Minireview Article

In Search Of Bioactivities Of Hyaluronan And Its Fragments: A Mini-Review

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ABSTRACT

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| Hyaluronic acid (hyaluronan, HA) plays a crucial role in various biological processes, including cancer resistance, pain modulation, and inflammation control. This study explores HA’s biomedical applications by examining its molecular mechanisms and therapeutic potential. Research on naked mole rats, conducted by Professors Vera Gorbunova and Andrei Seluanov, suggests that HA contributes to their cancer resistance, pain insensitivity, and reduced inflammation. Professor David Jackson identified the HA receptor LYVE-1, which facilitates leukocyte migration through the lymphatic system. Expanding on this, Dr. Matthew Hui demonstrated that low molecular weight HA (HA35, <220 nm) rapidly diffuses through lymphatic organs and enters circulation, promoting inflammatory cell clearance. Further investigations by Professors Elvira de la Peña and Carlos Belmonte revealed that high molecular weight HA (HMWHA) inhibits the TRPV1 calcium channel, while HA35 blocks TRPA1, providing dual analgesic effects. Dr. Hui’s team developed a recombinant human sperm hyaluronidase PH20-based HA35 injection, showing its efficacy in alleviating inflammatory, neuropathic, wound, and cancer pain, reducing inflammation, and accelerating wound healing. In vitro studies indicate that HMWHA and HA35 share receptor-binding properties, suggesting overlapping biological activities. The findings emphasize HA’s potential in therapeutic applications, with effective in vivo activity requiring high doses of LMWHA. |

*Keywords:* *Hyaluronic acid (HA); Low molecular weight HA (HA35); Cancer resistance; Anti-inflammation; Pain relief*

1. INTRODUCTION

HA is a naturally occurring polysaccharide with diverse biological functions, ranging from tissue hydration to cellular signaling (Hargittai, 2010; Johnson, et al, 2021). Over the past decades, extensive research has uncovered its critical roles in disease prevention (Hynnekleiv, et al, 2022), pain modulation (Sherif, et al, 2018; Diaz-Salmeron, et al, 2023), and tissue regeneration (Hargittai, et al,2010). From its early structural elucidation to modern large-scale production methods, HA has evolved into a key biomedical material with broad clinical applications (Hui, et al,2024; Xu, et al,2024; Trgger et al,2024). Recent studies have further expanded our understanding of HA, particularly in relation to its molecular weight-dependent functions (Jia, et al,2023), receptor interactions (Chaudhry, et al,2021), and therapeutic potential (Zhang, et al,2024). This review highlights significant advancements in HA research, including its role in cancer resistance, inflammation control, and pain relief, while also exploring cutting-edge biotechnological approaches for enhancing its medical applications.

2. Advancements in HA Research: From Molecular Insights to Clinical Applications

* 1. Thorbjörn Laurent & HA Structure

Thorbjörn Laurent (1930–2009) was a pioneering Swedish scientist who made groundbreaking contributions to the study of HA, particularly in elucidating its chemical structure (Hargittai, 2010). In 1991, he was elected President of the Royal Swedish Academy of Sciences, where he played a key role in overseeing the awarding of the Nobel Prizes in Physics and Chemistry, as well as the Alfred Nobel Memorial Prize in Economic Sciences (Hargittai, 2010). HA is a high-molecular-weight polymer composed of repeating disaccharide units of D-glucuronic acid and N-acetylglucosamine. It is abundantly distributed in human tissues, with concentrations reaching as high as 80% in the eyes and umbilical cord. However, its large molecular size significantly limits its ability to penetrate deep tissues, restricting its bioactivity primarily to localized applications. This limitation has prompted researchers to explore novel approaches to enhance the tissue permeability and bioavailability of HA, expanding its potential applications in medicine.

* 1. Endre A. Balazs & Advances in Medical Applications of HA

Endre A. Balazs, a scientist from the United States, revolutionized the medical use of HA by extracting it from chicken combs and developing injectable formulations for treating arthritis and as protective agents in ophthalmic surgeries. Together with his collaborators, he developed six major HA-based products, including low-dose (20 mg) joint cavity injections (Marshall et al., 1998), ophthalmic surgery formulations (Huerta et al., 2021), dermal fillers (Beasley et al., 2009), adhesion prevention products for abdominal surgeries (Kramer et al., 2002), treatments for bladder pain syndrome (Sherif, 2018; Diaz-Salmeron et al., 2023), and eye drops for dry eye syndrome (Hynnekleiv, 2022). Initially, medical-grade HA was predominantly extracted from bovine eyes and chicken combs. However, with the rising global demand, researchers began exploring more efficient production methods. In this regard, Dr. Peixue Ling from Shandong University in China played a pivotal role in advancing large-scale fermentation techniques for producing HA from Streptococcus bacteria. His contributions positioned China as a global leader in HA production, with the country now accounting for approximately 82% of the world's supply. The phrase “the world sees HA from China, and China sees it from Shandong” reflects his significant impact on the industry.

