ARTICLE
Journal of Cellular Biochemistry 115:967–976 (2014)

Journal of Cellular Biochemistry

Heparan Sulfate Proteoglycans and Human Breast Cancer Epithelial Cell Tumorigenicity

Rachel K. Okolicsanyi,[1] Andre J. van Wijnen,[2] Simon M. Cool,[3] Gary S. Stein,[4,5] Lyn R. Griffiths,[1] and Larisa M. Haupt[1]*

[1]*Genomics Research Centre, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia*

[2]*Department of Orthopedic Surgery and Biochemistry and Molecular Biology, Mayo Clinic, Rochester, Minnesota*

[3]*Glycotherapeutics Group, Institute of Medical Biology, Agency for Science, Technology and Research, Singapore, Singapore*

[4]*Department of Biochemistry, University of Vermont Medical School, Burlington, Vermont*

[5]*Vermont Cancer Center, University of Vermont Medical School, Burlington, Vermont*

ABSTRACT

Heparan sulfate proteoglycans (HSPGs) are key components of the extracellular matrix that mediate cell proliferation, invasion, and cellular signaling. The biological functions of HSPGs are linked to their co-stimulatory effects on extracellular ligands (e.g., WNTs) and the resulting activation of transcription factors that control mammalian development but also associated with tumorigenesis. We examined the expression profile of HSPG core protein syndecans (SDC1–4) and glypicans (GPC1–6) along with the enzymes that initiate or modify their glycosaminoglycan chains in human breast cancer (HBC) epithelial cells. Gene expression in relation to cell proliferation was examined in the HBC cell lines MCF-7 and MDA-MB-231 following treatment with the HS agonist heparin. Heparin increased gene expression of chain initiation and modification enzymes including EXT1 and NDST1, as well as core proteins SDC2 and GPC6. With HS/Wnt interactions established, we next investigated WNT pathway components and observed that increased proliferation of the more invasive MDA-MB-231 cells is associated with activation of the Wnt signaling pathway. Specifically, there was substantial upregulation (>5-fold) of AXIN1, WNT4A, and MYC in MDA-MB-231 but not in MCF-7 cells. The changes in gene expression observed for HSPG core proteins and related enzymes along with the associated Wnt signaling components suggest coordinated interactions. The influence of HSPGs on cellular proliferation and invasive potential of breast cancer epithelial cells are cell and niche specific. Further studies on the interactions between HSPGs and WNT ligands may yield clinically relevant molecular targets, as well as new biomarkers for characterization of breast cancer progression. J. Cell. Biochem. 115: 967–976, 2014.

KEY WORDS: HEPARAN SULFATE PROTEOGLYCANS; BREAST CANCER; MCF-7; MDA-MB-231; GENE EXPRESSION; Wnt SIGNALING

For breast cancers detected early, while still localized in the breast, chances of 5-year survival are 90%. Patient survival declines after 5 years, and, after 10 years is dependent on the stage at diagnosis [Kamangar et al., 2006]. The identification of the cellular origin of genetic and microenvironmental changes involved in breast tumor initiation, malignant conversion, and the processes occurring during the establishment of secondary tumor sites are of great medical and social interest.

Within the mammary gland, the epithelial cells are embedded within an adipose and mesenchymal stroma separated by the basement membrane. This combination of the surrounding connective tissue and basement membrane is termed the extracellular matrix (ECM) [Murphy and Gavrilovic, 1999; Brinckerhoff et al., 2000]. Disruption of the carefully ordered ECM microenvironment (niche), important for tissue specific function [McDonnell and Matrisian, 1990; Salamonsen and Woolley, 1999], can alter cellular and tissue morphology. In breast tumors, abnormal epithelial cell growth, differentiation, and invasion can aid in the development and expression of inherent genetic changes leading to tumorigenesis [Salamonsen and Woolley, 1999]. In addition, throughout the active

Grant sponsor: National Institutes of Health; Grant numbers: P01, CA082834, R01, AR049069.
*Correspondence to: Dr. Larisa M. Haupt, Genomics Research Centre, Institute of Health and Biomedical Innovation, Queensland University of Technology, Musk Ave, Kelvin Grove, QLD 4059, Australia. E-mail: larisa.haupt@qut.edu.au
Manuscript Received: 2 December 2013; Manuscript Accepted: 5 December 2013
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 19 December 2013
DOI 10.1002/jcb.24746 • © 2013 Wiley Periodicals, Inc.

process of metastasis, it is still unclear if the ability to degrade ECM constituents is mediated by factors produced by the tumor cells themselves or by the associated stromal cells [Stetler-Stevenson, 1990; Liotta, 1992; Salamonsen and Woolley, 1999].

Major constituents of the ECM, proteoglycans (PGs) structurally and functionally influence multiple cellular events including proliferation, differentiation and gene expression [Lamoureux et al., 2007]. PGs are characteristically composed of a core protein to which one or more glycosaminoglycan (GAG) side-chains attach. Heparan sulfate proteoglycans (HSPGs) are ubiquitous to the cell surface and ECM, in particular the subendothelial and epithelial basement membranes, where HS co-ordinates ECM structural integrity and barrier function [Hacker et al., 2005]. Moreover, the ECM is a reservoir for HSPGs that function to bind endogenous growth factors and support their bioactivity (http://www.ncbi.nlm.nih.gov/pubmed/17682830). HS sequences are not directly encoded by genes, but are created by an elaborate post-translational biosynthesis [Gallagher et al., 1992; Ling et al., 2006] with precursor HS chains synthesized in the Golgi as non-sulfated co-polymers attached to the HSPG core protein. The tissue specific complex HS sulfation pattern is produced following several co-ordinated temporal modification steps and determines ligand interactions including growth factors and morphogens (FGF, Wnts, BMPs), their receptors (FGFRs), and ECM structural molecules (collagen, fibronectin) [Habuchi et al., 2004; Hacker et al., 2005; Haupt et al., 2009].

The role of HSPG core proteins has been examined in a number of studies in different cancer types using in vitro and in vivo human and murine models with specific core proteins shown to have tissue and disease specific associations. Of the core proteins, several studies examining the modulation of growth factor interactions and cellular proliferation have identified concomitant alterations in glypican expression. Glypican-3 (GPC3) one of the most highly investigated members of the HSPG family, was identified as a novel tumor marker of melanoma [Nakatsura et al., 2004], and its expression associated with neuroblastoma and Wilms′ tumor [Saikali and Sinnett, 2000]. GPC3 is highly expressed in hepatocellular carcinoma (HCC), and is currently being evaluated as a target for antibody and cell-based therapies of HCC [Gao and Ho, 2011; Ho and Kim, 2011]. In a murine breast cancer model, GPC3 appears to have a dual role in mediating Wnt signaling and apoptosis [Buchanan et al., 2010]. In addition, glypican-1 (GPC1) was shown to be induced in pancreatic cancers and to be strongly expressed in breast cancers with the remaining glypicans (2, and 4–6) relatively undetected in these tumors [Matsuda et al., 2001; Ding et al., 2005]. Members of the syndecan family of HSPG core proteins are also postulated to have a role in breast tumors with overexpression of syndecan-1 (SDC1) and an absence of syndecan-4 (SDC4) previously correlated with poor prognosis and aggressive phenotype [Lendorf et al., 2011].

HSPG core protein and growth factor interactions are well documented, with promotion or inhibition of cell proliferation shown to be dependant on HS type, expression pattern and accessibility to ligands (reviewed in Haupt et al. [2009]). As HSPGs not only provide a storage depot for heparin-binding molecules in the cell microenvironment, but also decisively regulate their accessibility, function and mode of action [Vlodavsky et al., 2012], including growth factor interactions, a role for HS chains in the localized invasion and metastases in breast tumors is feasible. What has yet to be fully investigated is the role of the side chain initiation and modification enzymes, which determine the final niche specific sulfation profile, have in breast tumor initiation, progression and metastases. In this study, we have examined the gene expression profiles of key HSPG genes including side chain initiation and modification enzymes, along with the sulfation enzymes and core proteins (syndecans, glypicans) in two human breast cancer (HBC) cell culture models. This expression profile was assessed in response to the HS agonist heparin along with the Wnt pathway for insight into potential roles for HSPGs in breast cancer epithelial cell proliferation and migration.

