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Hyaluronan Endocytosis: Mechanisms of Uptake and Biological Functions

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1. Introduction

Hyaluronan (HA) is a non-sulfated linear glycosaminoglycan composed of multiple copies of the disaccharide unit of D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine (GlcNAc); [β-1,4-GlcA-β -1,3-GlcNAc-]$_n$ where n is the number of repeating disaccharide subunits. HA is synthesized by the HA synthase family of enzymes. Three HA synthases, termed HAS1 through HAS3, have been identified in humans and in mice. These enzymes differ from each other in their catalytic activities (HAS3 > HAS2 > HAS1) as well as in the sizes of their final products. HAS1 and HAS2 polymerize long stretches of GlcA-GlcNAc disaccharide chains, whereas HAS3 polymerizes relatively short stretches (<300 kDa). Biosynthesis of HA is regulated by exogenous stimuli. For example, HA synthesis in fibroblasts is upregulated by phorbol esters, tumor growth factor alpha, and platelet derived growth factor, whereas HA synthesis in keratinocytes is upregulated by retinoic acid, epidermal growth factor, and tumor growth factor alpha and suppressed by corticosteroids [1-4]. HA is unique among extracellular matrix components in that it is not synthesized within the cell and transported to the surface via vesicles. Hyaluronan synthase is an integral membrane protein on the surface of cells. It links together UDP-α-N-acetyl-D-glucosamine and UDP-α-D-glucuronate to spin out long strands of HA. Because, unlike other extracellular matrix carbohydrates, HA is spooled out from the cell surface, it can achieve molecular weights ranging from five thousand Daltons to twenty million Daltons.

Although HA was originally considered to be an inert filling material in the extracellular matrix and intercellular spaces, this simple carbohydrate is now known to have a number of functions in several different biological processes including development, cancer biology, wound healing and the immune response.

2. HA endocytic pathway

A scheme for the endocytosis of high molecular weight HA (HMW-HA) and its catabolism to HA oligomers have been suggested previously [5]. Figure 1 illustrates the current model for uptake of HMW-HA and its processing to bioactive oligomeric fragments.

HMW-HA in the extracellular matrix can be degraded to fragments that are 50-100 saccharides in length by the HA digesting enzyme, hyaluronidase 2 (Hyal2) [6]. Hyal2 is expressed in the lysosome and also as a GPI-linked cell surface protein [7]. This raises the interesting question of how cell surface Hyal2 retains catalytic activity for HA digestion given its requirement for an acid environment to function. Previous investigations have shown that binding of HA to the HA-receptor, CD44, leads to the interaction of CD44 with the NHE1 Na+/H+ exchanger. In turn, the NHE1 Na+-H+ exchanger creates an acidic environment facilitating Hyal2 activity [8]. The HA saccharides generated by Hyal2 can then be endocytosed by one of multiple pathways. To date, receptor mediated endocytosis of HA and macropinocytosis of bulk phase HA have been reported. Receptor mediated endocytosis can occur via lipid rafts or by the clathrin coated pit pathway. Receptors for HA endocytosis may be recycled to the cell surface or turned-over. HA saccharides are further digested to HA oligosaccharides by hyaluronidase 1 (Hyal1) in the endosome. The HA oligosaccharides could then potentially be degraded into its GlcA and GlcNAc building blocks by the concerted activities of β-D-glucuronidase and β-N-acetyl-D-hexosaminidase [9] or HA oligosaccharides could be exocytosed. The exocytosed HA fragments could have myriad biological functions which will be detailed below.

Although HA endocytosis and subsequent degradation may be important for generation of bioactive HA fragments, the HA endocytic pathway is also essential for HA homeostasis. In a 70 kilogram human there is approximately 15 grams of total HA [10]. Up to 50% of the total HA in the body is expressed in the skin [11]. HA is turned over at a rate of approximately 5 grams per day [10]. In the skin, HA has a metabolic half-life <1.5 days [11]. HA is turned-over locally in tissues while systemic HA is cleared mostly in the liver and to a lesser extent the kidneys and spleen [12,13]. HA in the tissue extracellular matrix is thought to be partially degraded and then enters the lymph nodes via the draining lymphatics. Specific HA receptors in the lymphatics will be discussed below.

3. Mechanisms of hyaluronan endocytosis

3.1. Receptor mediated endocytosis

Uptake of hyaluronan by various receptors has been widely studied, and several key receptors have been identified. Some receptors, such as CD44 and LYVE-1, serve dual purposes in that they not only facilitate the endocytosis of HA, but also trigger signaling events that generate cell specific responses to HA binding. ICAM-1 was initially believed to serve as a metabolic receptor for HA only but is now suspected to have cell signaling roles in response to HA binding [14,15].

