Heparanase, Hyaluronan, and CD44 in Cancers: A Breast Carcinoma Perspective

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Introduction

Breast carcinoma is the most frequently occurring cancer in women worldwide and the most common cause of malignancy-associated deaths (1). Accurate prediction of clinical outcome in patients is difficult and usually based on clinicopathologic variables, such as tumor stage, histologic grade, and the presence of metastases to regional lymph nodes and distant organs. Additional factors that have been shown to possess prognostic significance include overexpression of ERBB2, BCL2, and other oncogenes, mutations involving TP53, BRCA1, BRCA2, and other tumor suppressor genes, as well as the presence of estrogen and progesterone receptors in cancerous cells. The aim of this brief review is to present recent findings on the roles of heparanase, hyaluronan, and CD44 in breast cancer behavior, their use in disease prognosis, and their potential as therapeutic targets. As the functions of these molecules vary according to cancer type, we have tried, as far as possible, to include only data specifically generated from studies on breast cancer cells and tumors.

Up-regulation of Heparanase in Breast Cancer

Heparanase is an important regulator of breast cancer proliferation, invasion, and metastasis as well as a significant promoter of malignancy-associated angiogenesis. Analysis of the metastatic breast carcinoma cell lines MDA-MB-231 and MDA-MB-435 revealed up-regulated expression of HPSE transcripts and heparanase enzyme activity compared with the nonmetastatic MCF-7 breast cancer cell line (2). Tumors formed from MCF-7 cells transfected with HPSE cDNA that were injected into the mammary pad of nude mice showed enhanced growth, invasion, and survival together with a greater degree of vascularity compared with tumors produced by mock-transfected cells (4). In contrast, silencing HPSE in MDA-MB-435 cells significantly reduced their invasive ability (5).

Further corroborative evidence on the importance of heparanase in breast cancer has come from studies of clinical specimens. In situ hybridization of invasive ductal carcinoma tissue sections showed strong staining for HPSE transcripts, in contrast to its absence in normal breast tissues (2). Immunohistochemical detection of heparanase in breast cancer was associated with a larger primary tumor size and the presence of tumor metastases, together with a lack of heparan sulfate deposition (6). In addition, heparanase was more frequently found in invasive breast cancer than in noninvasive/preinvasive ductal carcinoma in situ of the breast (7). Within the latter group, heparanase expression was associated with subtypes that have a more aggressive behavior (8).

Heparanase seems to be involved in several fundamental events that regulate breast cancer behavior. First, it degrades heparan sulfate found on cancer cells and in the extracellular matrix (ECM). Heparan sulfate is a reservoir on which heparin-binding growth factors and angiogenic factors aggregate. Indeed, it has been reported that heparan sulfate produced by malignant breast cancer cells possesses a higher capacity to bind to fibroblast growth factor 2 (FGF2) and hepatocyte growth factor compared with heparan sulfate from normal breast cells (9). Thus, by breaking down heparan sulfate, heparanase releases these signaling molecules, which can then act to promote tumor growth and invasion and stimulate angiogenesis (10). Furthermore, heparan sulfate fragments generated by heparanase action are optimal in size and highly potent in promoting FGF2 binding to its receptor and subsequent receptor dimerization (11). Third, for cancer cells to invade into the underlying stroma and metastasize distally via vascular and lymphatic routes, the cells must degrade the constituents of the basement membrane and ECM, which include heparan sulfate proteoglycans, collagens, and laminins (12). This is achieved through the action of heparanase and other glycosidic and proteolytic enzymes. In addition, elevated heparanase expression in breast cancer cells promotes metastasis via stimulation of osteoclastogenesis, resulting in bone resorption (13). Enzymatically active heparanase has also been found in the nuclei of MDA-MB-435 breast cancer cells, where it may contribute to the phenotype of cancer cells by regulating topoisomerase I-mediated DNA relaxation and nuclear FGF2-induced transcriptional activities (14).
Recently, it was reported that heparanase can influence angiogenesis by bringing about endothelial cell migration through stimulation of AKT and phosphatidylinositol 3-kinase (PI3K) and by inducing vascular endothelial growth factor (VEGF) expression and p38 phosphorylation (15). These effects seem to be mediated by a heparanase receptor and are not dependent on the enzymatic activity of heparanase. In addition, heparanase can stimulate cyclooxygenase-2 expression, leading to induction of VEGF and FGF2 as well as angiogenesis (7).

