Review Article

Impact of the hyaluronan-rich tumor microenvironment on cancer initiation and progression

Naoki Itano,[1,2,5] Lisheng Zhuo[3] and Koji Kimata[4,5]

[1]Department of Molecular Oncology, Division of Molecular and Cellular Biology, Institute on Aging and Adaptation, Shinshu University Graduate School of Medicine, Nagano 390-8621; [2]Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Saitama 332-0012; [3]Institute for Molecular Science of Medicine, Aichi Medical University, Aichi 480-1195; [4]Research Complex for the Medicine Frontiers and Institute for Molecular Science of Medicine, Aichi Medical University, Aichi 480-1195, Japan

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Hyaluronan acts as a microenvironmental stimulus that can influence the malignant phenotype of cancer cells. During cancer progression, hyaluronan assembles an extracellular matrix that is favorable for both the motility and proliferation of cancer cells and the recruitment of inflammatory and bone marrow-derived progenitor cells. The varied roles of this polysaccharide are regulated via multiple mechanisms involving biosynthesis, degradation, binding with other extracellular molecules, and activation of signaling pathways. Recent animal studies have provided evidence that aberrant biosynthesis of hyaluronan accelerates tumor growth through a diverse repertoire of host–tumor interactions, such as stromal cell recruitment, angiogenesis, lymphangiogenesis, and inflammation. Hyaluronan in the tumor microenvironment thus significantly impacts cancer initiation and progression via stroma–cancer cell interactions. (*Cancer Sci* 2008; 99: 1720–1725)

It is now widely recognized that tumors are not autonomous cells developing independently, but rather are cellular masses formed by complex interactions with their microenvironment.[1–3] In fact, extensive remodeling of its adjacent microenvironment is closely associated with tumor development and progression.[4,5] Apart from the tumor cells themselves, the tumor microenvironment is composed of stromal cells, such as fibroblasts, vascular cells, and infiltrating immune cells, and of non-cellular compartments, including secreted soluble factors and solid-state structural extracellular matrix (ECM) (Fig. 1). Tumor cells sense signals from the tumor microenvironment and bilaterally communicate with host stromal cells. During cancer progression, these cellular communications dramatically alter the cellular and molecular composition of a particular tumor microenvironment to support cancer cell proliferation, migration, invasion, and metastasis,[6] pointing to a need to better understand the molecular basis of these influences on all stages of cancer for the design of targeted, molecular-based therapies.

Hyaluronan (HA) is a major constituent of ECM and provides a favorable microenvironment for cell proliferation and migration.[7,8] A wide variety of HA binding molecules contribute to the assembly of pericellular HA-rich ECM and tightly regulate HA functions.[9] Several crosslinkers such as serum-derived HA-binding protein (SHAP), TSG-6, and versican (also called PG-M) play crucial roles in the formation of HA meshwork,[10–12] and CD44, lymphatic vessel endothelial HA receptor (LYVE)-1, and RHAMM act as receptors for the anchorage of HA-rich ECM to the cell surface.[13] In addition to its solid-state structural features, HA-rich ECM activates intracellular signals through interaction with cell surface receptors. These downstream signals subsequently induce gene expression related to cell growth and survival, and stimulate active cell migration. HA itself is a simple polysaccharide composed of repeating disaccharide units of *N*-acetylglucosamine and glucuronic acid.[14] Despite its relatively simple chemical composition, HA functions are pleiotropic dependent on concentration and structure, and multilaterally regulated in a complex fashion based on biosynthesis, degradation, binding with other extracellular molecules, and activation of signaling pathways. The dynamic turnover of HA molecules is balanced by biosynthesis and catabolism, thereby maintaining a constant concentration in the normal tissues. In vertebrates, the

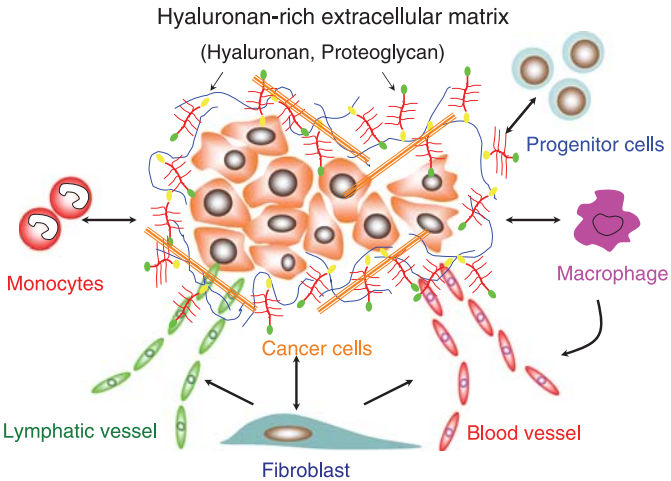

Fig. 1. Host–tumor cell interactions in the tumor microenvironment. Hyaluronan (HA) forms part of the tumor microenvironment by linking HA-binding partners into macromolecular aggregates. Tumor cells sense signals from the tumor microenvironment and crosstalk with host stromal cells. A HA-rich tumor microenvironment may accelerate the recruitment of monocytes and macrophages. These complex cellular interactions also accelerate tumor angiogenesis and lymphangiogenesis by stimulating endothelial cell infiltration and recruitment of bone marrow-derived progenitor cells.