3.1.1. CD44

CD44 is a cell surface glycoprotein that serves as the endocytic receptor for HA in keratinocytes, chondrocytes, and breast cancer cells [16-18]. It is important to stress that the binding of HA to CD44 and the uptake of HA by CD44 mediated endocytosis are two separate events that often do not take place at the same time [19]. Internalization of HA through CD44 mediated endocytosis has been shown to require acylation of the CD44 cytoplasmic tail [20]. CD44 associates with lipid rafts for internalization as determined by gradient ultracentrifugation. Palmitoylation of CD44 on two cysteine residues, Cys^{286} in the transmembrane domain and Cys^{295} in the cytoplasmic domain, was found to be essential for lipid raft association, but not for HA binding. These acylation reactions could be cell type specific, which may explain why CD44 does not endocytose HA in all CD44 expressing cell types, such as B16-F10 melanoma cells [19]. There is also evidence that CD44 interacts directly with endocytosis proteins such as coatomer protein complexes [21]. Previous investigations have suggested that endocytosed CD44 can be recycled to the cell surface provided it is not ubiquitinated after endocytosis [22]. Recent studies in fibroblasts have shown that clathrin-independent carriers (CLIC) form an endocytic sorting system at the leading edge of migrating cells. Adhesion molecules, including CD44, are recycled in the CLIC pathway. CD44 and other CLIC cargo are concentrated within flotillin-1 and cholesterol enriched microdomains. Actin and GRAF-1 form the initial carriers within 15 seconds. Next, Rab11 and Rab5 / EEA-1 complexes allow bulk membrane flow to early endosomes and plasma membrane recycling [23]. It is tempting to speculate that the CLIC pathway in CD44 recycling is also involved in HA endocytosis.

3.1.2. RHAMM

The **R**eceptor for **H**yaluronic **A**cid **M**ediated **M**otility (RHAMM) was discovered originally as a soluble protein that altered the migration of cells and could bind HA [24]. RHAMM has no cytoplasmic or transmembrane domain and has no signaling domains, but it has been implicated in ERK1/2 signaling through a complex with CD44 upon HA binding [25]. RHAMM is also found in the cytoplasm where it associates with the mitotic spindle apparatus, which is responsible for establishing cell polarity and distribution of chromosomes during mitosis [25]. RHAMM can be transported out of the cytoplasm to the cell surface. In terms of RHAMM endocytosis, very little is known. By contrast, a number of studies have shown a role for RHAMM in tumor progression and the differentiation of osteoblasts [26,27]. As it is known that CD44 and RHAMM can associate with each other and that RHAMM binds HA, it is possible that RHAMM/CD44/HA complexes can be endocytosed or leads to signaling. There is evidence that RHAMM and CD44 co-signal through the ERK1/2 pathway to increase basal motility in breast cancer cells and increase fibroblast migration and differentiation during wound repair [28,29].

3.1.3. LYVE-1

Lymphatic vessel endothelial-1 (LYVE-1) is expressed on the surface of lymphatic endothelial cells. Interestingly, LYVE-1 has a glycosylation domain on its extracellular

domain that renders it inactive. Cleavage of this glycosylated region allows LYVE-1 to bind HA [30]. The LYVE-1 binds HA which is then bound by leukocyte CD44 in order to facilitate their adhesion and entry into the lymphatics [31]. Not much is known about the mechanisms of LYVE-1 endocytosis of HA, but it is thought to occur similarly to CD44 mediated uptake of HA; i.e., LYVE-1 associates with lipid rafts before endocytosis [31]. LYVE-1 may be responsible for the transport of HA to the luminal side of the lymphatics. It has previously been shown that LYVE-1 binds to HA on the lymphatic endothelial cells, endocytosis of the complex occurs, and then the vesicles are released on the lumen side of the lymphatics allowing for release of HA which can possibly modulate immune responses or mediate removal of HA from the lymph for clearance [32]. Importantly, the lymph nodes are the first sites of clearance for total body HA turn-over. In fact, about 85% of total body HA is cleared by the lymph nodes. The remaining HA is largely turned over in the liver [33].

3.1.4. HARE

Hyaluronan Receptor for Endocytosis (HARE) is expressed on sinusoid hepatocytes, the venous sinuses of the red pulp in spleen and the medullary sinuses in lymph nodes where it is important for the turnover of systemic HA [34]. Indeed, blocking HARE results in an inhibition of HA clearance in the liver [35]. HARE also plays a role in chondroitin sulfate proteoglycan endocytosis [36]. Previous investigations infer that HARE endocytosis occurs by the clathrin coated pit pathway and it appears that HARE is recycled to the cell surface [37]. Binding of HARE to HA was observed using ligand blotting and immunohistochemistry which shows that the HARE HA binding event occurs prior to HA internalization [38]. Four putative AP-2 / clathrin mediated endocytosis signaling domains have been identified in the HARE cytoplasmic domain: $YSYFRI^{2485}$, $FQHF^{2495}$, $NPLY^{2519}$, and DPF^{2534} (315-HARE numbering). Deletion analyses of the signaling domains showed that three signal sequences (YSYFRI, FQHF, and NPLY) provide redundancy to mediate coated pit targeting and endocytosis of HARE. Importantly, the coated pit targeting domains did not impact binding of the HARE ectodomain to HA showing that HA binding to HARE and HARE mediated endocytosis of HA are separate events [39].

It is noted that HARE and LYVE-1 are both expressed in the lymph nodes. Interestingly, HARE and LYVE-1 show different and non-overlapping distributions [33]. How and if LYVE-1 and HARE coordinate HA turn-over remains to be determined.

3.1.5. ICAM-1

Intercellular adhesion molecule 1 (ICAM-1) is perhaps the least studied of the receptors for HA. It appears not to facilitate HA endocytosis but rather, it is a receptor for HA that has signaling capacity. Preliminary data suggests that ICAM-1 functions as a signaling molecule when HA binds to it. When HA is added to the macrophage cell line U937, it induces Akt phosphorylation which activates the nuclear factor-kappa B pathway, inducing interleukin-6 production, but blocking of ICAM-1 with an antibody stops this from occurring [14,15].