* 1. HA Receptors & Biological Activity

Although HMW-HA has limited tissue permeability, its biological activity in vivo remains a subject of extensive research. Studies have demonstrated that HA and its continuously degrading fragments (Laurent et al., 1991; Fraser et al., 1997; Lebel et al., 1991) interact with multiple cell surface binding proteins and receptors, including CD44 (Chaudhry et al., 2021), LYVE-1 (Johnson et al., 2021), RHAMM (Messam et al., 2021), HARE (Pandey et al., 2015; Harris et al., 2020), Siglec-9 (Mei et al., 2023), TLR2 (Jiang et al., 2015), CEMIP (Domanegg et al., 2022), and TMEM2 (Tobisawa et al., 2021). The widespread distribution of these receptors suggests that HA plays a crucial role in various biological processes, including inflammation regulation, immune response, and cell migration. However, due to the deep localization of many HA receptors within tissues and the poor penetration of HMW-HA, its in vivo biological activity has not been definitively confirmed. To address this limitation, scientists have turned to animal models to better understand HA’s potential biological functions. Among these, the naked mole rat has emerged as a particularly intriguing subject of study.

* 1. HA in Naked Mole Rats & Its Biological Significance

Professors Vera Gorbunova and Andrei Seluanov from the University of Rochester have spent the past 15 years investigating HA levels in naked mole rats (Tian et al., 2013; Takasugi et al., 2020; Taguchi et al., 2020). Certain tissues in these remarkable animals contain HA concentrations as high as 6% (6 grams per 100 grams of tissue), significantly higher than those found in other mammals. With an average lifespan of 32 years, naked mole rats are often referred to as “immortal creatures” and exhibit extraordinary traits, including lifelong cancer resistance, pain insensitivity, a lack of major inflammatory diseases, and minimal subcutaneous fat deposition. These characteristics suggest that highly concentrated HA may have biological activities or therapeutic effects related to cancer prevention, anti-inflammation, pain relief, and inhibition of subcutaneous adipogenesis (<https://en.wikipedia.org/wiki/Naked_mole-rat>). Building on these findings, researchers transferred the HA synthase gene from naked mole rats into laboratory mice. Under the regulation of the strongest known chick β-actin promoter, these transgenic mice expressed relatively high levels of HA in their tissues (Zhang et al., 2023). The results demonstrated that HA-enriched mice exhibited notable resistance to cancer and inflammation, alongside cosmetic benefits (Zhang et al., 2023). Consequently, the HA synthase gene from naked mole rats has been designated as a longevity gene. The work of Professors Gorbunova and Seluanov further indicates that gene transfer techniques—such as the use of viral or mRNA vectors combined with potent gene promoters—could overcome the limited tissue permeability of HMW-HA. These methods may facilitate the effective delivery of HA into human tissues, enabling interactions with various HA receptors to produce significant biological activities and therapeutic effects in clinical applications. This strategy opens new possibilities for HA-based therapies in medicine.

* 1. HA, the Lymphatic System, and Immune Cell Movement

Professor David Jackson from Oxford University was the first to identify LYVE-1, a key HA receptor in the lymphatic system, and its role in facilitating the return of leukocytes, including lymphocytes, to the bloodstream (Johnson et al., 2021; Jackson, 2019; Stanley et al., 2020). This discovery laid the foundation for further research on HA’s influence on immune cell circulation. Building on Jackson’s work, Dr. Matthew Hui, a graduate of the University of Toronto, demonstrated that HA35, with a size of less than 220 nm, rapidly diffuses through the lymph nodes and spleen before re-entering systemic circulation (Jia et al., 2023). Moreover, HA35 was shown to enhance leukocyte mobility, potentially reducing the accumulation of inflammatory cells in affected tissues (Gantumur et al., 2024; Hui et al., 2024). These findings suggest that HA35 may have significant implications in regulating immune responses, reducing chronic inflammation, and potentially alleviating inflammatory disorders.

* 1. HA’s Role in Pain and Itch Regulation

Expanding the scope of HA research, Professors Elvira de la Peña and Carlos Belmonte from Spain demonstrated that high concentrations (400 µg/mL) of HMW-HA inhibit the pain-related calcium channel TRPV1 (Caires et al., 2015; de la Peña et al., 2016). Inspired by their research, Dr. Hui discovered that both HA35 and HMW-HA at the same high concentration (400 µg/mL) inhibit another pain-related calcium channel, TRPA1. This dual antagonism of TRPV1 and TRPA1 (submitted for publication) produces a synergistic and potent analgesic effect. Moreover, Liu et al. proposed that itch and pain share largely overlapping receptors, including TRPV1 and TRPA1(Liu et al., 2013). Consequently, these findings suggest that HA35, as a dual TRPV1 and TRPA1 antagonist, may not only provide pain relief but could also be effective in alleviating itch caused by mosquito bites, gingivitis, and senile eczema. This expands the potential therapeutic applications of HA, particularly in dermatology and pain management.

