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Hyaluronan Regulation of Endothelial Barrier Function in Cancer

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Abstract

Vascular integrity or the maintenance of blood vessel continuity is a fundamental process regulated by endothelial cell–cell junctions. Defects in endothelial barrier function are an initiating factor in several disease processes including tumor angiogenesis and metastasis. The glycosaminoglycan, hyaluronan (HA), maintains vascular integrity through specific mechanisms including HA-binding protein signaling in caveolin-enriched microdomains, a subset of lipid rafts. Certain disease states, including cancer, increase enzymatic hyaluronidase activity and reactive oxygen species generation, which break down high molecular weight HA (HMW-HA) to low molecular weight fragments (LMW-HA). LMW-HA can activate specific HA-binding proteins during tumor progression to promote disruption of endothelial cell–cell contacts. In contrast, exogenous administration of HMW-HA promotes enhancement of vascular integrity. This review focuses on the roles of HA in regulating angiogenic and metastatic processes based on its size and the HA-binding proteins present. Further, potential therapeutic applications of HMW-HA in treating cancer are discussed.

1. INTRODUCTION

The vascular endothelium, which lines the inner surface of blood vessels, acts as a selectively permeable barrier to regulate the movement of liquid and solutes between blood and the surrounding tissue, particularly in the microvasculature (Curry, 2005; Dejana, Tournier-Lasserre, & Weinstein, 2009; Vandenbroucke, Mehta, Minshall, & Malik, 2008). The semipermeable nature of the endothelium allows plasma fluid, nutrients, and even cells to move out of the blood and into the tissues, while metabolic products may be taken up by the circulation. This exchange between the blood and the tissues is vital for organ function and tissue viability by maintaining fluid and metabolic homeostasis. Vascular barrier function is dependent on the integrity of the endothelial cell (EC) layer (Lennon & Singleton, 2011a, 2011b). Several mechanisms regulate basal vascular integrity including the endothelial glycocalyx, a meshwork of hyaluronan (HA), proteoglycans, glycolipids, and proteins between the vascular luminal space and the EC surface, endothelial cell–cell junctions which are controlled by tight junctions, adherens junctions, and caveolin-enriched microdomains (CEM) (Lennon & Singleton, 2011a, 2011b). Disruption or dysregulation of the endothelial layer can lead to altered permeability resulting in leakage of fluid, solutes

and proteins from the blood into the underlying tissue resulting in edema. Dysregulation of endothelial barrier function can occur in a wide range of human pathologies including tumor angiogenesis and metastasis (Pardue, Ibrahim, & Ramamurthi, 2008; Reymond, d'Agua, & Ridley, 2013; Singleton & Bourguignon, 2002, 2004; Slevin et al., 2007).

The major nonsulfated glycosaminoglycan in most tissues, HA, plays a fundamental role in the maintenance of vascular integrity (Singleton, Dudek, Ma, & Garcia, 2006; Singleton et al., 2010). HA is composed of a linear repeat of disaccharide units consisting of D-glucuronic acid and N-acetylglucosamine (Almond, 2007; Cantor, 2007; Cantor & Nadkarni, 2006; Fraser, Laurent, & Laurent, 1997; Gaffney, Matou-Nasri, Grau-Olivares, & Slevin, 2010; Girish & Kemparaju, 2007; Jiang, Liang, & Noble, 2007, 2011; Liao, Jones, Forbes, Martin, & Brown, 2005; Olczyk, Komosinska-Vashev, Winsz-Szczotka, Kuznik-Trocha, & Olczyk, 2008; Scott & Heatley, 2002; Singleton et al., 2010; Tammi, Day, & Turley, 2002; Toole, 2004; Wang, de la Motte, Lauer, & Hascall, 2011). The major form of HA *in vivo*, high molecular weight HA (HMW-HA), has a molecular weight >1 million Da. HMW-HA exhibits a random coil structure that can expand in aqueous solutions (Scott & Heatley, 2002). Aqueous HMW-HA is highly viscous and elastic, properties which contribute to its filtering functions in the glycocalyx (Furlan, La Penna, Perico, & Cesaro, 2005; Henry & Duling, 1999). HA is a dynamic molecule with a high rate of metabolism. In humans, up to one third of total HA is turned over in the body everyday (Fraser et al., 1997). The majority of HA within the vasculature is incorporated into the endothelial glycocalyx and the extracellular matrix of the underlying tissue (Nandi, Estess, & Siegelman, 2000; Olczyk et al., 2008; Wheeler-Jones, Farrar, & Pitsillides, 2010). The levels of soluble HA are low in normal human plasma due to rapid removal by the liver and kidneys (Fraser et al., 1997).