[5]To whom correspondence should be addressed.
E-mail: kimata@aichi-med-u.ac.jp;itano@sch.md.shinshu-u.ac.jp

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biosynthesis of HA molecules is regulated by three HA synthases (HAS) 1–3, and HA catabolism is regulated by an enzymatic degradation reaction involving several hyaluronidases.[15–17]

In many aggressive cancers, the close association of aberrant HA production with cancer progression is now being established,[18] and upregulation of *HAS* genes has been demonstrated in cells undergoing malignant transformation.[19] Significant compositional and structural alterations of HA-rich ECM have also been implicated in cancer malignancies.[20] In this review article, we will focus our discussion primarily on the role of the HA-rich tumor microenvironment in cancer initiation and progression, and will also discuss the emerging concept of HA-rich ECM as a target for therapeutic agents.

Evidence of HA acceleration of cancer initiation and progression

Increasing lines of evidence show that aberrant HA production influences the malignant behavior of cells; HA overproduction in non-malignant cells impairs the intercellular adhesion machinery and diminishes contact inhibition of cell growth and migration.[21] This is representative of the HA-induced precancerous state of cells. Likewise, forced expression of *HAS2* and *HAS3* genes results in excess HA production, which enhances the tumorigenic ability of fibrosarcomas and melanoma cells.[22,23] We have shown that induced expression of HAS1 restores the metastatic potential of mouse mammary carcinoma mutants previously having low levels of HA synthesis and metastatic ability.[24] Although the above clearly demonstrates the important role of HA in tumorigenesis, the tumor-promoting ability of excess HA is still somewhat controversial as HAS overexpression also suppresses the tumorigenesis of tumor cells under certain conditions.[25] Therefore, the mechanism by which HA drives alterations in tumor malignancy by modulating cellular interactions within the tumor microenvironment is largely unknown.

We previously generated a transgenic (Tg) mouse model allowing overexpression of murine Has2 in mammary glands to elucidate the *in vivo* action of HA on tumor initiation and progression.[26] In this model, the expression of exogenous Has2 was conditionally controlled by the expression of Cre-recombinase driven by a mammary epithelial cell-specific MMTV promoter (Fig. 2). By intercrossing with a mouse mammary tumor model expressing rat *c-neu* protooncogenes in mammary epithelial cells, the Tg mice developed mammary tumors with aggressive growth rates. Tumor incidence was also significantly elevated in Has2-overexpressing mice compared with the controls. Histologically, these tumors were classified as poorly differentiated adenocarcinomas, whereas control tumors had the characteristics of ductal carcinomas. The most prominent histological feature in the Has2-overexpressing mammary tumors was increased formation of intratumoral stroma (Fig. 3). HA staining demonstrated that this molecule was abundant in tumoral stromal compartments, as well as in the perivascular elastic structure of angiogenic microvessels (Fig. 3). It is therefore likely that microenvironmental HA in tumors provides a favorable scaffold for the interaction of cancer and host cells, which in turn accelerates tumor cell survival and angiogenesis. Interestingly, both downregulation of E-cadherin and increased nuclear translocation of β-catenin were evident in Has2-overexpressing tumor cells. These phenomena are hallmarks of epithelial–mesenchymal transition (EMT), supporting the notion that HA overproduction induces EMT-like epithelial changes of cancer cells toward a migratory fibroblastic phenotype.[27] EMT was originally defined as a morphological conversion during normal development, but has recently gained attention as a central mechanism for carcinoma progression and metastasis. This finding thus strengthens the need to elucidate how HA accelerates cancer progression *in vivo*.

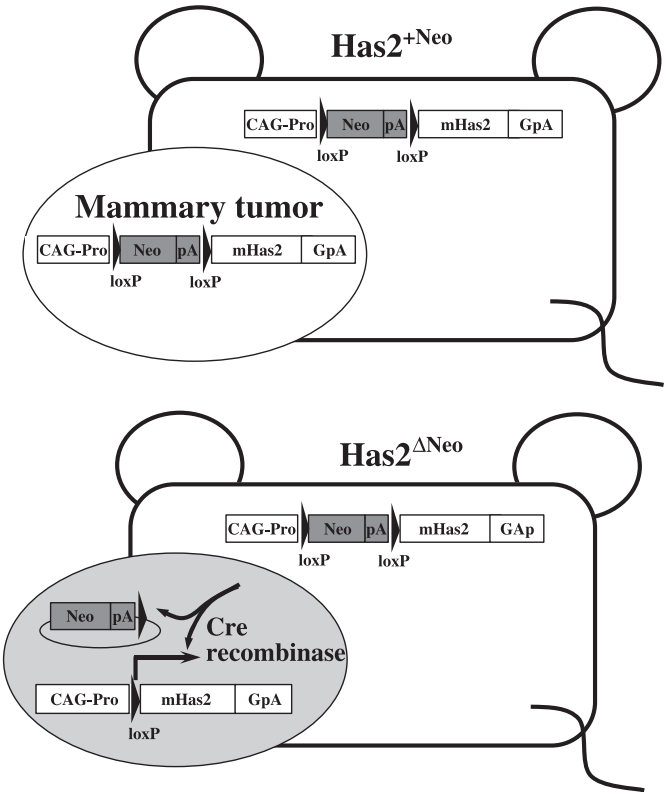

Fig. 2. Schematic of the transgene construct in Has2-overexpressing transgenic ($Has2^{\Delta Neo}$) and control ($Has2^{+Neo}$) mice. Murine Has2 cDNA was positioned downstream of the transgene unit including the CAG promoter (CAG Pro), a *lox*P sequence, the *Neo*-resistance gene (Neo), the SV40 poly(A) signal (pA), and a second *lox*P sequence. Upon recognition of the *lox*P site, Cre recombinase specifically expressed in $Has2^{\Delta Neo}$ mammary tumors deletes the Neo cassette along with one of the *lox*P sequences and then joins the CAG promoter and Has2 cDNA, leading to expression of Has2 mRNA.

