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Alarmins and Antitumor Immunity

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Abstract

Purpose: Alarmins are constitutively present endogenous molecules that essentially act as early warning signals for the immune system. We provide a brief overview of major alarmins and highlight their roles in tumor immunity.

Methods: We searched PubMed up to January 10, 2016, using *alarmins* and/or *damage-associated molecular patterns* (DAMPs), as key words. We selected and reviewed articles that focused on the discovery and functions of alarmin and their roles in tumor immunity.

Findings: Alarmins are essentially endogenous immunostimulatory DAMP molecules that are exposed in response to danger (eg, infection or tissue injury) as a result of degranulation, cell death, or induction. They are sensed by chemotactic receptors and pattern recognition receptors to induce immune responses by promoting the recruitment and activation of leukocytes, particularly antigen-presenting cells.

Implications: Accumulating data suggest that certain alarmins, High-mobility group nucleosome-binding protein 1 (HMGN1) in particular, contribute to the generation of antitumor immunity. Some alarmins can also be used as cancer biomarkers. Therefore, alarmins can potentially be applied for our fight against cancers.

Keywords

alarmin; biomarker; damage-associated molecular patterns; immune response; pattern recognition receptor; tumor immunity

INTRODUCTION

During the early 1990s, 2 competitive theories were proposed to explain the ability of the immune system to mount an effective immune response against infectious agents while

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CONFLICTS OF INTEREST

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maintaining tolerance toward self-antigens. The Infectious Non-Self theory put forward by Charles Janeway stated that antigen-presenting cells (APC) discriminate infectious nonself from noninfectious self by recognizing pathogen-associated molecular patterns (PAMPs) using pattern-recognition receptors (PRRs).¹ The interaction between PRRs and corresponding PAMPs activates APC, leading to upregulation of costimulatory signals and subsequent activation of T cells. Although insightful and experimentally well supported, this theory could not sufficiently explain the generation of immune responses in the absence of an infectious nonself, such as graft rejection, unresponsiveness to normal microflora, and autoimmunity. The more general danger model proposed by Polly Matzinger² suggested that the immune system is triggered to respond to dangerous signals known as damage-associated molecular patterns (DAMPs) as a result of cell death, tissue injury, or microbial attack. Research led to the identification of many exogenous PAMPs (eg, lipopolysaccharide, CpG DNA, and flagellin) and PRRs such as Toll-like receptors (TLRs) and RIG-I-like receptors in the late 1990s and early 2000s; however, the identification of endogenous DAMPs has lagged.¹⁻³ Because a prominent DAMP, namely high-mobility group box B protein 1 (HMGB1), was discovered in the circulation of patients with septic shock, these DAMPs were considered dangerous mediators.^{4,5}

In the late 1990s and early 2000s, extensive research into the role of several antimicrobial peptides and proteins (AMPs) and HMGB1 revealed that these structurally distinct multifunctional endogenous mediators shared certain common characteristics: they are present intracellularly in granules, the nucleus or cytosol, and are rapidly released as a result of degranulation, cell death, and/or induction; once they become extracellular, they are sensed by cellular receptors, resulting in recruitment and activation of immune cells, including the most potent APCs, which are known as dendritic cells (DCs); and they have the capacity to promote innate and adaptive immune responses. These constitutively present endogenous molecules essentially act as early warning signals for the immune system and therefore the term “alarmin” was coined by us to illustrate the unique function of these molecules.⁶ In essence, alarmins are immunostimulatory DAMPs that contribute to protective host defense. However, not all DAMPs are immunostimulatory. For example, phosphatidylserine exposed on the surface of dying cells can be considered a DAMP; however, it is anti-inflammatory and therefore certainly not an alarmin. DAMPs are considered dangerous. In contrast, alarmins function as intercellular signals and therefore behave as cytokines, and are dangerous only when they induce an excessive immune response known as a cytokine storm. Nevertheless, the terms “alarmins” and “DAMPs” are sometimes used interchangeably in the literature.

