Fragmented hyaluronan is an autocrine chemokinetic motility factor supported by the HAS2-HYAL2/CD44 system on the plasma membrane

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Abstract. Hyaluronan (HA) is synthesized by HA synthase (HAS) 1, HAS2 and HAS3, and degraded by hyaluronidase (HYAL) 1 and HYAL2 in a CD44-dependent manner. HA and HYALs are intricately involved in tumor growth and metastasis. Random cell movement is generally described as chemokinesis, and represents an important step at the beginning of tumor cell liberation from the primary site. To investigate the roles of HAS2 and HYAL2/CD44 in cell motility, we examined HeLa-S3 cells showing spontaneous chemokinesis. HeLa-S3 cells expressed HAS2 and HAS3. siRNA-mediated knockdown of HAS2 decreased spontaneous chemokinesis of HeLa-S3 cells. Although HeLa-S3 cells secreted 50 ng/ml of high molecular weight (HMW)-HA (peak: 990 kDa) into the culture supernatant after 6 h of culture, exogenously added HMW-HA did not enhance spontaneous chemokinesis of the cells. These observations suggested that HeLa-S3 cells may have a self-degrading system for HA to regulate their spontaneous chemokinesis. To examine this possibility, we investigated the effects of siRNA-mediated knockdown of HYAL2 or CD44 on the spontaneous chemokinesis of HeLa-S3 cells. Knockdown of either molecule decreased the spontaneous chemokinesis of the cells. Low molecular weight (LMW)-HA (23 kDa) reversed the HYAL2 siRNA-mediated reduction in spontaneous chemokinesis of HeLa-S3 cells to the level in control cells stimulated with the same HA. These findings indicate that the HAS2-HYAL2/CD44 system may support spontaneous chemokinesis of human cancer cells through self-degradation of HMW-HA to produce LMW-HA by an autocrine mechanism. Consequently, our study may further expand our understanding of HA functions in cancer.

Introduction

Hyaluronan (HA) is a non-sulfated linear glycosaminoglycan that consists of repeating disaccharide subunits of glucuronic acid and N-acetylgal醜osamine. It has a ubiquitous distribution and is the major type of glycosaminoglycan present in the extracellular matrix (1). High molecular weight (HMW)-HA exists in normal tissues and contributes to local homeostasis by suppressing cell proliferation, migration, angiogenesis, inflammation and immunologic reactions (2-4). On the other hand, low molecular weight (LMW)-HA is associated with inflammation and tumor progression (5,6). In fact, HA interacts with several cell-surface receptors, such as CD44 and RHAMM (7), and HA-CD44 interactions can lead to the activation of intracellular signaling pathways that affect the proliferation, migration and invasion of cancer cells (8-11). Recent reports have made it clear that the balance of HA synthesis and degradation is critical for tumor progression (12,13). HA is synthesized on the cytoplasmic surface of the plasma membrane by hyaluronan synthase (HAS) 1, HAS2 and HAS3, and extruded into the extracellular space (14,15). The catalytic activities of the three HAS enzymes are different. HAS1 and HAS2 produce HA of 200-2000 kDa, whereas HAS3 mainly synthesizes HA of 100-1000 kDa (14). Knockdown of the HAS genes in tumor cells was reported to inhibit their proliferation, invasion and motility in vitro and tumor growth and metastasis in vivo (16-22). In particular, it has been reported that the levels of HAS2 expression are often correlated with malignant behavior in various cancer cells such as breast cancer, fibrosarcoma and osteosarcoma cells (17,23-25). Furthermore, HAS2 is a key molecule for the induction of the epithelial-mesenchymal transition (EMT) with HA assembly (26-30).

In vivo, HMW-HA is degraded to LMW-HA via oxygen radicals and enzymatic degradation by hyaluronidases (HYALs).
Six HYAL-like genes have been identified in humans. Specifically, HYAL1, HYAL2 and HYAL3 are clustered on chromosome 3p21.3, while HYAL4, PHAL1 and SPAL1 are clustered on 7q31.3 (31,32). Notably, HYAL1 and HYAL2 are the major HYALs expressed in human somatic tissues (33). HYAL2 is linked to the plasma membrane by a glycosylphosphatidylinositol anchor (34). HYAL2 initially cleaves HMW-HA to ~20 kDa fragments in cooperation with CD44 (35-37). The levels of HYAL2 expression are correlated with the invasive potential of human breast cancer cells (38,39). Furthermore, Udabage et al (39) recently revealed that highly invasive breast cancer cell lines preferentially express HAS2, HYAL2 and CD44, while less invasive cells express HAS3 and HYAL3. Nevertheless, it has remained controversial whether HYAL2 functions as an oncogene or a tumor suppressor gene (33).