Hyaluronan regulates crosstalk between stroma and tumor cells

Stromal elements first influence tumor cells in the early stages of cancer establishment, then later have a strong effect on tumor progression.[28] As the carcinoma evolves, phenotypic changes also occur in the adjacent stroma. Despite the importance of tumor–stroma interactions, our overall understanding of how stromal cells are recruited and how they affect tumor initiation and progression are limited. Earlier *in vivo* investigations using tumor xenograft models have provided evidence for the importance of the stromal response in tumorigenesis. In one experiment, forced expression of transforming grwoth factor (TGF)-β1 in human melanoma cells led to a survival advantage and increased metastasis through the remodeling of neighboring stroma when xenografted into immunodeficient mice.[29] Additional insight into the stromal contribution to cancer initiation and progression has come from studies with genetically manipulated animal models. Analysis of knockout mice lacking the TGF-β type II receptor gene revealed that the loss of TGF-β responsiveness in mesenchymal fibroblasts resulted in rapid development of lethally aggressive prostate cancer through upregulation of TGF-α, and HGF-mediated signaling networks.[30] These data clearly support the significance of tumor stroma in providing a favorable microenvironment for tumor development and growth.

In clinicopathological studies, HA levels in stromal compartments correlate with tumor aggressiveness and adverse clinical outcome in human breast and prostate cancers.[31,32] Our study

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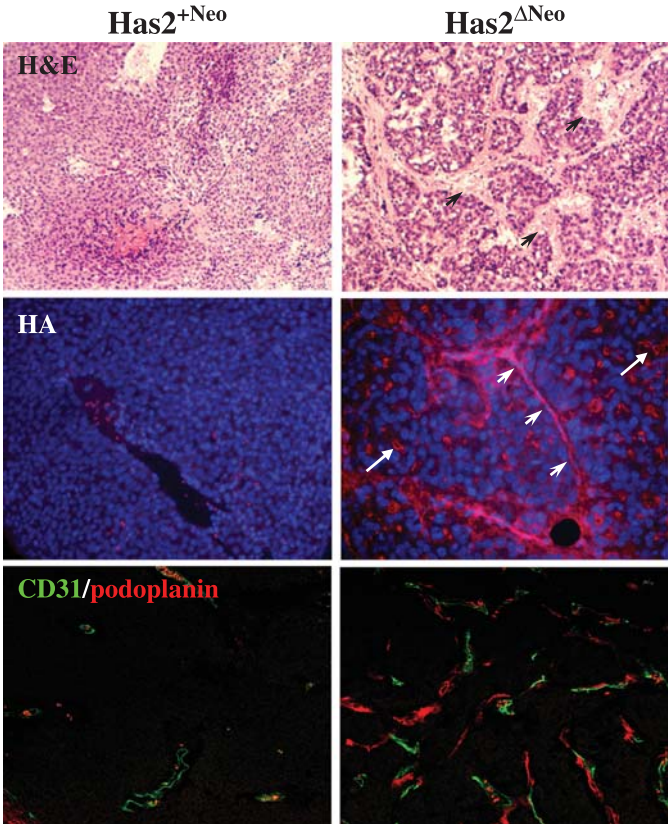

Fig. 3. Hyaluronan (HA) overproduction promotes the formation of intratumoral stroma. Tumor sections from Has2$^{\Delta Neo}$ and Has2^{+Neo} mice were stained with hematoxylin–eosin (HE, upper panels). Has2$^{\Delta Neo}$ tumors showed marked formation of intratumoral stroma (arrowheads). In contrast, control tumors had the characteristics of ductal carcinoma with much less stroma. Tissue sections from Has2$^{\Delta Neo}$ and Has2^{+Neo} tumors were stained with the biotynylated HA-binding domain of bovine cartilage aggrecan (HA, middle panels). Intense HA staining (red) was observed at the intercellular boundaries of tumor cells and particularly in the tumor stroma of Has2$^{\Delta Neo}$ (white arrowheads). HA-rich matrix was also prominent in the perivascular elastic structure of angiogenic microvessels (white arrows). In contrast, low deposition of HA was observed in Has2^{+Neo} mice. Immunofluorescence of blood vessel marker CD31 (green) and lymphatic vessel marker podoplanin (red) demonstrated that both microvessels and lymphatic vessels were more numerous in the Has2$^{\Delta Neo}$ tumors compared with control tumors (bottom panels).

