TÜBİTAK

Original Article

Turk J Med Sci
2012; 42 (6): 1020-1027
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1203-52

Hyaluronidase-1 expression is not a predictor of biochemical recurrence in radical prostatectomy specimens

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Aim: The purposes of this study were to evaluate the rate of biochemical recurrence among patients who underwent radical prostatectomy due to localized prostate cancer, assess the risk factors of biochemical recurrence, and investigate its relation with hyaluronidase-1 (HYAL-1).

Materials and methods: We retrospectively evaluated the data of 112 patients who underwent radical retropubic prostatectomy. HYAL-1 expression was evaluated immunohistochemically and staining was graded as mild, moderate, and severe. Two blinded pathologists evaluated the specimens. The relation among preoperative pathologic findings that were detected in transrectal ultrasound (TRUS) guided biopsy, specimens of radical prostatectomy in the final stage, and biochemical recurrence rate were also evaluated. Mean follow-up time was 59.8 months (range: 20 to 92 months).

Results: Tumor recurrence was detected in 29 patients. There was no relation between HYAL-1 expression and recurrence. In multivariate analysis, the percentage of tumors detected in TRUS biopsy specimens, positive surgical margin, capsular involvement, and seminal vesicle invasion detected in the final pathology were correlated with recurrence. The sensitivity, specificity, positive predictive value, and negative predictive value of HYAL-1 expression were respectively 38%, 63%, 30%, and 71%.

Conclusion: HYAL-1 expression was not found to be predictive for recurrence. Further molecular markers and genetic studies are necessary to predict the biochemical recurrence after radical prostatectomy.

Key words: Prostate, prostate cancer, recurrence, hyaluronic acid

Introduction

There has been rapid development regarding the treatment for men with localized prostate cancer in recent years. Surveillance, radical prostatectomy (RP), external beam radiotherapy, and brachytherapy are used to manage localized prostate cancer.

There was a randomized controlled trial in Sweden. In that trial, the results of the Scandinavian Prostate Cancer Group 4 suggested that RP may improve overall survival if the tumor is detected at an early stage (1). However, biochemical recurrence (BCR) was detected in 25% of all patients who underwent RP for localized prostate cancer during follow-up (2,3).

Hyaluronic acid (HA) is a glycosaminoglycan composed of repeating D-glucuronic acid and N-acetyl-D glucosamine units. Six HA genes were identified in human tissues. HA regulates several cellular functions and molecular mechanisms, including tumor adhesion, migration, and proliferation. HA level is high in several tumors

Received: 13.03.2012 – Accepted: 21.04.2012

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such as breast, colon, bladder, lung, ovary, and, recently, prostate (4-10). Increased expression of HA is associated with proliferation and progression of breast, colon, and ovarian tumors (4,5,10).

The aims of our study were to identify a profile for hyaluronidase-1 (HYAL-1) expression in RP specimens and to correlate it with BCR and other classical prognostic factors.

Material and methods

We analyzed 112 RP specimens of patients with clinical localized prostate cancer who underwent RP between the years 2004 and 2009. This study was approved by the İstanbul Göztepe Research and Training Hospital review board (Date: 21.04.2010, Number: B.10.4.ism.0.4.34.59.21. / 050.01.03). Computed tomography and bone scintigraphy were conducted when the prostate-specific antigen (PSA) level was over 10 ng/dL preoperatively. Partin and Kattan nomograms were calculated for all the patients and lymphadenectomy was performed only when the probability was over 10%. None of the patients received neoadjuvant or adjuvant androgen deprivation therapy or adjuvant radiotherapy or chemotherapy until BCR occurred. All but 12 tissue blocks from 112 patients were analyzed. The remaining tissue blocks from 12 patients could not be evaluated due to tissue loss during specimen staining. Each specimen was evaluated by 2 blinded and experienced pathologists unaware of the patients. BCR was defined as the case of PSA being 0.2 ng/dL or greater in 2 successive measurements after RP. It was found that 29 of 100 patients experienced BCR after RP and 71 were recurrence-free. We checked PSA, digital rectal examinations, and blood parameters (if necessary) every 3 months during follow-up.