METHODS

CELL CULTURE

The HBC lines MCF-7 and MDA-MB-231 were obtained from ATCC and grown routinely as a monolayer in culture. MCF-7 cells were maintained in 50% DMEM, 50% RPMI medium containing 10% fetal calf serum and 100 U/ml penicillin/streptomycin. MDA-MB-231 cells were maintained in RPMI medium containing 10% fetal calf serum and 100 U/ml penicillin/streptomycin. Cells were grown in a 5% CO_2 humidified atmosphere at 37°C. Cells were plated at $1 \times 10^4/cm^2$ in 100 mm culture dishes in maintenance or dosed media. Dosed conditions were maintained for one, three or five days with media supplemented with appropriate concentrations of heparin. Media was changed and cells re-dosed every 2 days. Experiments were conducted in triplicate. Cell culture medium was obtained from Lonza (Australia). Heparin and sodium chlorate were obtained from Sigma–Aldrich (Australia).

RNA ISOLATION AND REVERSE TRANSCRIPTION

For RNA isolation, cells were washed twice in 1× phosphate-buffered saline (PBS) and placed on ice. To each culture dish, 1 mL of TRIzol (Invitrogen, Australia) was added onto the cell monolayer, the monolayer homogenized, and samples stored at −80°C for at least 24 h. RNA was isolated using the Invitrogen TRIzol Extraction protocol and RNeasy MiniKit (Qiagen, Australia) for RNA clean up. The quality and quantity of isolated RNA was assessed using a Nanodrop (Thermo Scientific, Australia). For conversion to cDNA, 250 ng of RNA was reverse transcribed using 250 U Superscript III, 2 mM DTT, 1× First Strand buffer (Invitrogen), 300 ng random hexamers (Promega, Australia), 500 μM dNTPs (NEB, Australia), and 100 U RNaseOUT (Promega) in a 50-μl reaction. Resulting cDNA was diluted to a working concentration of 40 ng/μl.

Q-PCR

We investigated the gene expression changes of 30 genes involved within the biosynthesis and modification pathway of HSPGs as well as their core proteins and associated enzymes. For the genes of interest, mRNA levels were quantified at Days 1, 3, and 5 (D1, D3, D5) using Q-PCR. Briefly, 120 ng cDNA was amplified using the ABI7900 (Life Technologies). 100 μM primers were used with a 2× supermix (BioRad/Promega) in a 10 μl final reaction volume. Standard (50°C × 2 min, 95°C × 10 min, 50 cycles at 95°C × 1 min, 60°C × 30 s) or fast cycling conditions (50°C × 2 min, 95°C × 3 min, 50 cycles at 95°C × 1 s, 60°C × 3 s) were applied. Gene expression was normalized

TABLE I. Primer Sequences for Q-PCR

Gene	Forward primer	Reverse primer	Amplicon size	Ref seq. (GenBank)
NDST1	TGGTCTTGGATGGCAAACTG	CGCCAAGGTTTTGTGGTAGTC	107	NM_001543
NDST2	CCTATTTGAAAAAAGTGCCACCTACT	GCAGGGTTGGTGAGCACTGT	105	NM_003635
NDST3	ACCCTTCAGACCGAGCATACTC	CCCGGGACCAAACATCTCTT	151	NM_004784
NDST4	ATAAAGCCAATGAGAACAGCTTACC	GGTAATATGCAGCAAAGGAGATTGA	122	NM_022569
EXT1	TGACAGAGACAACACCGAGTATGA	GCAAAGCCTCCAGGAATCTGAAG	119	NM_000127.2
EXT2	CAGTCAATTAAAGCCATTGCCCTG	GGGATCAGCGGGAGGAAGAG	149	NM_000401
C5-Epimerase	AGCTGTCAAGCCAACCAAAATAA	CTTACTAGCCAATCACTAGCAGCAA	138	AY635582
HST2ST1	TCCCGCTCGAAGCTAGAAAG	CGAGGGCCATCCATTGTATG	80	NM_012262
HS6ST1	AGCGGACGTTCAACCTCAAGT	GCGTAGTCGTACAGCTGCATGT	139	NM_004807
HS6ST2	TCTGGAAAGTGCCAAGTCAAATC	ATGGCGAAATAAAGTTCATGTTGAA	116	NM_147175
SDC1	CTGGGCTGGAATCAGGAATATTT	CCCATTGGATTAAGTAGAGTTTTGC	76	BC008765.2
SDC2	AGCTGACAACATCTCGACCACTT	GCGTCGTGGTTTCCACTTTT	72	NM_002998.3
SDC3	CTTGGTCACACTGCTCATCTATCG	GCATAGAACTCCTCCTGCTTGTC	118	AF248634
SDC4	CCACGTTTCTAGAGGCGTCACT	CTGTCCAACAGATGGACATGCT	76	BC030805.1
GPC1	GGACATCACCAAGCCGGACAT	GTCCACGTCGTTGCCGTTGT	100	NM_002081
GPC2	TGATCAGCCCCAACAGAGAAA	CCACTTCCAACTTCCTTCAAACC	75	NM_152742
GPC3	GATACAGCCAAAAGGCAGCAA	GCCCTTCATTTTCAGCTCATG	71	NM_004484
GPC4	GGTGAACTCCCAGTACCACTTTACA	GCTTCAGCTGCTCCGTATACTTG	71	NM_001448
GPC5	GCTCACCTCAATGGACAAAAATT	GTTGGCAAGCGTCTCTTCACT	159	NM_004466
GPC6	CAGCCTGTGTTAAGCTGAGGTTT	GATGTGTGTGCGTGGAGGTATGT	71	NM_005708

against expression of *18S* and calculated using $2^{-\Delta\Delta Ct}$. Specific primer sequences for the genes investigated are summarized in Table I. Variation between dosed and control (undosed), at each timepoint, was assessed using a Student's *t*-test. Mean gene expression ($2^{-\Delta\Delta Ct}$) was calculated between 3 independent experiments and graphed with SEM. All experiments were performed in quadruplicate. Significance was set at $\alpha < 0.05$.

WNT GENE EXPRESSION ARRAYS

Expression analysis of the Wnt pathway was conducted using the 384-well format of the Lonza StellARray qPCR arrays (Lonza) and their validated gene primer sets focused on the Wnt pathway. Expression was determined in MCF-7 and MDA-MB-231 cells following heparin treatment, using four biological replicates of dose and control samples at D1, D3, and D5. cDNA was synthesized as described and amplified using StellARray protocols. Analysis of the resulting gene expression changes was conducted as described for Q-PCR. Gene expression was analyzed as per the manufacturer's formula with expression normalized relative to *18S* using their online Global Pattern Recognition (GPR)™ Analysis tool (version 2.0).

RESULTS

HEPARIN INCREASES THE PROLIFERATION OF MORE INVASIVE HBC CELLS

An initial dose response curve was conducted by the addition of the HS analog, heparin, to the cell cultures (1–25 μg/ml), resulting in a dose of 10 μg/ml identified for the remainder of the heparin treatments. At this dose (10 μg/ml) the MCF-7 cells had a 33% reduction in cell number between control and dosed cells at D1. This was followed by a 20% increase in cells in the dosed cultures above controls by D3 then another phase of slow cell growth in the dosed cells with a 2.7% decrease in cell number compared to controls by D5 (Fig. 1A). Under the same conditions, MDA-MB-231 cells demonstrated a 59% increase in cell number above controls at D1, and 24% at D3. There was a marked increase in cell proliferation and difference in cell number between D3 and D5 in the MDA-MB-231 treated cells, with a 75% increase in cell number compared to the control cultures (Fig. 1B). Under normal cell maintenance media conditions, both cell populations showed, as expected, an increase in cell number from D1 to D3. Interestingly, the MCF-7 cells show little increase in cell