Metastasis, referring to the spread of malignant cells from a primary tumor to distant sites, poses the biggest problem in cancer treatment and is the main cause of death of cancer patients. Cell motility is a principal requirement for tumor cells to undergo invasion during metastasis. There are three types of cell motility, namely random cell motility (chemokinesis), directional cell motility (chemotaxis) and haptotaxis. Chemokinesis is important in the initiation and maintenance of cell migration (40). Furthermore, chemokinesis may play a role during the EMT process, thereby facilitating the separation of tumor cells from their primary site (41). Although HA has previously been reported to induce tumor cell motility, the molecular mechanism underlying the role of HA in chemokinesis during tumor progression remains unclear. The objective of this study was to determine how alterations in HA turnover can affect the induction of chemokinesis in tumor cells. In this study, we examined the potential role of the HA synthesis/degradation system in tumor cell motility and found that the HAS2-HYAL2/CD44 system supports chemokinesis stimulated by fragmented HA on the plasma membrane in an autocrine manner.

Materials and methods

Materials. A human HYAL2 cDNA clone (GenBank accession no.: NM_033158.2) in pCMV6-XL4 was purchased from OriGene (Rockville, MD). The empty pCMV6-XL4 vector was used as a control. An anti-HYAL2 polyclonal antibody was raised in a rabbit against the amino acid sequence CFYRDGLYLPRDFSAGRVS. The obtained antibody was affinity-purified using the immunizing peptide. An anti-CD44 monoclonal antibody, Hermes 3, was purified from conditioned medium of hybridoma HB-9480 cells purchased from the American Type Culture Collection (ATCC, Rockville, MD) (10). An anti-HAS2 antibody (Y-14; sc-34068) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). An anti-actin antibody (A 2066) was purchased from Sigma (Tokyo, Japan). These HAs were free of protein and other glycosaminoglycans and had peak molecular weights of 23, 230 and 920 kDa, respectively. The HA fragments had an endotoxin content of <0.002 ng/ml as determined by Limulus amebocyte lysate assays. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide for the MTT assay obtained from Sigma.

Cell culture. A human cervical cancer cell line, HeLa-S3, and a human breast cancer cell line, MDA-MB-231, were obtained from the ATCC. HeLa-S3 cells were cultured in DMEM (Nissui, Tokyo, Japan) supplemented with 10% heat-inactivated FCS and 4 mM L-glutamine. MDA-MB-231 cells were cultured in DMEM supplemented with 10% heat-inactivated FBS and 4 mM L-glutamine. Both cell lines were maintained at 37°C in a 5% CO2 incubator.

Western blotting. Whole cell lysates were prepared with ice-cold RIPA buffer (50 mM Tris-HCl pH 7.5, 1% nonidet P-40, 150 mM NaCl, 0.1% SDS, 0.5% deoxycholic acid) containing 1 µg/ml leupeptin, 1 µg/ml pepstatin, 1 µg/ml aprotinin, 1 mM DTT, 1 mM NaVO4 and 0.5 mM PMSF. The supernatants were saved as total cell lysates following centrifugation. Aliquots of the cell lysates (50 µg of protein) were separated by 10% SDS-PAGE and transferred to PVDF membranes (Bio-Rad Laboratories, Hercules, CA). Primary antibodies that bound to their antigens on the membranes were detected using appropriate HRP-conjugated secondary antibodies (Amersham Bioscience, Piscataway, NJ) and a Super Signal chemiluminescence detection system (Pierce, Rockford, IL) or the Lumi-Light® Western blotting substrate (Roche Diagnostics, Basel, Switzerland) according to the manufacturer’s instructions.