using the Has2 Tg mouse model demonstrated this HA accumulation in stromal compartments within aggressive tumors as well,[26] although Has2 was overexpressed specifically in mammary tumor cells. HA was abundant in tumor stroma but less at the intercellular boundaries of tumor cells (Fig. 3). As the stromal cells established from primary tumors actively and endogenously synthesized HA, they appear to be responsible for stromal HA accumulation and partly support the hypothesis that increased stromal HA modulates tumor cell behavior by controlling crosstalk between stroma and tumor cells. The experiment did not address how tumor-derived HA recruits stromal cells within tumors, although several likely explanations can be considered. One can first speculate that extracellular HA accumulation in mammary carcinomas provides microenvironments amenable to easy penetration of stromal cells by increasing turgidity and hydration or by disruption of cell-to-cell junctions.[21] Moreover, HA appears to promote stromal cell motility by acting on intracellular signaling pathways through interaction with cell surface receptors, such as CD44 and RHAMM.[13] In concert with HA-binding molecules, HA may allow cells to prepare for proliferation and migration by enhancing cell detachment from the ECM or by participating in the assembly of intracellular machinery and transmitting signals.[9] HA-rich ECM may also mediate the recruitment of mesenchymal stem cells, which are progenitors of tumor-associated fibroblasts (TAF).[33] Lastly, HA overproduction in tumor cells may induce EMT, which can in turn convert tumor cells to fibroblasts.

Tumor-associated host cells support tumorigenesis and tumor angiogenesis

As major cellular components of tumor stroma, TAF are capable of modulating their local microenvironment during tumor formation and progression.[6] Olumi *et al.* reported that coinoculation of TAF with SV40 immortalized prostatic epithelial cells accelerated tumor growth in mice, whereas normal fibroblasts were incapable of such stimulation.[34] TAF also supply a variety of cytokines, growth factors, tissue-remodeling enzymes, and ECM components, all of which modulate host–tumor interactions. Using vascular endothelial growth factor (VEGF)-null fibrosarcomas, Dong *et al.* demonstrated that PDGFR signaling requires the recruitment of VEGF-producing stromal fibroblasts for tumor angiogenesis and growth.[35] Orimo *et al.* further demonstrated that TAF isolated from invasive human breast carcinomas promote angiogenesis by recruiting endothelial progenitor cells into carcinomas, an effect mediated in part by stroma-derived factor (SDF)-1.[36] Our Tg mouse studies have shown that HA overproduction in tumor cells accelerates stromal reactions accompanied by formation of intratumoral neovasculature.[26] In these mammary tumors, accumulation of microvessels was observed frequently within or near stromal structures of tumors (Fig. 3), supporting the idea that tumor-derived HA matrix promotes tumor angiogenesis via the induction of angiogenic factors from infiltrating stromal cells. As SDF-1 is transcriptionally upregulated in HA-rich tumors, the action of SDF-1 from infiltrating TAF may partly account for accelerated angiogenesis; indeed, TAF established from primary mammary tumors expressed high levels of SDF-1 and significantly promoted tumor angiogenesis in xenografted tumors (H. Koyama *et al.* unpublished results). HA accumulation may also accelerate the recruitment of monocytes and macrophages into tumor tissues and convert them to tumor-associated macrophages (TAM), which are pivotal for the promotion of angiogenesis. From the observations of de la Motte *et al.* cable-like structures of HA have been implicated in monocyte recruitment in vascular lesions via CD44.[37] Kuang *et al.* have further suggested that tumor-derived HA induces the conversion of recruited blood monocytes to immunosuppressive and angiopromotive TAM.[38] Taken together, the HA-rich microenvironment appears to be important for the recruitment of tumor-associated host cells that support tumorigenesis and tumor angiogenesis.

Angiogenesis is now recognized as a significant event in tumor progression and acquisition of a malignant phenotype, and the composition of the ECM surrounding the vasculature can affect angiogenesis either positively or negatively.[39,40] As mentioned above, HA was abundant in the perivascular elastic structure of angiogenic microvessels in Has2-overexpressing mammary tumors, and an analogous localization was observed for versican as a HA-bound matrix component.[26] Versican is a chondroitin sulfate proteoglycan capable of binding to HA via its N-terminal domain,[41] and its increased immunostaining is often associated with vascular and perivascular elastic structures in malignant tumors.[42] Our own matrigel plug angiogenesis assays have suggested that versican plays a role in HA-mediated angiogenesis by enhancing recruitment of host stromal cells.[26] Although the requirement of HA for versican activity is not fully resolved, these results suggest that this molecule acts as an angiogenic modulator via interaction with HA.

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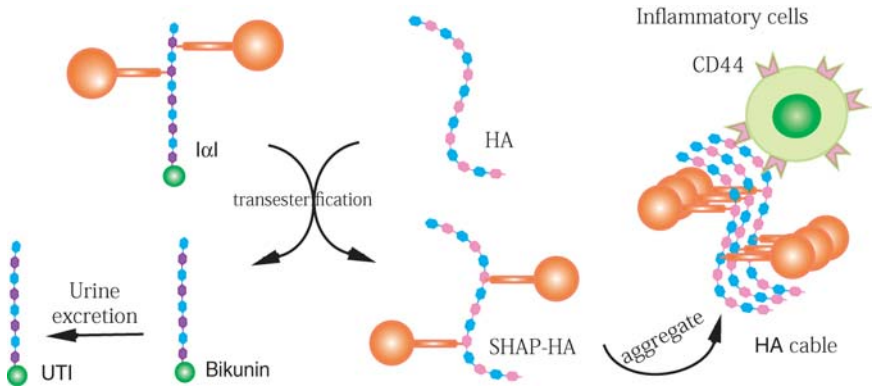

Fig. 4. Serum-derived hyaluronan-binding protein (SHAP)–hyaluronan (HA) complex formation and its possible role in cell activation. The interα-trypsin inhibitor (IαI) family of hepatocyte-secreted plasma proteins consist of a chondroitin sulfate proteoglycan (light chain or bikunin) and one or two heavy chains (HC) linking to the chondroitin sulfate chain. Upon a proper stimulus, circulating IαI family molecules are recruited to extravascular sites, where the HC are transferred to locally synthesized HA to form SHAP–HA complexes by transesterification. SHAP modification induces HA to aggregate, forming cable-like structures. Aggregated HA exhibits markedly increased avidity to CD44 and thus much firmer adhesion of infiltrating inflammatory cells. UTI, urinary trypsin inhibitor.