In several types of cancer, there is enhanced production of HA in the tumor stroma, which correlates with angiogenesis, metastasis, and poor patient outcome (Sironen et al., 2011; Tammi et al., 2008). HA is synthesized by hyaluronan synthases (HAS) (Weigel & DeAngelis, 2007). The three main HAS (HAS1, HAS2, and HAS3) differ in the Km values for their substrates (D-glucuronic acid and N-acetylglucosamine) leading to differential rates of hyaluronan synthesis and secretion from the plasma membrane (Weigel & DeAngelis, 2007). HAS1 and HAS2 produce HA with a molecular weight >500 kDa and HAS3 produces <500 kDa HA (Itano & Kimata, 2002). HAS2 upregulation is correlated with breast cancer invasion (Bernert, Porsch, & Heldin, 2011). Inhibition of HA synthesis with 4-methylumbelliferone has been shown to promote growth arrest, apoptosis, and metastasis in tumor cells (Arai et al., 2011; Futamura et al., 2013; Saito, Dai, & Asano, 2013). HA is degraded in cancer by hyaluronidases and reactive oxygen species (ROS) to produce lower molecular weight fragments (<500 kDa) (Girish & Kemparaju, 2007). HA fragments in the ~ 10–15 disaccharide range promote angiogenesis and have been observed in several cancer types. There are six hyaluronidase genes encoding HYAL-1,2,3,4, PHYAL1 (a pseudogene), and PH-20 (Csoka, Frost, & Stern, 2001; Stern, 2003; Stern, Asari, & Sugahara, 2006; Stern, Kogan, Jedrzejewski, & Soltes, 2007). HYAL enzymes have different cellular localization and optimal pH activity which can lead to generation of different sized HA fragments (Bourguignon, Singleton, Diedrich, Stern, & Gilad, 2004; Csoka et al., 2001; Stern, 2003, 2008; Stern et al., 2006, 2007). Although the correlation of specific hyaluronidases with

tumor progression varies, several aggressive cancer types have upregulated Hyal-1 and/or Hyal-2 expression (Tan, Wang, Li, et al., 2011; Tan, Wang, Su, et al., 2011; Udabage, Brownlee, Nilsson, & Brown, 2005). The differential mechanisms of HA's regulation of vascular integrity during cancer are discussed below.

2. HA REGULATION OF VASCULAR INTEGRITY

Vascular integrity or the maintenance of blood vessel continuity is a fundamental process in which HA plays numerous roles including regulation of endothelial cell–cell junctions (Lennon & Singleton, 2011a, 2011b; Singleton & Lennon, 2011). Our previous published data indicate that HMW-HA (~1 million Da) enhances vascular integrity both *in vitro* and *in vivo* (Singleton et al., 2006, 2007, 2010). Using a murine model of LPS-induced acute lung injury with pulmonary vascular hyperpermeability, we observed that intravenous administration of HMW-HA protected against impaired pulmonary vascular integrity (Singleton et al., 2007, 2010). This effect was also observed in a ventilator-induced model of acute lung injury (Lennon & Singleton, 2011b; Liu et al., 2008). The protective effects of HMW-HA were abrogated in CD44 or caveolin-1 knockout mice indicating the importance of these molecules in endothelial barrier enhancement (Singleton et al., 2007, 2010).

Addition of HA to human pulmonary microvascular EC monolayers and measuring transendothelial electrical resistance (TER) revealed that HMWHA promotes endothelial barrier enhancement while LMW-HA (~2500 Da) induces EC barrier disruption (see Fig. 7.1). This has accompanied by dramatic changes in the actin cytoskeleton. HMW-HA induced a cortical actin “ring” structure while LMW-HA caused stress fiber formation in EC (Singleton et al., 2006).