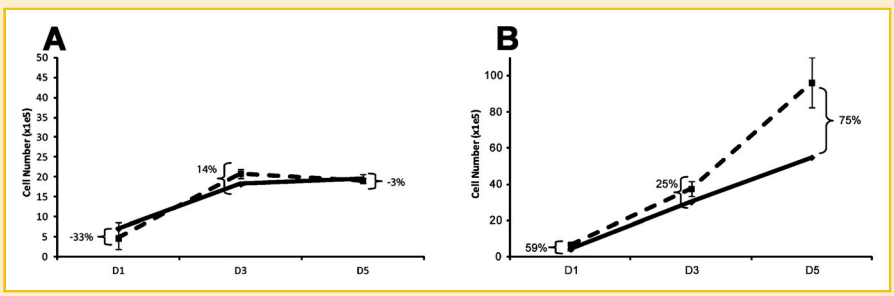

Fig. 1. Cell growth curves in the presence or absence of heparin (10 μg/ml). Solid line: control; dashed line: 10 μg/ml heparin. (A) MCF-7 and (B) MDA-MB-231.

number between D3 and D5, while the MDA-MB-231 cells demonstrated a significant increase in cell number between these two time points. The ER+ve and poorly invasive MCF-7 cells grew as distinct cobblestone colonies retaining an epithelial appearance. In contrast, the ER−ve, invasive and metastatic MDA-MB-231 cells maintained a more "fibroblast-like" morphology. These differences between the two cell populations, along with their ER status and invasive potential are also likely to influence the demonstrated proliferation rate in response to heparin treatment. Clearly the addition of the HS agonist to the cell cultures elicited a strong proliferative response in the MDA-MB-231 cells, with little observed difference and potentially a slight inhibition of proliferation evident in the MCF-7 cultures between D3 and D5. Having established these proliferation differences between the HBC cells both in control cells and in cultures treated with heparin, we next examined the expression of genes associated with HSPG production.

HS CHAIN INITIATION AND *N*-SULFATION EXPRESSION IS ALTERED FOLLOWING HEPARIN TREATMENT

HS chain initiation is catalyzed by the action of EXT enzymes (EXT1 and EXT2) and C5-epimerase. Following the co-ordinated action of these enzymes, the commitment to a HS chain is determined by NDSTs, through the introduction of critical *N*-sulfation at points along the side chain. Of the four NDSTs, NDST1 and 2 are more widely physiologically expressed, with NDST3 and 4 primarily expressed during development and in the brain. Following treatment with heparin, the gene expression profile of all the HS chain initiation enzymes (EXT1/2 and C5-Epimerase) in MCF-7 cells increased at D1 (EXT1: $P = 0.00016$; EXT2: $P = 0.055$; C5-Ep: $P = 0.00062$), followed by decreased expression by D3 (EXT1: $P = 9.92 \times 10^{-5}$; EXT2: $P = 3.75 \times 10^{-5}$; C5-Ep: $P = 0.23$) and increased expression levels by D5 (EXT1: $P = 0.081$; EXT2: $P = 0.019$; C5-Ep: $P = 0.203$). This expression profile was also observed for the NDST1 and 2 genes in these cells with expression significantly increased at D1 (NDST1: $P = 0.0163$; NDST2: $P = 0.0016$), reduced by D3 (NDST1: $P = 2.66 \times 10^{-9}$; NDST2: $P = 6.08 \times 10^{-6}$), and increased by D5 (NDST1: $P = 0.0153$; NDST2: $P = 0.0051$) (Fig. 2A,B). In the MDA-MB-231 cells, gene expression followed a similar trend in terms of their expression profile following treatment with heparin. The chain initiation enzymes EXT1 and 2 and C5-epimerase all increased between D1 and D3 (D1: EXT1: $P = 0.202$; EXT2: $P = 0.0071$; C5-Ep: $P = 0.190$; D3: EXT1: $P = 1.77 \times 10^{-8}$; EXT2: $P = 6.63 \times 10^{-8}$; C5-Ep: $P = 6.66 \times 10^{-5}$) followed by reduced gene expression by D5 (EXT1: $P = 4.82 \times 10^{-5}$; EXT2: $P = 0.00069$; C5-Ep: $P = 0.00013$). This same trend was observed for NDST1 gene expression (D1: $P = 0.0147$; D3: $P = 3.31 \times 10^{-5}$, D5: $P = 0.0144$). In contrast, the gene expression profile of NDST2 at D1 and D3, although at a significantly lower level when compared to MCF-7 cells, continued to gradually increase at each time point (D1: $P = 0.944$, D3: $P = 0.409$) (Fig. 2C,D). In MCF-7 cells, the observed gene expression level of the HS chain initiation and commitment enzymes was well above the level observed in MDA-MB-231 cells in both control and heparin treated cultures. In addition, most of these genes, with the exception of NDST2 in the MDA-MB-231 cultures, had their highest level of expression at D3, with the lowest level of expression observed in MCF-7 cells also occurring at D3.

O-SULFATION ENZYME GENE EXPRESSION CHANGES FOLLOWING HEPARIN TREATMENT

Following the initiation and commitment to HS side chains, further modifications include the addition of *O*-sulfation, primarily by 2-*O*-sulftotransferases followed by glucosamine 6-*O*-sulfation. *O*-sulfation of HS side chains is critical for interactions with ligands and downstream signaling including FGF2 where the binding of the FGF2/FGFR complex is dependent on the presence of 2-*O*- and 6-*O*-sulfation in the HS chain [Habuchi et al., 2004]. Once again, the overall level of gene expression of the sulfation enzymes examined was markedly higher in the MCF-7 (approx. 20-fold) than the MDA-MB-231 cells. HS2ST1 and HS6ST1 gene expression was significantly increased in MCF-7 cells at D1 (HS2ST1: $P = 2.088 \times 10^{-5}$; HS6ST1: $P = 0.0122$), decreased at D3 (HS2ST1: $P = 0.716$; HS6ST1: $P = 5.82 \times 10^{-6}$), and increased again by D5 (HS2ST1: $P = 0.00012$; HS6ST1: $P = 1.54 \times 10^{-6}$). Gene expression of HS6ST2 peaked at D1 ($P = 5.09 \times 10^{-5}$) followed by a decrease in expression level that remained steady relative to control cultures from D3 to D5 (D3: $P = 0.747$; D5: $P = 0.484$). In the MDA-MB-231 cells, a significant increase in HS2ST1 gene expression was observed at D3 when compared to control cultures ($P = 3.82 \times 10^{-5}$). The gene expression changes observed at D1 showed little variation between control and heparin treated cells, while at D5 there was a significant increase for HS6ST1 (Day 5; HS2ST1: $P = 0.0087$; HS6ST1: $P = 0.00128$) (Fig. 2E).

GPC5 AND 6 MAY HAVE A ROLE IN INVASIVE POTENTIAL

The glypicans are a family of six (GPC1–6) HSPG core proteins bound to the cell surface through a glycosylphosphatidylinositol (GPI) anchor with extensive interactions during cell signaling. Expression of glypicans has been associated with both the stromal and epithelial cellular compartments in HBCs [Buchanan et al., 2010]. Following heparin treatment of MCF-7 cells, only expression of GPC3, 4, and 6 were observed at all timepoints. Significantly increased gene expression was observed for GPC3 at D1 and D5 (D1: $P = 0.0393$, D5: $P = 7.68 \times 10^{-5}$) and GPC4 at D3 ($P = 2.03 \times 10^{-5}$) and GPC6 at D3 ($P = 1.66 \times 10^{-7}$). In the MDA-MB-231 cells, GPC1, 5, and 6 were observed at all timepoints and following treatment of the cultures with heparin, no significant changes in gene expression of these glypicans were observed at D1. Significantly increase expression at D3 was observed for all three of these glypicans (GPC1: $P = 4.39 \times 10^{-5}$; GPC5: $P = 0.0271$; GPC6: $P = 2.94 \times 10^{-9}$). This increased expression continued at D5 with a significant increase in expression of GPC1 ($P = 0.00867$) and GPC5 observed ($P = 0.00217$) (Fig. 3C,D). These data demonstrate a potential role for GPC3 in tempering MCF-7 proliferation in vitro and GPC5 and GPC6 in the significant proliferation and potentially increased migratory potential observed in the MDA-MB-231 cells at the D3 and D5 timepoints.