The HA-mediated acceleration of tumor angiogenesis may also be explained by the well-known fact that only HA degradation products of a specific size induce an angiogenic response.[43,44] Studies in chick chorioallantoic membranes and rat skin have demonstrated that HA degradation products of a specific size (3–10 disaccharide units) have the potential to induce neovascularization. Furthermore, HA oligosaccharides, together with the angiogenic factor VEGF, synergistically stimulate endothelial cell proliferation, migration, and capillary formation *in vitro*.[45] Has2-overexpressing tumors contained significant amounts of small HA species, suggesting that specific HA oligosaccharides at least partly influence tumor-induced angiogenesis. As studies have suggested that myofibroblasts are the major sources of hyaluronidase, it needs to be investigated whether TAF-derived hyaluronidases degrade high molecular weight HA to smaller angiogenic oligosaccharides.

Role of HA in lymphangiogenesis

Lymphangiogenesis has also gained attention for its potential involvement in cancer dissemination and metastasis.[46] VEGF-C and -D play a critical role in the induction of lymphangiogenesis, and influence conjunctive lymphatic vessels via their VEGFR-3 receptor to induce lymphangiogenesis in solid tumor masses. Recent studies have provided evidence that both stromal cells and cancer cells are capable of secreting many potential lymphangiogenic factors that likely lead to *de novo* formation of lymphatic vessels. Schoppmann *et al*. proposed the idea that a subpopulation of monocytes is exposed to activators in the stroma and then converted to TAM to switch on *de novo* synthesis of VEGF-C and -D.[47] Clinically, lymphatic microvessels are localized primarily within the peritumoral stroma in several human tumors.[48,49] Thus, tumoral lymphangiogenesis is likely governed by complex interactions between tumor cells and stroma, whose cells may serve as important mediators.

Similarly, a spontaneous cancer model using Has2-overexpressing Tg mice revealed that microenvironmental HA plays a pivotal role in intratumoral lymphangiogenesis by promoting the interactions between tumor cells and stroma.[50] In mammary tumors, HA overproduction resulted in the promotion of tumor lymphangiogenesis concurrently with the formation of stromal structures (Fig. 3). Additionally, lymphatic vessels frequently penetrated and accumulated in stromal compartments, and upregulation of VEGF-C and -D was detected at tumor–stroma interfaces. The contribution of stromal cells was further supported by tumor xenograft transplantation studies, where TAF were found to give rise to highly lymphatic tumors when coimplanted with tumor cells, suggesting the importance of targeting tumor stroma to prevent lymphangiogenesis and ensuing cancer progression.

Multiple mechanisms may be involved in HA-stimulated tumor lymphangiogenesis. HA-rich ECM may directly interact with cell surface HA receptors of lymphatic endothelial cells and accelerate tumor lymphangiogenesis. The HA receptor LYVE-1, which has a similarity to CD44, is expressed abundantly on the surface of lymphatic endothelium,[51] thereby implicating it in fundamental roles like maintaining either lymphatic architecture or normal lymphatic function. A recent report on LYVE-1 null mice, however, suggested that direct interaction of LYVE-1 with HA is not obligatory for normal or pathological lymphangiogenesis.[52]

Hyaluronan covalently associated with SHAP links chronic inflammation to cancer

Emerging evidence has suggested that chronic inflammation is frequently associated with malignant growth and is thought to enhance cancer progression.[53] Therefore, treatments are being sought to reduce chronic inflammation in the tumor microenvironment to limit this malignancy. We previously identified a SHAP, formed by the heavy chains of the inter-α-trypsin inhibitor (IαI) family, which is closely associated with chronic inflammatory diseases.[54] Among HA-associating proteins, SHAP is unique in its formation of covalent linkages with HA in contrast to the domain or motif-mediated non-covalent binding of other proteins, as well as in its origin of sytemic circulation (Fig. 4).[55] Screening of serum SHAP–HA complexes showed elevated levels in patients with cancers and inflammatory diseases such as rheumatoid arthritis.[56–58]

It is well recognized that cancer metastasis and leukocyte recruitment share common molecular mechanisms in cell adhesion and migration, and HA-CD44 is one such common adhesion molecule pair.[59,60] CD44-bearing cancer cells and leukocytes adhere to the HA-rich ECM on the endothelial surface or tissue stroma, where HA production is upregulated in response to tumorigenic or inflammatory conditions.[61,62] Accordingly, SHAP modification of HA may have a regulatory effect on CD44–HA interactions and mediate cancer cell metastasis and inflammatory cell extravasation. In related experiments, HA substrata were prepared with either free HA or SHAP–HA complexes,[63] to which adhesion of CD44-positive leukemia cells was compared under both static and flowing conditions. The results showed that the SHAP–HA substratum induced visibly more firm cell adhesion.[64] The presence of HA, but not SHAP protein IαI, interfered with cell adhesion. In addition, pretreatment of cells with blocking CD44 antibodies completely abolished cell adhesion. Therefore, the higher cell adhesiveness of SHAP–HA is likely due to the increased avidity of HA to CD44 after SHAP modification (Fig. 4).

