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Increased expression of non-sulfated chondroitin correlates with adverse clinicopathological parameters in prostate cancer

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Chondroitin sulfate is a structurally diverse glycosaminoglycan, which contains a variable degree of sulfation that helps to determine its biological function. It is involved in the regulation of cellular activity and has been implicated in carcinogenesis. To determine if the non-sulfated chondroitin backbone has a functional role in prostate cancer, we analyzed its expression by immunohistochemistry using the 1B5 monoclonal antibody and a set of tissue microarrays constructed with 227 prostate specimen cores from 81 cases of benign prostate tissue and 77 cases of prostate cancer, of which 69 of these cases are matched. Non-sulfated chondroitin was found in the secretory epithelial cells and stromal regions of both prostatic adenocarcinoma and benign prostatic tissues, as well as in the basal cells of benign glands. A higher percentage of cancerous cells were stained positively for non-sulfated chondroitin as compared with benign secretory cells of the same patient. Cancerous cells stained more intensely for non-sulfated chondroitin. This increase in percentage of cells stained and increase in staining intensity were associated with higher pathological T stage and extraprostatic extension. Non-sulfated chondroitin expression (either staining intensity or percentage of cells stained) in adenocarcinoma and its peritumoral stroma correlated significantly with several clinicopathological parameters of unfavorable outcome, including higher pathological T stage and Gleason score, presence of tumor in both prostatic lobes, extraprostatic extension, seminal vesicle involvement and preoperative prostate-specific antigen levels. These data suggest that non-sulfated chondroitin is a potentially useful biomarker for prostate cancer, and may be involved in regulating prostate cancer behavior.
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Carcinoma of the prostate is the most frequently diagnosed cancer among American men, and is second only to lung cancer in malignancy-related deaths.[1] Indeed, men have a one in six lifetime risk of developing prostate cancer. Although comparatively less common in Asian populations,[2] prostate cancer has seen a rise of 118% from 6.6 to 14.4 per 100 000 person-years among Chinese men in Singapore,[3] where it is now the fifth commonest malignancy among all Singapore men.[4] The natural history of prostate carcinoma is complex and not fully understood. The disease can be asymptomatic in some patients but present in a more aggressive form in others. This poses a challenge for urologists, as decisions must be made to either employ 'watchful waiting' or intervene therapeutically. It is thus important to identify molecular biomarkers that will be helpful in differentiating between quiescent cancers and aggressive ones. Several recent studies have demonstrated the use of Pin1,[5] Akt-1, 2, and 3[6] and cyclin D1[7] as predictive factors of clinical

894

outcome in prostate cancer. In this study, we aimed to determine if non-sulfated chondroitin, a glycosaminoglycan molecule, could potentially be used as a prognostic marker by analyzing its expression for correlations with clinicopathological parameters of prostate cancer.

Glycosaminoglycans are long, unbranched molecules composed of repeating disaccharide subunits of an amino sugar alternating with a uronic acid.[8] The chondroitin backbone consists of N-acetyl-galactosamine (GalNAc) and glucuronic acid (GlcA) residues, which are modified by the addition of sulfate groups at various positions, such as the 4- and 6-hydroxyl positions of GalNAc and the 2- and 3-hydroxyl positions of GlcA, to produce chondroitin sulfate.[9] Chondroitin sulfate chains are attached to core proteins to form chondroitin sulfate proteoglycans. These have been found to regulate cancer cell behavior and affect disease outcome in a number of malignancies, including cancers of the lung, breast, ovary, mouth, pharynx and skin.[10–15]

Previous studies have shown that expression of versican, a chondroitin sulfate proteoglycan, is increased in prostate cancer and inhibits cancer cell adhesion to fibronectin in the stromal matrix.[16,17] Furthermore, high performance liquid chromatography analysis of glycosaminoglycans extracted from prostate cancer samples revealed that chondroitin-6-sulfate and dermatan sulfate were the predominantly upregulated components.[18] Indeed, elevated levels of sulfated chondroitin were reported to be associated with poorer disease outcome in early stage prostate cancer.[19,20]

The biological activities of chondroitin sulfate, as well as its ability to bind to and interact with different growth factors and signaling molecules, are dependent on the presence of distinct sulfation motifs.[21–23] However, recent studies suggest that non-sulfated chondroitin is also biologically active and is essential for cell division.[22,24] Thus, we aimed to determine the expression pattern of this molecule in prostate cancer by immunohistochemistry using 1B5, a monoclonal antibody with established specificity for non-sulfated chondroitin.[25,26] We also evaluated the potential use of non-sulfated chondroitin as a prognostic marker in prostate cancer by comparing its expression against clinicopathological parameters.

