

Matrix Pathobiology

Prostate Cancer Increases Hyaluronan in Surrounding Nonmalignant Stroma, and This Response Is Associated with Tumor Growth and an Unfavorable Outcome

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Our objective was to investigate whether the presence of a tumor increases hyaluronan (HA) levels in surrounding prostate tissues and whether this extratumoral HA influences tumor growth and outcome. From a series of 287 men diagnosed with prostate cancer at transurethral resection and followed up with watchful waiting, tissue microarrays were constructed, stained, and scored for HA. A high HA staining score in the tumor stroma or in nonmalignant prostate tissue stroma were both associated positively with higher Gleason score and larger tumor volume, and was associated with a poor outcome. HA staining score was not an independent marker for outcome (multivariate Cox, with Gleason score, tumor volume, stage, and HA variables). In an orthotopic rat prostate cancer model, hyaluronic acid synthase-1 mRNA levels and HA staining were increased in normal prostate tissue surrounding prostate cancer. Orthotopic prostate cancer growth was increased by intraprostatic injection of HA. In conclusion, cancer in the prostate apparently stimulates HA synthesis both in tumor stroma and in the surrounding normal tissue. This promoted tumor growth and was associated with an unfavorable outcome. (*Am J Pathol* 2011, 179:1961–1968; DOI: 10.1016/j.ajpath.2011.06.005)

Prostate cancer has a highly variable and unpredictable course.^{1–3} Currently, prostate cancer is diagnosed and its aggressiveness is classified by tumor stage, Gleason

score, extent of tumor at biopsy, and serum levels of prostate-specific antigen (PSA).¹

Recent studies have indicated that alterations in the stroma adjacent to the tumor and in more remote nonmalignant parts of the prostate also are associated with treatment response and clinical outcome.⁴ Prostate cancer progression is associated with loss of smooth muscle cells and an increased number of fibroblasts and myofibroblasts (cancer-associated fibroblasts) in the tumor stroma. This process, described as a reactive stroma,^{5–7} is similar to the stroma reaction in wounds.⁸ Reactive tumor stroma in prostate cancer is accompanied by changes in gene expression,⁹ decreased androgen-receptor staining,¹⁰ increased angiogenesis,¹¹ accumulation of inflammatory cells,^{12–14} and alterations in the extracellular matrix,^{5,15} and the magnitude of these changes are all related to outcome.

Interestingly, the stroma in the nonmalignant parts of a prostate with cancer also is changed, indicating that the reactive stroma extends far beyond the borders of the tumor. The androgen receptor and smooth muscle markers are decreased,¹⁰ mast cells¹⁶ and platelet-derived growth factor-receptor β are increased,¹⁷ and these alterations are related to prostate cancer death, and for androgen receptor these alterations also are related to a limited response to castration therapy.¹⁰ The mechanisms explaining why the stroma is changed in prostate cancers and in the surrounding normal prostate tissue, and why this is related to disease aggressiveness and treatment outcome, are largely unknown. Alterations in tissues surrounding tumors could be the result of carcinogenic factors influencing an entire organ, termed a cancer field effect.¹⁸ In addition, tumor epithelial cells may secrete factors that adapt the closely adjacent tumor stroma and the more remote nonmalignant prostate tissues to the needs of

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an expanding tumor.^{19–21} The surrounding tissues are in this way tinted by the presence and nature of the tumor and because such changes could be of diagnostic importance we have suggested that this tissue can be called tumor-indicating normal tissue (TINT).^{10,22}

In many tumor types hyaluronan (HA) constitutes a major part of the extracellular matrix.^{15,23} HA is a glucosaminoglycan (polysaccharide) important for cell division, cell migration, and angiogenesis during embryogenesis, inflammation, and wound healing.^{24,25} Degradation of HA forms a matrix that favors tumor cell invasion, epithelial to mesenchymal transition, cell proliferation, angiogenesis, lymphangiogenesis, and it recruits bone marrow-derived inflammatory and progenitor cells to tumors.²³ In prostate cancer accumulation of HA in tumor stroma and altered hyaluronic acid synthase (HAS) and hyaluronidase in tumor epithelial cells, are associated with increased cell proliferation, invasion, metastasis, and poor outcome in men who have undergone radical prostatectomy.^{26–32} Manipulation of intratumoral HA levels in experimental animal models influences tumor growth.³¹

Against this background we wanted to test the hypothesis that HA levels are increased in the nonmalignant prostate tissue adjacent to prostate cancers and that the magnitude of this could be related to tumor aggressiveness.