Transfection with expression vectors and siRNAs. Expression vectors and siRNAs were introduced into cells using Lipofectamine® 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Stealth™ siRNAs specific for HYAL2 (sense, 5'-AAUAUUGGUGGCCAGCAGAC AUU-3'; antisense, 5'-AUUGUGUCCUGGCGCAACAA AUU-3'), HAS2 (sense, 5'-UUACUAUCCACAGACUG GCG-3'; antisense, 5'-CCCGCCCAUCAUGUGGAGAU GGUA-3') and CD44 (sense, 5'-UAUAUAAUCAACUGAUC UGCGCCAGG-3'; antisense, 5'-CCUGGGCCAGAUCGA UUUGAAUAUA-3') were purchased from Invitrogen. A scramble siRNA (sense, 5'-UAUAUAAUCAACUGAUCU CGCGUAGG-3'; antisense, 5'-CCUGGCGCAUAGUGA UGUGGUA-3') used as a control, and was also purchased from Invitrogen. A siRNA against HAS3 (sc-45295) and the corresponding scramble control siRNA (sc-37007) were obtained from Santa Cruz Biotechnology.

Real-time RT-PCR. Total RNA was extracted from cells using the TRIzol reagent (Invitrogen), and cDNAs were synthesized with a PrimeScript™ RT reagent kit (Takara Bio) according to the manufacturer's protocols. Real-time PCR was performed using SYBR Premix Ex Taq™ (Takara Bio) and an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) under the following conditions: 30 sec at 95°C; 40 cycles of 5 sec at 95°C and 31 sec at 60°C. Specific primer sets for HYAL2 (forward, 5'-ACCATGCAC TCCAGTCTACC-3'; reverse, 5'-TCGCGCAATGTTAGA CATGAGGC-3'), HAS1 (forward, 5'-ACGCAGCTGGCGA TAGTGGAAT-3'; reverse, 5'-CTTGTGTGAGCCGCTCAA GAA-3'), HAS2 (forward, 5'-TATCAGTCTGGGAACTT GAAC-3'; reverse, 5'-A ACATTTTCAAGCAGGATGCAC-3'),
HAS (forward, 5'-TCGCCGATTCCGGTGAATA-3'; reverse, 5'-CCTCAAGCTCTGAGATCA-3') and GAPDH (forward, 5'-GACCCGCTCAAGGGCTGAAC-3'; reverse, 5'-TGGTGAAGACCGCAGTGG-3') were purchased from Takara Bio. The specificity of each amplification was confirmed by a dissociation curve consisting of a single peak. All samples were amplified in triplicate in each experiment. The values were normalized by the expression of GAPDH.

Boydren chamber assay. To investigate the roles of HYAL2 and HAS2 in spontaneous chemokinesis, we employed the Boydren chamber migration assay (42). Expression vectors and siRNAs were transfected into HeLa-S3 or MDA-MB-231 cells using Lipofectamine™ 2000 (Invitrogen). After incubation for 30 h, the cells were starved overnight in DMEM supplemented with 2% CS or FBS. The cells were then harvested and subjected to Boydren chamber assays. HeLa-S3 cells (5x10^4 cells/well) or MDA-MB-231 cells (2.5x10^4 cells/well) were loaded in the upper chambers of polycarbonate membrane transwell inserts (Corning Inc., Corning, NY). Each set of lower and upper chambers was separated by an 8-µm pore size polycarbonate membrane. To evaluate the chemokinesis, each upper and lower chamber contained the same concentration of serum (2% CS for HeLa-S3 cells and 2% FBS for MDA-MB-231 cells) or 50 µg/ml of HA (23, 230 and 920 kDa). The cells were allowed to migrate for 4 or 6 h. The membranes were then fixed with formalin and stained with Meyer’s hematoxylin. The cells on the upper side of the membranes were removed with cotton swabs. The cells on the lower side of the membranes were counted under a light microscope at x200 magnification. Four random microscopic fields were counted.

Gel permeation chromatography assay. To determine the molecular weights of HA in the conditioned medium, gel permeation chromatography was performed as previously described (43). Briefly, a 100-µl aliquot of concentrated and desalted conditioned medium was loaded onto a column system consisting of serially connected TSK-G4000PWXL and G3000PW column (Tosoh Corporation, Tokyo, Japan). The columns were eluted with 0.2 M NaCl at a flow rate of 0.5 ml/min, and the eluted HA was monitored by measuring the UV absorbance at 215 nm. A calibration curve was created using molecular weight standards for HA (2310, 1410, 993, 847, 637, 460, 104 and 18 kDa).

