Accepted Manuscript

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PII: S0945-053X(17)30444-4
DOI: https://doi.org/10.1016/j.matbio.2018.01.021
Reference: MATBIO 1419

To appear in:

Received date: 4 December 2017
Revised date: 22 January 2018
Accepted date: 28 January 2018

Please cite this article as: Alexandra Hauser-Kawaguchi, Leonard G. Luyt, Eva Turley , Design of peptide mimetics to block pro-inflammatory functions of HA fragments. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Matbio(2017), https://doi.org/10.1016/j.matbio.2018.01.021

Title: Design of peptide mimetics to block pro-inflammatory functions of HA fragments

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Abstract

Hyaluronan is a simple extracellular matrix polysaccharide that actively regulates inflammation in tissue repair and disease processes. The native HA polymer, which is large (>500kDa), contributes to the maintenance of homeostasis. In remodeling and diseased tissues, polymer size is strikingly polydisperse, ranging from <10 kDa to >500 kDa. In a diseased or stressed tissue context, both smaller HA fragments and high molecular weight HA polymers can acquire pro-inflammatory functions, which result in the activation of multiple receptors, triggering pro-inflammatory signaling to diverse stimuli. Peptide mimics that bind and scavenge HA fragments have been developed, which show efficacy in animal models of inflammation. These studies indicate both that HA fragments are key to driving inflammation and that scavenging these is a viable therapeutic approach to blunting inflammation in disease processes. This mini-review summarizes the peptide-based methods that have been reported to date for blocking HA signaling events as an anti-inflammatory therapeutic approach.

Keywords:

Hyaluronan;
Receptor for hyaluronan mediated motility (RHAMM);
HMMR
CD44;
Peptide therapeutic;
HA receptor signaling;
Inflammation;

Abbreviations used:

HA, hyaluronan; RHAMM, receptor for hyaluronan mediated motility; HMMR: human motility mediated receptor; ROS/RNS, reactive oxygen species/reactive nitrogen species; DAMP, damage-associated molecular pattern molecules; IL, interleukin; FAK, focal adhesion kinase; IFN-γ, interferon-gamma; TLR, toll-like receptor; STAB2, stabilin 2; OBOC, one-bead one-compound; VLS, vascular leak syndrome; CTT, carboxy terminal tail; uPA, urokinase plasminogen activator; SPR, surface plasmon resonance

Acknowledgements:

Supported by Natural Sciences and Engineering Research Council of Canada (NSERC); Pamela Greenaway-Kohlmeier Translational Breast Cancer Unit; and Breast Cancer Society of Canada.

Conflict of Interest: None

Hyaluronan, inflammation and disease

Hyaluronan (HA) is a simple extracellular matrix polysaccharide that a wealth of experimental approaches has demonstrated actively regulates inflammation in tissue repair, fibrogenic disease processes and cancer progression. Thus, studies show that knockout/knockdown of the proteins involved in its production, catabolism, signaling and organization alter inflammation in repair and disease models [1-6]. Both these reports and additional *in culture* analyses point to size-dependent effects of HA on tissue and cell functions relevant to inflammation. In general, large HA polymers, which are mainly present in homeostatic tissues, are immunologically quiescent and contribute to enforcing homeostasis. HA fragments are generated by reactive oxygen/nitrogen species (ROS/RNS) and hyaluronidases produced during tissue stress and repair

[7-14]. HA fragments are considered to belong to the group of damage-associated molecular pattern molecules (DAMP) that activate innate immunity [2, 15]. Their functional consequences on inflammatory processes have the added complexity of being cell-context specific since responses depend not only on the precise polymer size but also on the stimulation status and type of cell responding to the fragments [15-18]. For example, 30 kDa endotoxin-free HA fragments stimulate the production of inflammatory cytokines (e.g. IL1ß) by B-lymphocytes [19] while 20 kDa HA fragments have no effect on the production by bone marrow macrophages [17]. Furthermore, since HA fragments activate multiple receptors (**Figure 1**), they can trigger different downstream events depending upon the injury context [1, 3, 20-23]. The inflammation and fibrotic stages of remodeling tissues occur in the presence of complex mixtures of high and low molecular weight HA polymers raising the possibility that a variety of sizes are required for manifestation of its pro-inflammatory properties. The lack of inflammation resulting from treatment of tissues with hyaluronidase (PEGPH20), which generates sizes of HA shown to be pro-inflammatory in cultured cells is interesting in this regard. PEGPH20 has a number of biological effects on HA signaling[24, 25] but has clearly been shown not to induce inflammation[24] and is currently in clinical trials for improving cancer therapy[26]. A likely explanation for the lack of inflammation is rapid clearance of fragments from the target tissue[27]. Given the overall complexity of HA fragment biology, it is perhaps not surprising that the effects of specific sizes of HA polymers on cell functions is currently controversial. The design of future experiments intended to clarify and categorize size-dependent functions will clearly have to take these complex variables into consideration[28, 29].

In homeostatic tissues, the majority of HA occurs in its high molecular weight and native form (e.g. >500 kDa), which is organized in the extracellular matrix by a variety of extracellular

binding proteins but it also occurs as coats around cells that are held in place by interactions with constitutively expressed cellular HA receptors such as CD44 [24, 30, 31]. The functions of these large HA polymers are understudied, but at a minimum promote tissue hydration, provide lubrication, protect against mechanical damage, reduce proliferation, modulate immune recognition, promote expression of anti-inflammatory cytokines, such as IL-10, and block macrophage functions, such as phagocytosis [4, 7, 9-11, 13, 32]. In contrast to homeostatic tissues, HA polymer size in remodeling and diseased tissues is strikingly polydisperse, ranging from <10 kDa to >500 kDa [33]. The inflammatory functions of short HA fragments have recently been intensely studied, and specific size ranges of these smaller HA fragments are reported to promote pro-inflammatory cytokine expression/release as well as regulate innate immune cell chemotaxis [3, 5, 34]. In a diseased or stressed tissue context, even high molecular weight HA can acquire pro-inflammatory functions by forming cables that provide adhesion sites for in-trafficking monocytes [35, 36]. Therefore, remodeling and diseased tissues contain a complex mixture of "activated" HA polymer sizes, and the categorization of the net consequence of this massive amount of bio-information is a challenge but has the potential to offer exquisite fine tuning in the therapeutic intervention of inflammatory processes.

Characterization of the key extracellular HA binding proteins and cellular receptors required for responses to HA polymers and development of reagents that block these interactions have greatly aided identification of the mechanisms by which HA and its fragments initiate and control innate immunity. These approaches have also identified effects of both HA and HA fragments beyond their roles in inflammation. These include mesenchymal differentiation [2, 37-39] and cancer

metastases [40-45]. This review will focus on the role of HA in inflammation and the consequence of blocking its activity, particularly with regard to innate immune cell functions.

There are at least 5 HA receptors expressed on innate immune cells that "sense" or bind to complex mixtures of HA polymer sizes and activate pathways required for an inflammatory response. These include CD44, receptor for hyaluronan mediated motility (RHAMM), toll-like receptors 2 and 4 (TLR2, TLR4), and stabilin 2 (STAB2) [5, 46-48]. CD44, LYVE [49] and Stab-2/HARE[50] are endocytic receptors that bind to HA and share a conserved ~100 amino acid residue region homologous to the link module of the extracellular HA-binding link protein. These HA receptors thus belong to the HA-binding link protein superfamily [51]. This compact region is made up of two antiparallel beta-sheets, which are composed of six beta-strands and two alpha-helices stabilized by two disulfide bridges. This region binds to a minimum of 6-8 HA residues [51, 52]. The CD44 link module is unique since it is extended by four beta-strands on the N- and C-terminal sequences flanking the link module, and is further stabilized by a third disulfide linkage [53]. LYVE-1 has also been hypothesized to contain an extended structure [54], but high resolution structural information is not available to confirm this. A group of proteins that bind to HA but lack the link module include RHAMM (gene name HMMR) and Layilin. Of these 'outlier" proteins, the mechanism by which RHAMM binds to HA is best characterized [55-57]. RHAMM binds to HA via two domains near the protein's carboxyl terminus, each of which has a BX_7B binding motif, where B represents any basic residue and X represents any non-acidic residue [56]. These clusters of basic residues allow for ionic interactions with the carboxylate ions of the polysaccharide [56]. CD44 has also been reported to contain two regions of RHAMM-like clustered basic amino acids which have been implicated in its HA-binding

activity [32, 56, 58]. RHAMM-like HA binding motifs are also present in Streptococcal HAS that affect polymer size[59]. However, the sequences required for binding HA to Layilin have to our knowledge not yet been reported.