Materials and methods

Clinical Materials

Archival prostate adenocarcinoma samples and paired benign prostatic tissue specimens from corresponding patients were obtained from the Department of Pathology, Singapore General Hospital. The samples were collected from 89 patients who underwent radical prostatectomy from 2001 to 2004. Targeted areas of the specimens were identified and used for tissue microarray construction using the Beecher arrayer as previously described.[27] The numbers and sizes of prostatic tissue microarray cores used in this study is shown in Table 1. Clinicopathological data were collected for statistical analysis. Ethics approval was obtained from the Institutional Review Board, Singapore General Hospital.

Immunohistochemistry

The monoclonal antibody 1B5 that recognizes chondroitinase ABC-digested non-sulfated chondroitin stubs was purchased from Seikagaku (Tokyo, Japan). Tissue microarray sections were processed for immunohistochemical staining as described previously.[28] Briefly, after deparaffinization and rehydration, endogenous peroxidase activity was quenched with 3% aqueous hydrogen peroxide for 30 min. After rinsing with Tris-buffered saline, the sections were treated with 0.025 units/ml chondroitinase ABC (Sigma-Aldrich) and incubated for 2 h at 37°C. They were then blocked with goat serum for 1 h at room temperature, and incubated at 4°C overnight with the 1B5 antibody (1:100 dilution). After washing, biotinylated secondary antibody was added and incubated for 1 h at room temperature, followed by visualization of the staining by the avidin-biotin-complex technique using diaminobenzidine as the substrate (ABC kit, Vector Laboratories). The sections were counterstained with hematoxylin. Control sections were processed using the same protocol, but the chondroitinase ABC incubation step was omitted.

Table 1 Distribution of prostate specimen core sizes used for study (excluding specimen cores that dropped off during immunohistochemical processing)

Tissue microarray core sizes	Benign prostate specimen		Prostate adenocarcinoma specimen	
	No. of cores	No. of cases represented	No. of cores	No. of cases represented
0.6 mm	28	13	23	11
1.0 mm	74	46	58	44
2.0 mm	22	22	22	22
Total	124	81	103	77

Immunohistochemical Evaluation

Sections were assessed for expression of non-sulfated chondroitin in secretory and basal cells of benign prostatic tissue, in malignant cells of adenocarcinoma specimens, and in the stromal components of both groups. The intensity of staining was noted as absent (0), weak $(1+)$, moderate $(2+)$ or strong $(3+)$. The percentage of cells stained was also recorded. For a tissue section stained heterogeneously with different intensities, a weighted average of intensity was computed as such: $\sum(\text{intensity}_1 \times \text{percentage of cells stained with intensity}_1) + (\text{intensity}_2 \times \text{percentage of cells stained with intensity}_2) \div \text{total percentage of cells stained}$. Sections from different cores of the same specimen were independently scored, and average values for weighted average staining intensity and percentage of cells stained calculated for each sample. Positive differences in the values of these measurements in prostate cancer sections compared against paired benign prostatic tissues from corresponding patients were taken as upregulated expression of non-sulfated chondroitin, while negative values denoted reduced expression.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows Version 12.0. Wilcoxon signed rank test was used to compare the means between variables. Associations between the immunohistochemical staining data and clinicopathological parameters were determined using Fisher's exact test. A P-value of <0.05 was considered to be statistically significant.

Results

Clinical and Demographic Data

Eighty-nine prostate cancer samples and paired benign prostatic tissue specimens from constructed tissue microarrays were used for immunohistochemical evaluation. The age of the patients ranged from 45 to 74 years, with a mean of 62.4 years and a median of 62 years. The study cohort predominantly consisted of Chinese patients (73 cases), while the remaining cases included Malays (six cases) and other minor ethnic groups (10 cases). Owing to loss of tissue sections during deeper cuts for immunohistochemical processing, expression of non-sulfated chondroitin was examined in a total of 227 prostate specimen cores from 81 cases of benign tissue and 77 cases of cancer as detailed in Table 1. Consequently, only 69 matched benign prostatic and adenocarcinoma cases were available to compare differences in means in immunohistochemical staining. Clinicopathological features of the 77 cancer samples are shown in Table 2.

