**Mechanisms of high-molecular-mass hyaluronan on anti-aging and cancer resistance in *Heterocephalus glaber***

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**Key Words:**

Cancer, naked mole rat, high-molecular-mass hyaluronan, fibroblasts, longevity

**The naked mole rat (NMR), *Heterocephalus glaber*, is a long-lived rodent species that is extremely resistant to anti-aging diseases (including cancer). While the exact methods of its cancer resistance remains unknown, recent research has identified high-molecular-mass hyaluronan (HMM-HA) as a contributor to *H. glaber*’s cancer resistance. The NMR HMM-HA differs from HA in other mammalian cells by its highly-supercoiled structure and ability to form robust macroscopic gels. It is hypothesized that HMM-HA’s unique traits help prevent the invasion of cancerous tumors into other tissues of the body. HMM-HA also prevents cancer by upregulating the p53 pathway and CD44 signaling, resulting in increased apoptosis. However, very high-molecular mass hyaluronan (vHMM-Ha) has an opposing effect of downregulation the p53 pathway and CD44 signaling, resulting in decreased apoptosis. HMM-HA is being looked into as a potential treatment for cancer, but more research into the anti-cancer mechanisms of HMM-HA and its opposing partner, vHMM-HA, is required first.**

**Introduction**

Naked mole rats (NMRs), or *Heterocephalus glaber*, have remarkable longevity and live up to ten times longer than similarly sized mammals with a maximum lifespan of 30 years. [1] Even more incredible is the NMR’s high resistance to cancer and other aging diseases such as metabolic decline, osteoporosis, and diabetes. [2] While the exact methods of this resistance remains unknown, high-molecular-mass hyaluronan (HMM-HA), an unbranched disaccharide polymer in the extracellular matrix (ECM), secreted by the NMR’s fibroblasts was theorized to contribute to this longevity and cancer resistance. [3] Much research has gone into examining the properties of HMM-HA, the biochemical pathways that it is involved in, the differences between the HA of NMRs and other species (such as humans, mice, and guinea pigs), and even the applications that can be made to cancer treatment with this knowledge.

**Recent Progress**

In the past decades mice and rats have remained the conventional model for researching cancer, due to their high cancer incidence and short lifespan of 12-24 months. While these models are beneficial for studying the causes and progression of cancer, they do not often lead to tumor/cancer resistance mechanisms because mice and rats tend to lack such traits. That is why Tian et al lab instead looked at the NMR, an aging resistant species that was known to be highly resistant to cancer, in an attempt to identify a novel anticancer mechanism. They found that NMR cells exhibit early contact inhibition (ECI), an anti-cancer mechanism that arrests cell growth when cells come into contact with one another. It was also discovered that his contact inhibition occurred at much lower cell densities in NMR than in mice. [3]

The next step was to determine the signals that trigger ECI in the NMR cells. Such a discovery was made when it was found that NMR fibroblasts secrete a viscous substance, identified to be HMM-HA, in culture media, while fibroblasts from other species and NMR embryonic fibroblasts, which do not exhibit ECI, do not cause this viscosity of culture media. This indicates that HMM-HA is an extracellular signal involved in the pathway stimulating ECI. [3]

Tian et al further investigated the involvement of HMM-HA in this pathway by testing NMR tissues for HMM-HA, culturing NMR fibroblasts with bacterial HAase, an enzyme capable of degrading HA, and by introducing a CD44-blocking antibody (CD44 is a receptor of HA in both mice and human cells). This led to multiple discoveries, the first of which being increased levels of HA in NMRs compared to other species due to an overexpression of HAS2 (one of the enzymes that synthesizes HMM-HA), and lower expression of HAase, leading to increased synthesis and decreased degradation of HMM-HA in NMRs. It was also found that HMM-HA triggers ECI through a signal that is transmitted by the CD44 receptor. Finally, it was determined that NMR cells have higher affinity for HA than do human and mouse cells, and as a result, exhibit increased sensitivity to HA signaling, likely causing ECI. Overall, these new findings established HMM-HA as a key player in the cancer resistance of naked mole rats. [3]

Kulaberoglu et al’s paper recognizes this role of HMM-HA in the cancer resistance and longevity of NMRs and contrasts the properties of NMR HA with that of other species such as mice and humans. First examined was the distinctive wrinkly and stretchy skin tissue of the NMR. It was found that NMR HA extends deeper into the epidermis than it does in mouse skin tissue, and is characterized by dense supercoils, which greatly contrast with the large, flat networks of HA in human skin. The cells from NMR brain and lung tissue also revealed densely packed supercoils, albeit with different morphologies. These supercoils were found to be more elastic than the fibers and flat networks seen in human and mouse cells, likely contributing to the elasticity of NMR skin. One other observation of this paper was that the HA of all NMR tissue types would spontaneously form robust macroscopic gels up to 1cm in length and 1 micron thick, a characteristic not seen in human or mouse HA. [1]