3.2. Non-receptor mediated endocytosis
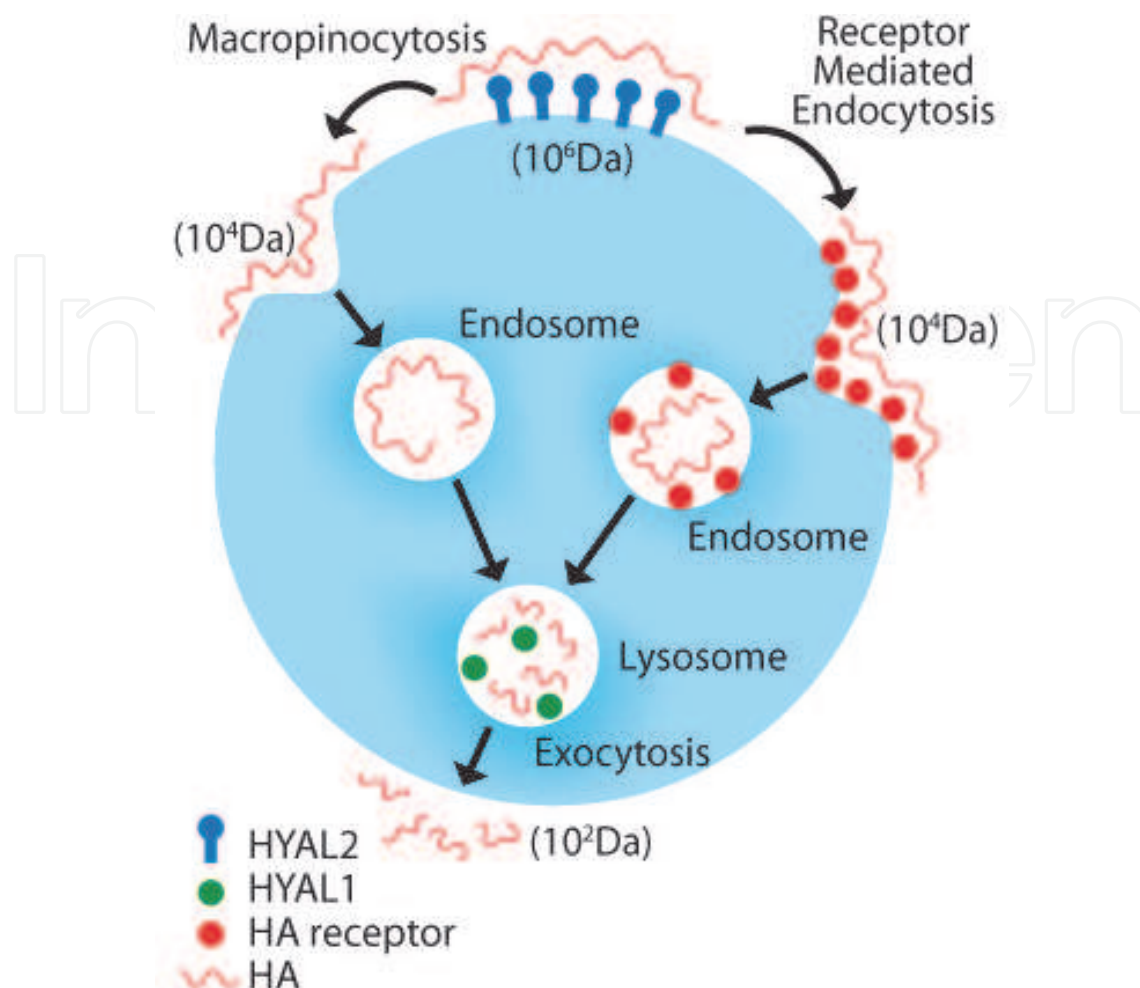
3.2.1. Macropinocytosis of hyaluronan

It has been demonstrated that HA uptake can occur without the aid of endocytic receptors. In [19], it was determined that B16-F10 melanoma cells endocytosed HA through macropinocytosis. The cells were observed to display membrane ruffling and localization of HA within vesicles as well as strong co-localization of HA with fluorescently labeled dextran, a macropinocytosis tracer. The uptake was also inhibited by amiloride, an inhibitor of macropinocytosis. Interestingly, the B16-F10 melanoma cells expressed surface CD44. Moreover, CD44 mediated adhesion of the melanoma cells to HA-coated plates. On the other hand, removal of CD44 from the B16-F10 melanoma cells by proteolytic cleavage failed to impact HA uptake. These results showed that CD44 did not play a significant role in the endocytosis of soluble HA. As mentioned in the previous section, the uptake of HA by CD44 requires CD44 to be in a specific state. It is possible, through splice variants of CD44, that these modifications were not present, and that there are distinct pathways in which HA can be taken up by cells. The ability of HA to induce macropinocytosis in B16-F10 melanoma cells is most likely due to its ability to non-specifically interact with the cell surface. HA has been shown to interact with and cause rearrangement of the cell surface [40]. [41] also showed that blocking of CD44 failed to inhibit the uptake of HA by 10T1/2 fibroblasts.

4. Potential biological functions of endocytosed HA

As shown in Figure 1, HA oligomers are produced by degradation of HA following its endocytosis. On the other hand, most studies on the biological activities of HA have been performed by adding exogenous HA oligomers to cell cultures and then determining their effect on biological activity. Presumably, HMW-HA co-polymers are endocytosed and digested and the resulting low molecular weight-HA (LMW-HA) fragments exocytosed under physiological conditions. Indeed, the hyaluronidase inhibitor apigenin resulted in accumulation of HA in pre-lysosomal endosomes in rat keratinocytes in vitro [18]. On the other hand, direct evidence for exocytosis of HA fragments are currently lacking. It is important to note that in some cases, the hyaluronidases may be secreted into the extracellular matrix or in the case of Hyal-2, expressed on the cell surface. HA degrading activity can also be exocytosed [42]. Obiviously, secreted hyaluronidase and cell surface Hyal-2 could circumvent the requirement for endocytosis.