Table 2 Clinicopathological features of 77 prostate cancer samples

Clinicopathological parameter	Frequency distribution
Age (years)	
Median	62
Minimum	45
Maximum	74
Pathological T staging	
T2a	4
T2b	5
T2c	31
T3a	25
T3b	8
At least T2c[a]	3
Not available	1
Gleason sum	
Gleason sum 5	5
Gleason sum 6	27
Gleason sum 7	41
Gleason sum 8	2
Gleason sum 9	2
Maximum tumor dimension	
$<2.5\,\text{cm}$	39
$\geq 2.5\,\text{cm}$	37
Not available	1
Tumor volume	
$<4.0\,\text{cm}^3$	31
$\geq 4.0\,\text{cm}^3$	29
Not available	17
Extraprostatic extension	
Yes	33
No	40
Cannot be assessed[b]	3
Not available	1
Lymphovascular invasion	
Present	10
Absent	63
Not available	4
Obturator lymph node metastasis	
Yes	0
No	45
Not sampled	32
Seminal vesicle involvement	
Yes	7
No	69
Not available	1
Perineural invasion	
Seen	62
Not seen	15
Resection margin involvement (other than at apex and bladder base/proximal margin)	
Involved	35
Not involved	41
Not available	1
Apex (distal) margin involvement	
Involved	34
Not involved	42[c]
Not available	1

Table 2 Continued

Clinicopathological parameter	Frequency distribution
Bladder base (proximal) margin involvement	
Involved	9
Not involved	68
Clinical staging	
T1c	36
T2a	3
T2b	4
T2c	1
Not available	33
Preoperative PSA	
≤8 ng/ml	22
>8 ng/ml	20
Not available	35
Lobe occurrence	
Both lobes	64
Single lobe	11
Not available	2

[a]Pathological T staging is classified as 'at least T2c' due to inability to ascertain the capsular status of the tumor.
[b]Capsular integrity cannot be ascertained, as the site of the positive surgical margin does not disclose the capsule of the gland.
[c]One case of absence of apex margin involvement consists of high-grade prostatic intraepithelial neoplasia (HG-PIN) found at the apex margin.

Immunohistochemical Staining

Localization of non-sulfated chondroitin in benign prostatic and adenocarcinoma tissues

In benign prostatic tissue, non-sulfated chondroitin was expressed at moderate to high intensity in basal cells and in the periglandular stroma (Figure 1a). Weaker staining intensity was detected in the cytoplasm of secretory cells and in the stromal connective tissue away from the immediate periglandular zones. In prostate cancer, immunohistochemical staining for non-sulfated chondroitin was seen in the cytoplasm of malignant cells and in the surrounding stroma (Figure 1b). The expression data of non-sulfated chondroitin in 81 cases of benign prostatic tissues and 77 cases of prostate adenocarcinoma samples are shown in Table 3.

Comparison of non-sulfated chondroitin expression in benign prostatic and adenocarcinoma tissues