Mechanistically, we observed that HMW-HA binds to and inhibits the EC barrier disrupting activity of the extracellular serine protease HABP2 (Mambetsariev et al., 2010). In addition, HMW-HA binds to the transmembrane receptor, CD44s (standard form), in specialized plasma membrane domains enriched in the scaffolding protein, caveolin-1, called CEM (Singleton et al., 2006, 2009; Singleton, Dudek, Chiang, & Garcia, 2005). CD44s then transactivates the barrier enhancing S1P₁ receptor (Singleton et al., 2006). This results in the serine/threonine kinase, Akt-mediated activation of the Rac1 guanine nucleotide exchange factor, Tiam1, and Rac1-GTP formation leading to cortical actin formation and strengthening of EC–EC contacts (Singleton et al., 2006, 2010). Further, HMW-HA recruits several other actin regulatory proteins to CEM including annexin A2, protein S100-A10, filamin-A, and filamin-B which enhance cortical actin formation and vascular integrity (see Fig. 7.2).

In contrast to HMW-HA, LMW-HA binds to and activates the HA receptor, CD44v10 (variant 10) in CEM (Singleton et al., 2006). CD44v10 then transactivates the barrier disruptive S1P₃ receptor (Singleton et al., 2006). In addition, LMW-HA binds to and activates the extracellular serine protease, HABP2, which induces protease-activated receptor activation in EC (Mambetsariev et al., 2010). These events promote RhoA guanine nucleotide exchange factor activation and RhoA-GTP formation which stimulates the serine/threonine kinase, rho kinase (ROCK) (Singleton et al., 2006). This leads to actin stress fiber

formation and EC barrier disruption (see Fig. 7.2). In addition, Flynn, Michaud, Canosa, & Madri (2013) have demonstrated that CD44 regulation of the EC barrier involves PECAM-1. Disruption of the endothelium is an important initiating event in tumor angiogenesis and cancer metastasis (Pardue et al., 2008; Reymond et al., 2013; Singleton & Bourguignon, 2002, 2004; Slevin et al., 2007).

3. HA REGULATION OF ENDOTHELIAL BARRIER FUNCTION DURING TUMOR ANGIOGENESIS

Angiogenesis is an essential phenotype in a number of physiologic and pathologic processes including growth and development (Flamme, Frolich, & Risau, 1997), wound healing (Arnold & West, 1991), and reproduction (Shimizu, Hoshino, Miyazaki, & Sato, 2012). Inadequate angiogenesis contributes to ulcer formation (Folkman et al., 1991), while excessive angiogenesis contributes to the pathology of arthritis (Semerano, Clavel, Assier, Denys, & Boissier, 2011), psoriasis (Leong, Fearon, & Veale, 2005), and neoplasia (Folkman, 1974). In a series of now classical experiments, Folkman and colleagues demonstrated that solid tumors cannot grow larger than 3–4 mm in diameter unless they induce their own blood supply (Hanahan & Folkman, 1996).

An initial step in the complex process of angiogenesis is the disruption of the barrier of a preexisting blood vessel to allow “budding” of EC to form new capillaries (Otrock, Mahfouz, Makarem, & Shamseddine, 2007; Potente, Gerhardt, & Carmeliet, 2011). We have previously reported that LMW-HA induces EC barrier disruption, which suggests a role for HA fragments in the initiation of tumor angiogenesis (Singleton et al., 2006). Several cancers produce HA fragments in the ~10–15 disaccharide range that have been shown to promote angiogenesis (Lataillade, Albanese, & Uzan, 2010). These HA fragments bind to EC surface receptors such as CD44, RHAMM, and TLR4 (Dang et al., 2013; Gao, Yang, Mo, Liu, & He, 2008; Sironen et al., 2011). Wang, Cao, et al. (2011) reported that CD44 regulates EC proliferation and tubule formation induced by HA fragments in HUVECs. In telomerase-immortalized foreskin microvascular EC, Olofsson, Porsch, and Heldin (2014) demonstrated that inhibiting CD44 or Hyal2 expression prevented tubular network formation in 3D matrices. In addition, CD44 and TLR4 can be physically associated in a signaling complex following exposure to HA and endothelial TLR4 regulates certain responses to HA fragments (Taylor et al., 2004, 2007). Further, Savani et al. (2001) demonstrated that HA fragment-induced HUVEC and H5V (murine EC line) angiogenesis involved the expression of both CD44 and RHAMM. Specifically, CD44 regulated HA-mediated adhesion, proliferation, and tube formation, while RHAMM regulated migration and *in vivo* angiogenesis. Gao et al. (2008) showed that RHAMM is the major receptor involved in HA fragment-induced angiogenesis during wound healing. Further, Matou-Nasri, Gaffney, Kumar, & Slevin (2009) demonstrated that both CD44 and RHAMM are involved in HA fragment-induced BAEC angiogenesis. Additional confirmation of a dual role of CD44 and RHAMM in HA fragment-mediated HUVEC angiogenesis was determined by Park et al. (2012), who demonstrated that HA induced RHAMM–TGF β receptor complexes through a CD44–PKC δ mechanism. Interestingly, in postmortem human coronary arteries that have endothelial injury and angiogenesis associated with