4.1. Cell activation via HA

A number of investigators have reported that LMW-HA can induce molecular pathways culminating in gene expression. [43] has shown that oligomeric HA stimulated various transcription factors in chondrocytes including Sp1 and NF-κB. The same group showed that HA oligosaccharides induce expression of matrix metalloproteinase 13 by p38 MAPK and transcriptional activation of NF-κB [44]. It has also been shown that HA oligosaccharides

HMW-HA (~10^6Da) is first degraded by hyaluronidase 2 (HYAL2) into smaller 10^4Da sized fragments before it is taken up by a cell. The cell can either utilize surface HA receptors for receptor mediated endocytosis or macropinocytosis. Once internalized the HA is degraded by hyaluronidase 1 (HYAL1) into small 10^2Da fragments and then exocytosed.

Figure 1. Schematic overview of HA endocytosis and processing.

inhibit the expression of runt-related gene 2 (Runx2) in chondrocytes. Runx2 is a transcription factor for chondrocyte differentiation in hypertrophic chondrocytes [45,46]. Thus, HA oligosaccharides may impact the differentiation of chondrocytes during endochondrol ossification. [46] showed that binding of HA fragments to the HA receptor CD44 induces the Nanog-Stat-3 signaling pathway culminating in expression of the multidrug resistance gene, MDR-1, in breast and ovarian tumor cells. Expression of MDR-1 in the tumor cells conferred resistance to chemotherapeutic drugs doxorubicin and paclitaxel. Finally, it has been shown that LMW-HA upregulated CD44 expression and increased the expression levels of PKCδ and PKCε [47].

4.2. Cancer cell invasion and metastasis

Controversy has surrounded the role of the hyaluronidase enzymes in tumor biology with initial reports suggesting that Hyal1 was a tumor suppressor [48]. Early positional cloning studies identified the Hyal1 locus on 3p21.3 with LuCa1 (Lung Cancer 1). Because LuCa1

was either deleted or there was a loss in heterozygosity in LuCa1 in most lung cancers, it was hypothesized that Hyal1 behaved as a tumor suppressor. However, subsequent studies showed that Hyal1 was not the relevant tumor suppressor at the examined locus [49]. Nonetheless, the suppressive activity of hyaluronidses was confirmed by investigations showing that administration of high doses (300 U) of PH20 to mice with human breast tumor xenografts showed a significant reduction in tumor growth [50]. The over-expression of Hyal1 in a rat colon carcinoma line also inhibited tumor growth further suggesting that Hyal1 is a tumor suppressor [51].

Groundbreaking work by [52] showed that Hyal1 promoted tumor growth, invasion and angiogenesis. On the other hand, overproduction of Hyal1 to high levels (100 mU / 10^6 cells) inhibited tumor growth. These results suggested that high concentrations of Hyal1 may result in tumor inhibition while at lower levels Hyal1 leads to tumor progression.

Previous studies have shown that anti-sense Hyal1 stably expressed in bladder and prostate cancer cells induced down regulation of cdc25c, cyclin B1, cdk1 and cdk1 kinase activity [52,53]. Expression of Hyal1 in oral carcinoma cells resulted in a dramatic increase in cells in S-phase and a decrease in the number of cells in the G0-G1 phase [54]. To date, the mechanism whereby Hyal1 promotes tumor cell growth remains unknown. On the other hand, treatment of mouse fibroblasts with PH20 leads to phosphorylation of JNK-1 and -2 as well as p42 / p44 ERK [55]. Importantly, ERK plays a role in the G1-S transition [56].

The activities of the hyaluronidase enzymes also appear to have roles in the biology of malignant melanoma (MM) tumors. [57] found that aberrant expression of PH20 by MM cells was correlated with their induction of angiogenesis in a mouse model. Histological studies of MM showed that tumor associated HA expression was correlated with patient survival with low HA levels showing poor prognosis [58]. Because the activities of HA synthesis (by the HAS enzymes) and HA degradation (by the hyaluronidase enzymes) is highly regulated and may be interconnected, these results might suggest that an imbalance in HA metabolism in MM tumors may lead to cancer progression in humans.

Hyaluronidase activity has also been evaluated as a biomarker in bladder cancer. Tumor associated Hyal1 is released into the urine of bladder cancer patients [59]. Urinary hyaluronidase activity was elevated in patients with intermediate and high grade bladder cancer as compared with patients with: a) low grade bladder cancer, b) patients with a history of bladder cancer, c) normal individuals, and d) patients with benign urologic conditions [60]. These findings underscore the potential utility of the hyaluronidases to serve as biomarkers for cancer grading.

4.3. Wound healing

Tissue contraction during wound healing is achieved by myofibroblasts. Fibroblasts differentiate into myofibroblasts which line up at the edges of the wound and adhere to each other with desmosomes. They then use their actin networks to contract the ECM around the wound and shrink the size of the wounded area. Fibroblasts in the area around the wound then secrete collagen to stabilize the contraction. The trigger for contraction is HA production,

and HA plays a key role in regulating this process by directing fibroblast migration and proliferation at wound sites [61].