Alarmins identified so far can roughly be classified into several categories (Table), including certain AMPs (eg, defensin, cathelicidin, eosinophil-derived neurotoxin [EDN], and granulysin), nuclear binding proteins (eg, HMGB1 and interleukin [IL]-1 α), heat shock proteins (HSPs) such as HSP60 and HSP96, ion-binders (eg, S100A8 and A9 and lactoferrin), nucleotides/metabolites (eg, adenosine triphosphate [ATP] and uric acid), and certain degradation products of the extracellular matrix.⁷⁻¹² A property common to all alarmins is their capacity to promote inflammation and immunity; however, they are sensed by distinct receptors and have distinct roles.¹³⁻¹⁷ With progress in immunology, the functions of alarmins have been further elucidated and their potential clinical use has

attracted more research enthusiasm.^{13,16,18–22} Based on their involvement in inflammation, antimicrobial defense, adaptive immunities, and wound healing, the potential clinical use of alarmins has become attractive.^{21,23–28} In this short review, we provide a general overview on the roles of well-characterized alarmins in immunity, followed by focusing on their roles in cancer.

OVERVIEW OF VARIOUS ALARMIN AMPs

Defensins consist of a family of small antimicrobial peptides with a characteristic β -sheet-rich fold and 6 cysteines forming 3 intrachain disulfide bonds that are classified into 3 (α , β , and θ) subfamilies.²⁷ There are multiple α - and β -defensins in mice and humans^{29–32}; however, only a few defensins have been studied at the protein level. Both α - and β -defensins are chemotactic for immature DCs, monocytes, and subsets of T cells.^{33–35} The receptors that β -defensins use to chemoattract DCs and macrophages are CC chemokine receptor 6 [CCR6] and CC chemokine receptor 2 [CCR2], respectively.³⁶ A few β -defensins have been shown to induce DC maturation,³⁷ with β -defensin 2 reported to use TLR4 as a receptor.³⁸ Certain β -defensins can also form complexes with DNA and activate plasmacytoid DCs using TLR9.³⁹ α -Defensins promote both Th1 and Th2 immune responses upon administration with antigen, whereas β -defensins predominantly stimulate a Th1 response.^{31,40,41}

Cathelicidins have a conserved N-terminal pro-sequence of approximately 100 residues known as the cathelin domain, and a C-terminal antimicrobial domain that is highly heterogeneous in terms of size and structure, which can be either an α -helix, β -hairpin with 1 or 2 intrachain disulfide bonds, or with extended polypro-line-type folding.⁴² Human cathelicidin (LL-37) and mouse cathelicidin (cathelin-related antimicrobial peptide [CRAMP]) can induce the migration of neutrophils and monocytes through human formyl peptide receptor like-1 [FPRL1] and mouse FPR2 (the ortholog of FPRL1).^{43–45} Cathelicidin can promote DC differentiation and maturation, and antigen-specific immune responses.⁴⁶ By forming complexes with DNA and RNA, cathelicidin can induce the activation of plasmacytoid and myeloid DCs through the use of TLR7, 8, or 9, all of which have been shown to contribute to autoimmune diseases and wound healing.^{47–50}

EDN present in the granules of eosinophils belongs to the ribonuclease family of proteins and shows a typical RNase V-shaped folding organized into 2 lobes, each consisting of 3 anti-parallel β -strands and 1 α -helix, and with 2 α -helices located between the 2 lobes.³¹ EDN is chemotactic for both immature and mature DCs, indicating it may regulate DC trafficking.⁵¹ EDN can activate myeloid DCs by triggering TLR2-MyD88 signaling pathway and promoting antigen-specific, preferentially Th2, immune responses.¹⁴