MTT assay. The MTT assay, which is widely used to measure cell proliferation and screen for anticancer drugs, is based on the reduction of a tetrazolium salt (44-46). We used the MTT assay to evaluate the effects of the level of HYAL2 expression on cell proliferation. HeLa-S3 cells transfected with HYAL2 expression vector/empty vector or siRNAs for HYAL2/scramble control were plated in 96-well plates at 1.5x10^4, 2.5x10^4 or 5x10^4 cells/well and the plates were incubated for 4 h. Subsequently, 100 µl of 10% SDS and 0.01 N HCl were added to each well. After overnight incubation, the absorbances were measured at 570 nm using a Vmax microplate reader (Molecular Devices, Tokyo, Japan). In these experiments, five replicate wells were used for each time point, and the results were calculated as the mean ± SD.
cells transfected with a scramble control siRNA. Similarly, we were able to reduce the HAS3 mRNA expression by siRNA-mediated knockdown (Fig. 2C). When we assessed spontaneous chemokinesis of the cells transfected with the HAS2 siRNA, we found that the cell motility was significantly reduced compared with the control siRNA-transfected cells (Fig. 2D). Both knockdown of HAS2 expression and reduction of spontaneous chemokinesis were induced by siRNAs with independent sequences derived from the HAS2 mRNA (data not shown). In contrast, knockdown of HAS3 mRNA expression increased spontaneous chemokinesis of HeLa-S3 cells (Fig. 2E). In agreement with a previous study performed with a different cell line (17), knockdown of HAS2 mRNA expression in HeLa-S3 cells induced a remarkable increase in HAS3 mRNA expression (Fig. 3A), while knockdown of HAS3 mRNA expression caused a significant elevation of HAS2 mRNA expression (Fig. 3B). These results indicate that the expression level of HAS2 is involved in the spontaneous chemokinesis of HeLa-S3 cells.

Cell-associated HA plays a critical role in the regulation of spontaneous chemokinesis. To further investigate the roles of the HAS enzymes in the spontaneous chemokinesis, we determined the molecular weights of HA produced by HeLa-S3...
cells by gel permeation chromatography assays. HeLa-S3 cells cultured in serum-free medium for 6 h produced ~50 ng/ml of HA with a peak molecular mass of 990 kDa (Fig. 4A). These results are consistent with the notion that the HA is generated by HAS2, which produces HMW-HA in HeLa-S3 cells. Since the expression of HAS2 appeared to be correlated with the cell motility of HeLa-S3 cells, we assessed the effects of an excessive amount of the 920 kDa HA on the spontaneous chemokinesis of the cells. However, exogenously added 920 kDa HA did not enhance the spontaneous chemokinesis of the cells within 6 h (Fig. 4B). These findings suggest that the HMW-HA, which is presumably generated by HAS2, does not directly induce the spontaneous chemokinesis of HeLa-S3 cells. The findings further indicate that HeLa-S3 cells may have a self-degrading system for HA to regulate their spontaneous chemokinesis.

Knockdown of HYAL2 or CD44 reduces the spontaneous chemokinesis of HeLa-S3 cells. It was previously shown that HMW-HA bound to the cell surface with the aid of CD44 is preferentially degraded during a culture period of 2-6 h in HEK293 cells overexpressing both HYAL2 and CD44 (36). Accordingly, our results indicated that the HA generated by HAS2 in HeLa-S3 cells may need to be degraded by HYAL2 at the cell surface to regulate spontaneous chemokinesis. To evaluate the effects of HYAL2 on spontaneous chemokinesis, we performed siRNA-mediated knockdown of HYAL2 in HeLa-S3 cells. In Western blot analyses, an anti-HYAL2 antibody recognized two bands with apparent molecular masses of ~54 kDa (Fig. 5A), as expected from the structure of the HYAL2 cDNA. Introduction of the HYAL2 siRNA, but not the control siRNA or an unrelated siRNA, resulted in almost complete reduction of the slower migrating band but not the faster migrating band (Fig. 5A). Unfortunately, we had a non-specific band near HYAL2 band by Western blot analysis. It did not change by the treatment of HYAL2 siRNA. As shown in Fig. 5B and C, this siRNA treatment did not change the mRNA expression levels of either HAS2 or HAS3 when measured by real-time RT-PCR. When we assessed spontaneous chemokinesis of the HeLa-S3 cells with reduced expression of HYAL2, we found that the motility was markedly decreased compared with the control siRNA-transfected cells (Fig. 5D). These findings are consistent with the idea that HYAL2 is involved in the HA-dependent spontaneous chemokinesis. Next, to assess the effects of CD44 on spontaneous chemokinesis, we examined siRNA-mediated knockdown of CD44 expression in HeLa-S3 cells. Introduction of a siRNA against CD44 resulted in a
remarkable decrease in the protein expression as detected by Western blot analysis (Fig. 5A). This treatment also decreased spontaneous chemokinesis compared with the control siRNA-transfected cells (Fig. 5D). These findings indicate that CD44...
is also involved in HA-dependent spontaneous chemokinesis and that catabolism of HA by HYAL2 and CD44 at the cell surface is a key event in the regulation of the spontaneous chemokinesis. To further validate the involvement of HYAL2 in the motility, we examined the MDA-MB-231 cell line, as a CD44-positive breast cancer cell line (39,47), which expressed lower levels of HYAL2 protein than HeLa-S3 cells (Fig. 6A). MDA-MB-231 cells transiently transfected with the HYAL2 cDNA robustly expressed HYAL2 protein (Fig. 6B). The HYAL2-overexpressing MDA-MB-231 cells showed a remarkable increase in spontaneous chemokinesis compared with control mock-transfected cells (Fig. 6C). These findings suggest that HYAL2/CD44 degrades HA and that the resulting fragmented HA stimulates cells to undergo spontaneous chemokinesis.