As shown in Table 4, when we compared staining differences in the means of 69 cases of matched benign and prostate adenocarcinoma tissues, we found that cancer cells were more intensely stained compared against benign secretory cells ($P = 0.030$). Furthermore, an average of 40% of cancer cells were positively stained for non-sulfated chondroitin, in contrast to an average of 25% of secretory cells in the benign prostatic group ($P = 0.013$). However, we did not observe any significant changes in staining intensity or percentage of cells stained in the peritumoral stroma, as compared to the periglandular stroma.
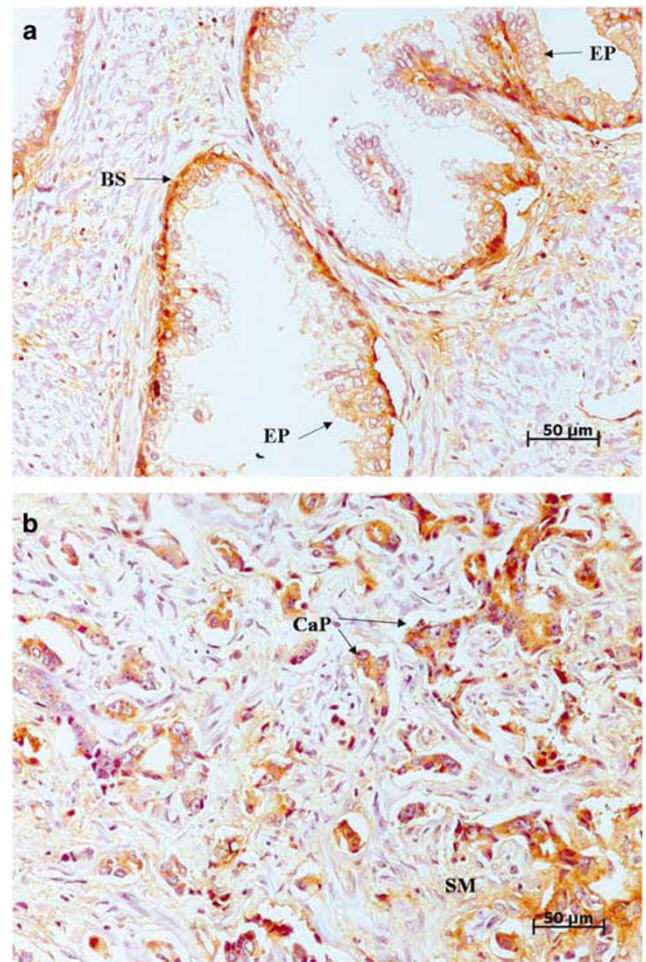

Figure 1 Expression of non-sulfated chondroitin in benign prostate tissues and prostate adenocarcinoma tissues. (**a**) The cytoplasm of the basal cells (BS) of the benign prostate tissue was stained strongly (3+) for non-sulfated chondroitin while there was weaker staining (1+) in the cytoplasm of benign secretory epithelial cells (EP). (**b**) The cytoplasm of prostate adenocarcinoma cells (CaP) was stained intensely (3+) for non-sulfated chondroitin while there was weak (1+) to moderate (2+) staining in the peritumoral stromal cells (SM).

Association of Immunohistochemical Staining with Clinicopathological Parameters

We examined the changes in staining intensity and percentage of cells stained for non-sulfated chondroitin in secretory epithelial cells of benign prostatic tissue and malignant cells of paired adenocarcinoma samples. When compared with clinicopathological parameters, increase in both staining intensity and percentage of cancer cells stained for non-sulfated chondroitin was found to be significantly associated with higher pathological T stage and presence of extraprostatic extension (Tables 5 and 6).

Analysis of absolute staining intensities for non-sulfated chondroitin in both prostate adenocarcinoma cells and peritumoral stroma showed significant associations with several clinicopathological parameters and known prognostic factors. Table 7

Table 3 Expression of non-sulfated chondroitin in benign prostatic and adenocarcinoma tissues

Stained tissue component	Total number	Number unstained	Number stained				
			Weighted average intensity (I)			Percentage of cells	
			≤ 1	$1 < I < 2$	≥ 2	≤ 50	> 50
Benign							
Secretory epithelial cells	81	29 (35.8%)	21 (25.9%)	9 (11.1%)	22 (27.2%)	37 (45.7%)	15 (18.5%)
Periglandular stromal cells	81	30 (37.0%)	17 (21.0%)	13 (16.0%)	21 (26.0%)	34 (42.0%)	17 (21.0%)
Adenocarcinoma							
Cancerous cells	77	17 (22.0%)	18 (23.4%)	15 (19.5%)	27 (35.1%)	33 (42.9%)	27 (35.1%)
Peritumoral stromal cells[a]	76	20 (26.3%)	29 (38.2%)	11 (14.4%)	16 (21.1%)	36 (47.4%)	20 (26.3%)

[a]One adenocarcinoma section has too few stromal cells for analysis.