DNA Binding Proteins

High mobility group (HMG) proteins consist of a superfamily of nucleosome-binding proteins that are classified into HMGA, HMGB, and HMGN subfamilies, each of which contains several members, such as HMGB 1–3 and HMGN 1–4.⁵² HMGB1 is a nonhistone, chromatin-binding protein that is present in all mammalian nucleated cells. HMGB1 can be released by necrotic or apoptotic cells or secreted by activated macrophages, DCs, bone

cells, or some tumor cells like melanoma cells.^{53–57} HMGB1 induces the migration of DCs, macrophages, and many nonleukocytes such as epithelial and neural/nerve cells.^{58–60} HMGB1 activates DC, monocytes, and macrophages, leading to upregulated expression of costimulatory molecules and inflammatory cytokines, including IL-6, IL-12, and tumor necrosis factor α .^{19,61–65} The effect of HMGB1 in partly complexing DNA and RNA is mediated by multiple receptors, including TLR2, 4, 7, 8, and 9; receptor for advanced glycation end-products (RAGE); and CD24.^{60,66,67} Aside from promoting adaptive immune responses, HMGB1 has been shown to contribute to inflammation, such as during endotoxic shock,^{4,64,68} trauma-induced inflammation,⁶⁹ and autoimmune disorders.^{70–73}

HMGN1 is composed of 2 major domains, a C-terminal chromatin-unfolding domain and an N-terminal nucleosomal binding domain, and is highly expressed in the nucleoli of proliferative tissues that undergo constant turnover, such as epithelial and stem cells.⁵² Like HMGB1, HMGN1 has critical biological functions in development, host defense and tissue repair.^{52,63,74–76} In 2012, HMGN1 was identified as a novel alarmin demonstrating its contribution to the promotion of APC/DC maturation and induction of antigen-specific, preferentially Th1, immune responses.⁷⁷ Despite the functional similarities of HMGB1 and HMGN1, the amino acid sequence of HMGN1 is distinct from that of HMGB1 and they are generated from distinct genes located on different chromosomes. HMGN1 lacks any cysteines in its sequence and is therefore not subject to oxidative inactivation as is HMGB1.⁷⁸

IL-1 α was initially identified and studied as a classical IL; however, recent studies have revealed that IL-1 α and IL-33 each has the properties of alarmins. Both have the capacity to act as transcription factors that bind chromatin like HMGB1. They are released by producing cells passively via cell death or actively through a nonclassical “release” pathway. IL-1 α transmits its signal by interacting with IL-1R, whereas IL-33 uses the ST2 receptor. IL-1 α is a critical proinflammatory cytokine that participates in many inflammatory and immune responses.^{79,80} IL-33 favors Th2 polarization and can stimulate the production of IL-5 and IL-13 by type 2 innate lymphoid cells, and therefore plays important roles in allergic inflammation.^{81,82} More recently, IL-33 was reported to promote regulatory T cell [Treg] function in the intestine by enhancing Treg differentiation and accumulation.⁸³

HSPs

HSPs act as intracellular chaperones to protect proteins against acute denaturation and aggregation. Extracellular HSPs induce maturation of DCs and promote the generation of innate and adaptive immune responses.⁸⁴ HSP70, HSP90, and HSP96 can bind to TLRs, CD14, or SRA1 to trigger the activation of target cells.⁸⁵ HSPs act as proinflammatory alarmins by promoting the production of proinflammatory cytokines.^{86,87} On the other hand, HSPs are also involved in the induction of Tregs in certain autoimmune situations.⁸⁸

Iron-binding proteins

Lactoferrin is an 80-kilodalton iron-binding protein that belongs to the transferrin superfamily.⁸⁹ It can be induced in neutrophils and epithelial cells and secreted into most exocrine fluids in an iron-free form.^{90,91} Lactoferrin has direct antimicrobial activity

independent of its iron-binding property.⁹² Based on its iron-binding property, lactoferrin can also have antimicrobial effect against bacteria, viruses, fungi, and some parasites.^{93–97} Gonzalo et al⁹⁸ reported in 2008 that lactoferrin acts as an alarmin capable in promoting the recruitment and activation of APCs and inducing antigen-specific immune responses.