HA receptors activate a variety of signaling pathways in innate immune cells that converge on NF*k*B expression/activation with consequent production of inflammatory cytokines. These receptors also coordinate signaling through growth factor receptors and other extracellular matrix receptors to control innate immune cell adhesion and chemotaxis. This coordination seems to be stimulus-specific [11, 23, 32, 45, 60]. For example, in response to HA fragments, CD44 forms complexes with TLR4 and MD2/LY96 to promote expression of TGF-ß2 and MMP13 that stimulate sterile inflammation [61]. On the other hand, signaling through surfactant protein A:TLR2 results in the production of TGF-ß1 production, which promotes HA-RHAMM-mediated macrophage chemotaxis [62]. TLR2 and 4 are also required for HA fragment:HA receptor-regulated NF*k*B expression and P2X7/inflammasome activation to express, activate and release IL1ß in response to tissue damage (**Figure 1**) [2, 19]. A variety of approaches for blocking the pro-inflammatory signaling initiated by HA fragments have been reported in experimental models of disease. These include inhibiting HA synthesis by small molecules (e.g. 4MU [63, 64]), gene knockdown/knockout, and blocking hyaluronidase expression/ROS formation [41, 65, 66], as well as development of hyaluronidase formulations (e.g. PEGPH20) [24, 25], modified HA polymers [67], small peptides that bind HA and small peptides that bind to HA receptors. At this stage in our as yet limited understanding of the complex biology of HA-stimulated inflammasome signaling, an approach that in animal models appears to be efficacious for most pro-inflammatory stimuli is to block signaling of receptor complexes by sequestering

the upstream pro-inflammatory HA polymer sizes. The development of peptide mimics that bind to HA fragments has provided proof-of-concept for this therapeutic approach in experimental models of inflammation and other diseases. This review focusses upon peptide mimics that bind directly to HA fragments and sequester them from activating receptors. Peptides that block the HA binding sites on HA receptors are also reviewed since these may be most useful in inflammatory conditions where the target HA receptor is known to be a driver of the disease.

Blocking HA signaling with peptide mimetics as a therapeutic approach

The goal of creating therapeutics that target HA fragments and HA receptors is challenging, in that this requires the targeting of a protein-polysaccharide interaction. Similar to that of targeting protein-protein interactions, the large surface area of interaction and the lack of well-defined binding pockets limits the ability to utilize small molecules to interfere with these binding interfaces. This is further complicated by the conformational changes that these HA receptor proteins undergo upon binding to HA [68]. Higher molecular weight entities such as peptides, proteins, and antibodies are more readily able to block protein-polysaccharide interactions due to their ability to interact over a larger surface area. Peptides are particularly attractive as drug candidates for this type of interaction as they are biocompatible with typically low toxicity, interact over a large surface area, and are structurally diverse permitting excellent selectivity [69].

The discovery of peptides to modulate HA receptor interactions has focused on two approaches, unbiased peptide library screening and rational design based upon known structures or binding sites. Unbiased peptide library screening has used phage display, which is a biochemical

approach to identify high affinity peptides displayed on a bacteriophage, and one-bead one-compound (OBOC) libraries, which is a chemical approach of screening peptides using polymer beads. These unbiased peptide libraries were primarily used for identifying peptide mimetics that scavenge HA and HA fragments, as discussed subsequently in the *Peptide library screening* section. The discovery approaches used for finding HA binding peptides that mimic HA receptors have primarily been based upon rational design with structural leads being known binding sites for HA.

Peptide library screening for HA- and HA receptor-binding

The P15-1 peptide (STMMSRSHKTRSHHV) is a 15mer peptide which was the first peptide mimetic that was reported to bind specifically to HA fragments of <10 kDa. It was identified by screening a recombinant phage display library with a complexity of approximately 10^{13} transformants for peptides that both bind to HA fragments (MW range 5-200 kDa) linked to Sepharose beads and that block cell motility [70]. Two peptide sequences were recovered in the screen and of these, P15-1 exhibited the highest affinity for HA fragments ($K_D = 10^{-7}$ M), and most strongly blocked cell motility. It has low homology with known HA receptors but contains a BX_7B motif similar to that required for binding of HA to RHAMM [56]. In a model of excisional skin injury, P15-1 blunted inflammation and fibrogenesis [70]. Consistent with the proposed possibility that P15-1 blocks RHAMM signaling though HA fragments, the consequences of this peptide mimetic on skin wound repair is similar to that of the genetic deletion of RHAMM [71], which results in blunted responses to HA fragments [72]. For example, both conditions block inflammation and fibrogenesis in excisional wounds but neither affect the course of incisional repair, which does not involve the massive waves of cellular

trafficking and migration that are required for healing of excisional wounds [71, 73, 74]. P15-1 synthesized entirely with D-amino acids (referred to as HABP42) also reduced bacterial burden in surgical skin wounds by modulating neutrophil responses [75, 76].

Pep-1, a 12mer peptide (GAHWQFNALTVR) was identified as an HA binding sequence by screening an M13 phage display library expressing random 12mer peptides fused to gene 3 (pIII) minor coat proteins with a complexity of approximately 10^9 transformants [77]. This peptide (Pep-1) was isolated by panning the library for sequences that bind to HA-coated plates. Pep-1 binds to HA with moderate affinity (K_D = 1.4 μM), inhibits HA binding to innate immune cells, and was shown to inhibit leukocyte attachment to HA substrates [77, 78]. The systemic, subcutaneous or topical administration of this peptide inhibited dinitrofluorobenzene/oxazolone induced-contact hypersensitivity by both blocking in-trafficking of inflammatory cells and migration of hapten-triggered langerhan (dendritic) cells out of the epidermis [77]. Skin dendritic cells utilize HA as a motogenic stimulus for migrating from the epidermis to lymph nodes, where they function as antigen-presenting cells, a process that is required for generating protective pro-inflammatory and tolerogenic immune responses during tissue injury [79, 80]. Aberrant activation of these cells contributes to inflammatory disease processes [80]. These results provided early evidence supporting the development of HA inhibitors for inflammatory disorders. Pep-1 was later shown to inhibit HA fragment-promoted MIP-2 production by bone marrow macrophages [81], reduce bronchial inflammation [82], reduce pro-inflammatory cytokine production (TNF-α, IL-6, MMP13 and iNOS), as well as preserve cartilage architecture in a mouse model of collagen-induced arthritis [79, 83], and like P15-1, reduced bacterial burden in surgical skin wounds of mice by modulating neutrophil response [75]. Pep-1 also dramatically

inhibited interleukin-2 (IL-2)-induced vascular leak syndrome (VLS) [84], which may be linked to its anti-inflammatory effects. Pep-1 targets multiple cell types that take part in inflammatory processes additional to macrophages. For example, this peptide blocks NF*k*B activation and cytokine production by chondrocytes *in culture* [85]. The efficacy of P15-1 and Pep-1 in blunting inflammation in animal models of disease/repair provided the original strong collective support for the development of peptide mimetics to block activity of HA fragments in inflammatory processes that lead to disease. Additionally, these early peptides have been useful in dissecting the signaling pathways that are regulated by HA fragments [70, 86]. Curiously, although they have similar functional effects, neither peptide is related to each other at a protein sequence level or to characterized HA receptors/binding proteins.