Table 4 Comparison of weighted average intensity and percentage of cells stained for non-sulfated chondroitin between 69 matched samples of benign/adenocarcinoma prostate sections

	Weighted average intensity			Percentage of cells stained (%)		
	Mean (s.e.m.)	Median	P-value	Mean (s.e.m.)	Median	P-value
Epithelial/cancer cells						
Benign secretory epithelial cells	0.96 (0.11)	1.0	0.030[a]	24.7 (3.7)	10.0	0.013[a]
Adenocarcinoma cells	1.29 (0.11)	1.2		39.8 (4.3)	40.0	
Stromal region[b]						
Periglandular stromal cells	1.03 (0.12)	1.0	0.516	28.3 (4.4)	10.0	0.109
Peritumoral stromal cells	1.09 (0.10)	1.0		35.6 (4.4)	20.0	

s.e.m., standard error of the mean.
[a]Statistically significant result.
[b]1 adenocarcinoma section has too few stromal cells for analysis. Thus, there are only a total of 68 matched benign/adenocarcinoma prostate stromal sections available for analysis.

Table 5 Association between changes in weighted average intensity of epithelial/cancerous cells in 69 paired benign and adenocarcinoma samples and clinicopathological parameters

Clinicopathological parameter	Total[a]	Change in weighted average intensity of paired benign/cancer samples		P-value
		No change/decrease	Increase	
Pathological T staging				
T2a to T2c	39	22 (56.4%)	17 (43.6%)	0.045[b]
T3a to T3b	27	8 (29.6%)	19 (70.4%)	
Total	66[c]			
Extraprostatic extension				
Yes	27	8 (29.6%)	19 (70.4%)	0.047[b]
No	38	21 (55.3%)	17 (44.7%)	
Total	65[d]			

[a]A total of 69 matched pairs of benign prostatic and adenocarcinoma samples were available for analysis.
[b]Statistically significant result.
[c]Two cases classified as 'at least T2c' were excluded from analysis and there was unavailable information for one case.
[d]Extraprostatic extension for three cases cannot be ascertained and there was unavailable information for one case.

shows that pathological T stage, presence of extraprostatic extension, involvement of seminal vesicles, and pre-operative PSA levels were all correlated with strong expression of non-sulfated chondroitin in the peritumoral stroma. A strong expression of chondroitin in adenocarcinoma cells was also associated with involvement of both prostatic lobes.

Table 6 Association between changes in percentage of epithelial/cancerous cells stained in 69 paired benign and adenocarcinoma samples and clinicopathological parameters

Clinicopathological parameter	Total[a]	Change in percentage of cells stained of paired benign/cancer samples		P-value
		No change/decrease	Increase	
Pathological T staging				
T2a to T2c	39	23 (59.0%)	16 (41.0%)	0.012[b]
T3a to T3b	27	7 (25.9%)	20 (74.1%)	
Total	66[c]			
Extraprostatic extension				
Yes	27	7 (25.9%)	20 (74.1%)	0.011[b]
No	38	23 (60.5%)	15 (39.5%)	
Total	65[d]			

[a]A total of 69 matched pairs of benign prostatic and adenocarcinoma samples were available for analysis.

[b]Statistically significant result.

[c]Two cases classified as 'at least T2c' were excluded from analysis and there was unavailable information for one case.

[d]Extraprostatic extension for three cases cannot be ascertained and there was unavailable information for one case.

Table 7 Association between weighted average staining intensity of non-sulfated chondroitin in 77 prostate cancer samples and clinicopathological parameters

Stained tissue component	Clinicopathological parameter	Total	Weighted average intensity <2	Weighted average intensity ≥ 2	P-value
Adenocarcinoma cells	*Lobe occurrence*				
	Single lobe	11	11 (100.0%)	0 (0.0%)	0.013[a]
	Both lobes	64	38 (59.4%)	26 (40.6%)	
	Total	75[b]			
Peritumoral stroma[c]	*Pathological T staging*				
	T2a to T2c	39	34 (87.2%)	5 (12.8%)	0.049[a]
	T3a to T3b	33	22 (66.7%)	11 (33.3%)	
	Total	72[d]			
	Extraprostatic extension				
	Yes	33	22 (66.7%)	11 (33.3%)	0.049[a]
	No	39	34 (87.2%)	5 (12.8%)	
	Total	72[e]			
	Seminal vesicle involvement				
	Involved	7	3 (42.9%)	4 (57.1%)	0.034[a]
	Not involved	68	56 (82.4%)	12 (17.6%)	
	Total	75[f]			
	Pre-op PSA				
	≤ 8 ng/ml	21	21 (100.0%)	0 (0.0%)	0.021[a]
	>8 ng/ml	20	15 (75.0%)	5 (25.0%)	
	Total	41[g]			

[a]Statistically significant result.