A recent study has shown that early growth response gene 1 (EGR1), which plays an essential role in breast cancer growth, invasion, and metastasis, regulates heparanase expression in breast cancer cells (16). EGR1 binds to the heparanase promoter, leading to a dose-dependent increase in promoter activity (16). Acting through histone deacetylases, wild-type tumor suppressor TP53 binds to the heparanase promoter and inhibits its activity in breast cancer cells (17). In contrast, mutant TP53 is not inhibitory. Mutations in TP53 are associated with increased risk of breast carcinoma and a higher rate of cancer cell proliferation (18). Four ETS transcription factor binding sites have also been described in the heparanase promoter (19). Binding of ETS1 or ETS2 leads to transactivation of HPRS. Both ETS1 and ETS2 are overexpressed in breast cancer and may contribute to tumor invasion and metastasis (20). Besides binding sites for transcription factors, the heparanase promoter contains four estrogen response elements (21). Estrogen stimulates mammary cell proliferation and is an important risk factor for breast cancer. Estrogen treatment of estrogen receptor (ER)-positive MCF-7 breast cancer cells leads to increased heparanase promoter activity (21). Epigenetically, the heparanase promoter is inactivated by methylation in mammalian cells (22), whereas it is unmethylated in heparanase-expressing metastatic MDA-MB-435 breast cancer cells.

Considering its multiple malignancy-associated roles, targeting of heparanase holds promise for treatment of breast cancer. PI-88 is phosphomannopentaose sulfate, a sulfated oligosaccharide and structural mimic of heparan sulfate capable of inhibiting heparanase activity and the binding of growth factors to heparan sulfate (23). PI-88 significantly reduced growth, vascularity, and metastasis of highly invasive mammary carcinoma in rats. Laminarin sulfate, a sulfated polysaccharide and heparanase inhibitor, dramatically reduced lung colonization by breast cancer cells (24). Other heparanase inhibitors with antineoplastic and antiangiogenic activities include suramin and its analogues as well as low molecular weight (LMW) heparins (25, 26). Heparin has been available for clinical use as an anticoagulant for many years, whereas PI-88 and other heparanase inhibitors are currently in different stages of development and clinical trials.

Roles of Heparanase and CD44 in Breast Cancer

Heparanase, also known as heparunic acid, is a nonsulfated, linear glycosaminoglycan consisting of 2,000 to 25,000 repeating disaccharide subunits of glucuronic acid and N-acetylgalactosamine. It is ubiquitously distributed and is the major type of glycosaminoglycan present in the ECM. Its viscosity and hydrodynamic properties enable it to participate in maintenance of tissue hydration and structural integrity (27).

Heparanase is well recognized as an important determinant of cancer cell behavior. Interaction of heparanase with its cell surface receptors CD44 (see below) and RHAMM induces signaling events that promote anchorage-independent tumor cell growth, survival, and migration, thereby increasing metastatic spread (27). Increased hyaluronan synthesis in cancer cells may lead to formation of a less dense matrix that enhances tumor cell motility and invasion. In addition, hyaluronan appears to form a coat that protects cancer cells from cytotoxic cells and chemotherapeutic agents (27, 28). Furthermore, hyaluronan oligosaccharides promote tumor-associated angiogenesis (27).