SDC2 AND SDC4 EXPRESSION MAY CORRELATE WITH TUMOROGENEITY

The HSPG core protein family of syndecans carry a number of HS side chains at their distal ends. Syndecans play roles in cell–cell adhesion, cell motility and cell–ECM interactions in a tissue specific manner. Interestingly, the level of gene expression of the syndecan genes was similar between the two cell lines examined. In the MCF-7 cultures, following heparin treatment, cells displayed significantly increased

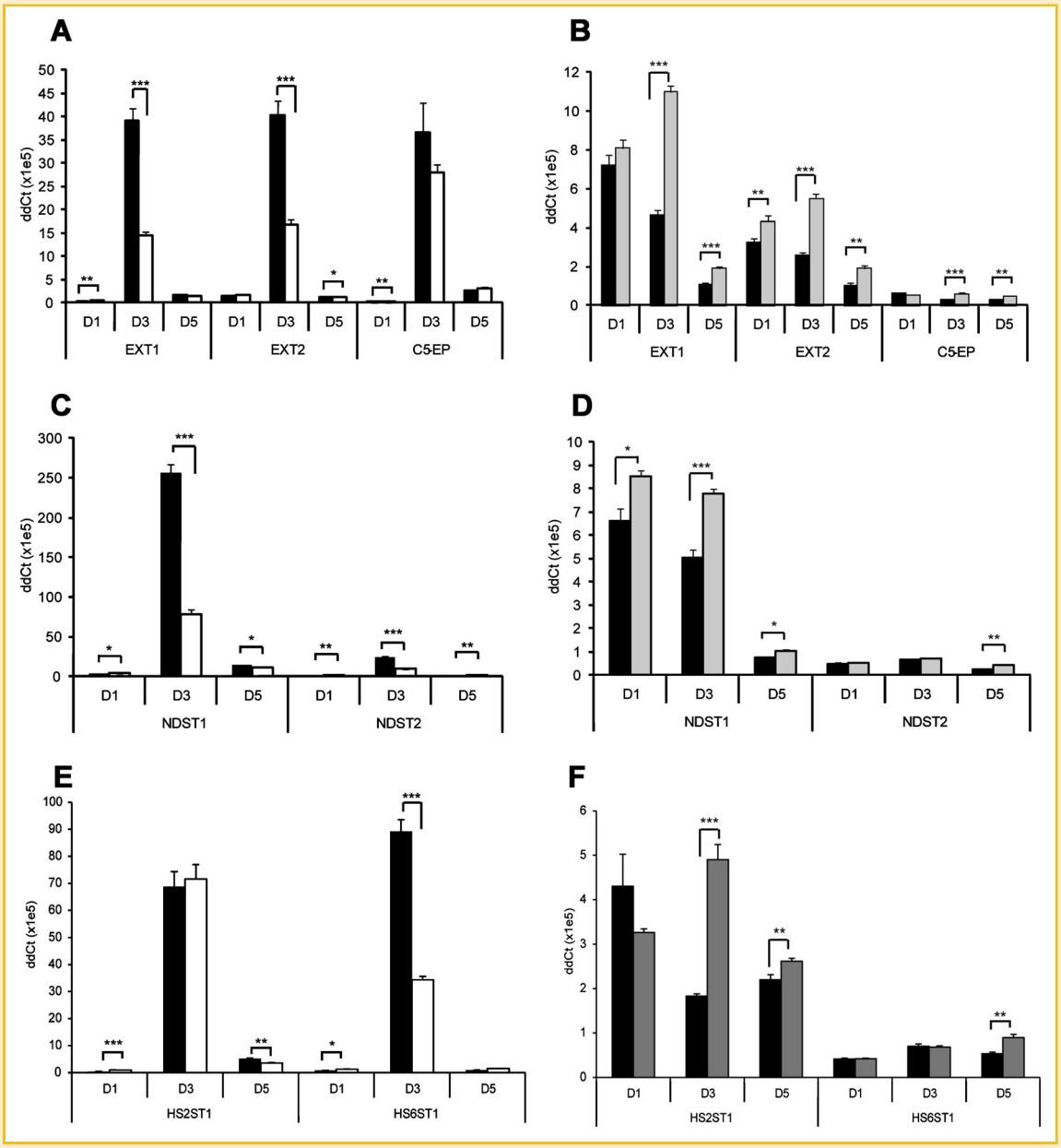

Fig. 2. Expression of glycosaminoglycan chain initiation and modification enzymes following heparin treatment. Solid bars: control; open bars: MCF-7 heparin (10 μg/ml); gray bars: MDA-MB-231 heparin (10 μg/ml). (A) MCF-7 NDST1 and NDST2; (B) MDA-MB-231NDST1 and NDST2; (C) MCF-7 EXT1, EXT2, and C5-Epimerase; (D) MDA-MB-231 EXT1, EXT2, and C5-Epimerase; (E) MCF-7 HS2ST1 and HS6ST1; and (F) MDA-MB-231 HS2ST1 and HS6ST1.

gene expression in SDC1 and SDC4 at each of the timepoints examined (D1: $P = 0.02038$, $P = 0.04545$, D3: $P = 0.0119$, $P = 2.20 \times 10^{-6}$, and D5: $P = 0.01617$, $P = 0.04776$). In addition, both SDC2 and SDC3 demonstrated significant increases in gene expression at D3 ($P = 9.21 \times 10^{-7}$, $P = 0.03896$) and D5 ($P = 0.00176$, $P = 0.00307$). Following heparin treatment of the MDA-MB-231 cells, gene expression of SDC1 remained relatively unchanged when compared to control cultures at all timepoints. In contrast, the remaining syndecans (SDC2, 3, and 4) all demonstrated a significant increase in gene expression at D3 (SDC2: $P = 1.56 \times 10^{-5}$, SDC3: $P = 3.24 \times 10^{-5}$, SDC4: $P = 0.00029$), with SDC2 expression levels highest at D5 ($P = 0.00179$) (Fig. 3). Although the syndecan gene expression profile in both cell lines examined did not show the distinctive D3 up/downregulation, distinct differences in the gene expression profile were observed. SDC4 was observed to be the dominant syndecan expressed in MCF-7 cells, and SDC2 the dominantly expressed syndecan in the MDA-MB-231 cells.

WNT PATHWAY ANALYSIS

The interaction of HSPGs with several growth factor families, including the Wnts, is well established. We utilized a Wnt pathway gene expression array to examine the two HBC lines under our culture

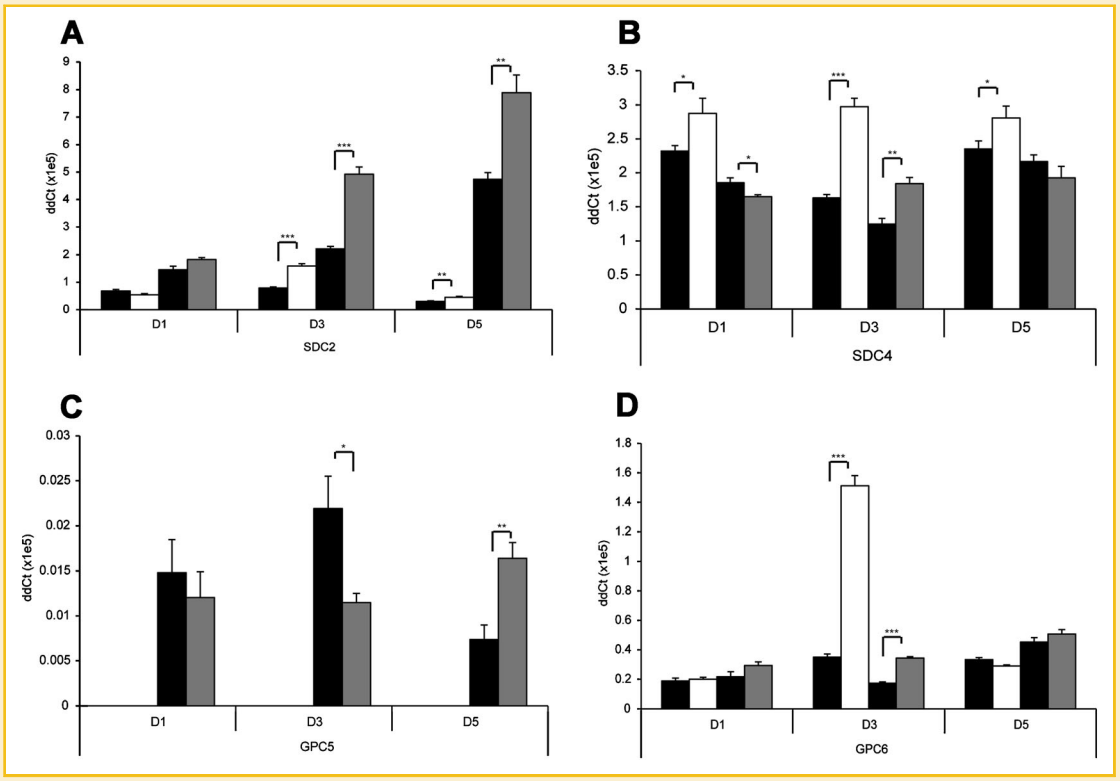

Fig. 3. Expression of core proteins following treatment with heparin. Solid bars: control; open bars: MCF-7 heparin (10 μg/ml); gray bars: MDA-MB-231 heparin (10 μg/ml). (A) SDC2, (B) SDC4, (C) GPC5, and (D) GPC6.