HA's role in wound healing is not clearly understood, but it is known that in a fetal state, as compared to an adult state, the extracellular matrix is composed of primarily HA. This abundance of HA rather than collagen reduces scar formation. CD44 mediated endocytosis of HA fragments also aids fibroblasts in migration to wound sites [62].

5. Applications of hyaluronan endocytosis

5.1. Drug delivery

HA may allow the targeted delivery of chemotherapeutic reagents to tumor cells via CD44. HA have a number of functional groups for "decoration" including a carboxylate on the glucuronic acid, the N-acetylglucosamine hydroxyl and the reducing end. Thus, a wide array of different chemotherapeutic reagents can be chemically conjugated to HA. A review of the chemical derivatization of HA and the potential applications of HA to disease treatment is beyond the scope of this chapter but has been recently reviewed [63,64]. In brief, previous investigations in vitro showed that a taxol-HA bioconjugate was cytotoxic to a panel of tumor cell lines (breast, colon and ovarian) but not human fibroblasts [65]. Although there are relatively few in vivo studies that have evaluated the efficacy of HA bioconjugates, previous investigations with a paclitaxol-HA bioconjugate have shown that it inhibits tumor growth of RT-112/84 human transitional cell carcinomas in mice and increases the survival of mice that had been inoculated with the NMP-1 or SK-OV-3ip human ovarian carcinoma lines [66,67]. Similarly, a butyrate-HA bioconjugate was found to inhibit tumor growth and reduced lung metastasis in mice inoculated with LL3 murine lung carcinoma cells [68]. Thus, a number of reports reinforce the concept that HA may be useful as a drug carrier / ligand targeting delivery agent. Other investigators conjugated doxorubicin, a chemotherapeutic agent, to HA and administered it topically to B16-F10 melanoma tumors in mice [69]. They found that doxorubicin-conjugated HA selectively targeted the tumor cells and reduced tumor growth. These findings open the door for future work with drug delivery to tumor cells using HA.

HA may also be a useful carrier of carboranes for boron neutron capture therapy for tumors. Previous investigations have shown that a water soluble HA-polycarborane derivative was taken up and showed toxicity to a number of tumor cell lines in vitro [70].

5.2. Imaging

HA may also be derivatized for imaging tumor cells. An activatable HA molecular probe, called FRET-HA, designed to detect hyaluronidase activity was recently reported [71]. Briefly, the HA co-polymer was chemically labeled with donor and acceptor fluorescent probes. Energy transfer from the donor to acceptor probe resulted in quenching of the acceptor (i.e., fluorescence resonance energy transfer or FRET). Because energy transfer is distance dependent, an increase in the distance between the donor and acceptor probes, for

example due to HA degradation, results in de-quenching of the donor. The rate of donor fluorescence change was used to determine the enzyme kinetics of bovine testes hyaluronidase with high precision and accuracy. FRET-HA has also been used to detect hyaluronidase activity in B16-F10 melanoma cells in vitro (unpublished results). The potential utility for FRET-HA to detect increased hyaluronidase activities in vivo is currently under investigation.

Recently, HA has been coated onto superparamagnetic iron oxide nanoparticles [72]. These HA-coated nanoparticles were endocytosed by cancer cells allowing their magnetic resonance imaging in vitro.

6. Conclusion

HA is a glycosaminoglycan with diverse biological functions. The molecular weight of HA is important for dictating its biological functions with HA fragments inducing distinct responses from high molecular weight co-polymers. Endocytosis of HA via receptor dependent and independent pathways is likely required for digestion of HA to biologically active fragments. In addition, endocytosis of HA may be exploited for the uptake of chemotherapeutic drugs for cancer treatment or imaging probes for the detection of metastatic tumors. Future directions include, 1) better understanding of the endocytic mechanism for HA metabolism, 2) better understanding of HA receptor signaling and interactions and 3) the development of second generation HA scaffolds for delivery and medical imaging.

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7. References

[1] Ogawa M, Hirano H, Tsubaki H, Kodama H, Tanaka T. The role of cytokines in cervical ripening: correlations between the concentrations of cytokines and hyaluronic acid in

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cervical mucus and the induction of hyaluronic acid production by inflammatory cytokines by human cervical fibroblasts Am J Obstet Gynecol 1998 Jul;179(1):105-110.

[2] Tammi R, Ripellino JA, Margolis RU, Maibach HI, Tammi M. Hyaluronate accumulation in human epidermis treated with retinoic acid in skin organ culture J Invest Dermatol 1989 Mar;92(3):326-332.

[3] Agren UM, Tammi M, Tammi R. Hydrocortisone regulation of hyaluronan metabolism in human skin organ culture J Cell Physiol 1995 Aug;164(2):240-248.

[4] Lamberg SI, Yuspa SH, Hascall VC. Synthesis of hyaluronic acid is decreased and synthesis of proteoglycans is increased when cultured mouse epidermal cells differentiate J Invest Dermatol 1986 Jun;86(6):659-667.

[5] Stern R. Devising a pathway for hyaluronan catabolism: are we there yet? Glycobiology 2003 Dec;13(12):105R-115R.

[6] Stern R. Hyaluronidases in cancer biology. Semin Cancer Biol 2008 Aug;18(4):275-280.

[7] Rai SK, Duh FM, Vigdorovich V, Danilkovitch-Miagkova A, Lerman MI, Miller AD. Candidate tumor suppressor HYAL2 is a glycosylphosphatidylinositol (GPI)-anchored cell-surface receptor for jaagsiekte sheep retrovirus, the envelope protein of which mediates oncogenic transformation. Proc Natl Acad Sci U S A 2001 Apr 10;98(8):4443-4448.

