

Hyaluronan-CD44 interactions and tumor progression

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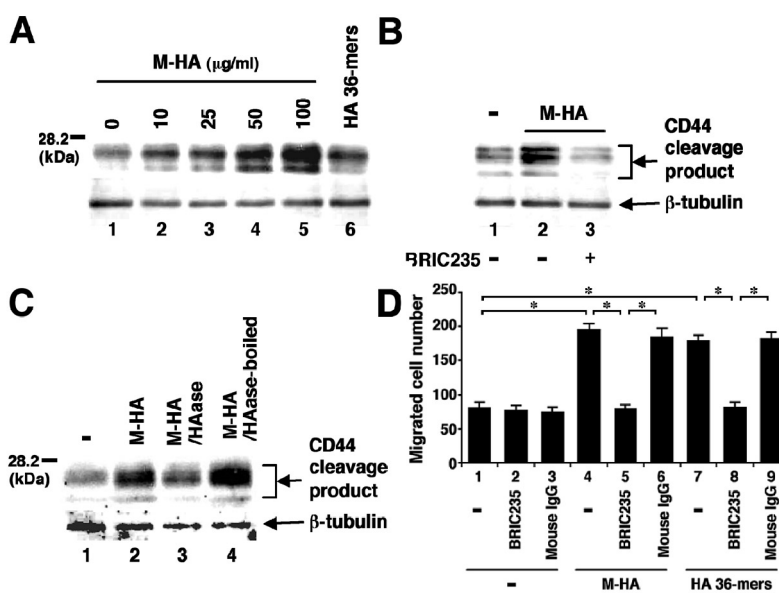
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HA oligosaccharides isolated from MIA PaCa-2 culture supernatant (M-HA) up-regulate CD44 cleavage and promote tumor cell migration by interacting with CD44 in MIA PaCa-2 cells.

A, M-HA enhanced CD44 cleavage in MIA PaCa-2 cells in a dose dependent manner, as evidenced by an increase in the membrane-bound 25-kDa CD44 cleavage products in a Western blotting analysis. B, The enhanced CD44 cleavage was inhibited by an anti-CD44 neutralizing antibody BRIC235. C, Treatment of M-HA with hyaluronidase but not with heat-inactivated hyaluronidase down-regulated the ability of the oligosaccharides to enhance CD44 cleavage. D, M-HA promoted MIA PaCa-2 migration in a Transwell migration assay. Note that the enhanced migration was inhibited by BRIC235, indicating that it was due to CD44-HA interaction.

Hyaluronan (HA) participates not only in physiological events such as cell adhesion, migration, and proliferation, but also in pathological conditions including cancer. Accumulating evidence indicates that low-molecular weight (LMW)-HAs have distinct functions from high-molecular-weight (HMW)-HAs, and that HA molecules of different sizes have different functions. For instance, HA 4-16-mers induce cytokine gene expression in dendritic cells and promote their maturation, HA 6-20-mers induce chemokine gene expression in endothelial cells, and HA 8-50-mers induce angiogenesis. We have previously shown that HA 6-36-mers significantly enhance the proteolytic cleavage of CD44 from the surface of tumor cells, a phenomenon that has been suggested to play an important role in tumor cell migration along ECM components. The HA 36-mers that strongly enhanced CD44 cleavage in tumor cells, also induced morphological changes and promoted tumor-cell migration in a CD44-dependent manner, in accordance with the idea that LMW-HAs can activate tumor cells *via* the cell-surface receptor CD44.

Because high levels of LMW-HAs have been found in several human tumors, and tumor tissues express hyaluronidases (HAases), we hypothesized that tumor cells themselves may generate LMW-HAs of a certain size range, and that the tumor-related LMW-HAs may in turn activate the tumor cells by interacting with CD44. We found that a human pancreatic tumor cell line MIA PaCa-2 secretes HAases including Hyal-1 and Hyal-2. When we examined the size profiles of the HAs generated by MIA PaCa-2 cells, we found that the sizes ranged from approximately 10-mers to 40-mers, which corresponded to those of the LMW-HAs

that enhanced CD44 cleavage and cell migration in tumor cells (6-36-mers). Interestingly, the HA oligosaccharides isolated from the MIA PaCa-2 culture supernatant could enhance CD44 cleavage and cell motility. As shown in Fig. A, the MIA PaCa-2-derived HA oligosaccharides induced the up-regulation of CD44 cleavage in MIA PaCa-2 cells in a concentration-dependent manner. The enhancement was almost completely inhibited by the anti-CD44 mAb BRIC235 (Fig. B), indicating that the cleavage was induced by the interaction between CD44 and the HA oligosaccharides. When completely digested by the HAase, the MIA PaCa-2-derived HA oligosaccharides lost their ability to enhance CD44 cleavage, whereas the same treatment with heat-inactivated HAase did not (Fig. C). In addition, the MIA PaCa-2-derived HA oligosaccharides enhanced filopodia and actin filament formation and tumor-cell migration *in vitro* (Fig. D). These changes in cell morphology and motility were abrogated by BRIC235, confirming that they were dependent on the interaction between CD44 and HA. Collectively, these results strongly indicate that the MIA PaCa-2 cells themselves constitutively generate HA oligosaccharides that can enhance CD44 cleavage and tumor cell motility *via* the action of HA-degrading enzymes that the tumor cells also produce. In conclusion, a mechanism consisting of constitutively high HAase expression, constitutive generation of HA oligosaccharides, sustained CD44 stimulation, and the sustained induction of tumor motility and invasion may represent a novel autocrine/paracrine activation mechanism in tumor cells, which would lead to the promotion and maintenance of their own malignant properties.

Keywords: hyaluronan, oligosaccharides, CD44, cleavage, tumor