Materials and Methods

Patients

TMA's were generated from formalin-fixed, paraffin-embedded samples from a consecutive series of 404 patients with voiding symptoms diagnosed with prostate

cancer between 1975 and 1995 after transurethral resection (TUR) of the prostate (see Hammarsten et al²² for details). The TMAs contained 5 to 8 tumor cores and 4 nonmalignant tissue cores per patient. The mean age at diagnosis was 74 years (range, 53 to 95 years). Staging was performed at the time of surgery; local clinical stage was determined by digital rectal examination and radio-nuclide bone scan was performed for detection of metastases, but no lymph node staging was performed. Gleason score was re-assessed when the TMA was constructed. Because this series was collected before the PSA era, information on serum PSA was not available. A substantial number of our patients ($n = 295$) had not received any cancer therapy before the TUR of the prostate and were managed with watchful waiting (287 of these patients followed with watchful waiting had enough tissue in the TMAs to be evaluated). The clinicopathologic characteristics of the patients can be found in Table 1. The median overall follow-up period was 5.9 years (range, 0 to 25.5 years). The cause of death was determined by examination of patient records. Prostate cancer-specific and relative survival was very similar, indicating a correct evaluation of the cause of death.³³ The Research Ethics committee in Umeå, Sweden, approved the study.

Animal Experiments

The ventral prostate lobe in adult male Copenhagen rats were injected with 2000 rat Dunning AT-1, MatLyLu prostate cancer cells, or with saline as described earlier.^{19–21} Animals with AT-1 tumors and controls were sacrificed after 7, 10, or 14 days ($n = 5$ to 7, in each group) and

Table 1. Clinicopathological Characteristics and Relation to HA Variables

	Analyzed cohort ($n = 287$)	Mean HA in tumor stroma	Mean HA in tumor epithelium*	Mean HA in normal stroma
Gleason score				
4 to 6	184 (64%)	6.3 (± 2.7)	0.4 (± 0.7)	6.0 (± 2.5)
7	49 (17%)	8.0 (± 2.9)	0.7 (± 0.7)	7.0 (± 2.6)
8 to 10	54 (19%)	8.3 (± 2.9)	0.9 (± 1.0)	7.2 (± 2.3)
Tumor stage		$P < 0.001^\dagger$	$P < 0.001^\dagger$	$P < 0.001^\dagger$
1	183 (64%)	6.7 (± 2.8)	0.5 (± 0.7)	6.4 (± 2.6)
2	73 (25%)	7.0 (± 2.7)	0.5 (± 0.8)	6.5 (± 2.5)
3 to 4	30 (10%)	8.9 (± 3.5)	1.2 (± 1.1)	6.5 (± 2.3)
Estimated tumor volume		$P < 0.001^\dagger$	$P < 0.001^\dagger$	NS [‡]
<20%	170 (59%)	6.5 (± 2.8)	0.5 (± 0.7)	6.2 (± 2.5)
>20%	117 (41%)	7.7 (± 2.8)	0.6 (± 0.9)	6.8 (± 2.4)
Ki-67 index in tumor areas		$P < 0.001^\dagger$	$P = 0.05^\ddagger$	$P < 0.05^\ddagger$
≤ 0.027	165 (60%)	6.5 (± 2.8)	0.5 (± 0.6)	6.1 (± 2.5)
>0.027	108 (40%)	7.8 (± 2.9)	0.7 (± 1.0)	6.9 (± 2.5)
Vascular count in tumor		$P < 0.001^\dagger$	NS [‡]	$P < 0.05^\ddagger$
≤ 13.4	154 (57%)	6.7 (± 2.7)	0.4 (± 0.6)	6.4 (± 2.6)
>13.4	114 (43%)	7.6 (± 3.0)	0.7 (± 0.9)	6.6 (± 2.5)
Vascular count in normal tissue		$P < 0.05^\ddagger$	NS [‡]	NS [‡]
≤ 10.75	143 (52%)	6.9 (± 2.8)	0.5 (± 0.8)	6.5 (± 2.7)
>10.75	130 (48%)	7.3 (± 3.0)	0.6 (± 0.9)	6.4 (± 2.3)
		NS [‡]	NS [‡]	NS [‡]

The analyzed cohort of patients did not receive any treatments (followed with watchful waiting) and had no metastases at diagnosis.