The above *in vitro* observations imply a novel molecular mechanism for the regulation of inflammatory responses; adhesion of CD44-bearing leukocytes to HA substrates are enhanced via the formation of SHAP–HA complexes. This hypothesis has been

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validated in an increasing number of pathological conditions. In inflammatory bowel disease, smooth muscle cells of the hyperplastic muscularis mucosae interact with infiltrating mononuclear leukocytes to cause disease development and propagation. HA in the ECM of smooth muscle cells forms disease-specific 'cable' structures that specifically mediate the binding of mononuclear leukocytes, and SHAP was found to be present and required for the construction of this structure.[37,65] Neutrophil adhesion to liver sinusoidal endothelium was recently found to be mediated by CD44–HA interactions in endotoxemic conditions and central to lipopolysaccharide-induced hepatic injury. Although endotoxemia did not alter the endothelial HA-binding avidity of the neutrophil CD44, it did induce the modification of endothelial HA by SHAP. In fact, it was the latter modification that dramatically induced neutrophil adhesion.[66] These observations indicate that SHAP modification of HA is a new molecular mechanism for the regulation of inflammatory responses by enhancing leukocyte–endothelium and -stroma interactions. Leukocyte infiltration is a cardinal feature of virtually all cancers, and many inflammatory mediators, such as cytokines and chemokines, are secreted from inflammatory cells and are capable of stimulating tumor proliferation and angiogenesis. Prevention of SHAP–HA complex formation is thus a promising pharmacological target for anti-inflammation and anticancer drugs.

Conclusion and perspectives

The tumor microenvironment plays a key role in cancer progression. Specifically, HA-rich tumor microenvironments regulate important host–tumor interactions and have a significant impact on many aspects of cancer initiation and malignancy. The use of Tg animal models have allowed us to improve our understanding of the pathogenesis of HA-rich cancers. These experiments have led to the finding that tumor-derived HA induces stromal reactions and subsequent promotion of tumor angiogenesis and lymphatic penetration within intratumoral stromal compartments. In addition, HA-rich tumor microenvironments also accelerate the recruitment of inflammatory cells, thus providing cytokines and chemokines for tumor growth and angiogenesis. As such, targeting microenvironmental HA is becoming an increasingly promising therapeutic approach in cancer treatment.