LMW-HA, but not HMW-HA reverses the HYAL2 siRNA-mediated reduction of spontaneous chemokinesis to the level in cells stimulated with the corresponding HA. In this study, we found that the knockdown of HYAL2, HAS2 or CD44 resulted in a decrease in spontaneous chemokinesis of HeLa-S3 cells. To determine which sizes of HA are involved in spontaneous chemokinesis of these cells, we obtained HAs with different molecular weights (23, 230 and 920 kDa) and evaluated how they affected the decrease in spontaneous chemokinesis of HeLa-S3 cells after the siRNA-mediated knockdown of HYAL2. Addition of the 23 kDa HA reversed the HYAL2 siRNA-dependent reduction in spontaneous chemokinesis to the level of control cells stimulated with the same HA (Fig. 7A). However, the two HMW-HAs (230 and 920 kDa) had no effect on the reduction in the spontaneous chemokinesis of HeLa-S3 cells after siRNA-mediated knockdown of HYAL2. Addition of the 23 kDa HA reversed the HYAL2 siRNA-dependent reduction in spontaneous chemokinesis to the level of control cells stimulated with the same HA (Fig. 7A). However, the two HMW-HAs (230 and 920 kDa) had no effect on the reduction in the spontaneous chemokinesis of HeLa-S3 cells after siRNA-mediated knockdown of HYAL2 (Fig. 7B and C). These findings indicate that HeLa-S3 cells autonomously generate HYAL2/CD44-mediated HA fragments, which enhance their spontaneous chemokinesis. Therefore, our findings suggest that the HAS2-HYAL2/CD44 system supports HA fragment-dependent cell motility by an autocrine mechanism.

Figure 7. HA (23 kDa) reverses the siHYAL2-mediated reduction of spontaneous chemokinesis to the control level. The effects of different molecular weight HAs (23, 230 and 920 kDa) on the HYAL2 siRNA (siHYAL2)-mediated reduction of spontaneous chemokinesis of HeLa-S3 cells were assessed by Boyden chamber assays. (A-C) The siHYAL2-transfected HeLa-S3 cells were incubated in the absence or presence of 50 µg/ml of the 23 kDa HA (A), 230 kDa HA (B) or 920 kDa HA (C). The data shown are means ± SD (n=5). *p<0.01, **p<0.05, vs. the control by Student’s t-test.
HYAL2 does not affect the proliferation of HeLa-S3 cells. To eliminate the possibility that HYAL2 affected the cell proliferation during the motility assays, we assessed the effects of the alterations in HYAL2 expression on the proliferation of HeLa-S3 cells by MTT assays. As shown in Fig. 8A and B, neither upregulation nor downregulation of HYAL2 expression affected the cell proliferation during a culture period of 4 days. Therefore, the HYAL2-mediated changes in the spontaneous chemokinesis seem to be independent of cell proliferation. These results are consistent with a previous study using different cell lines (48).