Immunohistochemistry

Sections of 3 μm were taken from selected paraffin blocks that were previously coated with poly-L-lysine. Specimen slides were then deparaffinized with xylene for 10 min and rehydrated. Hydrogen peroxide, phosphate buffered saline (PBS), and Ultra V block nonspecific blocking reagent (ScyTek Laboratories, USA) were applied as the antigen retrieval solution. Rabbit HYAL-1 antibody (Atlas Antibodies, Sweden) was then localized at room temperature for approximately 90 min using diluted 1:150 HYAL-1 IgG binding protein purified rabbit nasal cartilage. After incubation with primary reagents, the slides were incubated with Ultra Tek antipolyvalent biotinylated antibody (ScyTek Laboratories) as the secondary antibody for 15 min. After rehydration, Ultra Tek HRP (ScyTek Laboratories) was added to the specimens. After rehydration with PBS, the AEC chromogen system (AEC substrate kit, ScyTek Laboratories) was added to the specimens. Slides were counterstained with hematoxylin. All prostatectomy slides were stained twice and scored independently to determine staining reproducibility.

Slide grading

Two blinded pathologists independently evaluated all slides in a blinded fashion. Tumor-associated stroma and tumor cells were graded for HYAL-1 staining in each slide. Staining was observed for all slides. The intensity of staining of HYAL-1 was graded as 0 (no staining), 1, 2, and 3 for each slide (Figure). The overall staining grade for each slide was assigned based on the staining intensity of the tumor tissue in the specimen, and the average of the scores of the 2 readers was accepted as the specimen score. However, if 50% of the tumor tissue showed grade 1 and the other 50% showed grade 3 staining, the overall staining grade was assigned to be 2. On the other hand, if 50% of the tumor showed grade 2 and the remaining showed grade 3 staining, the overall staining was assigned as 3. The staining scale was then subcategorized into low and high grades. Grade 2 and grade 3 staining represented high grade staining and the others were defined as low grade staining. The readers agreed with each other 90% of the time. Any discrepancy about the grading of slides was resolved by the 2 readers.

Statistical analysis

Statistical analysis was done with NCSS 2007 (version 07.1.20) software. Independent t-tests and chi-square tests were used to compare qualitative data. Cox regression and Kaplan-Meier analysis were used to determine the effects of recurrence and survival rate, respectively. Log rank analysis was used to determine the affecting factors of recurrence and its relation with HYAL-1. The statistical significance level was accepted as 0.05.

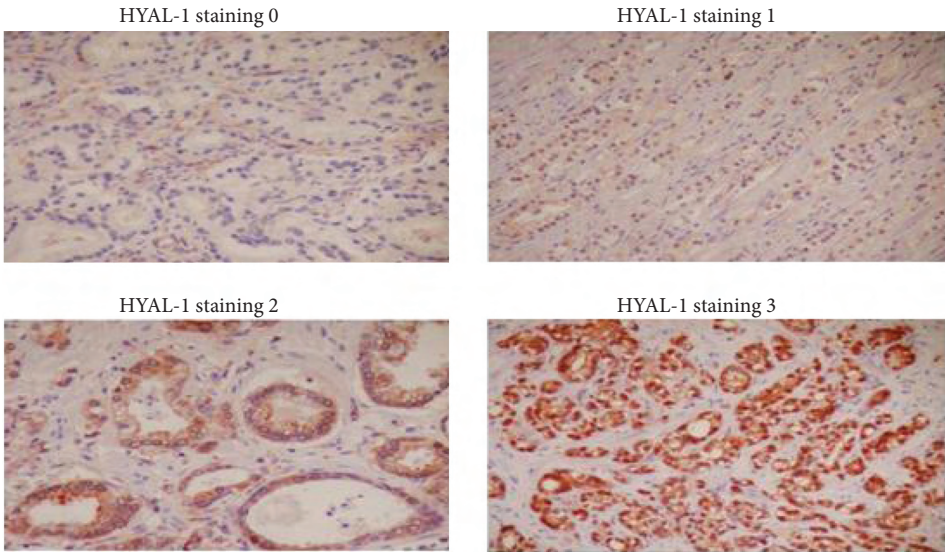

Figure. Examples of staining results for HYAL-1 scores of 0, 1, 2, and 3.