Acknowledgments

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References

1 Mueller MM, Fusenig NE. Friends or foes – bipolar effects of the tumour stroma in cancer. *Nat Rev Cancer* 2004; **4**: 839–49.
2 Bhowmick NA, Moses HL. Tumor–stroma interactions. *Curr Opin Genet Dev* 2005; **15**: 97–101.
3 Li H, Fan X, Houghton J. Tumor microenvironment: the role of the tumor stroma in cancer. *J Cell Biochem* 2007; **101**: 805–15.
4 Kopfstein L, Christofori G. Metastasis: cell-autonomous mechanisms versus contributions by the tumor microenvironment. *Cell Mol Life Sci* 2006; **63**: 449–68.
5 Cretu A, Brooks PC. Impact of the non-cellular tumor microenvironment on metastasis: potential therapeutic and imaging opportunities. *J Cell Physiol* 2007; **213**: 391–402.
6 Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature* 2004; **432**: 332–7.
7 McDonald JA, Camenisch TD. Hyaluronan: genetic insights into the complex biology of a simple polysaccharide. *Glycoconj J* 2002; **19**: 331–9.
8 Laurent TC, Fraser JR. Hyaluronan. *FASEB J* 1992; **6**: 2397–404.
9 Day AJ, Prestwich GD. Hyaluronan-binding proteins: tying up the giant. *J Biol Chem* 2002; **277**: 4585–8.
10 Matsumoto K, Shionyu M, Go M *et al*. Distinct interaction of versican/PG-M with hyaluronan and link protein. *J Biol Chem* 2003; **278**: 41 205–12.
11 Zhuo L, Kimata K. Cumulus oophorus extracellular matrix: its construction and regulation. *Cell Struct Funct* 2001; **26**: 189–96.
12 Day AJ, de la Motte CA. Hyaluronan cross-linking: a protective mechanism in inflammation? *Trends Immunol* 2005; **26**: 637–43.
13 Turley EA, Noble PW, Bourguignon LY. Signaling properties of hyaluronan receptors. *J Biol Chem* 2002; **277**: 4589–92.
14 McDonald J, Hascall VC. Hyaluronan minireview series. *J Biol Chem* 2002; **277**: 4575–9.
15 Itano N, Kimata K. Mammalian hyaluronan synthases. *IUBMB Life* 2002; **54**: 195–9.
16 Weigel PH, Hascall VC, Tammi M. Hyaluronan synthases. *J Biol Chem* 1997; **272**: 13 997–4000.
17 Stern R, Asari AA, Sugahara KN. Hyaluronan fragments: an information-rich system. *Eur J Cell Biol* 2006; **85**: 699–715.
18 Toole BP. Hyaluronan: from extracellular glue to pericellular cue. *Nat Rev Cancer* 2004; **4**: 528–39.
19 Itano N, Sawai T, Atsumi F *et al*. Selective expression and functional characteristics of three mammalian hyaluronan synthases in oncogenic malignant transformation. *J Biol Chem* 2004; **279**: 18 679–87.
20 Theocharis AD, Vynios DH, Papageorgakopoulou N. *et al*. Altered content composition and structure of glycosaminoglycans and proteoglycans in gastric carcinoma. *Int J Biochem Cell Biol* 2003; **35**: 376–90.
21 Itano N, Atsumi F, Sawai T *et al*. Abnormal accumulation of hyaluronan matrix diminishes contact inhibition of cell growth and promotes cell migration. *Proc Natl Acad Sci USA* 2002; **99**: 3609–14.
22 Kosaki R, Watanabe K, Yamaguchi Y. Overproduction of hyaluronan by expression of the hyaluronan synthase Has2 enhances anchorage-independent growth and tumorigenicity. *Cancer Res* 1999; **59**: 1141–5.
23 Liu N, Gao F, Han Z, Xu X, Underhill CB, Zhang L. Hyaluronan synthase 3 overexpression promotes the growth of TSU prostate cancer cells. *Cancer Res* 2001; **61**: 5207–14.
24 Itano N, Sawai T, Miyaishi O, Kimata K. Relationship between hyaluronan production and metastatic potential of mouse mammary carcinoma cells. *Cancer Res* 1999; **59**: 2499–504.
25 Bharadwaj AG, Rector K, Simpson MA. Inducible hyaluronan production reveals differential effects on prostate tumor cell growth and tumor angiogenesis. *J Biol Chem* 2007; **282**: 20 561–72.
26 Koyama H, Hibi T, Isogai Z *et al*. Hyperproduction of hyaluronan in neu-induced mammary tumor accelerates angiogenesis through stromal cell recruitment: possible involvement of versican/PG-M. *Am J Pathol* 2007; **170**: 1086–99.
27 Zoltan-Jones A, Huang L, Ghatak S, Toole BP. Elevated hyaluronan production induces mesenchymal and transformed properties in epithelial cells. *J Biol Chem* 2003; **278**: 45 801–10.
28 Desmouliere A, Guyot C, Gabbiani G. The stroma reaction myofibroblast: a key player in the control of tumor cell behavior. *Int J Dev Biol* 2004; **48**: 509–17.
29 Löhr M, Schmidt C, Ringel J *et al*. Transforming growth factor-β1 induces desmoplasia in an experimental model of human pancreatic carcinoma. *Cancer Res* 2001; **61**: 550–5.
30 Cheng N, Chytil A, Shyr Y *et al*. Enhanced hepatocyte growth factor signaling by type II transforming growth factor-beta receptor knockout fibroblasts promotes mammary tumorigneesis. *Cancer Res* 2007; **67**: 4869–77.
31 Auvinen P, Tammi R, Parkkinen J *et al*. Hyaluroanan in peritumoral stroma and malignant cells associates with breast cancer spreading and predicts survival. *Am J Pathol* 2000; **156**: 529–36.
32 Lipponen P, Aaltomaa S, Tammi R, Tammi M, Agren U, Kosma VM. High stromal hyaluronan level is associated with poor differentiation and metastasis in prostate cancer. *Eur J Cancer* 2001; **37**: 849–56.
33 Zhu H, Mitsuhashi N, Klein A *et al*. The role of the hyaluronan receptor CD44 in mesenchymal stem cell migration in the extracellular matrix. *Stem Cells* 2006; **24**: 928–35.
34 Olumi AF, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD, Cunha GR. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res* 1999; **59**: 5002–11.
35 Dong J, Grunstein J, Tejada M *et al*. VEGF-null cells require PDGFRα signaling-mediated stromal fibroblast recruitment for tumorigenesis. *EMBO J* 2004; **23**: 2800–10.
36 Orimo A, Gupta PB, Sgroi DC *et al*. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* 2005; **121**: 335–48.
37 de la Motte CA, Hascall VC, Calabro A, Yen-Lieberman B, Strong SA. Mononuclear leukocytes preferentially bind via CD44 to hyaluronan on