A novel combination of *ex vivo* and *in vivo* biopanning of peritoneal disseminated gastric cancer cells using a constrained CX_7C peptide library displayed on bacteriophage coupled with high throughput sequencing, identified a peptide (IP3, CKRDLSRRC) that targets to areas of peritoneal tumors rich in macrophages [87]. This peptide contains a RHAMM-like HA binding motif [56] and binds to HA-coated plates. IP-3 decorated with silver nanoparticles efficiently target these to peritoneal tumors suggesting it may be useful in delivering nanoparticle payloads to tumors and likely to areas of chronic inflammation.

Peptide-displaying phage and peptide library technology have also been used to identify peptides that mimic carbohydrates [88]. HA peptide mimics that bind with high affinity to recombinant RHAMM containing hyaluronan binding sequences, were originally identified by Ziebell, M. et al [89, 90]. Two libraries of 8mer peptides were designed to target recombinant RHAMM

fragments, with one library consisting of peptides made of entirely random sequences. The second library was biased, with alternating acidic residues being incorporated in every other position of the sequence, with the intention of mimicking the placement of the glucuronic acid moieties of HA [89]. Peptides from the unbiased (random) library that bound in an HA fragment-dependent manner to recombinant RHAMM with μM to nM affinity, exhibited some similarities with respect to regions of hydrophobic residues (e.g. PVY), but contained very few negatively charged amino acids. These peptides were then computationally modeled to evaluate their binding to an NMR-based model of RHAMM, from which residues within RHAMM were identified that were theorized to stabilize RHAMM-HA interactions [90]. However, these peptides have not yet been reported to affect cellular functions relevant to inflammation.

Recombinant CD44 protein has also been used to screen peptide libraries [91]. In this study, a Ph.D.-12mer phage display peptide library with a peptide complexity of $2.7x10^{-9}$ was screened using recombinant CD44 as bait. The screen isolated several peptides, one of which exhibited a K_D = 7.5 pM for recombinant CD44. However, none of the isolated peptides were tested for their ability to bind to the CD44 HA binding region or assayed for functional effects. Nevertheless, these studies show that isolating peptides that bind to HA receptors is a viable approach for potentially developing novel inhibitors of HA receptor signaling.

Rational design of peptide mimetics

RHAMM-based peptide design

RHAMM is the protein product of the HMMR gene [92]. RHAMM mRNA and protein expression is limited during homeostasis but high during injury and in diseased states, such as

inflammatory diseases and cancer [22, 32, 93, 94]. Although RHAMM is a cytoplasmic protein, it is unconventionally exported during cellular stress, where it partners with CD44 and growth factor receptors to activate focal adhesion kinase (FAK) [70], Src [95, 96], PI3K [97, 98], ERK1,2 [99-103], and motogenic signaling pathways. As an intracellular protein, it binds to microtubules, kinases, such as ERK1,2 [102], AURKA/TPX2 complexes [104-106] and transcriptional proteins such as E2F1 [107]. In this multifunctional capacity, intracellular RHAMM contributes to the dynamic organization and orientation of mitotic spindles and interphase microtubules, subcellular compartmentalization of the above kinases and expression of E2F1 regulated target gene expression (e.g. fibronectin [107]). RHAMM is unique amongst characterized cellular HA receptors in its relatively high binding affinity for hyaluronan and hyaluronan fragments. The binding affinity of purified RHAMM protein for HA was originally documented to be in the nM range [108] and a chemically synthesized 7 kDa fragment of RHAMM containing the HA binding region has more recently been quantified with a K_D of 0.84 nM for 5-10 kDa HA (Hauser-Kawaguchi, A., et al., submitted). This is a stronger affinity for HA than has been reported for other cellular receptors including, CD44 (65.7 μM) [109, 110] and LYVE-1 (35.6 μM) [111, 112]. This property as well as the clear role of RHAMM in disease processes and its highly regulated/restricted expression, which predicts limited toxic and off-target effects of therapeutic intervention, make RHAMM an ideal candidate for designing peptides that either sequester HA fragments or bind to and block RHAMM signaling.

To date, several RHAMM-sequence based peptide mimetics have been rationally designed to bind to HA fragments and have been shown to have therapeutic effects in a number of processes, including inflammation, wound repair, and fibrosis/adipogenesis. One of the first rationally

designed HA-binding peptides was based on the RHAMM BX_7B HA binding motif, and like P15-1, it does not otherwise have any amino acid sequence homology with RHAMM. This peptide strongly reduced BAL macrophages in bleomycin-induced lung injury and blunted destruction of lung architecture [113], reduced surfactant protein A-induced macrophage chemotaxis [62] and ozone induced lung hyper-responsiveness [114]. Another peptide, pep-35 has 70% homology with RHAMM sequence and essentially joins four RHAMM HA binding sequences together. This peptide reduced *Staphyloccus aureus* burden in infected surgical wounds and increased the production of CXCL1,2 by inflammatory cells, which subsequently increased neutrophil influx into the wound [76]. Other peptides have been designed to mimic the three BX_7B motifs of CD44 and although these have not been reported to affect inflammation, were shown to block tumor cell growth [115]. Finally, RHAMM sequence mimics (NPI-0102, NPI-0104), which do not appear to directly bind to HA but disrupt HA binding to RHAMM, have been reported to promote adipogenesis and reduce tissue fibrosis [37]. The effect on fibrosis may not directly result from blunting innate immune cell function, as these peptides increased the production of adiponectin, which is an anti-fibrogenic adipokine [116, 117].

In another rationally designed approach, Esguerra et al. developed HA peptide mimics from C-terminal region of α- and β-tubulin that bind to RHAMM [118]. Novel 12mer peptide ligands were identified that bind with high affinity (nM) to RHAMM and compete with HA for RHAMM binding [118]. The strongest binding compounds were those that were taken from the negatively charged carboxy terminal tail (CTT) and helix H12 regions of tubulins, and that contained a repeating amino acid motif of EEXEE, suggesting both electrostatic forces and conformational effects may be important for the development of RHAMM-binding ligands.

These compounds block HA binding to tumor cells but have not yet been reported to affect inflammatory processes.

Other RHAMM peptide-based therapies could reasonably be developed from varying the peptide backbone and/or altering the peptide structure, which may confer improved specificity and affinity towards its target. Such strategies include the development of stapled or cyclized peptides. These more drug-like peptides could then be optimized for their ability to block inflammation by use of a screening funnel such as is modelled in **Figure 2**, with the intent of blocking the inflammasome/NF*k*B signaling axis.

CD44-based design of small molecule and peptide mimetics

CD44 is often considered to be the major HA-binding receptor [16, 46, 66] as it is the most ubiquitously expressed HA receptor in homeostatic tissues. Like RHAMM and other HA receptors, CD44 has multiple functions in innate immunity [5, 11, 23, 119] and is frequently highly expressed in inflammation-based [46, 66] and other diseases [45]. Blocking CD44 function with antibodies has been successful in controlling inflammation in animal models but in clinical trials, blocking CD44 variant function with antibodies has had off-target and toxic effects [120, 121]. CD44 is activated in order to bind HA, which is a highly regulated process [5, 122], and therefore blocking its HA binding functions may be more efficacious and less toxic than targeting its variant or standard forms. RHAMM does not always partner with CD44 to mediate responses to HA fragments [22, 45, 62], thus, the development of therapies that directly block the HA binding properties of CD44 are desirable and represent a feasible alternative and/or additional approach to controlling inflammation. However, the development of small molecule

or peptidomimetics to directly block HA:CD44 interactions is challenging due the large surface area that mediates the interaction between this polysaccharide and CD44 [123]. Nevertheless, a number of reports have identified molecules that achieve this. One of these, A6 peptide, is a capped 8mer peptide derived from the connecting peptide domain of human urokinase plasminogen activator (uPA). This was shown to block HA:CD44 interactions [124] and cellular functions such as migration, but clinical trials showed no efficacy as a cancer treatment [125]. Using a combination of binding assays, fragment screening, and crystallographic characterization of complexes of the CD44:HA binding domain, one group has reported the formation of a small inducible "pocket" adjacent to the binding groove and through fragment screening identified a series of small molecules that reduce HA binding to CD44 [123]. Protein-ligand interaction studies indicated that the small molecule ligands competed with HA for CD44 binding by surface plasmon resonance (SPR); however, optimization of these lead compounds is required before advancing to animal models. Nevertheless, further research into the optimization of these compounds could result in identification of small molecule, peptide or hybrid peptide/small molecule inhibitors capable of disrupting HA binding to CD44 and blocking signaling that leads to inflammation.