*The natural logarithm was used in the statistical test.

†Analysis of variance was used.

‡Binary logistic regression was used.

NS, not significant ($P > 0.05$).

animals with MatLyLu tumors and controls were sacrificed after 7 days ($n = 7$, in each group). Prostate lobes were fixed in buffered formalin or frozen in liquid nitrogen.

To examine whether injected HA affects prostate cancer growth, 2000 AT-1 cells were injected into the ventral prostate and at day 8 the ventral prostates were injected with either 400 μg HA in 40 μL saline (Hyalgan; Nycomed, Stockholm, Sweden) or 40 μL saline alone and examined 4 days later. Tumor size was determined by measuring the volume density of tumor in the ventral prostate using a stereologic approach and then multiplying the volume density by the ventral prostate lobe weight as previously described.¹⁹ All animal work was approved by the local ethical committee for animal research.

HA Staining

The TMAs and rat prostate samples were sectioned and to localize HA in the tissue sections we used a HA binding protein probe (Corgenix, Inc., Westminster, CO). The isolation and biotin labeling of the HA binding protein and the staining method used, including controls, have been described elsewhere.³⁴ This method detects different molecular sizes of HA.

HA Scoring

In patient samples, staining of the stroma (within tissue infiltrated by cancer and in the surrounding nonmalignant prostate tissue) was evaluated for distribution and intensity and the product of staining intensity and staining distribution was calculated (with score values between 0 and 16). Distribution was evaluated as none (0), < 10% (1), 10% to 50% (2), 50% to 90% (3), or more than 90% (4). Intensity was evaluated as none (0), faint (1), moderate (2), strong (3), or very strong (4). Each patient was represented by the mean value from the tumor and nonmalignant tissue cores respectively. HA staining in the epithelium was only scored by intensity. In rat samples stroma HA staining was scored for distribution (the fraction of stroma volume stained, from 0 to 1) and the intensity of staining (none = 0, moderate = 1, or strong = 2). The intensity and distribution values were multiplied to obtain a HA staining score. The HA staining scores were related to previously published data of vascular density, tumor cell proliferation, estimated tumor size (percentage of the TUR chip area containing cancer), and Gleason score in this set of patients.³⁵

RNA Preparation and Quantitative RT-PCR Analysis of HAS-1 Expression

Total RNA, from AT-1 and MatLyLu tumor tissue, intact ventral prostate tissue, normal prostate tissue adjacent to tumor, and AT-1 cells (grown *in vitro*), were extracted using TRIzol reagent (Invitrogen, Stockholm, Sweden) following the manufacturer's instructions. The concentration of each total RNA sample was measured with a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). RNA integrity was determined using the Agilent 2100 BioAnalyzer (Agilent, Wilmington,

DE). The RNA was reverse transcribed using random hexamers (Applied Biosystems, Sundbyberg, Sweden) and Superscript II reverse transcriptase (Invitrogen). All cDNA reactions were run in duplicate. mRNA quantity was assessed by using TaqMan gene expression assays (Applied Biosystems) for HAS1 (Rn00597231_m1) and ribosomal protein S27a (Rn00821295_g1), according to the manufacturer's description. The thermal profile of the quantitative PCR was 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

Statistics

Values are given as means and SD. Correlations between continuous variable were analyzed using the Pearson correlation test. Associations between HA variables and categoric variables (Gleason score and clinical stage) were analyzed using one-way analysis of variance and associations with the dichotomous variables were analyzed using binary logistic regression. The distribution of the values indicating HA staining was distributed normally in the tumor stroma but in the normal epithelial cells this was not the case and these values were therefore log transformed. The Mann-Whitney *U* test was used to compare tumor weights in rats. Kaplan-Meier analysis was performed with death from prostate cancer as event, and deaths from other causes were censored and the difference in survival was tested with the log-rank test. Univariate and multiple Cox regression analysis were performed to control for other prognostic factors. Statistical analysis was performed using SPSS 17.0.0 software (SPSS, Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