conditions. In the MCF-7 cells, significantly increased gene expression at D1 following the addition of heparin to the cultures was observed in genes generally associated with the canonical pathway (APC2, DISDC1, DKK1, FRAT1, FZD5, WNT2, WNT4), as well as the cell cycle (APC2, RHOU). Perhaps most interesting however, was the down regulation of gene expression at D3 of genes associated with the canonical Wnt pathway (APC, DIXDC1, NKD2, TCF7L2) but also those associated with cell migration (APC, RHOA) and the cell cycle (APC, JUN, RHOA, TCF7L2). By D5 there was clear down regulation of all genes within the array, in particular Wnt5a (Fig. 4). This gene was the first Wnt gene found to be overexpressed in HBCs [Lejeune et al., 1995], where it has been shown to have elevated expression in both benign and primary tumors, and low expression in cell lines and normal breast tissue [Lejeune et al., 1995].

In contrast to the results seen in MCF-7 cells, MDA-MB-231 cells showed a variety of significant changes at D1 and widespread changes throughout the Wnt pathway at D5. Significant down-regulation of several canonical (DVL1, FRAT2, WNT3A) and growth and proliferation genes (KREMEN1) was observed at D1 in the MDA-MB-231 cells following the addition of heparin to the cultures. The only gene to demonstrate significant upregulation was CTNNBIP1, which plays a role in canonical Wnt signaling and process of cell proliferation with a threefold increase ($P = 0.002$) in gene expression observed. The most striking difference between the two cell types however, was the almost universal significant increase in gene expression at the D5 timepoint in the MDA-MB-231 cultures. The gene with the greatest increase in gene expression at D5 was WNT4, with a 15-fold increase ($P = 0.006$). These data suggest that MDA-MB-231 cells are more susceptible to the influence of heparin, resulting in increased proliferation mediated by the Wnt pathway.

DISCUSSION

HSPGS AND PROLIFERATION VS. MIGRATION

The two HBC cell lines examined in this study represent different ends of the spectrum of HBCs, one highly invasive, highly metastatic (MDA-MB-231) and one lowly invasive, poorly metastatic (MCF-7). By examining the differences between these two cell types, we hoped to identify some significant gene expression changes associated with the postulated role of the matrix or localized niche in the regulation of breast epithelial cell tumorigeneity. The HSPGs are central to a number of cellular processes and it is feasible that these proteins may play a role in breast tumor initiation and progression. Tissue or cell specific regulation of HS biosynthesis (reviewed in Ledin et al. [2004], Kreuger et al. [2006], and Vlodavsky et al. [2012]) dictates subtle and selective changes in the interactions between HS and its ligands [Lindahl, 2007; Gray et al., 2008; Vlodavsky et al., 2012]. HSPGs can play a role in both the inhibition and the promotion of all aspects of cell functioning including proliferation and migration. HSPGs have been shown to inhibit cellular invasion by promoting tight cell–cell and cell–ECM interactions, and by maintaining the structural

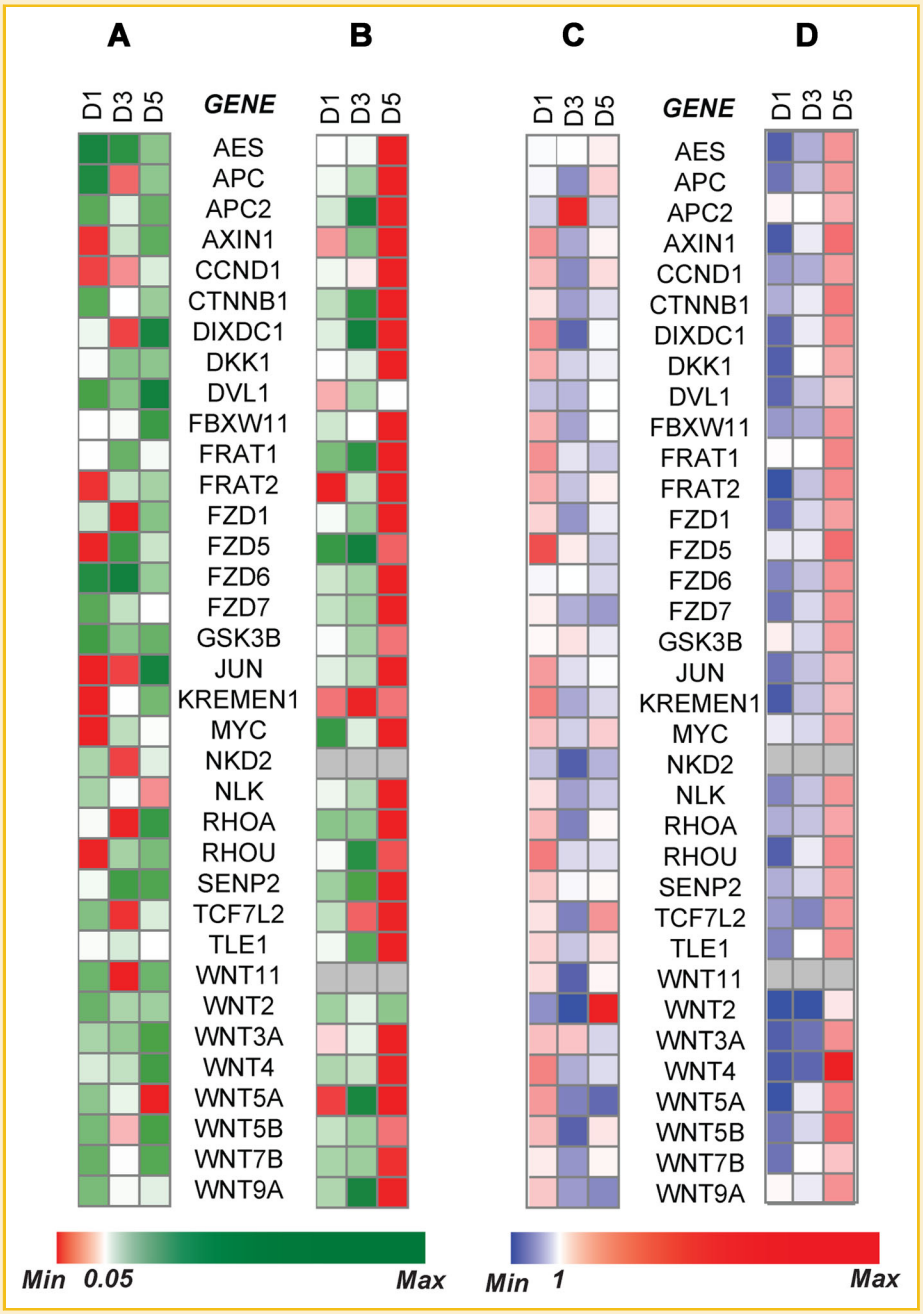

Fig. 4. Heat map representation of gene expression changes of genes involved in the Wnt pathway. Panels (A) and (B) significance; red: significant *P* values, green: non-significant *P* values. The darker red the more significant the *P* value. Panels (C) and (D) fold change; blue: decreased expression, white: no change, red: increased expression. The darker the red the greater the fold change. (A) MCF-7 *P* values, (B) MDA-MB-231 *P* values, (C) MCF-7 fold change, and (D) MDA-MB-231 fold change.

integrity and self-assembly of the ECM [Sanderson, 2001; Timar et al., 2002; Vlodavsky et al., 2012]. Indeed, a characteristic of malignant transformation is downregulation of GAG biosynthesis, especially HS chains [Sanderson, 2001; Timar et al., 2002; Vlodavsky et al., 2012]. Previous studies clearly demonstrate a role for HSPGs in promoting cell proliferation and migration that is both cell and niche specific [Lindahl et al., 1998; Cool and Nurcombe, 2006; Vlodavsky et al., 2012]. In this study, with the exception of the HSPG core proteins (syndecans and glypicans), the level of gene expression of HSPG genes examined associated with chain initiation, *N*-sulfation and *O*-sulfation enzymes was greater in MCF-7 cells when compared to MDA-MB-231 cells. Although the expression level was significantly higher in the MCF-7 cultures, addition of the HS analog heparin to the cultures decreased the gene expression of these chain initiation and sulfation enzymes in the MCF-7 cells but stimulated their expression in the MDA-MB-231 cells.