[8] Bourguignon LY, Singleton PA, Diedrich F, Stern R, Gilad E. CD44 interaction with Na+-H+ exchanger (NHE1) creates acidic microenvironments leading to hyaluronidase-2 and cathepsin B activation and breast tumor cell invasion. J Biol Chem 2004 Jun 25;279(26):26991-27007.

[9] Roden L, Campbell P, Fraser JR, Laurent TC, Pertoft H, Thompson JN. Enzymic pathways of hyaluronan catabolism. Ciba Found Symp 1989;143:60-76; discussion 76-86, 281-5.

[10] Laurent T, Fraser J. Catabolism of Hyaluronan. In: Henriksen J, editor. Degradation of Bioactive Substances: Physiology and Pathophysiology Boca Raton, FL: CRC Press; 1991. p. 249-265.

[11] Tammi R, Saamanen AM, Maibach HI, Tammi M. Degradation of newly synthesized high molecular mass hyaluronan in the epidermal and dermal compartments of human skin in organ culture. J Invest Dermatol 1991 Jul;97(1):126-130.

[12] Fraser JR, Appelgren LE, Laurent TC. Tissue uptake of circulating hyaluronic acid. A whole body autoradiographic study. Cell Tissue Res 1983;233(2):285-293.

[13] Fraser JR, Laurent TC, Pertoft H, Baxter E. Plasma clearance, tissue distribution and metabolism of hyaluronic acid injected intravenously in the rabbit. Biochem J 1981 Nov 15;200(2):415-424.

[14] Yasuda T. Hyaluronan inhibits Akt, leading to nuclear factor-kappaB down-regulation in lipopolysaccharide-stimulated U937 macrophages. J Pharmacol Sci 2011;115(4):509-515.

[15] Yasuda T. Activation of p38 mitogen-activated protein kinase is inhibited by hyaluronan via intercellular adhesion molecule-1 in articular chondrocytes stimulated with type II collagen peptide. J Pharmacol Sci 2012;118(1):25-32.

[16] Culty M, Shizari M, Thompson EW, Underhill CB. Binding and degradation of hyaluronan by human breast cancer cell lines expressing different forms of CD44: correlation with invasive potential. J Cell Physiol 1994 Aug;160(2):275-286.

[17] Hua Q, Knudson CB, Knudson W. Internalization of hyaluronan by chondrocytes occurs via receptor-mediated endocytosis. J Cell Sci 1993 Sep;106 (Pt 1)(Pt 1):365-375.

[18] Tammi R, Rilla K, Pienimaki JP, MacCallum DK, Hogg M, Luukkonen M, et al. Hyaluronan enters keratinocytes by a novel endocytic route for catabolism. J Biol Chem 2001 Sep 14;276(37):35111-35122.

[19] Greyner HJ, Wiraszka T, Zhang LS, Petroll WM, Mummert ME. Inducible macropinocytosis of hyaluronan in B16-F10 melanoma cells. Matrix Biol 2010 Jul;29(6):503-510.

[20] Thankamony SP, Knudson W. Acylation of CD44 and its association with lipid rafts are required for receptor and hyaluronan endocytosis J Biol Chem 2006 Nov 10;281(45):34601-34609.

[21] Skandalis SS, Kozlova I, Engstrom U, Hellman U, Heldin P. Proteomic identification of CD44 interacting proteins. IUBMB Life 2010 Nov;62(11):833-840.

[22] Eyster CA, Cole NB, Petersen S, Viswanathan K, Fruh K, Donaldson JG. MARCH ubiquitin ligases alter the itinerary of clathrin-independent cargo from recycling to degradation. Mol Biol Cell 2011 Sep;22(17):3218-3230.

[23] Howes MT, Kirkham M, Riches J, Cortese K, Walser PJ, Simpson F, et al. Clathrin-independent carriers form a high capacity endocytic sorting system at the leading edge of migrating cells. J Cell Biol 2010 Aug 23;190(4):675-691.

[24] Turley EA. Purification of a hyaluronate-binding protein fraction that modifies cell social behavior Biochem Biophys Res Commun 1982 Oct 15;108(3):1016-1024.

[25] Maxwell CA, Keats JJ, Crainie M, Sun X, Yen T, Shibuya E, et al. RHAMM is a centrosomal protein that interacts with dynein and maintains spindle pole stability Mol Biol Cell 2003 Jun;14(6):2262-2276.

[26] Li H, Guo L, Li JW, Liu N, Qi R, Liu J. Expression of hyaluronan receptors CD44 and RHAMM in stomach cancers: relevance with tumor progression. Int J Oncol 2000 Nov;17(5):927-932.

[27] Hatano H, Shigeishi H, Kudo Y, Higashikawa K, Tobiume K, Takata T, 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 2011 Sep 27.

[28] Hamilton SR, Fard SF, Paiwand FF, Tolg C, Veiseh M, Wang C, et al. The hyaluronan receptors CD44 and Rhamm (CD168) form complexes with ERK1,2 that sustain high basal motility in breast cancer cells. J Biol Chem 2007 Jun 1;282(22):16667-16680.

[29] Tolg C, Hamilton SR, Nakrieko KA, Kooshesh F, Walton P, McCarthy JB, et al. Rhamm-/- fibroblasts are defective in CD44-mediated ERK1,2 motogenic signaling, leading to defective skin wound repair. J Cell Biol 2006 Dec 18;175(6):1017-1028.