Results

HA Staining in Malignant and Nonmalignant Human Prostate Tissues

HA staining generally was observed in the tumor stroma and some patients also had staining in tumor epithelial cells (Figure 1). High-grade tumors had more widespread and more intense HA staining than low-grade tumors. In the surrounding nonmalignant prostate tissue HA staining was present mostly in stroma, was less common in basal cells, and was scarce in luminal epithelial cells. Basal cell staining was seen mainly in prostates containing low-grade tumors, whereas it generally was absent in cases harboring high-grade tumors. HA staining in the nonmalignant prostate tissue stroma was considerably more widespread and intense in prostates containing high-grade tumors, than in cases with low-grade variants (Table 1).

HA Staining Score Is Associated with Markers of Tumor Aggressiveness

HA staining in tumor epithelial cells and tumor stroma were correlated to Gleason score, estimated tumor volume (% cancer in the TUR chips), tumor vascular density, and tumor

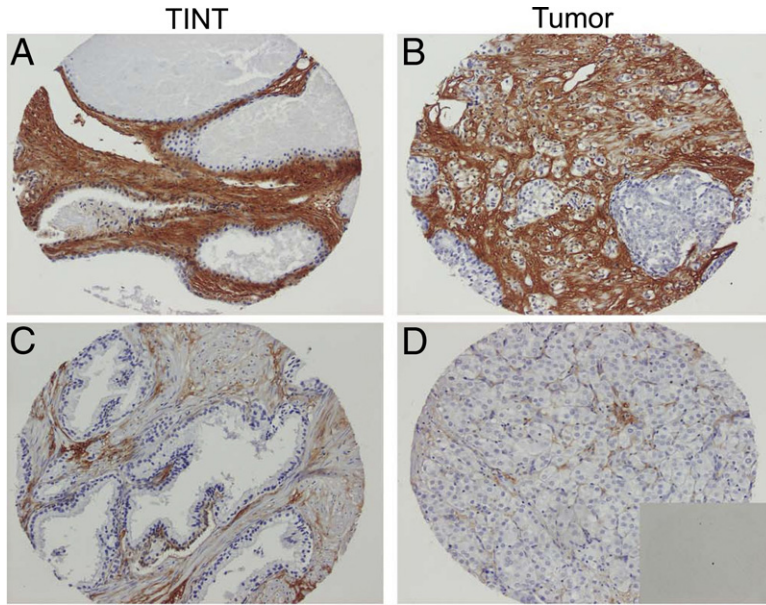


Figure 1. Tissue samples stained for HA; each tissue core is 0.6 mm in diameter. **A:** High stroma staining score in normal (TINT) tissue. **B:** High stroma staining score in tumor tissue. **C:** Low HA staining score in normal (TINT) tissue stroma (staining of the basal cells is focally present). **D:** Low stroma staining score in tumor stroma tissue. **Inset:** Lack of HA staining in a section pretreated with hyaluronidase, as described earlier in more detail.³⁴

cell proliferation (with correlation coefficients ranging from 0.18 to 0.37; Table 1). The HA staining score in tumor epithelium and tumor stroma were correlated ($r = 0.32, P < 0.001$). To evaluate if HA staining was related to metastasis at diagnosis, the whole series of 404 men were included (treated and untreated). Patients with bone scan-detected metastasis at diagnosis ($n = 46$) had a higher HA staining score in tumor epithelium (data not shown) and tumor stroma (10.1 ± 2.9 versus 7.2 ± 2.9 ; $P \leq 0.001$) than patients without metastasis at diagnosis ($n = 358$).

HA staining scores in the stroma of the nonmalignant prostate tissue also was correlated with Gleason score, estimated tumor volume, and tumor cell proliferation index, but not with clinical stage or vascular density (Table 1). HA staining in the tumor stroma was correlated to HA staining score in the nonmalignant prostate tissue stroma ($r = 0.33, P < 0.001$).

