, Stellenbosch. Anna-Mart Engelbrecht is currently professor in the Department of Physiological Sciences at Stellenbosch University. She completed a BSc (Hons) in Physionces at Stellenbosch University. She completed a BSc (Hons) in Physiology at Stellenbosch University, a MMedSc at the University of the Free State and received her PhD in 2005 at Stellenbosch University. She received several prestigious awards which include the Dean's and Senate's Medals as well as the Gencor Bronze Medal from the University of the Free State, the Marie Curie Scholarship of the European Union, the Rector's award for Excellence in Research and the Vice-Rector's Research Award for exceptional achievement from Stellenbosch University as well as the Lasec Award for Excellence in Physiology Research from the Physiological Society of Southern Africa (PSSA). Seventeen MSc and fifteen PhD students completed the degrees under her supervision; she currently serves as promotor and co-promotor for eight PhD students. She has published 76 peer reviewed, research articles and presented invited lectures at national and international conferences. She established the Cancer Research Group (CRG) where they investigate chemo-resistance and mechanisms to counteract chemotherapy-induced damage to the heart and skeletal muscle; as well as metabolic pathways in the cancer micro-environment.