doi: 10.1111/j.1349-7006.2008.00885.x
© 2008 Japanese Cancer Association

human intestinal mucosal smooth muscle cells after virus infection or treatment with poly (IC). *J Biol Chem* 1999; **274**: 30 747–55.
38 Kuang DM, Wu Y, Chen N, Cheng J, Zhuang SM, Zheng L. Tumor-derived hyaluronan induces formation of immunosuppressive macrophages through transient early activation of monocytes. *Blood* 2007; **110**: 587–95.
39 Carmeliet P. Angiogenesis in health and disease. *Nat Med* 2003; **9**: 653–60.
40 Sottile J. Regulation of angiogenesis by extracellular matrix. *Biochim Biophys Acta* 2004; **1654**: 13–22.
41 Kimata K, Oike Y, Tani K *et al*. A large chondroitin sulfate proteoglycan (PG-M) synthesized before chondrogenesis in the limb bud of chick embryo. *J Biol Chem* 1986; **261**: 13 517–25.
42 Nara Y, Kato Y, Torii Y *et al*. Immunohistochemical localization of extracellular matrix components in human breast tumours with special reference to PG-M/versican. *Histochem J* 1997; **29**: 21–30.
43 West DC, Hampson IN, Arnold F, Kumar S. Angiogenesis induced by degradation products of hyaluronic acid. *Science* 1985; **228**: 1324–6.
44 Sattar A, Rooney P, Kumar S *et al*. Application of angiogenic oligosaccharides of hyaluronan increases blood vessel numbers in rat skin. *J Invest Dermatol* 1994; **103**: 576–9.
45 Montesano R, Kumar S, Orci L, Pepper MS. Synergistic effect of hyaluronan oligosaccharides and vascular endothelial growth factor on angiogenesis *in vitro*. *Laboratory Invest* 1996; **75**: 249–62.
46 Alitalo K, Tammela T, Petrova TV. Lymphangiogenesis in development and human disease. *Nature* 2005; **438**: 946–53.
47 Schoppmann SF, Birner P, Stockl J *et al*. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. *Am J Pathol* 2002; **161**: 947–56.
48 Williams CS, Leek RD, Robson AM *et al*. Absence of lymphangiogenesis and intratumoural lymph vessels in human metastatic breast cancer. *J Pathol* 2003; **200**: 195–206.
49 Vleugel MM, Bos R, van der Groep P *et al*. Lack of lymphangiogenesis during breast carcinogenesis. *J Clin Pathol* 2004; **57**: 746–51.
50 Koyama H, Kobayashi N, Harada M *et al*. Significance of tumor-associated stroma in promotion of intratumoral lymphangiogenesis: pivotal role of a hyaluronan-rich tumor microenvironment. *Am J Pathol* 2008; **172**: 179–93.
51 Jackson DG, Prevo R, Clasper S, Banerji S. LYVE-1, the lymphatic system and tumor lymphangiogenesis. *Trends Immunol* 2001; **22**: 317–21.
52 Gale NW, Prevo R, Espinosa J *et al*. Normal lymphatic development and function in mice deficient for the lymphatic hyaluronan receptor LYVE-1. *Mol Cell Biol* 2007; **27**: 595–604.
53 Ben-Baruch A. Host microenvironment in breast cancer development: inflammatory cells, cytokines and chemokines in breast cancer progression: reciprocal tumor–microenvironment interactions. *Breast Cancer Res* 2003; **5**: 31–6.
54 Zhuo L, Shen L, Wu J *et al*. Biological function of hyaluronan-SHAP covalent complex. In: Garg HG, Hales CA, eds. *Chemistry and Biology of Hyaluronan*. Amsterdam: Elsevier, 2004; 205–22.
55 Zhuo L, Hascall VC, Kimata K. Inter-α-trypsin inhibitor, a covalent protein–glycosaminoglycan–protein complex. *J Biol Chem* 2004; **279**: 38 079–82.
56 Shen L, Zhuo L, Okumura A *et al*. The SHAP-hyaluronan complex in serum from patients with chronic liver diseases caused by hepatitis virus infection. *Hepatol Res* 2006; **34**: 178–86.
57 Kida D, Yoneda M, Miyaura S *et al*. The SHAP–HA complex in sera from patients with rheumatoid arthritis and osteoarthritis. *J Rheumatol* 1999; **26**: 1230–8.
58 Obayashi Y, Yabushita H, Kanyama K *et al*. Role of serum-derived hyaluronan-associated protein–hyaluronan complex in ovarian cancer. *Oncol Report* 2008; **19**: 1245–51.
59 Gunthert U. CD44: a multitude of isoforms with diverse functions. *Curr Top Microbiol Immunol* 1993; **184**: 47–63.
60 Lesley J, Hyman R, Kincade PW. CD44 and its interaction with extracellular matrix. *Adv Immunol* 1993; **54**: 271–335.
61 DeGrendele HC, Estess P, Picker LJ *et al*. CD44 and its ligand hyaluronate mediate rolling under physiologic flow: a novel lymphocyte–endothelial cell primary adhesion pathway. *J Exp Med* 1996; **183**: 1119–30.
62 Mohamadzadeh M, DeGrendele H, Arizpe H *et al*. Proinflammatory stimuli regulate endothelial hyaluronan expression and CD44/HA-dependent primary adhesion. *J Clin Invest* 1998; **101**: 97–108.
63 Yingsung W, Zhuo L, Morgelin M *et al*. Molecular heterogeneity of the SHAP–hyaluronan complex. Isolation and characterization of the complex in synovial fluid from patients with rheumatoid arthritis. *J Biol Chem* 2003; **278**: 32 710–18.
64 Zhuo L, Kanamori A, Kannagi R *et al*. SHAP potentiates the CD44-mediated leukocyte adhesion to the hyaluronan substratum. *J Biol Chem* 2006; **281**: 20 303–14.
65 de la Motte CA, Hascall VC, Drazba J *et al*. Mononuclear leukocytes bind to specific hyaluronan structures on colon mucosal smooth muscle cells treated with polyinosinic acid: polycytidylic acid: inter-α-trypsin inhibitor is crucial to structure and function. *Am J Pathol* 2003; **163**: 121–33.
66 McDonald B, McAvoy E, Lam F *et al*. CD44 and hyaluronan interaction is the dominant mechanism for neutrophil sequestration in inflamed liver sinusoids. *J Exp Med* 2008; **205**: 915–27.

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