HA staining in basal epithelial cells in nonmalignant glands was present in 30% of the cases and was corre-

lated negatively to Gleason score ($r = -0.12, P < 0.05$), local clinical stage ($r = -0.18, P < 0.01$), and estimated tumor volume ($r = -0.16, P < 0.01$).

HA Staining Score in Tumor and Nonmalignant Prostate Tissue Is Associated with Prostate Cancer Death in Patients Managed by Watchful Waiting

A HA staining score above the median in the tumor epithelium (0.4), tumor stroma (7.4), and nonmalignant prostate tissue stroma (6.25) all were associated with increased risk of prostate cancer death (Figure 2). HA staining score in stroma predicted prostate cancer death when using other possible cut-off values as the mean, and quartiles (data not shown). The presence of basal cell HA staining appeared to be associated with a lower risk of prostate cancer death, but this did not reach statistical significance ($P = 0.1$).

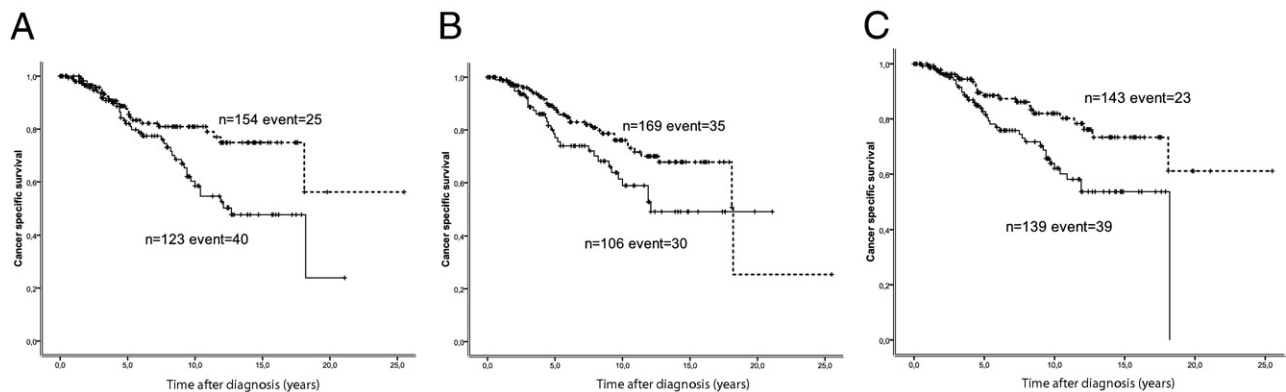


Figure 2. Kaplan–Meier curves of the analyzed cohort, divided by each variables median (solid line, above median; dashed line, under median). Patients with a Gleason score of 4 to 10 were managed with watchful waiting. **Cross:** Censored patients are shown. **A:** HA staining score in tumor stroma (log-rank test, 7.1; $P < 0.01$). **B:** HA staining intensity in tumor epithelium (log-rank test, 4.3; $P < 0.05$). **C:** HA staining score in nonmalignant stroma (log-rank test, 8.0; $P < 0.01$).

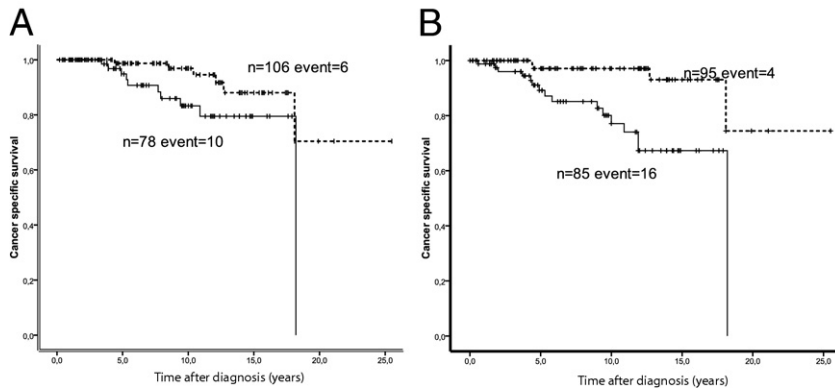


Figure 3. Kaplan-Meier curves of the analyzed subgroups of patients managed with watchful waiting, divided by median of HA staining score in nonmalignant tissue (**solid line**, above median; **dashed line**, under median). **A:** A total of 184 patients with Gleason score of 6 or less (log-rank test, 4.7; $P < 0.05$). **B:** A total of 180 patients with clinical T1 stage, this group also included 30 patients with a Gleason score of 7, and 13 patients with a Gleason score of 8 to 10 (log-rank test, 13.2; $P < 0.001$).