S100 proteins or calgranulins are a family of more than 20 cytosolic proteins capable of binding calcium or zinc ion. They are predominantly expressed by neutrophils, macrophages, and cells of epithelial origin.^{99–101} S100A8 and A9 form heterodimers that can bind to TLR4 and RAGE to induce the production of proinflammatory cytokines via activation of nuclear factor kappa B.^{102–104} S100A7, 8, 12, and 15 are chemotactic for neutrophils, mast cells, and monocytes/macrophages likely via the use of RAGE and some G protein-coupled receptors.^{100,105} S100A8 and A9 heterodimers behave as alarmins that enhance many inflammatory and autoimmune conditions.^{106–108}

Aside from the alarmins discussed above, ATP, uric acid, and certain degradative products of the extracellular matrix have also been classified as alarmins based on their functions in promoting maturation of APCs and induction of innate and adaptive immune responses.^{7,10–12} Ample amounts of ATP are released following cell death due to its high concentration inside cells. ATP can induce the migration of granulocytes, macrophages, and DC using P2Y2, P2Y6, and P2Y12 purinergic receptors.¹⁰⁹ ATP activates macrophages and DCs by interacting with the P2X7 receptor and promoting the production of IL-1 β by activating the NACHT, LRR and PYD domains-containing protein 3 [NALP3] inflammasome.^{110–112} Uric acid, a metabolite of purine nucleotides, forms urate microcrystals upon release from the cells.¹¹³ It induces inflammatory cytokines and activation of APCs, resulting in enhanced adaptive immune responses.¹¹⁴ Uric acid also activates the NALP3 inflammasome¹¹⁵ and is the major pathogenic factor of gout.^{116,117}

ALARMIN AS POTENTIAL TUMOR BIOMARKERS

Certain alarmins may be used as biomarkers for cancer diagnosis or as indicators of prognosis. Clinical studies indicated that the expression profile of HMGB1 in patients with different cancers can provide diagnostic and prognostic insights as well as new HMGB1-targeted therapeutic agents. Elevated levels of HMGB1 in patients with malignant mesothelioma or colorectal carcinoma indicate poor prognosis.^{118,119} Combined positivity for microtubule-associated protein 1 light chain 3B and nuclear HMGB1 is a positive predictor for longer breast cancer survival.¹²⁰ Metastatic breast cancer patients with high circulating HMGB1 level have a poor prognosis and a very poor response to cytotoxic therapies.¹²¹ The alarmin S100A9 was recently demonstrated to be a sensitive and specific marker for the activity of tumor-associated immune cells and represents a first in vivo imaging approach for prediction of local and systemic tumor development.¹²² Serum levels of HMGN1 have been shown to be closely associated with the clinical stages of non-small-cell lung cancer, indicating that HMGN1 may also be used as a biomarker.¹²³

These preliminary clinical studies suggested that alarmins play a role in many kinds of cancers, including malignant mesothelioma and ovarian cancer.^{119,124}

ALARMIN IN TUMOR IMMUNITY

The complicated interplay between the host immune system and cancer has been studied for decades. Since the early 20th century, many concepts concerning the development of cancer have been proposed. The most popular concepts that are now accepted widely are immunosurveillance,¹²⁵ genetic changes,¹²⁶ and immuno-editing.¹²⁷ Immunoediting is a dynamic process that not only involves tumor prevention, but also shapes the immunogenicity of developing tumors. In the course of tumor development, many immune cells are provoked, such as T cells, natural killer cells, macrophages, and DCs. As professional APCs, DCs play a critical role in the induction of antitumor responses due to their ability to present antigens to T cells.¹²⁸ An effective antitumor immune response by T cells requires efficient antigen presentation by activated mature DCs. The maturation of DCs depends on the local microenvironment and is influenced by various inflammatory stimulants. Unstimulated resting DCs are often in an immature/tolerant status induced by suppressive cells and their cytokines present in the tumor microenvironment.^{129,130} These immature DCs not only have poor ability to stimulate T cells, but also promote tumor growth by producing proangiogenic factors and enhancing endothelial cell migration that results in vasculogenesis.¹³¹ The development of large number of phenotypically and functionally matured DCs is important in the battle against tumors. Alarmins, based on their capacity to promote, recruit, activate, and mature DCs, can potentially play important roles in cancer progression, diagnosis, and potentially treatment.