The gene expression changes demonstrated in this study emphasize the role of HSPGs in mediating cell proliferation and

migration through alterations in matrix interactions resulting in changes in cell growth. Increased gene expression of EXT1 and 2 along with C5-epimerase was clearly observed as expected, in the early growth phase of both the MCF-7 and MDA-MB-231 cells. Interestingly, the addition of HS to the cultures significantly downregulated expression of all three genes in the MCF-7 cells at D3. Conversely, the addition of the HS agonist to the MDA-MB-231 cells increased gene expression at all timepoints in all three genes. The EXT genes are responsible for side-chain initiation, followed by *N*-sulfation, catalyzed by the NDSTs, resulting in commitment of the side-chains to the HS rather than the CS family of proteins. Reduced NDST1 and EXT gene expression at the D3 timepoint in the MCF-7 cells may reflect decreased migratory potential, suggested previously to occur through reduced EXT/ERK interaction and decreased collagen I binding (reviewed in Martin et al. [2011]). In contrast, the increased expression of these genes seen in MDA-MB-231 cells may reflect the increased migratory potential of these HBC cells. Importantly these diverse cellular mechanisms are clearly mediated by the addition of HS to the cultures.

This converse pattern of gene expression between the two cell lines is also apparent in the gene expression profile of the 2-*O* and 6-*O* HS sulfation enzymes. It is now commonly accepted that FGF2-FGFR-mediated signaling is dependent on specific HS fine structure, particularly *N*- or 6-*O*-sulfation of the glucosamine units and 2-*O*-sulfation of the iduronic acid units (reviewed in Guimond et al. [1993], Lundin et al. [2000], Sugaya et al. [2008], Jia et al. [2009], and Raman and Kuberan [2010]). HSPGs are integral in localizing FGF2 near its receptor and stabilizing the ligand-receptor complex allowing signal transduction [Plotnikov et al., 1999; Stauber et al., 2000; Raman and Kuberan, 2010]. The increased gene expression profile of the N-sulfation, 2-*O*- and 6-*O*-sulfation enzymes (NDST, HS2ST, HS6ST) associated with the addition of heparin to the MDA-MB-231 cultures support the increased activation of FGF2 signaling. In addition, along with increased growth, the binding of FGF2 to FGFR has been shown to stimulate migration and differentiation in several cancers [Adatia et al., 1997; Su et al., 2006; Raman and Kuberan, 2010], and specific FGF2 isoforms have been demonstrated to influence tumorigeneity of MDA-MB-231 cells [Korah et al., 2000]. Interestingly, previous data from our group have demonstrated that *N*-sulfation is more crucial than *O*-sulfation for heparin-Wnt3a interactions in the osteogenic model, although both forms of sulfation contribute to both binding affinity and biological activity [Ling et al., 2010].

HSPG CORE PROTEINS AND BREAST CANCER EPITHELIAL CELL PROLIFERATION AND MIGRATION

The glypicans are GPI-linked membrane bound proteins carrying several HS chains near the tip of their ectodomain [Fransson et al., 2004; Vlodavsky et al., 2012], which through growth factor and ligand interactions, mediate cellular differentiation and proliferation. Overexpression of glypicans has been reported in a number of cancers with their expression profile often correlated with the syndecans. Previous work by Matsuda et al. [2001], demonstrated significantly increased gene expression of GPC1 in HBC, with GPC3 and GPC4 moderately increased [Matsuda et al., 2001]. In the same study, the overexpression of GPC1 correlated with increased SDC1 expression and GPC2 and GPC5 were undetected [Matsuda et al., 2001]. This was reflected in our study with GPC1 and GPC6 strongly expressed in both cell lines, although expression in MCF-7 cells was higher for both genes than in MDA-MB-231. In the highly invasive/highly metastatic MDA-MB-231 cells, expression of GPC2, GPC3, GPC4, and GPC5 was too low to detect. However, in the MCF-7 cells, we observed very low expression of GPC3 and moderate expression of GPC4.

The syndecans regulate cell adhesion, proliferation, and differentiation, key aspects of tumor growth and associated metastases, through sequential adhesion, motility, and proliferation of the tumor cells [Hanahan and Weinberg, 2000; Wolf and Friedl, 2006]. SDC1 is the best characterized of the syndecans and is mainly expressed in epithelial cells [Bernfield et al., 1999; Couchman, 2003] with in vitro models suggesting SDC1 promotes tumorigenesis through the regulation of tumor cell adhesion [Beauvais and Rapraeger, 2003, ; Burbach et al., 2004], proliferation [Maeda et al., 2004], and angiogenesis [Maeda et al., 2006]. In breast cancer, upregulation of SDC1 has been previously observed to correlate with poor prognosis and aggressive phenotype [Barbareschi et al., 2003; Lofgren et al., 2007; Lendorf et al., 2011], while expression of SDC4 has been associated with better prognosis in breast cancer patients [Lendorf et al., 2011]. SDC4 is widely expressed, though usually at low levels in normal tissue, and is a key adhesion molecule [Couchman, 2003; Morgan et al., 2007], unique among the syndecan family members to localize at sites of cell–matrix adhesions, including focal adhesions [Woods and Couchman, 1994; Baciu and Goetinck, 1995]. When overexpressed, SDC4 promotes excess focal adhesion formation resulting in reduced cell migration [Longley et al., 1999]. Often, SDC1 is associated with an absence of SDC4 and vice versa, consistent with the distinct expression, localization, and regulation of the two syndecans [Lendorf et al., 2011]. Both SDC1 and SDC4 expression levels have been demonstrated to correlate with FGF2/FGFR1 receptor signaling, suggesting elevated co-receptor activity in breast carcinomas [Mundhenke et al., 2002]. In our study, we observed higher SDC4 gene expression in MCF-7 cells than in the MDA-MB-231 cells, supporting previous observations that syndecans are required for increased cell proliferation in breast cancers. The increased expression level of SDC2 in the MDA-MB-231 cells, upregulated by the addition of heparin to the cultures at all timepoints, supports previous studies demonstrating the importance of SDC2 in mediating cell proliferation, adhesion, and tumorigeneity in colon, lung, and breast cancer cells [Dobra et al., 2000; Park et al., 2002; Beauvais and Rapraeger, 2004; Tkachenko et al., 2005; Kousidou et al., 2008]. In contrast, SDC4 has been shown to have an anti-migratory/anti-invasive effect [Kousidou et al., 2008], supported here by the increased expression level of SDC4 in MCF-7 cells and the increase in the expression level of this gene following the addition of heparin to the cultures.