atherosclerotic plaques, there is increased expression of RHAMM and Hyal-1 but not CD44 in the neovessels suggesting differential roles of RHAMM and CD44 in angiogenesis from small versus large blood vessels (Krupinski et al., 2008).

A final step in the process of angiogenesis is the resealing of the endothelial barrier of the newly formed blood vessels from preexisting vessels (Otrock et al., 2007; Potente et al., 2011). We have reported that HMW-HA enhances EC barrier function (Singleton et al., 2006, 2010). The HA in the stroma of several cancers has been implicated in numerous processes including inhibition of apoptosis, stimulation of tumor cell proliferation, migration and invasion, epithelial mesenchymal transition, protection from immune cells, and regulation of intercellular space (Auvinen et al., 2013; Bollyky et al., 2009; Cho et al., 2012; Dickinson & Gerecht, 2010; Sironen et al., 2011; Tammi et al., 2008). Whether this stroma-associated HA also promotes resealing of new capillaries remains to be determined.

4. HA REGULATION OF ENDOTHELIAL BARRIER FUNCTION DURING CANCER METASTASIS

During cancer metastasis, there is a disruption of the endothelial barrier first during tumor cell intravasation from the tumor into a blood vessel and second during tumor cell extravasation out of a blood vessel and into a target tissue (Reymond et al., 2013). For intravasation, the tumor cell must invade through the tumor stroma (Chiang & Massague, 2008; Joyce & Pollard, 2009). HA, such as that found in the HA-rich stroma of tumors, can induce expression of several ECM degrading enzymes on tumor cells including the matrix metalloproteinases MT1-MMP, MMP-2, MMP-7, and MMP-9 as well as EMMPRIN and the cysteine proteases cathepsin D and cathepsin K (Abecassis, Olofsson, Schmid, Zalcman, & Karniguian, 2003; Bourguignon et al., 2004; Chetty et al., 2012; Droller, 2003; Kim et al., 2008; Kosunen et al., 2007; Marrero-Diaz et al., 2009; Mitchell & King, 2014; Murray, Morrin, & McDonnell, 2004; Nalla, Gorantla, Gondi, Lakka, & Rao, 2010; Veeravalli et al., 2010). Once at the blood vessel, the tumor cell can secrete a host of factors to induce disruption of EC junctions and EC retraction including MMP1, ADAM12, and TNF1 α to allow for paracellular transendothelial migration (Mierke, 2011). In addition, we have demonstrated that LMW-HA induces EC barrier disruption and can potentially contribute to this process (Singleton et al., 2006).

Within a blood vessel, the tumor cells are subjected to sheer stress and the host immune system (Reymond et al., 2013). Interestingly, Hirose et al. (2012) reported that inhibition of the scavenger receptor, Stabilin-2, in mice increases circulating HA which does not affect primary tumor size but inhibits tumor metastasis. The increased circulating HA was found to be ~40 kDa in size which did not affect tumor cell proliferation, migration, or invasion (Hirose et al., 2012). This size of HA was not tested in our experiments on EC barrier function. These results are intriguing since higher molecular weight HA can promote immune tolerance. While the exact mechanism for this effect is unknown, it illustrates the complex relationship between HA and cancer metastasis.