ALARMIN AS ENHANCERS OF ANTITUMOR IMMUNITY

Numerous studies have shown that alarmins play an important role in the generation of antitumor immune responses.^{132,133} Immunization of mice with a DNA plasmid harboring a fusion of mouse β -defensin 2 or 3 with mouse B-cell lymphoma epitope sFv38 can stimulate the generation of antilymphoma immunity and protection.^{133,134} Inoculation of mice with acute lymphoid leukemia L1210 cells transfected to produce mouse β -defensin 2 results in the generation of L1210-specific protective immunity with enhanced activation of natural killer cells and CD8 T cells.¹³² Immunization of mice with a fusion protein of mouse β -defensin 2 and gp100 (a mouse melanoma antigen) promotes the induction of B16 melanomaspecific CD8 T cells and protective immunity.¹³⁵ HSPs are also reported to be capable of promoting antitumor effector CD8 T cells and inhibiting tumor growth.^{136,137}

IL-1 α has been known for a long time to potentiate cell-mediated immunity and to play an essential role in the generation of antitumor immune defense.^{138,139} In 2014, the role of IL-33 in antitumor immunity was reported by Weiner et al.¹⁴⁰ They demonstrated that both of the 2 isoforms of IL-33 can significantly expand the magnitude of antigen-specific CD8 T cells responses and elicit potent effector-memory CD8 T cells.¹⁴⁰ Expression of IL-33 in the tumors is also reported to inhibit tumor growth by activating natural killer cells and CD8 T cells.¹⁴¹

HMGB1 is also an important alarmin for the generation of antitumor immunity in both mice and humans.¹⁴²⁻¹⁴⁴ Apetoh et al¹⁴⁴ found that HMGB1 released by dying tumor cells and the TLR4-MyD88 signaling pathway are required for the antitumor responses in mice. In

humans, TLR4 mutation has a negative prognostic influence on patients with breast cancer by affecting the binding of HMGB1 to its receptor.¹⁴⁴ Blocking HMGB1 activity or knockdown of HMGB1 expression inhibited cancer growth and metastasis by inhibiting lymphangiogenesis.^{145,146} HMGB1 can be considered a central target in the diagnosis and treatment of a number of human cancers due to the important role it has in the promotion of cancer growth and metastasis.^{121,146,147} This suggests that alarmins capable of promoting Th1 immune response possess the capacity to enhance antitumor immunity.

HMG1 was reported to have the capacity to promote antitumor immune responses as a vaccine adjuvant by Wei et al.²¹ They found that vaccination with a plasmid encoding the expression of a fusion protein consisting of gp100, a melanoma tumor-associated antigen, and HMG1, could induce gp100-specific CD8 response and immune protection in mice against B16 melanoma.²¹

The importance of alarmins in tumor immunity has been demonstrated in another way. HMG1 knockout mice develop more spontaneous tumors and are more susceptible to develop tumors in response to carcinogenic agents. Furthermore, in comparison with wild-type mice, malignant EG7 thymoma grows much faster in HMG1 knockout mice.²¹ The increase in tumor growth in HMG1 knockout mice is also accompanied by a reduction in the generation of EG7-specific CD8 T cells.²¹ Conversely, EG7 tumor cells transfected with HMG1 that is secreted grow much less well in normal mice than untransfected EG7 cells. Therefore, intrinsic HMG1 may be critical for the development of protective antitumor immunity.