HSPGS AND WNT SIGNALING IN BREAST CANCER EPITHELIAL CELL PROLIFERATION AND MIGRATION

The WNT family of secreted growth factors regulates developmental processes of cell fate and polarity, as well as general cell maintenance processes including homeostasis and cell cycle regulation. Currently, there are 19 WNT ligands in humans, which bind to the Frizzled (FZD) family of receptors and the co-receptors LRP5 and LRP6. WNT signaling comprises three pathways: the canonical pathway and two

non-canonical pathways: planar cell polarity (PCP) and a calcium ion-dependent pathway. The well-studied canonical WNT pathway signals through β-catenin and regulates the cell cycle, cell growth, and proliferation. Both non-canonical pathways mediate their cellular events independent of β-catenin, with the PCP pathway regulating cytoskeletal dynamics and cell motility, and the WNT/calcium pathway promoting NFAT transcription [Yiu et al., 2011]. HSPGs have been demonstrated to mediate both canonical and non-canonical Wnt signaling events including through *N*- and *O*-sulfation by mediating the physical binding and optimal co-stimulation of Wnt3a, promoting osteoblast differentiation [Ling et al., 2010]. The manifestation of cancer by aberrant Wnt signaling most likely results in degradation of β-catenin prior to nuclear translocation and Wnt signaling activation [Polakis, 2000]. Several target genes of β-catenin signaling have now been identified and some of their functions are consistent with control of cellular growth, differentiation and survival [Polakis, 2000]. With extensive and varied roles of HSPGs in activation and inhibition of many signaling pathways responsible for cellular growth and differentiation it is reasonable to assume the deregulation of some of these critical pathways is due to changes in HSPG gene expression. For instance, GPC3 is able to inhibit both canonical (morphology and migration) and non-canonical (morphogenesis and polarity) Wnt signals [Schambony et al., 2004; Stigliano et al., 2009]. In addition, previous work by Yiu et al. [2011] demonstrated GPC6 to have a key role in promoting the invasive migration of MDA-MB-231 cells through inhibition of canonical-β-catenin and Wnt signaling, and up regulation of non-canonical Wnt5a signaling through the activation of JNK (c-Jun-N-terminal kinase) and p38 MAPK (mitogen-activated protein kinase) [Yiu et al., 2011]. The data presented support this with increased GPC6 in MDA-MB-231 cells following treatment with heparin along with decreased expression of Wnt5a in MCF-7 cells and increased non-canonical expression in the MDA-MB-231 cultures. Here, we provide evidence supporting differing roles for specific HSPGs on breast cancer epithelial cell tumorigenic potential. In addition, this role utilizes the Wnt pathway and is dependent upon the HSPG and the epithelial cell characteristics. Hence, we suggest that the influence of HSPGs on breast cancer epithelial cell proliferation and invasive potential is cell and niche specific.

In conclusion, the observed gene expression differences between the two breast cancer epithelial cell models demonstrated clear differences in the ability of HSPG genes to influence cell proliferation, viability and potentially motility and tumorigeneity in response to the HS analog heparin. In addition, these effects are mediated through interactions with specific members of the Wnt pathway to differentially mediate breast cancer epithelial cell proliferation and migration. Further understanding of these key interactions between specific HSPGs and Wnts may provide new biomarkers and perhaps an opportunity to target abnormal cells during breast cancer progression.

ACKNOWLEDGMENTS

R.K.O. is supported by an Australian Postgraduate Award stipend, and the authors wish to acknowledge the support of the Estate of the late Clem Jones AO. A.Jv.W. and G.S.S. were supported by National Institutes of Health grant P01 CA082834 and R01 AR049069.

REFERENCES

Adatia R, Albini A, Carlone S, Giunciuglio D, Benelli R, Santi L, Noonan DM. 1997. Suppression of invasive behavior of melanoma cells by stable expression of anti-sense perlecan cDNA. Ann Oncol 8:1257–1261.

Baciu PC, Goetinck PF. 1995. Protein kinase C regulates the recruitment of syndecan-4 into focal contacts. Mol Biol Cell 6:1503–1513.

Barbareschi M, Maisonneuve P, Aldovini D, Cangi MG, Pecciarini L, Angelo Mauri F, Veronese S, Caffo O, Lucenti A, Palma PD, Galligioni E, Doglioni C. 2003. High syndecan-1 expression in breast carcinoma is related to an aggressive phenotype and to poorer prognosis. Cancer 98:474–483.

Beauvais DM, Rapraeger AC. 2003. Syndecan-1-mediated cell spreading requires signaling by alphavbeta3 integrins in human breast carcinoma cells. Exp Cell Res 286:219–232.

Beauvais DM, Rapraeger AC. 2004. Syndecans in tumor cell adhesion and signaling. Reprod Biol Endocrinol 2:3.

Bernfield M, Götte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M. 1999. Functions of cell surface heparan sulfate proteoglycans. Annu Rev Biochem 68:729–777.

Brinckerhoff CE, Rutter JL, Benbow U. 2000. Interstitial collagenases as markers of tumor progression. Clin Cancer Res 6:4823–4830.

Buchanan C, Stigliano I, Garay-Malpartida HM, Rodrigues Gomes L, Puricelli L, Sogayar MC, Bal de Kier Joffé E, Peters MG. 2010. Glypican-3 reexpression regulates apoptosis in murine adenocarcinoma mammary cells modulating PI3K/Akt and p38MAPK signaling pathways. Breast Cancer Res Treat 119:559–574.

Burbach BJ, Ji Y, Rapraeger AC. 2004. Syndecan-1 ectodomain regulates matrix-dependent signaling in human breast carcinoma cells. Exp Cell Res 300:234–247.

Cool SM, Nurcombe V. 2006. Heparan sulfate regulation of progenitor cell fate. J Cell Biochem 99:1040–1051.

Couchman JR. 2003. Syndecans: Proteoglycan regulators of cell-surface microdomains? Nat Rev Mol Cell Biol 4:926–937.

Ding K, Lopez-Burks M, Sanchez-Duran JA, Korc M, Lander AD. 2005. Growth factor-induced shedding of syndecan-1 confers glypican-1 dependence on mitogenic responses of cancer cells. J Cell Biol 171:729–738.

Dobra K, Andang M, Syrokou A, Karamanos NK, Hjerpe A. 2000. Differentiation of mesothelioma cells is influenced by the expression of proteoglycans. Exp Cell Res 258:12–22.

Fransson LA, Belting M, Cheng F, Jönsson M, Mani K, Sandgren S. 2004. Novel aspects of glypican glycobiology. Cell Mol Life Sci 61:1016–1024.

Gallagher JT, Turnbull JE, Lyon M. 1992. Patterns of sulphation in heparan sulphate: Polymorphism based on a common structural theme. Int J Biochem 24:553–560.

Gao W, Ho M. 2011. The role of glypican-3 in regulating Wnt in hepatocellular carcinomas. Cancer Rep 1:14–19.

Gray E, Mulloy B, Barrowcliffe TW. 2008. Heparin and low-molecular-weight heparin. Thromb Haemost 99:807–818.

Guimond S, Maccarana M, Olwin BB, Lindahl U, Rapraeger AC. 1993. Activating and inhibitory heparin sequences for FGF-2 (basic FGF). Distinct requirements for FGF-1, FGF-2, and FGF-4. J Biol Chem 268:23906–23914.

Habuchi H, Habuchi O, Kimata K. 2004. Sulfation pattern in glycosaminoglycan: Does it have a code? Glycoconj J 21:47–52.

Hacker U, Nybakken K, Perrimon N. 2005. Heparan sulphate proteoglycans: The sweet side of development. Nat Rev Mol Cell Biol 6:530–541.

Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. Cell 100:57–70.

Haupt LM, Murali S, Mun FK, Teplyuk N, Mei LF, Stein GS, van Wijnen AJ, Nurcombe V, Cool SM. 2009. The heparan sulfate proteoglycan (HSPG) glypican-3 mediates commitment of MC3T3-E1 cells toward osteogenesis. J Cell Physiol 220:780–791.

Ho M, Kim H. 2011. Glypican-3: A new target for cancer immunotherapy. Eur J Cancer 47:333–338.

Jia J, Maccarana M, Zhang X, Bespalov M, Lindahl U, Li JP. 2009. Lack of l-iduronic acid in heparan sulfate affects interaction with growth factors and cell signaling. J Biol Chem 284:15942–15950.

Kamangar F, Dores GM, Anderson WF. 2006. Patterns of cancer incidence, mortality, and prevalence across five continents: Defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 24:2137–2150.

Korah RM, Sysounthone V, Golowa Y, Wieder R. 2000. Basic fibroblast growth factor confers a less malignant phenotype in MDA-MB-231 human breast cancer cells. Cancer Res 60:733–740.

Kousidou OCh, Berdiaki A, Kletsas D, Zafiropoulos A, Theocharis AD, Tzanakakis GN, Karamanos NK. 2008. Estradiol-estrogen receptor: A key interplay of the expression of syndecan-2 and metalloproteinase-9 in breast cancer cells. Mol Oncol 2:223–232.