Conclusions and Future directions

Peptides that scavenge HA fragments offer considerable promise in regulating inflammation-based diseases and disorders. The opportunities for the development of such peptidomimetics is considerable since in addition to HA receptors, a number of extracellular proteins, which bind to HA and fragments (e.g. versican [126, 127], TSG-6 [128-130]) could be suitable candidates for

peptidomimetic design. In addition, directly targeting HA receptors that activate pro-inflammatory signaling cascades in response to HA fragments holds promise. Nevertheless, the biology of HA receptors appears to be complex in terms of stimuli-specific activation of these receptors and use of HA scavenging peptides likely holds more immediate therapeutic promise. The possibility of designing small molecule and/or hybrid small molecule/peptide therapies is an exciting novel approach. This review did not cover vaccine therapies but reports that RHAMM R3 peptide vaccines for the treatment of multiple myeloma and myelodysplastic syndrome [131, 132] are currently being evaluated in phase I/II clinical trials, predicts that alternate RHAMM-based peptide therapies can be successfully developed.

FIGURE LEGENDS

Figure 1. Model for initiation of inflammation by HA fragment signaling in macrophages. HA fragments bind to HA receptors that are expressed by macrophages (CD44, RHAMM shown). Depending upon the initial stimulus, these coordinate with TLR2,4 and P2X7 to activate the inflammasome so that activated caspase 1 is produced. HA fragment:HA receptor interactions also activate NF*k*B to produce pro-IL1B and other pro-inflammatory cytokines. Pro-IL1B is then processed by caspase 1 to a mature form that is released by macrophages.

Figure 2. Schematic for isolating peptide mimetics to block HA fragment-stimulated pro-inflammatory signaling. A. Funnel for screening anti-inflammatory peptide mimetics. Peptides isolated from peptide libraries or designed from known HA binding proteins are first assessed for their ability to bind to HA fragments (or to receptors such as CD44 and RHAMM, or extracellular HA binding proteins), then modified to increase their stability in serum. Peptides that can potentially block inflammation are then identified by their ability to reduce NF*k*B activation in response to TLR2 or 4 agonists by macrophages in culture as well as TNF-α production induced by LPS *in vivo*. **B.** The active peptides identified in (A) are predicted to block inflammation by either sequestering HA fragments or blocking the binding of HA to HA receptors (RHAMM shown). This prevents activation of the inflammasome and NF*k*B so that IL1B is not produced and either inflammation is not initiated, or an existing inflammation is suppressed.

References:

1. Avenoso, A., et al., *Hyaluronan in experimental injured/inflamed cartilage: In vivo studies.* Life Sci, 2017.
2. Anders, H.J. and L. Schaefer, *Beyond tissue injury-damage-associated molecular patterns, toll-like receptors, and inflammasomes also drive regeneration and fibrosis.* J Am Soc Nephrol, 2014. **25**(7): p. 1387-400.
3. Wight, T.N., et al., *Interplay of extracellular matrix and leukocytes in lung inflammation.* Cell Immunol, 2017. **312**: p. 1-14.
4. Schwertfeger, K.L., et al., *Hyaluronan, Inflammation, and Breast Cancer Progression.* Front Immunol, 2015. **6**: p. 236.
5. Lee-Sayer, S.S., et al., *The where, when, how, and why of hyaluronan binding by immune cells.* Front Immunol, 2015. **6**: p. 150.
6. Jackson, D.G., *Lymphatic Regulation of Cellular Trafficking.* J Clin Cell Immunol, 2014. **5**.
7. Kavasi, R.M., et al., *HA metabolism in skin homeostasis and inflammatory disease.* Food Chem Toxicol, 2017. **101**: p. 128-138.
8. Cowman, M.K., et al., *Viscoelastic Properties of Hyaluronan in Physiological Conditions.* F1000Res, 2015. **4**: p. 622.
9. Hull, R.L., et al., *Hyaluronan: A Mediator of Islet Dysfunction and Destruction in Diabetes?* J Histochem Cytochem, 2015. **63**(8): p. 592-603.
10. Lauer, M.E., et al., *The Rise and Fall of Hyaluronan in Respiratory Diseases.* Int J Cell Biol, 2015. **2015**: p. 712507.
11. Litwiniuk, M., et al., *Hyaluronic Acid in Inflammation and Tissue Regeneration.* Wounds, 2016. **28**(3): p. 78-88.
12. Monslow, J., P. Govindaraju, and E. Pure, *Hyaluronan - a functional and structural sweet spot in the tissue microenvironment.* Front Immunol, 2015. **6**: p. 231.
13. Schiraldi, C., et al., *Hyaluronan viscosupplementation: state of the art and insight into the novel cooperative hybrid complexes based on high and low molecular weight HA of potential interest in osteoarthritis treatment.* Clin Cases Miner Bone Metab, 2016. **13**(1): p. 36-7.
14. de la Motte, C.A. and S.P. Kessler, *The role of hyaluronan in innate defense responses of the intestine.* Int J Cell Biol, 2015. **2015**: p. 481301.
15. Schaefer, L., *Complexity of danger: the diverse nature of damage-associated molecular patterns.* J Biol Chem, 2014. **289**(51): p. 35237-45.
16. D'Agostino, A., et al., *Is molecular size a discriminating factor in hyaluronan interaction with human cells?* Carbohydr Polym, 2017. **157**: p. 21-30.
17. Dong, Y., et al., *Endotoxin free hyaluronan and hyaluronan fragments do not stimulate TNF-alpha, interleukin-12 or upregulate co-stimulatory molecules in dendritic cells or macrophages.* Sci Rep, 2016. **6**: p. 36928.
18. Werb, Z. and P. Lu, *The Role of Stroma in Tumor Development.* Cancer J, 2015. **21**(4): p. 250-3.
19. Ghosh, S., et al., *Hyaluronan stimulates ex vivo B lymphocyte chemotaxis and cytokine production in a murine model of fungal allergic asthma.* Immunobiology, 2015. **220**(7): p. 899-909.