Zhao et al also recognizes the ability of HMM-HA (termed EHMW-HA in this paper) to induce anti-cancer effects in NMRs and sets out to discover if the same effects can be produced in mice and humans. They began by producing human and mouse breast cancer cell lines that produced HMM-HA similar to the HA found in NMRs through the overexpression of the *nmrHas2* gene. They then tested HMM-HA against breast cancer cell growth in vitro, in 2D and 3D models, and in a mouse model, resulting in inhibition of breast cancer cell growth, apoptosis of cancer cells, upregulation of the CD44 receptor expression, and increased p53 (and p53 target) gene expression. This revealed that HMM-HA is a cancer resistant mechanism that can be used to treat cancer is many species rather than just in NMRs where it was initially found. [4]

Takasugi et al’s paper makes a distinction between HMM-HA and vHMM-HA (very high-molecular-mass hyaluronan), a distinction that the previous two papers did not make. They defined vHMM-HA as >6.1 MDa and HMM-HA as >1 MDa, while previous studies had grouped all of the HA >1 MDa in the HMM-HA category (or EHMW-HA in the case of the Zhao et al study). This study sought out to discover if this newly identified HA of very long polymer length in in NMRs differed in function from the regular HMM-HA studied in the previously mentioned papers. They found that vHMM-HA exhibits increased cytoprotective properties compared to HMM-HA. Unlike HMM-HA, vHMM-HA reduces the interaction of CD44 with other proteins, reducing the expression of p53 target genes and attenuating (at least partially) the p53 pathway. This results in the coping of cellular stress rather than a repression of the damage through apoptosis which HMM-HA typically causes. This cytoprotective effect through the mediating of the p53 pathway was seen in mouse and human cells in addition to NMR cells when vHMM-HA was introduced. [2]

**Discussion**

Tian et al’s study that found HMM-HA to be contributor to the NMR’s cancer resistance has led to many applicational possibilities. The overexpression of HAS2 , the enzyme responsible for the accumulation of HMM-HA, is caused by two small mutations. Compared to the *Has2* gene sequence of other mammals, the NMR *Has2* gene has two asparagines that were replaced with serines. When this genomic change was introduced into human HEK293 cells (a cell line from an embryonic kidney), they also began to secrete HMM-HA. [3] This raises the possibility that human cells can be altered to produce HMM-HA, which can then be used to increase cancer resistance in humans. However, much more research is needed before such a plan could be realized. The results from this study also open new paths for cancer prevention with the idea of using HMM-HA clinically to target the HA-CD44 signaling pathway in cancer patients.

 Kulaberoglu et al’s findings further explain how NMR HMM-HA can be used to prevent or treat cancer. Since the densely-folded HA structures leave few cell-sized pores in the NMR tissue, it was hypothesized that the HMM-HA can form a barrier against tumor growth and cancer metastasis. Also hypothesized was that the HA supercoiled structure prevents the cleavage of CD44 receptors in the ECM (which are commonly cleaved with matrix metalloproteinases in cancer cells to prevent apoptosis). Finally, since it is known that ECMs from younger individuals are more resistant to cancer invasion, it is thought that NMR HA is able to keep the ECM young due to its high water-retaining properties (which are a result of densely-folded and tightly-packed HA structures). [1]

 Zhao et al’s paper builds off of Tian et al’s findings and further explains how the HA-CD44 signaling pathway is mediated by HMM-HA. They explained that accumulation of HMM-HA can upregulate the p53 pathway, causing p53 to bind to the CD44 promotor, resulting in stress-induced apoptotic signals, or can upregulate proapoptotic proteins upstream of p53 (most notably p21 and Bax). The paper then theorizes that HMM-HA could be injected into the cancerous tissue of cancer patients to induce apoptosis. However, HMM-HA is quickly degraded when injected, so further research on how to modify HMM-HA to prevent its breakdown while preserving its anti-cancer activity is needed before it can be looked at realistically as a cancer treatment. [4]

 While Takasugi et al’s findings revealed a mechanism through which HA contributes to the longevity of NMRs, it raises the question as to how NMRs remain resistant to cancer. While preventing apoptosis and increasing the cytoprotective mechanisms of the cell contribute to organism longevity, it is almost counterintuitive to the prevention of cancer (which relies heavily upon apoptosis for tumor suppression). However, this paper offered a possible explanation as to how NMR remains resistant to carcinogenesis. They theorized that the vHMM-HA only partially attenuated the p53 pathway, allowing it to still perform its tumor suppressor duties. [2] Nevertheless, more research is needed to prove this hypothesis.

 Overall we see that the HMM-HA produced by NMR fibroblast plays an important role in cancer resistance. While its exact methods of doing so are unknown, recent studies have indicated that it upregulates the CD44 signaling and p53 pathway to induce apoptosis, and strengthen the ECM in different NMR tissues to prevent tumor invasion and metastasis. HMM-HA shows similar results when introduced to human and mouse cells, suggesting that HMM-HA could be used in the future to treat or prevent cancer in humans. Nevertheless, we are still a far way off from that possibility and require much more investigation into the anti-cancer mechanisms of HMM-HA before that treatment can become realized.

**Figure 1. Maximum Molecular Mass of HA by Species**

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**Fig 1.** Shows the maximum size of the HA secreted by mouse & guinea pig, human, and NMR fibroblasts. NMR fibroblasts secrete HMM-HA, as indicated by the much heavier HA seen in NMR than the other species

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