A final step of tumor cell metastasis is extravasation from a blood vessel, which is a process fundamentally different from intravasation (Reymond et al., 2013). Tumor cells initially

adhere to the HA-rich glycocalyx of EC in the vasculature and make initial cell–cell connections through various receptors including E-selectin, N-cadherin, and the HA receptor, CD44 (Draffin, McFarlane, Hill, Johnston, & Waugh, 2004; Kobayashi, Boelte, & Lin, 2007; Mine et al., 2003; Mitchell & King, 2014; Orian-Rousseau, 2010; St Hill, 2011). Then, stable adhesions are formed between tumor cells and EC, which are mediated by integrins, mucins, and CD44 (Fujisaki et al., 1999; Rahn et al., 2005; Wang et al., 2005). Tumor cells secrete various factors to disrupt endothelial cell–cell junctions and form protrusions to migrate between ECs, processes dependent on Rac1, RhoA, ROCK, MLCK, Src, PI3 kinase, and other signaling molecules (Aytes et al., 2013; Chatterjee et al., 2011; Chen, 2008; Khuon et al., 2010; Peinado, Lavotshkin, & Lyden, 2011; Reymond, Riou, & Ridley, 2012). The contribution of tumor cell hyaluronidases could be important in the breakdown of the glycocalyx, which can generate HA fragments and contribute to EC barrier disruption through activation of HA-binding proteins including HABP2 (see Fig. 7.3).

5. POTENTIAL THERAPEUTIC EFFECTS OF HMW-HA IN INHIBITING ENDOTHELIAL BARRIER DISRUPTION DURING CANCER PROGRESSION

Although HMW-HA (1 million Da) is produced endogenously and is an integral component of the extracellular matrix, synovial fluid, and vitreous humor, recent attention has been focused on the use of exogenously administered HMW-HA in a variety of diseases including cancer (Benitez et al., 2011; Cantor, 2007; Gaffney, Matou-Nasri, Grau-Olivares, & Slevin, 2010; Shay, He, Sakurai, & Tseng, 2011). *In vitro*, exogenous administration of HMW-HA inhibits ROS, nitrotyrosine, and inflammatory cytokine production (Bollyky et al., 2009; Miki et al., 2010; Yasuda, 2007). As discussed earlier, HA forms a major part of the vascular glycocalyx (Reitsma, Slaaf, Vink, van Zandvoort, & oude Egbrink, 2007). Loss or disruption of the glycocalyx leads to increased vascular permeability and edema, and may be a contributing factor to tumor angiogenesis and metastasis. Repair of the glycocalyx and restoration of vascular barrier function could potentially be beneficial in the treatment of cancer. Previous studies have shown restoration of the glycocalyx by plasma resuscitation in animal models of hemorrhagic shock (Kozar et al., 2011). Other studies have shown that per-fusion of HA can restore the glycocalyx following degradation by hyaluronidase or in response to ischemia/reperfusion injury (Henry & Duling, 1999; Rubio-Gayosso, Platts, & Duling, 2006). In models of pulmonary vascular hyperpermeability, exogenous administration of HMW-HA inhibited vascular barrier disruption (Liu et al., 2008; Singleton et al., 2007, 2010). In addition, HMW-HA and HA conjugates have been successfully utilized to target chemotherapeutics to cancer cells (Karbownik & Nowak, 2013; Misra et al., 2011; Ricciardelli et al., 2013; Wang et al., 2013; Yang et al., 2013). The ability of exogenously administered HMW-HA to restore damaged glycocalyx function and enhance EC barrier integrity make it a novel potential therapeutic strategy for diseases with defects in vascular integrity including cancer progression (Fig. 7.4).

6. CONCLUSIONS

HA is an important regulator of angiogenic and metastatic processes based on its size and the HA-binding proteins present. HMW-HA promotes vascular integrity, while LMW-HA induces EC barrier disruption, angiogenesis, and cancer metastasis. Several HA-binding proteins have been identified to regulate these processes including CD44, RHAMM, TLR4, and hyaluronidases. While, much is known about HA regulation of vascular integrity during cancer progression, several mechanisms have yet to be explained. The role of other HA-binding proteins in EC barrier function during cancer remains poorly defined. Further research into the interactions of HA and tumor cells within blood vessels can lead to novel chemotherapeutic interventions. In addition, the differential roles of HA and HA-binding proteins in angiogenesis from small and large blood vessels represent an intriguing area of research. Thus, HA and HA-binding proteins exhibit a complex regulation of EC barrier disruption, angiogenesis, and cancer metastasis. Understanding the mechanism(s) of these processes can lead to novel therapeutic targets for cancer.