[b]There was unavailable information for two cases.

[c]One adenocarcinoma section has too few stromal cells for analysis. Thus, there was a total of 76 prostate peritumoral stromal sections available for analysis.

[d]Three cases classified as 'at least T2c' were excluded from analysis. Unavailable information for one case.

[e]Extraprostatic extension could not be assessed in three cases. Unavailable information for one case.

[f]Unavailable information for one case.

[g]Unavailable information for 35 cases.

Examination of the percentage of tumor cells and peritumoral stromal cells stained positively for non-sulfated chondroitin revealed correlations with pathological T stage, Gleason scores and extraprostatic extension (Table 8).

No significant correlations were found when the expression patterns of non-sulfated chondroitin were analyzed with the other clinicopathological parameters listed in Table 2 ($P>0.05$), namely clinical stage, tumor size and volume, resection

Table 8 Association between percentage of cells positively stained for non-sulfated chondroitin in 77 prostate cancer samples and clinicopathological parameters

Stained tissue component	Clinicopathological parameter	Total	Percentage of positively stained cells ≤50%	Percentage of positively stained cells >50%	P-value
Adenocarcinoma cells	*Pathological T stage*				
	T2a to T2c	40	30 (75.0%)	10 (25.0%)	0.050
	T3a to T3b	33	17 (51.5%)	16 (48.5%)	
	Total	73[a]			
	Extraprostatic extension				
	Yes	33	17 (51.5%)	16 (48.5%)	0.050
	No	40	30 (75.0%)	10 (25.0%)	
	Total	73[b]			
Peritumoral stroma[c]	*Gleason sum*				
	5–6	31	27 (87.1%)	4 (12.9%)	0.035[d]
	7–9	45	29 (64.4%)	16 (35.6%)	
	Total	76			

[a]Three cases classified as 'at least T2c' were excluded from analysis. Unavailable information for one case.

[b]Extraprostatic extension could not be assessed in three cases. Unavailable information for one case.

[c]One adenocarcinoma section has too few stromal cells for analysis. Thus, there are only a total of 76 prostate peritumoral stromal sections available for analysis.

[d]Statistically significant result.

margin including distal and proximal margin involvement, lymphovascular invasion and perineural invasion. We were unable to correlate with obturator lymph node involvement as none of our patients demonstrated metastases to nodes (Table 2).

Discussion

In this study, we have examined the expression patterns of non-sulfated chondroitin in benign prostatic tissue and paired prostate cancer samples from corresponding patients. Non-sulfated chondroitin was overexpressed in adenocarcinoma cells compared against corresponding benign secretory cells, and this upregulation was associated with higher pathological T stage and extraprostatic extension. Strong expression of non-sulfated chondroitin in the cancer cells as well as a large number of positively stained cancer cells, correlated with occurrence of cancer in both prostate lobes, pathological T stage and extraprostatic extension. Similarly, a strong expression of non-sulfated chondroitin in cancer stroma and a large number of positively stained peritumoral stromal cells, correlated with Gleason scores, pathological T stage, extraprostatic extension, seminal vesicle involvement and pre-operative PSA levels. All these pathologic parameters augur a worse prognosis for patients, and underscore the association of non-sulfated chondroitin expression with conventional adverse predictors.