Results

Preoperative mean PSA level was 11.7 ng/dL (ranging from 1.4 to 33) and the mean follow-up time was 59.8 months (ranging from 20 to 92). Mean time to recurrence was 45.7 months (ranging from 36 to 92) (Table 1). Lymphadenectomy was performed in 8 patients based on Partin and Kattan nomograms.

The median age was 66.5 years (ranging from 51 to 72). Twenty-nine of all patients had BCR. Four patients died due to prostate cancer during the follow-up period. The HYAL-1 scores of the patients who died were 1 in 2 patients and 3 in the remaining 2 patients. Among the surviving patients, 7 had grade 1 scores, 37 had grade 2 scores, and 56 had grade 3 scores for HYAL-1.

Sensitivity, specificity, positive predictive value, and negative predictive value to predict BCR for HYAL-1 were 38%, 63%, 30%, and 71%, respectively. In a multivariate analysis that included clinical (age, preoperative PSA, and clinical stage) and pathological (Gleason sum, extraprostatic extension, capsule involvement, seminal vesicle invasion, lymph node involvement, and surgical margin status) parameters and HYAL-1 staining scores, the preoperative PSA level, clinical stage, Gleason score and primary Gleason pattern on biopsy, percentage of tumor on biopsy, Gleason score on RP, primary and secondary Gleason patterns on RP, positive surgical margin, capsule involvement, extraprostatic expansion, and seminal vesicle invasion were found to be significant predictors of BCR (Tables 2 and 3).

Only the preoperative PSA level was significant for recurrence in subgroup analysis including extraprostatic expansion, preoperative PSA, and HYAL-1 staining (Table 4).

We performed Cox proportional hazards analysis to determine clinical and pathological parameters to predict BCR. Gleason score, primary pattern on biopsy, preoperative PSA, percentage of tumor on biopsy, final specimen Gleason score, and primary and secondary Gleason pattern on RP were found statistically significant for recurrence (Tables 2 and 3).

Recurrence-free survival rates were 82.2%, 68.6%, and 68.6% for years 1, 5, and 8, respectively. There was no statistically significant difference between survival rates of cT1 and cT2. Median survival rate was not different between patients with and without BCR (Table 5).

HYAL-1 staining was not found to be related to overall, disease-free, and recurrence-free survival rates.

Table 1. Patient characteristics.

		n	%
Clinical stage	cT1	65	65
	cT2	35	35
Evidence recurrence	Positive	29	29
	Negative	71	71
Surgical margin	Negative	75	75
	Positive	25	25
RP Gleason score	<7	74	74
	≥7	26	26
Capsule involvement	Negative	65	65
	Positive	35	35
Extraprostatic expansion	Negative	84	84
	Positive	16	16
Seminal vesicle invasion	Negative	90	90
	Positive	10	10
Lymph node involvement	Negative	99	99
	Positive	1	1
Progression	Progression (-)	97	97
	Progression (+)	3	3
HYAL-1	Low grade	62	62
	High grade	38	38

Table 2. Factors related to biochemical recurrence.

	BCR (-)	**BCR (+)**	**P-value**
Age (years)	66.3 ± 6.04	67.1 ± 5.77	0.540
Preoperative PSA (ng/dL)	9.18 ± 9.70	18.16 ± 15.53	**0.001**
Gleason score on biopsy	5.65 ± 0.97	6.17 ± 1.20	**0.024**
Primary pattern on biopsy	2.75 ± 0.55	3.07 ± 0.70	**0.017**
Secondary pattern on biopsy	2.9 ± 0.57	3.1 ± 0.72	0.139
Percentage core of tumor on biopsy	0.24 ± 0.14	0.39 ± 0.23	**0.0001**
Gleason score on RP	5.69 ± 1.01	6.76 ± 1.35	**0.0001**
Primary Gleason pattern on RP	2.75 ± 0.53	3.31 ± 0.76	**0.0001**
Secondary Gleason pattern on RP	2.94 ± 0.63	3.59 ± 1.24	**0.001**
Duration of follow-up (months)	58.34 ± 21.95	63.48 ± 21.62	0.288

Table 3. Hazard ratio of related factors to biochemical recurrence.