Expression levels of hyaluronan and hyaluronan synthase (HAS) 2, as well as the rate of hyaluronan synthesis, were shown to be increased in highly metastatic breast carcinoma cells (29). Antisense inhibition of HAS2 reduced breast carcinoma cell proliferation and migration in vitro and blocked metastasis in vivo, leading to prolonged animal survival (30). In contrast, i.v. administration of hyaluronidase to severe combined immunodeficient mice bearing human breast cancer xenografts significantly reduced the tumor volumes and amount of hyaluronan in the cancerous growths (31). Heparan sulfate, a hyaluronidase-mediated cleavage differ from intact hyaluronan in their angiogenic, antiapoptotic, and inflammation-related activities (32). Consequently, changes in the expression of hyaluronidases have context-dependent effects in promoting and inhibiting breast cancer. Furthermore, hyaluronan seems to mediate chemoresistance in breast cancer cells through the PI3K signaling pathway (33).

In clinical breast carcinoma samples, expression of hyaluronan is up-regulated both in the cancer cells themselves and in the surrounding stroma and is an independent prognostic factor for patient survival (34). The presence of high levels of hyaluronan in stromal myxoid changes in breast cancer was strongly associated with high tumor grade, tumor emboli with lymph node involvement, and increased mortality (35). Sonographic examination of invasive breast cancer showed that the tumor shape correlated with that of the hyaluronan ECM (36). Furthermore, serum hyaluronan levels were significantly elevated in women with metastatic breast cancer compared with those with nonmetastatic carcinoma and also among patients in the latter group compared with those having benign breast diseases (37).

The major receptor for hyaluronan is CD44, a family of transmembrane glycoproteins encoded by a single gene with at least 19 exons that maps to band 11p13 on the human chromosome (38). Structural and functional diversities arise from alternative splicing and variation in N- and O-glycosylation, which is reflected by differential roles of CD44 isoforms in breast cancer. CD44s, the standard and most widely expressed isoform of CD44, is up-regulated in both in situ and invasive breast ductal carcinoma, colocalizing with hyaluronan in the same cells (39). Unlike hyaluronan, increased expression of CD44s correlates with overall patient survival (40, 41). Furthermore, CD44s is expressed at a higher level in tubular carcinoma, a subtype of breast ductal carcinoma that rarely metastasizes, compared with invasive micropapillary carcinoma, a variant of breast ductal carcinoma with a high metastatic potential and poor clinical outcome (42). Indeed, loss of CD44 in vivo resulted in a marked promotion of cancer metastasis to the lung in a mouse model of breast carcinoma, whereas tumor onset and tumor size were unaffected (43).

In contrast to CD44s, the presence of CD44v3 significantly correlates with tumor infiltration by T lymphocytes and cancer metastases to draining lymph nodes, together with a loss of TP53 protein expression (44). In a 10-year follow-up study of 91 breast cancer patients, expression of CD44v7–v8 was reported to be associated with significantly poorer disease-free and overall
Figure 1. Heparanase, hyaluronan, and CD44 regulate multiple aspects of the breast cancer cell phenotype, modulating cancer cell proliferation, migration, invasion, metastasis, and angiogenesis. Estrogen antagonism down-regulates heparanase expression. Heparanase activity is inhibited by PI-88, LMW heparins, laminarin sulfate, and suramin. Inhibition of HAS2 by 4-MU leads to decreased hyaluronan biosynthesis. Both soluble CD44 (CD44s) and hyaluronan oligomers (HA oligos) interfere with CD44-mediated signaling, resulting in synergistic effects with conventional chemotherapy (CT). Hyaluronidases (HYAL) degrade hyaluronan, leading to complementary effects with conventional chemotherapy agents, which can be delivered to cancer cells more efficiently (see text). GF/AF, growth factors, angiogenic factors, and signaling molecules; HS, heparan sulfate; SERM, selective estrogen receptor modulator.
survival rates (45). CD44v6 expression was also up-regulated in breast cancer and associated with hyaluronan expression (39, 40). However, it was not an independent prognostic factor and did not correlate with clinical outcome.