[30] Nightingale TD, Frayne ME, Clasper S, Banerji S, Jackson DG. A mechanism of sialylation functionally silences the hyaluronan receptor LYVE-1 in lymphatic endothelium J Biol Chem 2009 Feb 6;284(6):3935-3945.

[31] Jackson DG. Immunological functions of hyaluronan and its receptors in the lymphatics Immunol Rev 2009 Jul;230(1):216-231.

[32] Jackson DG, Prevo R, Clasper S, Banerji S. LYVE-1, the lymphatic system and tumor lymphangiogenesis. Trends Immunol 2001 Jun;22(6):317-321.

[33] Weigel PH, Yik JH. Glycans as endocytosis signals: the cases of the asialoglycoprotein and hyaluronan/chondroitin sulfate receptors. Biochim Biophys Acta 2002 Sep 19;1572(2-3):341-363.

[34] Harris EN, Kyosseva SV, Weigel JA, Weigel PH. Expression, processing, and glycosaminoglycan binding activity of the recombinant human 315-kDa hyaluronic acid receptor for endocytosis (HARE) J Biol Chem 2007 Feb 2;282(5):2785-2797.

[35] Weigel JA, Raymond RC, McGary C, Singh A, Weigel PH. A blocking antibody to the hyaluronan receptor for endocytosis (HARE) inhibits hyaluronan clearance by perfused liver J Biol Chem 2003 Mar 14;278(11):9808-9812.

[36] Smedsrod B, Malmgren M, Ericsson J, Laurent TC. Morphological studies on endocytosis of chondroitin sulphate proteoglycan by rat liver endothelial cells. Cell Tissue Res 1988 Jul;253(1):39-45.

[37] McGary CT, Raja RH, Weigel PH. Endocytosis of hyaluronic acid by rat liver endothelial cells. Evidence for receptor recycling. Biochem J 1989 Feb 1;257(3):875-884.

[38] Harris EN, Weigel JA, Weigel PH. Endocytic function, glycosaminoglycan specificity, and antibody sensitivity of the recombinant human 190-kDa hyaluronan receptor for endocytosis (HARE) J Biol Chem 2004 Aug 27;279(35):36201-36209.

[39] Pandey MS, Harris EN, Weigel JA, Weigel PH. The cytoplasmic domain of the hyaluronan receptor for endocytosis (HARE) contains multiple endocytic motifs targeting coated pit-mediated internalization. J Biol Chem 2008 Aug 1;283(31):21453-21461.

[40] Kultti A, Rilla K, Tiihonen R, Spicer AP, Tammi RH, Tammi MI. Hyaluronan synthesis induces microvillus-like cell surface protrusions. J Biol Chem 2006 Jun 9;281(23):15821-15828.

[41] Collis L, Hall C, Lange L, Ziebell M, Prestwich R, Turley EA. Rapid hyaluronan uptake is associated with enhanced motility: implications for an intracellular mode of action. FEBS Lett 1998 Dec 4;440(3):444-449.

[42] Bastow E, Last K, Golub S, Stow J, Stanley A, Fosang A. Evidence for lysosomal exocytosis and release of aggrecan-degrading hydrolases from hypertrophic chondrocytes, *in vitro* and *in vivo*. Biology Open 2012 February 15, 2012.

[43] Ohno S, Im HJ, Knudson CB, Knudson W. Hyaluronan oligosaccharide-induced activation of transcription factors in bovine articular chondrocytes. Arthritis Rheum 2005 Mar;52(3):800-809.

[44] Ohno S, Im HJ, Knudson CB, Knudson W. Hyaluronan oligosaccharides induce matrix metalloproteinase 13 via transcriptional activation of NFkappaB and p38 MAP kinase in articular chondrocytes. J Biol Chem 2006 Jun 30;281(26):17952-17960.

[45] Tanne Y, Tanimoto K, Tanaka N, Ueki M, Lin YY, Ohkuma S, et al. Expression and activity of Runx2 mediated by hyaluronan during chondrocyte differentiation. Arch Oral Biol 2008 May;53(5):478-487.

[46] Bourguignon LY, Peyrollier K, Xia W, Gilad E. Hyaluronan-CD44 interaction activates stem cell marker Nanog, Stat-3-mediated MDR1 gene expression, and ankyrin-regulated multidrug efflux in breast and ovarian tumor cells. J Biol Chem 2008 Jun 20;283(25):17635-17651.

[47] Campo GM, Avenoso A, Campo S, D'Ascola A, Traina P, Calatroni A. Differential effect of molecular size HA in mouse chondrocytes stimulated with PMA. Biochim Biophys Acta 2009 Oct;1790(10):1353-1367.

[48] Csoka AB, Frost GI, Heng HH, Scherer SW, Mohapatra G, Stern R. The hyaluronidase gene HYAL1 maps to chromosome 3p21.2-p21.3 in human and 9F1-F2 in mouse, a conserved candidate tumor suppressor locus. Genomics 1998 Feb 15;48(1):63-70.

[49] Zabarovsky ER, Lerman MI, Minna JD. Tumor suppressor genes on chromosome 3p involved in the pathogenesis of lung and other cancers. Oncogene 2002 Oct 7;21(45):6915-6935.

[50] Shuster S, Frost GI, Csoka AB, Formby B, Stern R. Hyaluronidase reduces human breast cancer xenografts in SCID mice. Int J Cancer 2002 Nov 10;102(2):192-197.