20. Maytin, E.V., *Hyaluronan: More than just a wrinkle filler.* Glycobiology, 2016. **26**(6): p. 553-9.
21. Garantziotis, S., et al., *The role of hyaluronan in the pathobiology and treatment of respiratory disease.* Am J Physiol Lung Cell Mol Physiol, 2016. **310**(9): p. L785-95.
22. Veiseh, M., et al., *Cellular heterogeneity profiling by hyaluronan probes reveals an invasive but slow-growing breast tumor subset.* Proc Natl Acad Sci U S A, 2014. **111**(17): p. E1731-9.
23. Albeiroti, S., A. Soroosh, and C.A. de la Motte, *Hyaluronan's Role in Fibrosis: A Pathogenic Factor or a Passive Player?* Biomed Res Int, 2015. **2015**: p. 790203.
24. Wong, K.M., et al., *Targeting the Tumor Stroma: the Biology and Clinical Development of Pegylated Recombinant Human Hyaluronidase (PEGPH20).* Curr Oncol Rep, 2017. **19**(7): p. 47.
25. Kang, L., et al., *Hyaluronan accumulates with high-fat feeding and contributes to insulin resistance.* Diabetes, 2013. **62**(6): p. 1888-96.
26. Infante, J.R., et al., *Phase 1 trials of PEGylated recombinant human hyaluronidase PH20 in patients with advanced solid tumours.* Br J Cancer, 2017.
27. Huang, Z., et al., *Recombinant human hyaluronidase PH20 does not stimulate an acute inflammatory response and inhibits lipopolysaccharide-induced neutrophil recruitment in the air pouch model of inflammation.* J Immunol, 2014. **192**(11): p. 5285-95.
28. Cowman, M.K., *Hyaluronan and Hyaluronan Fragments.* Adv Carbohydr Chem Biochem, 2017. **74**: p. 1-59.
29. Weigel, P.H., *Planning, evaluating and vetting receptor signaling studies to assess hyaluronan size-dependence and specificity.* Glycobiology, 2017. **27**(9): p. 796-799.
30. Bohaumilitzky, L., et al., *A Trickster in Disguise: Hyaluronan's Ambivalent Roles in the Matrix.* Front Oncol, 2017. **7**: p. 242.
31. Avenoso, A., et al., *Hyaluronan in the experimental injury of the cartilage: biochemical action and protective effects.* Inflamm Res, 2017.
32. Misra, S., et al., *Interactions between Hyaluronan and Its Receptors (CD44, RHAMM) Regulate the Activities of Inflammation and Cancer.* Front Immunol, 2015. **6**: p. 201.
33. Tolg, C., et al., *Hyaluronan modulates growth factor induced mammary gland branching in a size dependent manner.* Matrix Biol, 2017. **63**: p. 117-132.
34. Viola, M., et al., *Extracellular Matrix in Atherosclerosis: Hyaluronan and Proteoglycans Insights.* Curr Med Chem, 2016. **23**(26): p. 2958-2971.
35. Petrey, A.C. and C.A. de la Motte, *Hyaluronan, a crucial regulator of inflammation.* Front Immunol, 2014. **5**: p. 101.
36. Wang, A., et al., *Hyaluronan matrices in pathobiological processes.* Febs j, 2011. **278**(9): p. 1412-8.
37. Bahrami, S.B., et al., *Receptor for hyaluronan mediated motility (RHAMM/HMMR) is a novel target for promoting subcutaneous adipogenesis.* Integrative Biology, 2017. **9**(3): p. 223-237.
38. Astachov, L., et al., *Hyaluronan and mesenchymal stem cells: from germ layer to cartilage and bone.* Front Biosci (Landmark Ed), 2011. **16**: p. 261-76.
39. Pre, E.D., G. Conti, and A. Sbarbati, *Hyaluronic Acid (HA) Scaffolds and Multipotent Stromal Cells (MSCs) in Regenerative Medicine.* Stem Cell Rev, 2016. **12**(6): p. 664-681.
40. Lokeshwar, V.B., S. Mirza, and A. Jordan, *Targeting hyaluronic acid family for cancer chemoprevention and therapy.* Adv Cancer Res, 2014. **123**: p. 35-65.

41. Heldin, P., et al., *HAS2 and CD44 in breast tumorigenesis.* Adv Cancer Res, 2014. **123**: p. 211-29.
42. Mattheolabakis, G., et al., *Hyaluronic acid targeting of CD44 for cancer therapy: from receptor biology to nanomedicine.* J Drug Target, 2015. **23**(7-8): p. 605-18.
43. Safdar, M.H., et al., *New developments and clinical transition of hyaluronic acid-based nanotherapeutics for treatment of cancer: reversing multidrug resistance, tumour-specific targetability and improved anticancer efficacy.* Artif Cells Nanomed Biotechnol, 2017: p. 1-14.
44. Senbanjo, L.T. and M.A. Chellaiah, *CD44: A Multifunctional Cell Surface Adhesion Receptor Is a Regulator of Progression and Metastasis of Cancer Cells.* Front Cell Dev Biol, 2017. **5**: p. 18.
45. Turley, E.A., D.K. Wood, and J.B. McCarthy, *Carcinoma Cell Hyaluronan as a "Portable" Cancerized Prometastatic Microenvironment.* Cancer Res, 2016. **76**(9): p. 2507-12.
46. Jiang, D., J. Liang, and P.W. Noble, *Hyaluronan in tissue injury and repair.* Annu Rev Cell Dev Biol, 2007. **23**: p. 435-61.
47. Khaldoyanidi, S.K., et al., *Hyaluronan in the healthy and malignant hematopoietic microenvironment.* Adv Cancer Res, 2014. **123**: p. 149-89.
48. Prosdocimi, M. and C. Bevilacqua, *Exogenous hyaluronic acid and wound healing: an updated vision.* Panminerva Med, 2012. **54**(2): p. 129-35.
49. Milner, C.M., V.A. Higman, and A.J. Day, *TSG-6: a pluripotent inflammatory mediator?* Biochem Soc Trans, 2006. **34**(Pt 3): p. 446-50.
50. Harris, E.N., et al., *N-Glycans on the link domain of human HARE/Stabilin-2 are needed for hyaluronan binding to purified ecto-domain, but not for cellular endocytosis of hyaluronan.* Glycobiology, 2010. **20**(8): p. 991-1001.
51. Higman, V.A., et al., *A Refined Model for the TSG-6 Link Module in Complex with Hyaluronan USE OF DEFINED OLIGOSACCHARIDES TO PROBE STRUCTURE AND FUNCTION.* Journal of Biological Chemistry, 2014. **289**(9): p. 5619-5634.
52. Banerji, S., et al., *LYVE-1, a New Homologue of the CD44 Glycoprotein, Is a Lymph-specific Receptor for Hyaluronan.* The Journal of Cell Biology, 1999. **144**(4): p. 789-801.
53. Teriete, P., et al., *Structure of the Regulatory Hyaluronan Binding Domain in the Inflammatory Leukocyte Homing Receptor CD44.* Molecular Cell, 2004. **13**(4): p. 483-496.
54. Banerji, S., et al., *Distinctive properties of the hyaluronan-binding domain in the lymphatic endothelial receptor Lyve-1 and their implications for receptor function.* J Biol Chem, 2010. **285**(14): p. 10724-35.
55. Ziebell, M.R. and G.D. Prestwich, *Interactions of peptide mimics of hyaluronic acid with the receptor for hyaluronan mediated motility (RHAMM).* J Comput Aided Mol Des, 2004. **18**(10): p. 597-614.
56. Yang, B., et al., *Identification of a common hyaluronan binding motif in the hyaluronan binding proteins RHAMM, CD44 and link protein.* EMBO J, 1994. **13**(2): p. 286-96.
57. Yang, B., L. Zhang, and E.A. Turley, *Identification of two hyaluronan-binding domains in the hyaluronan receptor RHAMM.* J Biol Chem, 1993. **268**(12): p. 8617-23.
58. Liao, H.-X., et al., *N-terminal and central regions of the human CD44 extracellular domain participate in cell surface hyaluronan binding.* The Journal of Immunology, 1995. **155**(8): p. 3938-3945.