* 1. HA35 in Human Studies and Clinical Applications

In the United States, researchers Carol de la Motte and Laura Nagy were among the first to identify the biological activity of the HA35 fragment in human colostrum (Kessler et al., 2018; Saikia et al., 2017). They also conducted initial human safety studies for HA35 (Bellar et al., 2019), providing a foundation for its clinical use. Inspired by these findings, Dr. Matthew Hui and his collaborators developed a uniform injectable formulation of HA35 using sperm acrosome hyaluronidase PH20 (B-HA injection, 100 mg/5 mL, L20200708MP07707, Ministry of Health, Mongolia; EP3479830; US11839625B2; US11826380B2; US11826381B2; CA3049286; AU2017255833). Through a series of clinical studies, Dr. Hui demonstrated that high-dose HA35 injections (100 mg) are highly effective in managing various pain conditions, including inflammatory, neuropathic, wound-related, and cancer-related pain (Hui et al., 2024; Dashnyam et al., 2023; Xu et al., 2024; Treger et al., 2024; Zhang et al., 2024; Purevsuren et al., 2025). Beyond pain relief, HA35 injections have been shown to significantly reduce inflammation-related redness and swelling while accelerating wound healing (Hui et al., 2024; Treger et al., 2024). Further research by Dr. Hui revealed that both HMW-HA and HA35 exhibit comparable effects in cell culture studies, where tissue permeability is not a limiting factor (Jia et al., 2023; Gantumur et al., 2024). This finding suggests that HA’s receptor-binding capacity is primarily derived from its disaccharide units, meaning that both forms interact with the same receptors to modulate inflammatory responses (Huang et al., 2014; Huang et al., 2015). Moreover, Dr. Hui’s latest study, currently under review, suggests that different low molecular weight HA fragments (within the 100 kDa range and capable of passing through a 220 nm filter) exhibit varying affinities for red blood cell surfaces, influencing their ability to induce erythrocyte aggregation. The study ranks their affinity as 24 kDa > 35 kDa > 70 kDa. This finding indicates that, similar to influenza virus hemagglutinin, different molecular weights of low molecular weight HA can trigger red blood cell aggregation, potentially inhibiting influenza virus hemagglutinin activity. These insights could open new possibilities for HA in antiviral research (Skehel et al, 2000).

* 1. HA35 in Oral Applications and Fat Metabolism Regulation

In addition to injectable HA formulations, Dr. Hui successfully commercialized a food-grade beverage containing a high dose (5 grams) of 35kDa molecular weight HA fragments. These fragments, measuring <220 nm, are rapidly absorbed through the mesenteric lymphatic system, offering an effective alternative to high-dose HA35 injections. Clinical studies have demonstrated the beverage’s efficacy in treating various diseases and conditions (PCT priority patent application 2024113443177.7, submitted from China). This innovation highlights the potential of HA-based nutraceuticals for systemic health benefits. Furthermore, Dr. S. Bahram Bahrami from Lawrence Berkeley National Laboratory discovered that the expression of the HA receptor RHAMM inhibits subcutaneous adipogenesis, suggesting that HA may play a role in regulating fat accumulation (Bahrami et al., 2017). This hypothesis is further supported by observations in naked mole rats, where high HA concentrations are associated with minimal subcutaneous fat deposition (https://en.wikipedia.org/wiki/Naked\_mole-rat). These findings suggest that HA may have applications in metabolic health and fat regulation, potentially influencing future obesity-related treatments.

3. Conclusion

HA has undergone significant advancements in both fundamental research and clinical applications. From its early structural characterization to modern large-scale fermentation production, HA has become a crucial biomedical material. Studies on naked mole rats have provided insights into its roles in cancer resistance, pain modulation, and inflammation control, while research on HA receptors has deepened our understanding of its diverse biological functions. The development of HA35 has further expanded its therapeutic potential, demonstrating enhanced tissue permeability and efficacy in pain relief and wound healing. Recent breakthroughs in gene transfer techniques and innovative HA-based formulations, including high-dose oral and injectable therapies, highlight promising avenues for future medical applications. As research continues to bridge the gap between academia and industry, HA-based therapies may soon lead to groundbreaking medical innovations with significant clinical and commercial impact. By fostering collaboration between these two fields, it aspires to drive breakthroughs that could achieve prestigious recognitions such as the Lasker Award or the Nobel Prize in Medicine within the next decade.

Consent (where ever applicable)

It is not applicable.

Ethical approval (where ever applicable)

It is not applicable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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