HMG1 has so far been shown to preferentially promote Th1-polarized immune responses. Additionally, it is essential for the generation of protective immune defense against tumors. HMG1 also inhibit tumorigenesis by promoting DNA repair and genome stability.^{148,149} Finally, we are obtaining data showing that HMG1 can act as a therapeutic vaccine that enhances immunity to implanted tumors in mice (unpublished observations). Therefore, it appears that HMG1 exerts only protective antitumor effects (Figure), and can potentially be developed as a therapeutic agent for the treatment of cancers.

ALARMIN AS FACILITATORS OF TUMOR PROGRESSION

Besides their antitumor functions, certain alarmins have been shown to promote tumor progression. HMGB1 acts in an autocrine-regulated feedback loop to promote tumor angiogenesis through RAGE and TLR4 signaling in endothelial cells, which itself leads to increased HMGB1 secretion by endothelial cells themselves.¹⁵⁰ HMGB1 can enhance cancer cell growth by upregulating expression of the micro-RNAs MiR-221 and MiR-222 in papillary thyroid cancer cells by controlling cell proliferation through inhibition of p27kip1, a cell-cycle regulator.¹⁵¹ HMGB1 can induce production of IL-10, which in turn promotes the generation of Treg and facilitates tumor progression.¹⁵² HMGB1 produced by cancer cells is also reported to promote tumor progression by hampering the activation of plasmacytoid DCs.¹⁵³

β -Defensin 29 is reported to induce the recruitment of DC precursors to developing tumors and promote tumor progression by facilitating tumor angiogenesis.¹⁵⁴ Other alarmins, due to their capacity to activate immune cells such as macrophages and DCs may also facilitate tumor progression by promoting inflammatory responses in the developing tumors. For a given alarmin molecule, whether it is beneficial for resistance to cancer is probably dependent on multiple factors, including concentration.

CLINICAL PERSPECTIVE

Alarmins or immunostimulatory DAMPS are endogenous mediators rapidly released in response to danger signals, and are sensed through interaction with receptors to initiate or promote a variety of responses (eg, inflammation, immunity, and wound healing) aimed at the elimination of danger and restoration of homeostasis. In the context of tumor immunity, it appears that alarmins exhibit both beneficial and harmful effects (Figure). Many alarmins can promote the generation of antitumor immunity through activation of APCs, including DC and macrophages.^{21,26,32,34,35,37–39} Yet, some alarmins can also promote tumor progression via enhancement of inflammation with production of growth factors and angiogenesis.^{121,145,146,150–154}

CONCLUSIONS

Targeting alarmins or their sensing receptors has the potential to be used for the treatment of cancer patients. Alarmins, particularly those capable of enhancing Th1 responses, can be used as immunoadjuvants for the development of tumor vaccines or as therapeutic agents. Conversely, inhibition of alarmin-induced tumor-associated inflammation may slow down tumor progression, which would be beneficial for cancer patients. To achieve these approaches, the roles of individual alarmins in the development of cancer and anticancer immunity in humans need to be further defined.