59. Baggenstoss, B.A., et al., *Hyaluronan synthase control of synthesis rate and hyaluronan product size are independent functions differentially affected by mutations in a conserved tandem B-X7-B motif.* Glycobiology, 2017. **27**(2): p. 154-164.
60. Sherman, L.S., et al., *Hyaluronan Synthesis, Catabolism, and Signaling in Neurodegenerative Diseases.* Int J Cell Biol, 2015. **2015**: p. 368584.
61. Taylor, K.R., et al., *Recognition of hyaluronan released in sterile injury involves a unique receptor complex dependent on Toll-like receptor 4, CD44, and MD-2.* J Biol Chem, 2007. **282**(25): p. 18265-75.
62. Foley, J.P., et al., *Toll-like receptor 2 (TLR2), transforming growth factor-beta, hyaluronan (HA), and receptor for HA-mediated motility (RHAMM) are required for surfactant protein A-stimulated macrophage chemotaxis.* J Biol Chem, 2012. **287**(44): p. 37406-19.
63. Morera, D.S., et al., *Hyaluronic acid family in bladder cancer: potential prognostic biomarkers and therapeutic targets.* Br J Cancer, 2017. **117**(10): p. 1507-1517.
64. Collum, S.D., et al., *Inhibition of hyaluronan synthesis attenuates pulmonary hypertension associated with lung fibrosis.* Br J Pharmacol, 2017. **174**(19): p. 3284-3301.
65. Osterholt, H.C., et al., *Antioxidant protects against increases in low molecular weight hyaluronan and inflammation in asphyxiated newborn pigs resuscitated with 100% oxygen.* PLoS One, 2012. **7**(6): p. e38839.
66. Misra, S., et al., *Hyaluronan-CD44 interactions as potential targets for cancer therapy.* Febs j, 2011. **278**(9): p. 1429-43.
67. Jordan, A.R., et al., *Antitumor activity of sulfated hyaluronic acid fragments in pre-clinical models of bladder cancer.* Oncotarget, 2017. **8**(15): p. 24262-24274.
68. Banerji, S., et al., *Structures of the Cd44-hyaluronan complex provide insight into a fundamental carbohydrate-protein interaction.* Nat Struct Mol Biol, 2007. **14**(3): p. 234-9.
69. Nevola, L. and E. Giralt, *Modulating protein–protein interactions: the potential of peptides.* Chemical Communications, 2015. **51**(16): p. 3302-3315.
70. Tolg, C., et al., *A RHAMM mimetic peptide blocks hyaluronan signaling and reduces inflammation and fibrogenesis in excisional skin wounds.* Am J Pathol, 2012. **181**(4): p. 1250-70.
71. Tolg, C., et al., *Rhamm-/- fibroblasts are defective in CD44-mediated ERK1,2 motogenic signaling, leading to defective skin wound repair.* J Cell Biol, 2006. **175**(6): p. 1017-28.
72. Tolg, C., P. Telmer, and E. Turley, *Specific sizes of hyaluronan oligosaccharides stimulate fibroblast migration and excisional wound repair.* PLoS One, 2014. **9**(2): p. e88479.
73. Zhou, H., et al., *The progress and challenges for dermal regeneration in tissue engineering.* J Biomed Mater Res A, 2017. **105**(4): p. 1208-1218.
74. Sorg, H., et al., *Skin Wound Healing: An Update on the Current Knowledge and Concepts.* Eur Surg Res, 2017. **58**(1-2): p. 81-94.
75. Zaleski, K.J., et al., *Hyaluronic acid binding peptides prevent experimental staphylococcal wound infection.* Antimicrob Agents Chemother, 2006. **50**(11): p. 3856-60.
76. Lee, J.C., et al., *Modulation of the local neutrophil response by a novel hyaluronic acid-binding peptide reduces bacterial burden during staphylococcal wound infection.* Infect Immun, 2010. **78**(10): p. 4176-86.

77. Mummert, M.E., et al., *Development of a peptide inhibitor of hyaluronan-mediated leukocyte trafficking.* Journal of Experimental Medicine, 2000. **192**(6): p. 769-780.
78. Mummert, M.E., et al., *Synthesis and surface expression of hyaluronan by dendritic cells and its potential role in antigen presentation.* The Journal of Immunology, 2002. **169**(8): p. 4322-4331.
79. Mummert, M.E., *Immunologic roles of hyaluronan.* Immunol Res, 2005. **31**(3): p. 189-206.
80. Worbs, T., S.I. Hammerschmidt, and R. Forster, *Dendritic cell migration in health and disease.* Nat Rev Immunol, 2017. **17**(1): p. 30-48.
81. Morioka, Y., et al., *Cathelicidin antimicrobial peptides inhibit hyaluronan-induced cytokine release and modulate chronic allergic dermatitis.* J Immunol, 2008. **181**(6): p. 3915-22.
82. Uchakina, O.N., et al., *The role of hyaluronic acid in SEB-induced acute lung inflammation.* Clin Immunol, 2013. **146**(1): p. 56-69.
83. Campo, G.M., et al., *Combined treatment with hyaluronan inhibitor Pep-1 and a selective adenosine A2 receptor agonist reduces inflammation in experimental arthritis.* Innate Immun, 2013. **19**(5): p. 462-78.
84. Guan, H., P.S. Nagarkatti, and M. Nagarkatti, *Blockade of hyaluronan inhibits IL-2-induced vascular leak syndrome and maintains effectiveness of IL-2 treatment for metastatic melanoma.* The Journal of Immunology, 2007. **179**(6): p. 3715-3723.
85. Campo, G.M., et al., *Inhibition of small HA fragment activity and stimulation of A2A adenosine receptor pathway limit apoptosis and reduce cartilage damage in experimental arthritis.* Histochem Cell Biol, 2015. **143**(5): p. 531-43.
86. Campo, G.M., et al., *Beta-arrestin 1 is involved in the catabolic response stimulated by hyaluronan degradation in mouse chondrocytes.* Cell Tissue Res, 2015. **361**(2): p. 567-79.
87. Ikemoto, H., et al., *Hyaluronan-binding peptide for targeting peritoneal carcinomatosis.* Tumour Biol, 2017. **39**(5): p. 1010428317701628.
88. Fukuda, M.N., *Peptide-displaying phage technology in glycobiology.* Glycobiology, 2012. **22**(3): p. 318-25.
89. Ziebell, M.R., et al., *Peptides that mimic glycosaminoglycans: high-affinity ligands for a hyaluronan binding domain.* Chemistry & biology, 2001. **8**(11): p. 1081-1094.
90. Ziebell, M.R. and G.D. Prestwich, *Interactions of peptide mimics of hyaluronic acid with the receptor for hyaluronan mediated motility (RHAMM).* Journal of computer-aided molecular design, 2004. **18**(10): p. 597-614.
91. Park, H.Y., et al., *Screening of peptides bound to breast cancer stem cell specific surface marker CD44 by phage display.* Mol Biotechnol, 2012. **51**(3): p. 212-20.
92. Spicer, A.P., et al., *The human and mouse receptors for hyaluronan-mediated motility, RHAMM, genes (HMMR) map to human chromosome 5q33.2-qter and mouse chromosome 11.* Genomics, 1995. **30**(1): p. 115-7.
93. Maxwell, C.A., J. McCarthy, and E. Turley, *Cell-surface and mitotic-spindle RHAMM: moonlighting or dual oncogenic functions?* J Cell Sci, 2008. **121**(Pt 7): p. 925-32.
94. Shepard, H.M., *Breaching the Castle Walls: Hyaluronan Depletion as a Therapeutic Approach to Cancer Therapy.* Front Oncol, 2015. **5**: p. 192.