Studies on chondroitin sulfate proteoglycans have shown that they play important roles in cancer biology, and may be useful prognostic factors in breast, lung, ovarian and skin cancers.[10,13,14,29–31] Several immunohistochemical studies focusing on the sulfated chondroitin chain have shown that high stromal levels of this molecule is associated with a poor outcome in patients with early-stage prostate cancer.[19,20] Furthermore, increased expression of the core proteins of versican in the peritumoral stroma, and PTEN (two chondroitin sulfate proteoglycans) has also been reported to be predictive of poor prognosis in early-stage prostate cancer patients.[16,32]

To date, the role of the non-sulfated chondroitin backbone in prostate cancer has not been examined. Although it is well established that sulfate groups on chondroitin sulfate, heparan sulfate and other sulfated glycosaminoglycans are important determinants of the binding and biological actions of these molecules, some studies suggest that non-sulfated chondroitin may be biologically active as well. This has been highlighted by several recent reports on inhibiting biosynthesis of non-sulfated chondroitin by RNA interference, which demonstrated that the molecule is essential for cell proliferation, cytokinesis and migration, and may underlie the mechanistic processes leading to the association of non-sulfated chondroitin with adverse pathologic parameters as observed in our study.[22,24,33]

Chondroitin may regulate prostate cancer cell behavior and influence patient prognosis through several mechanisms. Platelet-derived growth factors (PDGFs) are involved in regulating proliferation of cancer cells and stimulating tumor angiogenesis, and chondroitin sulfate has been shown to enhance PDGF action by enhancing signaling of the tyrosine kinase receptors.[34] Indeed, PDGF and its receptor have been reported to be upregulated in prostate adenocarcinoma, and expression of PDGF receptor has been shown to mark prostate cancer cells with the greatest disposition for bony metastasis.[35,36]

Stromal cell-derived factor-1 (SDF-1) is a chemoattractant for prostate carcinoma cells.[37] Chondroitin sulfate has been shown to be required for cellular binding of SDF-1.[38] In prostate carcinoma, treatment of cancer cells with SDF-1 upregulated expression of $\alpha_v\beta_3$ integrin receptors, resulting in increased cell adhesion and invasion.[39] Furthermore, SDF-1 has been shown to be critical for spread of prostate cancer, and a positive correlation has been reported between SDF-1 signaling and bony metastasis.[40,41]

Pleiotrophin, also known as heparin affin regulatory peptide or heparin-binding growth-associated molecule, is an important regulator of cancer growth and angiogenesis.[42] Pleiotrophin expression has been reported in prostate cancer cells, but not in epithelial cells derived from normal or hyperplastic prostate.[43] Overexpression of pleiotrophin resulted in anchorage-independent growth. The importance of this molecule on prostate cancer cell growth and migration was further confirmed using antisense inhibition of pleiotrophin expression.[44] Recent studies have shown that pleiotrophin is able to bind to chondroitin sulfate in addition to heparan sulfate, and that chondroitin sulfate helps in the transfer of the growth factor to its receptor on the cell surface.[45] Pleiotrophin has also been demonstrated to function downstream of fibroblast growth factor-2 (FGF2) in increasing prostate cancer cellular proliferation and migration.[46] Inhibition of pleiotrophin expression in human prostate cancer LNCaP cells rendered them unable to respond to the FGF2 stimulus, resulting in an absence of increased cell proliferation and migration.

The prostate adenocarcinoma samples that were analyzed in this study were limited in their representation of the entire range of cancers especially for some of the clinicopathological parameters. For instance, no obturator lymph node metastases were observed in the patient cohort studied and therefore makes it unavailable for studying the association of this parameter with non-sulfated chondroitin staining. Besides, the small number of cases observed for positive lymphovascular invasion, presence of seminal vesicle involvement and proximal margin involvement contributes to the difficulty of making a strong association between these parameters and non-sulfated chondroitin staining. In addition, many of these prostate adenocarcinoma samples are of intermediate tumor grade (Gleason sum of 6 or 7) while there is a very small group of low-grade or high-grade tumors. In our study, we were unable to determine any associations of non-sulfated chondroitin staining with PSA relapse and survival due to the short period of patient follow-up. As such, future work will include collecting prostate adenocarcinoma samples that encompass a broader spectrum of prostate cancer disease, and a longer period of patient follow-up with data acquisition to further determine whether non-sulfated chondroitin will be a good indicator of prostate cancer progression.

Nevertheless, we have shown a significant increase in expression of non-sulfated chondroitin in prostate adenocarcinoma compared against paired benign prostatic tissue samples from corresponding patients, and that this is associated with several important clinicopathological parameters of poor prognosis. This suggests that non-sulfated chondroitin may be useful as a biomarker and predictive factor of patient outcome in prostate cancer, and may also serve as a potential therapeutic target to improve patient treatment and outcome.

Acknowledgements

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