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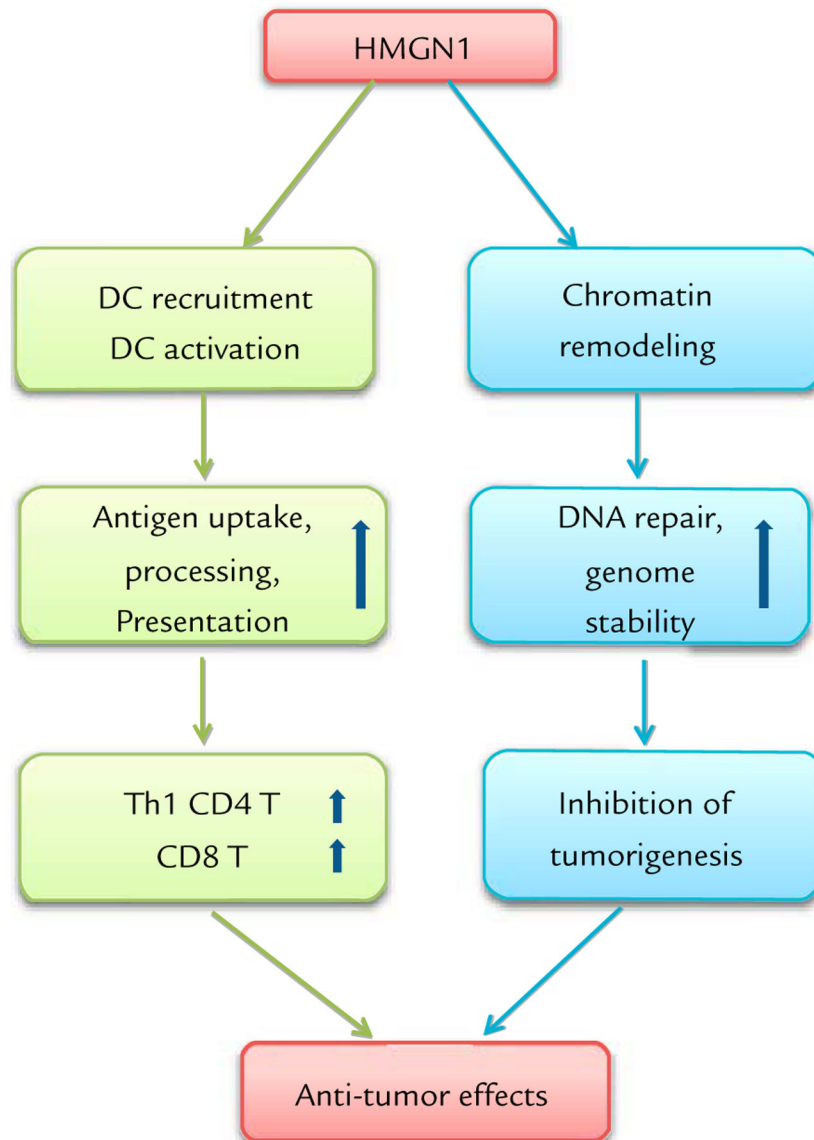


Figure. Potent antitumor effect of alarmin high-mobility groupnucleosome-binding protein1 [HMGN1]. HMGN1 manifests anti-tumor effects in at least 2 ways: promoting antitumor immunity by polarizing Th1 immune response and inhibiting tumorigenesis by promoting DNA repair and genome stability. DC = dendritic cell.

Receptors and roles of well-characterized alarmins.

Table.

Alarmins and their receptors	Biological activities and functions
AMPs	Antimicrobial effects
Defensins (α , β , and θ)	Leukocytes recruitment
Cathelicidins (eg. LL-37 and CRAMP)	Cytokine induction
EDN and others	APC/DC activation Promotion of immune responses Inflammation
DNA binding proteins	Regulation of gene expression
HMGNI	Leukocytes recruitment
HMGBI	Cytokine induction
IL-1 α	APC/DC activation
IL-33	Promotion of immune responses Inflammation
HSP	Protein protection
HSP70, 90, 96	APC/DC activation Treg polarization Inflammation
Ion-binding proteins	Leukocytes recruitment
Lactoferrin	Cytokine induction
S100 A8, A9	APC/DC activation Promotion of immune responses Inflammation
Others	Leukocytes recruitment
ATP and others	Cytokine induction APC/DC activation Inflammation

APC/DC = antigen-presenting cells/dendritic cells; AMP = antimicrobial peptides and proteins; ATP = adenosine triphosphate; CCR = CC chemokine receptor; CD = cluster of differentiation; CRAMP = cathelin-related antimicrobial peptide; CXCR = CXC chemokine receptor; EDN = eosinophil-derived neurotoxin; FPR = formyl peptide receptor; HMGB = High-mobility group protein with a 'Box' domain; HMGNI = High-mobility group protein with a 'nucleosome-binding' domain; IL = interleukin; RAGE = receptor for advanced glycation end-products; TLR = Toll-like receptor; Treg = regulatory T cell.