95. Hall, C.L., F.S. Wang, and E. Turley, *Src-/- fibroblasts are defective in their ability to disassemble focal adhesions in response to phorbol ester/hyaluronan treatment.* Cell Commun Adhes, 2002. **9**(5-6): p. 273-83.
96. Hall, C.L., et al., *pp60(c-src) is required for cell locomotion regulated by the hyaluronanreceptor RHAMM.* Oncogene, 1996. **13**(10): p. 2213-24.
97. Mascaro, M., et al., *Low molecular weight hyaluronan induces migration of human choriocarcinoma JEG-3 cells mediated by RHAMM as well as by PI3K and MAPK pathways.* Histochem Cell Biol, 2017. **148**(2): p. 173-187.
98. Goueffic, Y., et al., *Hyaluronan induces vascular smooth muscle cell migration through RHAMM-mediated PI3K-dependent Rac activation.* Cardiovasc Res, 2006. **72**(2): p. 339-48.
99. Kouvidi, K., et al., *Receptor for hyaluronic acid- mediated motility (RHAMM) regulates HT1080 fibrosarcoma cell proliferation via a beta-catenin/c-myc signaling axis.* Biochim Biophys Acta, 2016. **1860**(4): p. 814-24.
100. Katona, E., et al., *PP2B and ERK1/2 regulate hyaluronan synthesis of HT168 and WM35 human melanoma cell lines.* Int J Oncol, 2016. **48**(3): p. 983-97.
101. Jiang, J., P. Mohan, and C.A. Maxwell, *The cytoskeletal protein RHAMM and ERK1/2 activity maintain the pluripotency of murine embryonic stem cells.* PLoS One, 2013. **8**(9): p. e73548.
102. Tolg, C., et al., *RHAMM promotes interphase microtubule instability and mitotic spindle integrity through MEK1/ERK1/2 activity.* J Biol Chem, 2010. **285**(34): p. 26461-74.
103. Hatano, H., et al., *Overexpression of receptor for hyaluronan-mediated motility (RHAMM) in MC3T3-E1 cells induces proliferation and differentiation through phosphorylation of ERK1/2.* J Bone Miner Metab, 2012. **30**(3): p. 293-303.
104. Chakraborty, J., et al., *MoMuLV-ts-1: A Unique Mouse Model of Retrovirus-Induced Lymphoma Transmitted by Breast Milk.* Adv Virol, 2011. **2011**: p. 813651.
105. Kouvidi, K., et al., *Hyaluronan/RHAMM interactions in mesenchymal tumor pathogenesis: role of growth factors.* Adv Cancer Res, 2014. **123**: p. 319-49.
106. Blanco, I., et al., *Assessing associations between the AURKA-HMMR-TPX2-TUBG1 functional module and breast cancer risk in BRCA1/2 mutation carriers.* PLoS One, 2015. **10**(4): p. e0120020.
107. Meier, C., et al., *Association of RHAMM with E2F1 promotes tumour cell extravasation by transcriptional up-regulation of fibronectin.* J Pathol, 2014. **234**(3): p. 351-64.
108. Turley, E.A., D. Moore, and L.J. Hayden, *Characterization of hyaluronate binding proteins isolated from 3T3 and murine sarcoma virus transformed 3T3 cells.* Biochemistry, 1987. **26**(11): p. 2997-3005.
109. Lesley, J., et al., *Hyaluronan binding by cell surface CD44.* J Biol Chem, 2000. **275**(35): p. 26967-75.
110. Dubacheva, G.V., et al., *Designing multivalent probes for tunable superselective targeting.* Proc Natl Acad Sci U S A, 2015. **112**(18): p. 5579-84.
111. Banerji, S., et al., *Homodimerization of the Lymph Vessel Endothelial Receptor LYVE-1 through a Redox-labile Disulfide Is Critical for Hyaluronan Binding in Lymphatic Endothelium.* J Biol Chem, 2016. **291**(48): p. 25004-25018.
112. Lawrance, W., et al., *Binding of Hyaluronan to the Native Lymphatic Vessel Endothelial Receptor LYVE-1 Is Critically Dependent on Receptor Clustering and Hyaluronan Organization.* J Biol Chem, 2016. **291**(15): p. 8014-30.

113. Savani, R.C., et al., *A role for hyaluronan in macrophage accumulation and collagen deposition after bleomycin-induced lung injury.* American journal of respiratory cell and molecular biology, 2000. **23**(4): p. 475-484.
114. Garantziotis, S., et al., *Hyaluronan mediates ozone-induced airway hyperresponsiveness in mice.* J Biol Chem, 2009. **284**(17): p. 11309-17.
115. Xu, X.-M., et al., *A peptide with three hyaluronan binding motifs inhibits tumor growth and induces apoptosis.* Cancer research, 2003. **63**(18): p. 5685-5690.
116. Marangoni, R.G. and T.T. Lu, *The roles of dermal white adipose tissue loss in scleroderma skin fibrosis.* Curr Opin Rheumatol, 2017. **29**(6): p. 585-590.
117. Park, P.H., C. Sanz-Garcia, and L.E. Nagy, *Adiponectin as an anti-fibrotic and anti-inflammatory adipokine in the liver.* Curr Pathobiol Rep, 2015. **3**(4): p. 243-252.
118. Esguerra, K.V., et al., *Identification, design and synthesis of tubulin-derived peptides as novel hyaluronan mimetic ligands for the receptor for hyaluronan-mediated motility (RHAMM/HMMR).* Integr Biol (Camb), 2015. **7**(12): p. 1547-60.
119. McDonald, B. and P. Kubes, *Interactions between CD44 and Hyaluronan in Leukocyte Trafficking.* Front Immunol, 2015. **6**: p. 68.
120. Menke-van der Houven van Oordt, C.W., et al., *First-in-human phase I clinical trial of RG7356, an anti-CD44 humanized antibody, in patients with advanced, CD44-expressing solid tumors.* Oncotarget, 2016. **7**(48): p. 80046-80058.
121. Riechelmann, H., et al., *Phase I trial with the CD44v6-targeting immunoconjugate bivatuzumab mertansine in head and neck squamous cell carcinoma.* Oral Oncol, 2008. **44**(9): p. 823-9.
122. Siegelman, M.H., H.C. DeGrendele, and P. Estess, *Activation and interaction of CD44 and hyaluronan in immunological systems.* J Leukoc Biol, 1999. **66**(2): p. 315-21.
123. Liu, L.K. and B.C. Finzel, *Fragment-based identification of an inducible binding site on cell surface receptor CD44 for the design of protein-carbohydrate interaction inhibitors.* J Med Chem, 2014. **57**(6): p. 2714-25.
124. Piotrowicz, R.S., et al., *A6 peptide activates CD44 adhesive activity, induces FAK and MEK phosphorylation, and inhibits the migration and metastasis of CD44-expressing cells.* Mol Cancer Ther, 2011. **10**(11): p. 2072-82.
125. Gold, M.A., et al., *A phase II study of a urokinase-derived peptide (A6) in the treatment of persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma: a Gynecologic Oncology Group study.* Gynecol Oncol, 2012. **125**(3): p. 635-9.
126. Suwan, K., et al., *Versican/PG-M Assembles Hyaluronan into Extracellular Matrix and Inhibits CD44-mediated Signaling toward Premature Senescence in Embryonic Fibroblasts.* J Biol Chem, 2009. **284**(13): p. 8596-604.
127. Foulcer, S.J., A.J. Day, and S.S. Apte, *Isolation and purification of versican and analysis of versican proteolysis.* Methods Mol Biol, 2015. **1229**: p. 587-604.
128. Heng, B.C., et al., *Hyaluronan binding to link module of TSG-6 and to G1 domain of aggrecan is differently regulated by pH.* J Biol Chem, 2008. **283**(47): p. 32294-301.
129. Baranova, N.S., et al., *The inflammation-associated protein TSG-6 cross-links hyaluronan via hyaluronan-induced TSG-6 oligomers.* J Biol Chem, 2011. **286**(29): p. 25675-86.

130. Blundell, C.D., et al., *Determining the molecular basis for the pH-dependent interaction between the link module of human TSG-6 and hyaluronan.* J Biol Chem, 2007. **282**(17): p. 12976-88.
131. Schmitt, M., et al., *RHAMM-R3 peptide vaccination in patients with acute myeloid leukemia, myelodysplastic syndrome, and multiple myeloma elicits immunologic and clinical responses.* Blood, 2008. **111**(3): p. 1357-65.
132. Greiner, J., et al., *High-dose RHAMM-R3 peptide vaccination for patients with acute myeloid leukemia, myelodysplastic syndrome and multiple myeloma.* Haematologica, 2010. **95**(7): p. 1191-7.