[51] Jacobson A, Rahmanian M, Rubin K, Heldin P. Expression of hyaluronan synthase 2 or hyaluronidase 1 differentially affect the growth rate of transplantable colon carcinoma cell tumors. Int J Cancer 2002 Nov 20;102(3):212-219.

[52] Lokeshwar VB, Cerwinka WH, Lokeshwar BL. HYAL1 hyaluronidase: a molecular determinant of bladder tumor growth and invasion. Cancer Res 2005 Mar 15;65(6):2243-2250.

[53] Lokeshwar VB, Cerwinka WH, Isoyama T, Lokeshwar BL. HYAL1 hyaluronidase in prostate cancer: a tumor promoter and suppressor. Cancer Res 2005 Sep 1;65(17):7782-7789.

[54] Lin G, Stern R. Plasma hyaluronidase (Hyal-1) promotes tumor cell cycling. Cancer Lett 2001 Feb 10;163(1):95-101.

[55] Chang NS. Hyaluronidase activation of c-Jun N-terminal kinase is necessary for protection of L929 fibrosarcoma cells from staurosporine-mediated cell death. Biochem Biophys Res Commun 2001 May 4;283(2):278-286.

[56] Liu X, Yan S, Zhou T, Terada Y, Erikson RL. The MAP kinase pathway is required for entry into mitosis and cell survival. Oncogene 2004 Jan 22;23(3):763-776.

[57] Liu D, Pearlman E, Diaconu E, Guo K, Mori H, Haqqi T, et al. Expression of hyaluronidase by tumor cells induces angiogenesis in vivo. Proc Natl Acad Sci U S A 1996 Jul 23;93(15):7832-7837.

[58] Karjalainen JM, Tammi RH, Tammi MI, Eskelinen MJ, Agren UM, Parkkinen JJ, et al. Reduced level of CD44 and hyaluronan associated with unfavorable prognosis in clinical stage I cutaneous melanoma. Am J Pathol 2000 Sep;157(3):957-965.

[59] Hautmann SH, Lokeshwar VB, Schroeder GL, Civantos F, Duncan RC, Gnann R, et al. Elevated tissue expression of hyaluronic acid and hyaluronidase validates the HA-HAase urine test for bladder cancer. J Urol 2001 Jun;165(6 Pt 1):2068-2074.

[60] Pham HT, Block NL, Lokeshwar VB. Tumor-derived hyaluronidase: a diagnostic urine marker for high-grade bladder cancer. Cancer Res 1997 Feb 15;57(4):778-783.

[61] Slavkovsky R, Kohlerova R, Jiroutova A, Hajzlerova M, Sobotka L, Cermakova E, et al. Effects of hyaluronan and iodine on wound contraction and granulation tissue formation in rat skin wounds. Clin Exp Dermatol 2010 Jun;35(4):373-379.

[62] Maharjan AS, Pilling D, Gomer RH. High and low molecular weight hyaluronic acid differentially regulate human fibrocyte differentiation PLoS One 2011;6(10):e26078.

[63] Yadav AK, Mishra P, Agrawal GP. An insight on hyaluronic acid in drug targeting and drug delivery. J Drug Target 2008 Feb;16(2):91-107.

[64] Gaffney J, Matou-Nasri S, Grau-Olivares M, Slevin M. Therapeutic applications of hyaluronan. Mol Biosyst 2010 Mar;6(3):437-443.

[65] Luo Y, Prestwich GD. Synthesis and selective cytotoxicity of a hyaluronic acid-antitumor bioconjugate. Bioconjug Chem 1999 Sep-Oct;10(5):755-763.

[66] Rosato A, Banzato A, De Luca G, Renier D, Bettella F, Pagano C, et al. HYTAD1-p20: a new paclitaxel-hyaluronic acid hydrosoluble bioconjugate for treatment of superficial bladder cancer. Urol Oncol 2006 May-Jun;24(3):207-215.

[67] Auzenne E, Ghosh SC, Khodadadian M, Rivera B, Farquhar D, Price RE, et al. Hyaluronic acid-paclitaxel: antitumor efficacy against CD44(+) human ovarian carcinoma xenografts. Neoplasia 2007 Jun;9(6):479-486.

[68] Coradini D, Pellizzaro C, Abolafio G, Bosco M, Scarlata I, Cantoni S, et al. Hyaluronic-acid butyric esters as promising antineoplastic agents in human lung carcinoma: a preclinical study. Invest New Drugs 2004 Aug;22(3):207-217.

[69] Zhang LS, Greyner HJ, Mummert ME, Petroll WM. Development of a hyaluronan bioconjugate for the topical treatment of melanoma. J Dermatol Sci 2009 Jul;55(1):56-59.

[70] Di Meo C, Panza L, Campo F, Capitani D, Mannina L, Banzato A, et al. Novel types of carborane-carrier hyaluronan derivatives via "click chemistry". Macromol Biosci 2008 Jul 7;8(7):670-681.

[71] Zhang LS, Mummert ME. Development of a fluorescent substrate to measure hyaluronidase activity. Anal Biochem 2008 Aug 1;379(1):80-85.

[72] El-Dakdouki MH, Zhu DC, El-Boubbou K, Kamat M, Chen J, Li W, et al. Development of Multifunctional Hyaluronan-Coated Nanoparticles for Imaging and Drug Delivery to Cancer Cells. Biomacromolecules 2012, Apr 9; 13(4):1144-1151.