HA Staining Score Is Not an Independent Marker for Prostate Cancer Death

In univariate Cox analysis high HA staining in tumor epithelium, tumor stroma, and nonmalignant stroma was associated with an increased risk of prostate cancer death in patients with Gleason score 4 to 10 (relative risk, 1.4; relative risk, 1.2; relative risk, 1.1, respectively; $P < 0.01$), and for the Gleason score 4 to 6 subgroup in tumor stroma (relative risk, 1.26; $P < 0.05$). However, in multivariate analysis including estimated tumor volume, tumor stage, and Gleason score, HA staining was not an independent prognostic marker (data not shown).

High HA Staining Score in Nonmalignant Prostate Tissue Is Associated with Prostate Cancer Death in Patients with Low-Grade or T1 Tumors

In a subanalysis of the 184 patients with Gleason score 4 to 6 tumors, the HA staining score in the nonmalignant stroma was associated with prostate cancer death (Figure 3A; log-rank test, 4.7; and $P < 0.05$). This association also was seen in the 188 patients with stage T1 tumors (log-rank test, 13.2; $P < 0.001$; Figure 3B). Note, however, that the number of patients dying of prostate cancer in these groups are low and, although significant, the results therefore must be considered with some caution.

HAS-1 and HA Are Increased in Growing Rat Prostate Tumors and in Surrounding Normal Tissue and HA Stimulates Tumor Growth

Immunostaining showed strong HA staining in the rat prostate Dunning AT-1 and MatLyLu tumor stroma, but also a moderate to strong staining in the stroma of the surrounding nonmalignant prostate tissue (Figure 4). Scoring of HA staining in the nonmalignant tissue surrounding AT-1 tumors showed that it increased with tumor size from 0.33 ± 0.10 at 7 days to 0.51 ± 0.24 at 14 days, but the difference was not statistically significant ($P = 0.12$; $n = 5$ to 7). The HA staining score in the nonmalignant prostate tissue surrounding the MatLyLu tumors at day 7 ($n = 7$) was 0.62 ± 0.25 and thus significantly higher ($P = 0.04$) than in prostate tissue surrounding an AT-1 tumor (Figure 4, B versus C). HAS-1 mRNA levels were higher in the nonmalignant prostate tissue surrounding AT-1 tumors than in controls (Figure 5), and, similarly, HAS-1 mRNA levels were higher in the tissue surrounding MatLyLu tumors than in controls (data not shown). Intraprostatic injection of HA stimulated the growth of AT-1 tumors. The average tumor weight (gram) was significantly higher in the HA-treated animals than in controls (9.4 ± 2.3 versus 5.5 ± 2.3 ; $n = 5$ to 6; $P < 0.01$).

Discussion

The three key findings in this study were as follows: (i) an increased HA staining score in the tumor stroma was

Figure 4. Photographs of rat tissues from the ventral prostate (VP), in control (A), the border zone between an AT-1 tumor (left) and adjacent VP tissue (B), and from the border zone between a MatLyLu tumor (top) and adjacent VP tissue (C) stained to visualize HA. Original magnification, $\times 200$. Note the intense staining around the MatLyLu tumor.