Table 1 – Functional properties of HA binding peptide mimics

Disease model or context	Function of HA fragments blocked by peptides	Peptide studied for inhibitory function	Reference
Excisional skin wound model in mice	M1 macrophage influx and production of TGF-ß1, angiogenesis, and fibroplasia/fibrosis	P15-1	[70]
IL-2-induced vascular leak syndrome (VLS) in mice	HA-induced endothelial cell permeability	Pep-1	[84]
Collagen-induced arthritis in a mouse model	Reduced cartilage architecture	Pep-1	[83]
Mouse bone marrow derived macrophages	Macrophage release of MIP-2	Pep-1	[81]
Mouse model of SEB-induced lung injury in mice	Peri-bronchial inflammation	Pep-1	[82]
Contact hypersensitivity in mice	Inhibits leukocyte homing to injury and hapten-induced langerhan cell migration	Pep-1	[77]
Bleomycin-induced lung injury in mice	Inflammation and fibrosis-inducing	RG peptide	[113]
Staphlococcal aureus colonization of surgical skin wound	Increased CXCL1, CXCL2 production and neutrophil influx	Pep-35	[75, 76]
Subcutaneous mammary fat pads in female rats	Inflammation and obesity	NPI-0102	[37]
		NPI-0104	[37]

Table 2 - Functional peptides and their affinities for their targets

Target	**Peptide studied for inhibitory function**	**Peptide sequence(s)**	**Binding affinity**	**Reference**
HA	P15-1	STMMSRSHKTRSHHV	1×10^{-7} M	[70]
	Pep-1	GAHWQFNALTVR	1.4×10^{-6} M	[77]
	NPI-0102	KLKDENSQLKSEVSK	ND	[37]
	NPI-0104	KSEVSK	ND	[37]
RHAMM	Unbiased (random) library	SGRPYKPP YXSSNKPG EGEWPVYP WNYTEAKG QAMNKFTF NTDSNKNM NPVFNDGY FLRWFIMI EMAQMLLE PFLMKFPI IYIYPQPQ	μM to nM affinity	[89]
	Rationally designed (biased) library	MDYEPEQE YDSEYESE FDFDSEYE EDAENDEE	μM to nM affinity	[89]
	Tubulin-derived peptides	VEGEGEEEGEEY FTEAESNMNDLV EAFEDEEEEIDG EEDFGEEAEEEA GEFEEEAEEEVA SVEAEAEEGEEY	nM affinities	[118]
CD44	Ph.D. 12mer Phage display library	WHPWSYLWTQQA	7.5×10^{-12} M	[91]

Title: Design of peptide mimetics to block pro-inflammatory functions of HA fragments

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HIGHLIGHTS

- Hyaluronan (HA) fragments trigger pro-inflammatory signaling in stressed/injured and diseased tissues
- Peptides that inhibit HA signaling have been developed and show anti-inflammation efficacy
- Peptide mimetics of HA, as well as peptide mimetics of the HA receptors RHAMM and CD44, are reviewed

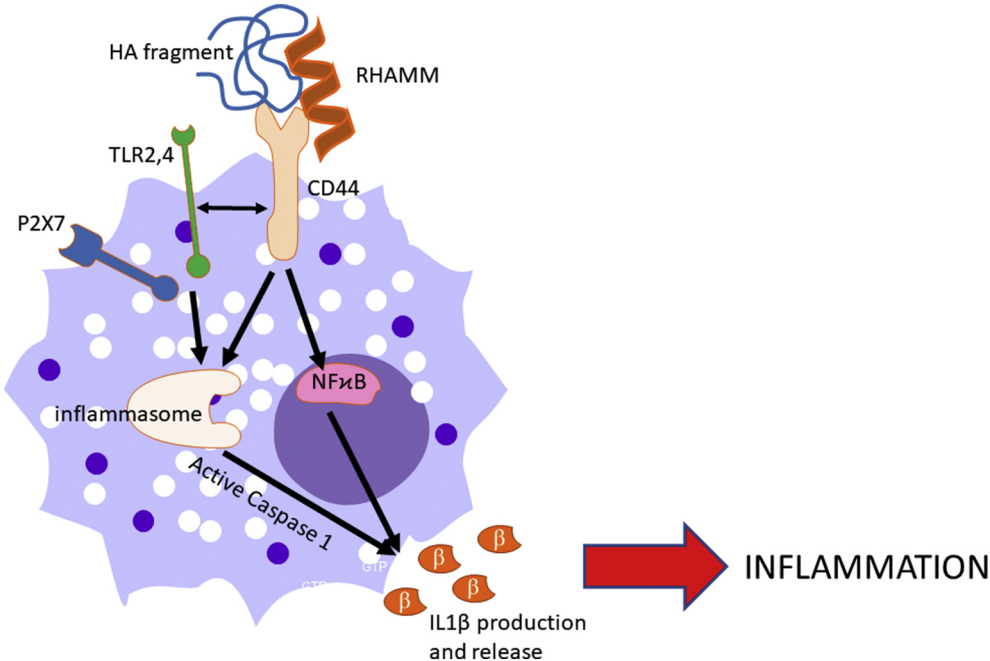
HA fragment
RHAMM
TLR2,4
CD44
P2X7
NFκB
inflammasome
Active Caspase 1
β
β
β
β
IL1β production
and release
INFLAMMATION

Figure 1

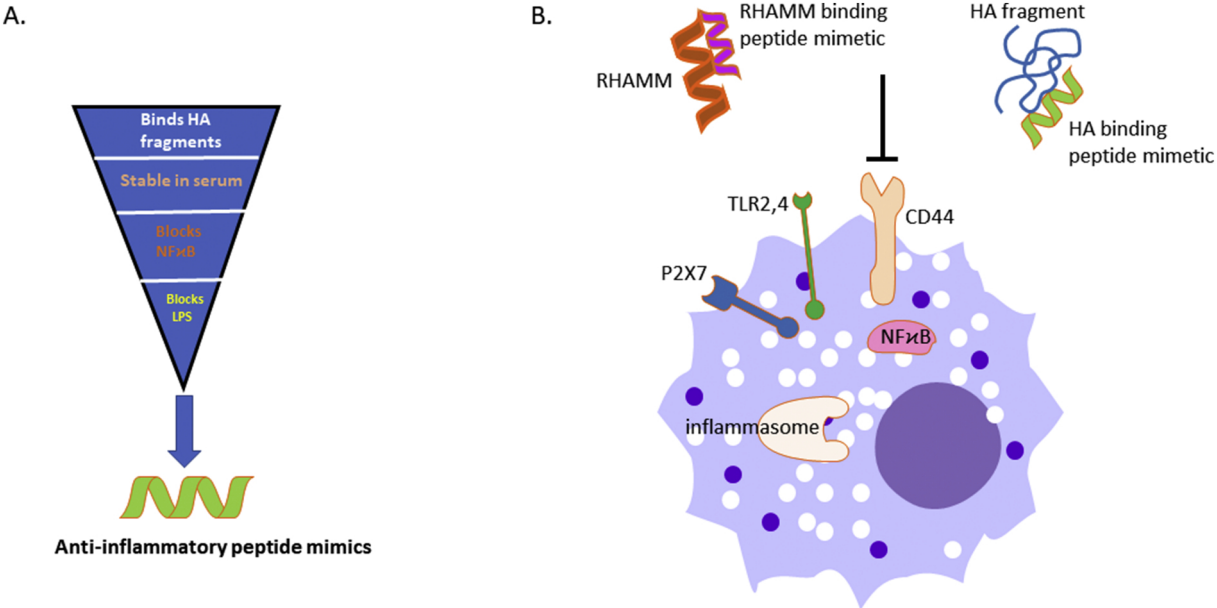
A.
Binds HA fragments
Stable in serum
Blocks NFκB
Blocks LPS
Anti-inflammatory peptide mimics
B.
RHAMM binding peptide mimetic
RHAMM
HA fragment
HA binding peptide mimetic
TLR2,4
CD44
P2X7
NFκB
inflammasome

Figure 2