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Figure 7.1. Hyaluronan regulation of endothelial barrier function

Left: HMW-HA (~1 million Da, 100 nM) induces an increase in human pulmonary microvascular EC barrier function using transendothelial electrical resistance (TER). An increase in resistance (y-axis) over time (x-axis) indicates increased barrier function. The *upper* line indicates HMW-HA treatment and the *lower* line indicates control (no treatment). Right: LMW-HA (~2500 Da, 100 nM) induces a decrease in human pulmonary microvascular EC barrier function using TER. A decrease in resistance (y-axis) over time (x-axis) indicates a decrease in barrier function. The *lower* line indicates LMW-HA treatment and the *upper* line indicates control (no treatment).

Figure 7.2. Hyaluronan regulation of normal and impaired vascular integrity

(A) HMW-HA (~1 million Da) binds to and inhibits the EC barrier disrupting activity of the extracellular serine protease HABP2. In addition, HMW-HA binds to the transmembrane receptor, CD44s (standard form), in specialized plasma membrane domains enriched in the scaffolding protein, caveolin-1, called caveolin-enriched microdomains (CEM). CD44s then transactivates the barrier enhancing S1P₁ receptor. This results in Akt-mediated activation of the Rac1 guanine nucleotide exchange factor, Tiam1, and Rac1-GTP formation leading to cortical actin formation and strengthening of EC-EC contacts. Further, HMW-HA recruits several other actin regulatory proteins to CEM including annexin A2, protein S100-A10, filamin-A, and filamin-B, which enhance cortical actin formation and vascular integrity. (B) In contrast to HMW-HA, LMW-HA (~2500 Da) binds to and activates the HA receptor, CD44v10 (variant 10) in CEM. CD44v10 then transactivates the barrier disruptive S1P₃ receptor. In addition, LMW-HA binds to and activates the extracellular serine protease, HABP2, which induces protease-activated receptor (PAR) activation in EC. These events promote RhoA guanine nucleotide exchange factor (RhoGEF) activation and RhoA-GTP formation, which stimulates the serine/threonine kinase, rho kinase (ROCK). This leads to

actin stress fiber formation and EC barrier disruption. Disruption of the endothelium is an important initiating event in tumor angio-genesis and cancer metastasis.

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Figure 7.3.

Overexpression of the HA-binding extracellular serine protease, HABP2, in human cancer cells promotes extravasation through endothelial monolayers. Human pulmonary microvascular EC were grown to confluency on gold plated electrodes and changes in electrical resistance (y-axis) over time (x-axis) were measured with addition of no cancer cells (control), 5000 or 15,000 vector control or 5000 or 15,000 HABP2 overexpressing (O/E) SKLU-1 human lung cancer cells. As cancer cells extravasate through the EC monolayers, a decrease in resistance is observed which is indicative of EC barrier disruption. HABP2 overexpressing SKLU-1 human lung cancer cells demonstrated a faster and greater magnitude of EC barrier disruption than equal numbers of vector control SKLU-1 cells.

Figure 7.4.

The role of hyaluronan regulation of endothelial barrier function in cancer. (A) Tumors that grow larger than 3–4 mm in diameter must undergo angiogenesis. Several types of tumor cells degrade HMW-HA, often found in the tumor stroma, to LMWHA through hyaluronidase activity and/or ROS. LMW-HA binds to and activates certain HA-binding proteins on EC including CD44v10, HABP2, RHAMM, and TLR4 which, along with other factors, disrupts the EC barrier of a preexisting blood vessel to allow “budding” of EC to form new capillaries and a vascularized tumor. (B) During cancer metastasis, there is a disruption of the endothelial barrier first during tumor cell intravasation from the tumor into a blood vessel and second during tumor cell extravasation out of a blood vessel and into a target tissue. For intravasation, HA fragments along with other factors produced by tumor cells promotes tumor cell invasion through the stroma and disruption of the EC barrier of a blood vessel. Tumor cells then undergo paracellular transendothelial migration into a blood vessel and travel through the bloodstream. For extravasation, tumor cells within blood vessels attach to the HA-rich glycocalyx of the endothelium and subsequently to the surface of ECs in a process regulated by several factors including the HA-binding receptor, CD44. Tumor cells then disrupt EC junctions and undergo paracellular transendothelial migration through the blood vessel wall to distal sites. HA* indicates steps during tumor cell metastasis in which HA and/or HA-binding proteins are involved.