CD44 acts through several mechanisms to regulate breast carcinoma behavior. Binding of CD44s to hyaluronan promoted breast cancer cell adhesion and inhibited invasion (43). On the other hand, tumor cell invasion was increased when CD44a interaction with hyaluronan was inhibited by a CD44 blocking antibody. Interaction of hyaluronan with CD44v3 stimulated breast cancer cell growth, survival, and invasion through the Rho and PI3K-AKT signaling pathways (46). Rho kinase promoted the interaction of CD44v3-8-10 with ankyrin and increased the migration of metastatic breast cancer cells (47). Hyaluronan-mediated stimulation of CD44v3 phosphorylation by transforming growth factor-β receptor I kinase also resulted in enhanced binding of CD44v3 to ankyrin and promotion of breast cancer migration. In contrast, tumor cell migration was inhibited by treatment with an anti-CD44v3 antibody (46, 47). CD44 isoforms that contain exon V3 can be modified by heparan sulfate (48). The latter can regulate cancer cell proliferation and invasion as well as angiogenesis via binding of FGF2, VEGF, and other heparin-binding growth factors (9).

Much interest has been generated by the recent discovery that CD44+CD24-CD49f-CD90+CD133+ (CD44+h) lineage marks breast cancer stem cells (49). These actively dividing cells make up only a small proportion of cells in breast cancer but are the only cells that can form tumors when injected into immunocompromised mice. In a study of 136 breast cancer specimens, a high prevalence of cancer stem cells in patients with distant, especially osseous, metastases was reported (50). However, no significant correlation with tumor progression, response to various treatment modalities, and clinical outcome was found.

Hyaluronan and CD44 are both potential candidates for cancer treatment, although not much is known about their use as therapeutic targets in breast cancer per se. The chemical 4-methylumbelliferone (4-MU) is a suppressor of HAS (51). It was found that much is not known about their use as therapeutic targets in breast cancer per se. The chemical 4-methylumbelliferone (4-MU) is a suppressor of HAS (51). It decreases hyaluronan content of melanoma cells and inhibits their metastasis to the liver, lung, spleen, and other organs. S.c. injection of hyaluronan oligomers into nude mice disrupts hyaluronan-CD44 interaction and markedly reduced melanoma growth (52). Hyaluronan oligomers have also been shown to sensitize breast cancer cells to chemotherapeutic drugs (53). Hyaluronan has been conjugated to chemotherapeutic agents and used to target breast and other cancer cell lines in vitro, exploiting the affinity of CD44 for hyaluronan (54). Indeed, a preliminary trial of this approach on 30 patients with metastatic cancer showed that the tumor responded to the treatment and that the procedure was well tolerated (55).

**Conclusion**

As summarized in Fig. 1, heparanase, hyaluronan, and CD44 regulate the breast cancer cell phenotype and seem to predict the clinical outcome in breast carcinoma patients. A synergistic combination of established chemotherapeutic agents with targeting of hyaluronan, CD44, and heparanase may be a promising approach for improving breast cancer treatment. Because increased hyaluronan production leads to cancer cell resistance to several chemotherapeutic drugs, including doxorubicin, cisplatin, methotrexate, and paclitaxel (53, 56), disruption of hyaluronan-CD44 signaling may result in better tumor response to conventional anticancer drugs. In addition, application of hyaluronidase to remove the immunoprotective and chemoprotective hyaluronan coat of cancer cells and to reduce interstitial fluid pressure may improve delivery of anticancer drugs and therapeutic antibodies to the tumor (57). Moreover, coupling of conventional chemotherapeutic drugs with hyaluronan allows for selective targeting of CD44-expressing cancers, thus lowering the dosages of anticancer medication administered and reducing unwanted side effects (28). Estrogen-antagonistic breast cancer therapy using tamoxifen, fulvestrant, or aromatase inhibitors may lead to a down-regulation of heparanase expression (Fig. 1). As heparanase, hyaluronan, and CD44 promote tumor cell proliferation, migration, and angiogenesis through different molecular mechanisms, simultaneous targeting of these three molecules may be expected to produce synergistic effects and prevent cancer cells from using alternative salvage pathways during treatment. Future studies will provide further insight into the pathophysiological roles of these molecules and may offer important tools for breast cancer management and prognostication.

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