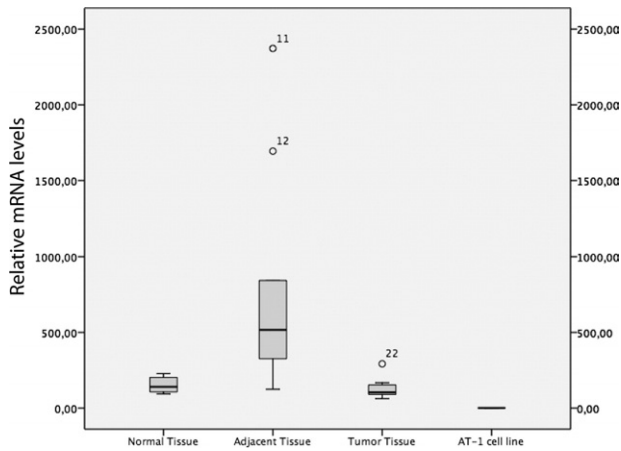


Figure 5. Box-plot showing relative HAS-1 mRNA levels in rat prostate tissues. Copenhagen rats were injected with 2000 AT-1 prostate tumor cells or saline into the ventral prostates (VPs) and examined 10 days later. The HAS-1 expression was notably increased in VP tissue adjacent to tumor, whereas the AT-1 tumor tissue showed no difference in HAS-1 expression compared with the normal VP tissue from animals without tumors. Note that HAS-1 mRNA was not expressed in AT-1 cells *in vitro*.

associated with unfavorable tumor characteristics and an increased risk of prostate cancer death, (ii) exogenous administration of HA stimulated tumor growth in an animal model, and (iii) the presence of a tumor increased the HA staining score in the surrounding morphologically normal prostate tissue and the magnitude of this was associated with tumor aggressiveness and the risk of prostate cancer death. The first observation confirms previous studies but the other two are novel.

A high HA staining score in the stroma of prostate cancers was associated with high tumor cell proliferation, high vascular density, high Gleason score, high stage, and increased risk of prostate cancer death. HA, however, is, because of its correlation with Gleason score, not an independent prognosticator of prostate cancer death, although it could, if verified in other cohorts, be of some use to identify low-grade cancer that have excellent prognosis also without active treatment. It should be noted, however, that our TUR-diagnosed prostate cancers could differ from current PSA-detected tumors and studies in more contemporary cohorts are needed to evaluate the role of HA as a prognostic marker. Our observations on HA in tumor stroma in a TUR-diagnosed watchful waiting cohort are in line with previous findings in contemporary cohorts including PSA-detected tumors in men receiving active treatments, and HA is predictive of PSA recurrence after radical prostatectomy when evaluated in preoperative biopsies.^{26–30,32,36} These observations suggest that there are no apparent differences in tumor HA between biopsy- and TUR-diagnosed prostate cancer.

Receptors for HA are present on epithelial cells (CD44, CD168, and Toll-like receptors 2 and 4) and on lymph vessels in the prostate [lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1)] and these are related to tumor aggressiveness.^{26,32,37–40} Different molecular HA sizes (polysaccharide lengths) have different biological functions.²⁴ Hyaluronidase degrades HA into angiogenic fragments³² and HA directly stimulates tumor cell prolif-

eration, epithelial to mesenchymal transition, and invasion.^{23,32,41} Together, these previous studies and the current observations clearly suggest that HA may influence prostate cancer growth by affecting several different cell types in the tumor and its surroundings. Accumulation of HA thus could, in addition to decreased smooth muscle markers and androgen receptor, and increased platelet-derived growth factor receptor β (see later), be an additional marker of a tumor stroma phenotype associated with aggressive disease.

To test if injection of HA stimulated tumor growth in our orthotopic rat model we injected Hyalgan (Nycomed). This HA compound is purified from cockscomb and may, although purified, contain proteins bound to HA, such as versican. Versican is known to act in synergy with HA.⁴² In this experiment HA was injected when a small tumor already was formed but because HA administered simultaneously with the tumor cells also stimulated tumor growth (our own unpublished data), it is likely that HA is stimulatory at various phases of tumor establishment and growth. Further studies are needed, however, to explore in more detail how different molecular forms of HA affect tumor growth, and how HA synthesis is regulated in the tumor and in the surrounding tissue.