Discussion

HA is a ubiquitous extracellular matrix component of the tumor environment, especially in the stroma where its accumulation is observed in various carcinomas (6,49-53). In particular, cell-associated HA is found in the plasma membrane of poorly differentiated invasive cells of advanced carcinomas (51). The increased levels of HA indicate that its metabolism is altered in carcinomas, and that this perturbation of HA synthesis and/or degradation may play important roles in tumor progression. Recently, it has become increasingly clear that LMW-HA can induce a variety of biological events, such as matrix metalloproteinase expression (54,55), angiogenesis (56) and tumor cell migration (57). Our findings suggest that HAS2 synthesizes HMW-HA, which is then degraded by the HYAL2/CD44 complex to LMW-HA on the plasma membrane in an autocrine manner to regulate cancer cell motility.

Previous studies have shown that HAS2 contributes to the initiation and progression of cancer (17,23-25,29,39). Our findings further showed that siRNA-mediated silencing of HAS2 expression decreased the spontaneous chemokinesis of HeLa-S3 cells. This observation supports the notion that HAS2 expression plays important roles in cancer progression and metastasis. We also found that HAS3 appeared to play a minor role in the regulation of the spontaneous chemokinesis of HeLa-S3 cells. Furthermore, the downregulation of HAS3 mRNA expression resulted in increased HAS2 mRNA expression, and vice versa. These findings suggest the implementation of a back-up cellular mechanism, and these events are likely to occur often in HA-related genes (17,23). HA synthesized by HAS2 may preferentially regulate the cell motility compared with HAS3-synthesized HA in HeLa-S3 cells, since the levels of HAS2 expression but not HAS3 expression were correlated with the rate of enhancement of chemokinesis.

It has remained controversial whether HYAL2 is a tumor promoter or a tumor suppressor. Duterme et al (58) reported that stable overexpression of HYAL2 with no detectable enzymatic activity reduced the cell motility of rat fibroblast cell lines in wound healing assays. In contrast, Udabage et al (39) reported that highly invasive human breast cancer cells preferentially expressed the mRNAs of HAS2, HYAL2 and CD44.

Our results support the latter observations that HYAL2 plays a critical role in the increase in cell motility, and that this effect is dependent on the expression levels of HAS2 and CD44. HYAL enzymes and their attendant control systems appear to be of critical importance in the expression of biological HA functions. It was previously shown that HMW-HAs associated with the cell surface are more preferentially degraded during cultures of HEK293 cells overexpressing both HYAL2 and CD44 compared with soluble HMW-HAs in the medium (36). Our findings indicate that the HYAL2/CD44 complex degrades HAS2-synthesized HMW-HA and that the resultant LMW-HA may act on hyaladherin receptors including CD44.

There have been a number of reports regarding CD44 ligation-induced cellular responses, including enhancement of cell motility (59,60), expression of adhesion molecules (61,62) and a growth factor receptor (62), cytokine and chemokine secretion (63-66), and protection from apoptosis (10,67-69). At the biochemical level, CD44 ligation has been shown to activate Rac1 (70-72) and nuclear factor-κB (73). An emerging concept in signal transduction is that cell adhesion molecules can function as co-receptor (74). CD44 is known to serve as a co-receptor for growth factors and associate with their receptors, such as tyrosine kinase receptor ErbB or c-Met (75,76). Some of these CD44 ligation-induced cellular responses would be related to LMW-HA production by the HAS2-HYAL2/CD44 system during neoplastic transformation.

The major signaling pathways relevant to cancer are involved in HA metabolism. Essentially, EGF receptor signaling increases HA production via elevation of HAS2 expression (77,78), p53 protein suppresses the expression of hyaladherins, such as CD44 (79), and Wnt signaling regulates the expression of CD44 (80). Accordingly, these lines of evidence, together with the findings of the present study, support the notion that altera-

![Figure 8. HYAL2 does not affect the proliferation of HeLa-S3 cells.](Image 87x456 to 246x765)
tions of HA metabolism in cancer cells could open a gateway for invasion and metastasis. The present findings indicate that HMW-HA synthesized by HAS2 is degraded by the HYAL2/CD44 complex to ~20 kDa LMW-HA on the membrane, which then acts on hyaladherin receptors. Subsequently, when a molecular interaction with an appropriate signaling receptor occurs, the resultant signaling activates Rho GTPases, which induce cell motility through dynamic cytoskeletal reorganization. All of these steps are controlled by the cells. Our study suggests that this autocrine LMW-HA-induced stimulation may be a novel activation mechanism that could facilitate cancer cell motility during epithelial-mesenchymal transition at the invasive front of metastatic cancer.

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