Kreuger J, Spillmann D, Li JP, Lindahl U. 2006. Interactions between heparan sulfate and proteins: The concept of specificity. J Cell Biol 174:323–327.

Lamoureux F, Baud'huin M, Duplomb L, Heymann D, Redini F. 2007. Proteoglycans: Key partners in bone cell biology. Bioessays 29:758–771.

Ledin J, Staatz W, Li JP, Götte M, Selleck S, Kjellén L, Spillmann D. 2004. Heparan sulfate structure in mice with genetically modified heparan sulfate production. J Biol Chem 279:42732–42741.

Lejeune S, Huguet EL, Hamby A, Poulsom R, Harris AL. 1995. Wnt5a cloning, expression, and up-regulation in human primary breast cancers. Clin Cancer Res 1:215–222.

Lendorf ME, Manon-Jensen T, Kronqvist P, Multhaupt HA, Couchman JR. 2011. Syndecan-1 and syndecan-4 are independent indicators in breast carcinoma. J Histochem Cytochem 59:615–629.

Lindahl U. 2007. Heparan sulfate–protein interactions—A concept for drug design? Thromb Haemost 98:109–115.

Lindahl U, Kusche-Gullberg M, Kjellen L. 1998. Regulated diversity of heparan sulfate. J Biol Chem 273:24979–24982.

Ling L, Murali S, Dombrowski C, Haupt LM, Stein GS, van Wijnen AJ, Nurcombe V, Cool SM. 2006. Sulfated glycosaminoglycans mediate the effects of FGF2 on the osteogenic potential of rat calvarial osteoprogenitor cells. J Cell Physiol 209:811–825.

Ling L, Dombrowski C, Foong KM, Haupt LM, Stein GS, Nurcombe V, van Wijnen AJ, Cool SM. 2010. Synergism between Wnt3a and heparin enhances osteogenesis via a phosphoinositide 3-kinase/Akt/RUNX2 pathway. J Biol Chem 285:26233–26244.

Liotta LA. 1992. Cancer cell invasion and metastasis. Sci Am 266:54–59,62–53.

Lofgren L, Sahlin L, Jiang S, Von Schoultz B, Fernstad R, Skoog L, Von Schoultz E. 2007. Expression of syndecan-1 in paired samples of normal and malignant breast tissue from postmenopausal women. Anticancer Res 27:3045–3050.

Longley RL, Woods A, Fleetwood A, Cowling GJ, Gallagher JT, Couchman JR. 1999. Control of morphology, cytoskeleton and migration by syndecan-4. J Cell Sci 112(Pt20):3421–3431.

Lundin L, Larsson H, Kreuger J, Kanda S, Lindahl U, Salmivirta M, Claesson-Welsh L. 2000. Selectively desulfated heparin inhibits fibroblast growth factor-induced mitogenicity and angiogenesis. J Biol Chem 275:24653–24660.

Maeda T, Alexander CM, Friedl A. 2004. Induction of syndecan-1 expression in stromal fibroblasts promotes proliferation of human breast cancer cells. Cancer Res 64:612–621.

Maeda N, Fukazawa N, Hata T. 2006. The binding of chondroitin sulfate to pleiotrophin/heparin-binding growth-associated molecule is regulated by chain length and oversulfated structures. J Biol Chem 281:4894–4902.

Martin JW, Zielenska M, Stein GS, van Wijnen AJ, Squire JA. 2011. The role of RUNX2 in osteosarcoma oncogenesis. Sarcoma 2011:282745.

Matsuda K, Maruyama H, Guo F, Kleeff J, Itakura J, Matsumoto Y, Lander AD, Korc M. 2001. Glypican-1 is overexpressed in human breast cancer and modulates the mitogenic effects of multiple heparin-binding growth factors in breast cancer cells. Cancer Res 61:5562–5569.

McDonnell S, Matrisian LM. 1990. Stromelysin in tumor progression and metastasis. Cancer Metastasis Rev 9:305–319.

Morgan MR, Humphries MJ, Bass MD. 2007. Synergistic control of cell adhesion by integrins and syndecans. Nat Rev Mol Cell Biol 8:957–969.

Mundhenke C, Meyer K, Drew S, Friedl A. 2002. Heparan sulfate proteoglycans as regulators of fibroblast growth factor-2 receptor binding in breast carcinomas. Am J Pathol 160:185–194.

Murphy G, Gavrilovic J. 1999. Proteolysis and cell migration: Creating a path? Curr Opin Cell Biol 11:614–621.

Nakatsura T, Kageshita T, Ito S, Wakamatsu K, Monji M, Ikuta Y, Senju S, Ono T, Nishimura Y. 2004. Identification of glypican-3 as a novel tumor marker for melanoma. Clin Cancer Res 10:6612–6621.

Park H, Kim Y, Lim Y, Han I, Oh ES. 2002. Syndecan-2 mediates adhesion and proliferation of colon carcinoma cells. J Biol Chem 277:29730–29736.

Plotnikov AN, Schlessinger J, Hubbard SR, Mohammadi M. 1999. Structural basis for FGF receptor dimerization and activation. Cell 98:641–650.

Polakis P. 2000. Wnt signaling and cancer. Genes Dev 14:1837–1851.

Raman K, Kuberan B. 2010. Chemical tumor biology of heparan sulfate proteoglycans. Curr Chem Biol 4:20–31.

Saikali Z, Sinnett D. 2000. Expression of glypican 3 (GPC3) in embryonal tumors. Int J Cancer 89:418–422.

Salamonsen LA, Woolley DE. 1999. Menstruation: Induction by matrix metalloproteinases and inflammatory cells. J Reprod Immunol 44:1–27.

Sanderson RD. 2001. Heparan sulfate proteoglycans in invasion and metastasis. Semin Cell Dev Biol 12:89–98.

Schambony A, Kunz M, Gradl D. 2004. Cross-regulation of Wnt signaling and cell adhesion. Differentiation 72:307–318.

Stauber DJ, DiGabriele AD, Hendrickson WA. 2000. Structural interactions of fibroblast growth factor receptor with its ligands. Proc Natl Acad Sci USA 97:49–54.

Stetler-Stevenson WG. 1990. Type IV collagenases in tumor invasion and metastasis. Cancer Metastasis Rev 9:289–303.

Stigliano I, Puricelli L, Filmus J, Sogayar MC, Bal de Kier Joffé E, Peters MG. 2009. Glypican-3 regulates migration, adhesion and actin cytoskeleton organization in mammary tumor cells through Wnt signaling modulation. Breast Cancer Res Treat 114:251–262.

Su G, Meyer K, Nandini CD, Qiao D, Salamat S, Friedl A. 2006. Glypican-1 is frequently overexpressed in human gliomas and enhances FGF-2 signaling in glioma cells. Am J Pathol 168:2014–2026.

Sugaya N, Habuchi H, Nagai N, Ashikari-Hada S, Kimata K. 2008. 6-O-sulfation of heparan sulfate differentially regulates various fibroblast growth factor-dependent signalings in culture. J Biol Chem 283:10366–10376.

Tímár J, Lapis K, Dudás J, Sebestyén A, Kopper L, Kovalszky I. 2002. Proteoglycans and tumor progression: Janus-faced molecules with contradictory functions in cancer. Semin Cancer Biol 12:173–186.

Tkachenko E, Rhodes JM, Simons M. 2005. Syndecans: New kids on the signaling block. Circ Res 96:488–500.

Vlodavsky I, Beckhove P, Lerner I, Pisano C, Meirovitz A, Ilan N, Elkin M. 2012. Significance of heparanase in cancer and inflammation. Cancer Microenviron 5:115–132.

Wolf K, Friedl P. 2006. Molecular mechanisms of cancer cell invasion and plasticity. Br J Dermatol 154(Suppl1):11–15.

Woods A, Couchman JR. 1994. Syndecan 4 heparan sulfate proteoglycan is a selectively enriched and widespread focal adhesion component. Mol Biol Cell 5:183–192.

Yiu GK, Kaunisto A, Chin YR, Toker A. 2011. NFAT promotes carcinoma invasive migration through glypican-6. Biochem J 440:157–166.