The mechanisms responsible for the development of a reactive stroma and increased HA levels in particular are largely unknown, but tumor cell secretion of tumor growth factor- β is one likely mechanism.^{7,43} The expression of tumor growth factor- β is increased in human prostate cancers⁴⁴ and in our animal model (our own unpublished observations). Tumor growth factor- β also regulates HAS 1 to 3.²⁴ Other growth factors produced in prostate cancers, such as insulin-like growth factor-1, fibroblast growth factor-2, and platelet-derived growth factor, also are known to stimulate HA production.³²

HA has been shown to increase the recruitment of cancer-associated fibroblasts⁴² and macrophages,⁴⁵ and they stimulate prostate cancer growth.^{12,20,21,46} Macrophages, which accumulate inside and around prostate tumors, in turn secrete IL-1 β and tumor necrosis factor- α ,²⁰ factors known to increase HA synthesis,³² and cancer-associated fibroblasts produce HA.³⁶ Prostate cancers are hypoxic; hypoxia makes tumors more aggressive,⁴⁷ and hypoxia stimulates HA synthesis.⁴⁸ Multiple mechanisms consequently may explain why HA is increased in prostate tumors and their surroundings, and why the magnitude of this is linked to disease aggressiveness. Although prostate tumors apparently increase HA in surrounding tissues, this is probably not the only mechanism by which prostate HA could be increased, other pathologies such as concomitant inflammation and benign prostate hyperplasia also could be involved.

One important finding in our study was that the presence of a tumor up-regulated the HA staining score, not only inside the tumor, but in the surrounding nonmalignant prostate tissue, and that the magnitude of this response was associated with tumor aggressiveness and outcome in patients. HA staining in normal tissue was tightly associated with tumor characteristics and thus not a marker providing prognostic information independently of Gleason score, clinical stage, and estimated tumor

size. To be of potential clinical use markers measured inside tumors preferably should provide information independent of tumor grade and stage, but the opposite is true for markers measured in the surrounding normal tissue (see later). Because of the lack of spatial information in TUR chips we cannot presently determine how far this response reaches into adjacent tissues (see Halin et al⁴⁹ for discussion). Further studies in radical prostatectomy specimens are needed to clarify this. Similarly in rats, the HA staining score was higher in the surroundings of the metastatic MatLyLu tumor than around the nonmetastatic AT-1 variant. The reasons for this are unknown but aggressive tumors are known to secrete factors with systemic effects, for example, substances recruiting inflammatory cells and cancer-associated fibroblasts from the bone marrow and factors creating premetastatic niches in other organs.⁵⁰ It is therefore not unlikely that they also could have specific effects in more adjacent tissues. In line with this, implantation of tumor cells into the rat prostate resulted in adaptive changes in the surrounding ipsilateral but also in the contralateral prostate lobe.^{19,20} Adaptions in the surrounding stroma, in part mediated by macrophages recruited to the tissue around tumors,²⁰ such as growth of the supporting vasculature and tissue remodeling are probably necessary for subsequent tumor growth and metastasis.^{19–21} Such responses in tumor-adjacent nonmalignant tissues are morphologically similar to those seen around wounds,^{8,51} and the gene expression in the normal rat prostate tissue surrounding an implanted AT-1 prostate cancer is similar to that in wounding and inflammation (Adamo et al, unpublished data). Tumors apparently are able to stimulate HA synthesis, either directly or through recruiting inflammatory cells, in surrounding cells and as the tumor expands previously nonmalignant stroma becomes integrated into the tumor stroma, probably explaining why the tumor stroma and nonmalignant tissue stroma show similar changes in HA staining. Interestingly, an increased HA staining score in the nonmalignant tissue was prognostic in low-grade and low-stage disease, suggesting that aggressive tumors may have a particular effect on adjacent tissues already at an early stage of the disease.

Because the nonmalignant prostate tissue apparently senses the presence and nature of tumors elsewhere in the organ this information probably can be used for diagnostic and prognostic purposes. For example, in negative biopsies such markers potentially could indicate the risk that aggressive tumors are present elsewhere in the prostate. We have proposed that this type of prostate tissue can be termed TINT, and that prostate tissue is tinted in a particular way by the presence of aggressive tumors.⁴⁹ In line with this, in TINT stroma surrounding aggressive tumors the androgen receptors are decreased,¹⁰ and mast cell numbers¹⁶ and platelet-derived growth factor-receptor β are increased.¹⁷ In TINT epithelium surrounding aggressive tumors phosphorylated epidermal growth factor receptors and phosphorylated activated protein kinase is increased.²² Increased HA staining score apparently could be an additional TINT

marker associated with prostate cancer aggressiveness as suggested in this study.

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