		BCR (-)		BCR (+)			OR (95% CI)
Clinical stage	T1	53	81.50%	12	18.50%	**P = 0.002**	**4.17**
	T2	18	51.40%	17	48.60%		1.67-10.38
Surgical margin	Negative	58	81.70%	17	58.60%	**P = 0.016**	**3.15**
	Positive	13	18.30%	12	41.40%		1.21-8.17
Gleason sum	<7	59	83.1%	15	51.7%	**P = 0.002**	**4.58**
	≥7	12	16.9%	14	48.3%		1.76-11.95
Capsule involvement	Negative	54	76.10%	11	37.90%	**P = 0.0001**	**5.20**
	Positive	17	23.90%	18	62.10%		2.06-13.14
Extraprostatic expansion	Negative	67	94.40%	17	58.60%	**P = 0.0001**	**11.82**
	Positive	4	5.60%	12	41.40%		3.38-41.30
Seminal vesicle invasion	Negative	69	97.20%	21	72.40%	**P = 0.0001**	**13.14**
	Positive	2	2.80%	8	27.60%		2.59-66.75
HYAL-1	Negative	45	63.38%	18	62.1%	P = 0.902	1.05
	Positive	26	36.62%	11	37.9%		0.43-2.58

Table 4. Subgroup analysis of factors related with BCR.

	P	HR	95.0% CI	
			Lower	Upper
Preoperative PSA	**0.045**	2.52	0.99	6.44
Extraprostatic expansion	0.021	1.15	0.70	2.33
HYAL-1	0.053	1.02	1.00	1.04

Table 5. Survival rates of all patients.

	Overall survival	Disease-free survival
Year 1	0.989 ± 0.011	0.989 ± 0.011
Year 2	0.989 ± 0.011	0.989 ± 0.011
Year 5	0.974 ± 0.019	0.970 ± 0.022
Year 8	0.936 ± 0.032	0.970 ± 0.022

Discussion

Tumor cell penetration through the basement membrane is an important component of tumor progression. Disruption of basement membrane integrity by hyaluronidase involves a complex sequence of cellular and biochemical events.

HA is an important component of the extracellular matrix (ECM), particularly in rapidly proliferating tissues (11). HYAL-1 and HYAL-2 are the major hyaluronidases in human tissues (12,13). HA performs several functions, such as supporting cartilage integrity and maintaining tissue hydration and osmotic balance in normal physiology (14,15). Currently, there is much evidence for the role of HA in tumor invasion and metastasis. HA is elevated in various tumors including bladder, breast, colon, prostate, lung, endometrial, and ovary (4-10,16). The localization of HA is in either tumor-associated stroma or tumor cells (4-7,10,17). Increased expression of HA is associated with malignant progression in several tumors such as breast, colon, and ovarian tumors (5,6,9,10,14). In these tumors, stimulation of independent growth and proliferation of tumor cells may be encouraged with HA (18,19). Moreover, HA may support tumor metastasis by promoting tumor cell proliferation and may also protect against immune surveillance (20-28). Increased hyaluronidase may facilitate ECM degradation and its action may also promote tumor angiogenesis. HYAL-1 is the major hyaluronidase expressed in bladder and prostate cancer tissues (17,25-27).

The aim of our study was to examine the effects of HYAL-1 expression, whose relation with other tumors had been revealed, on the prognosis and progression of prostate cancer. Current nomograms that are used to predict prognosis are not useful. This is why the addition of molecular markers due to heterogeneity of cancer in the nomograms, which consist of clinical and pathological parameters, may result in better prediction. Whether HYAL-1 might be among these molecular markers was one of the starting questions of this study.

In 2009, Gomez et al. reported the relation between BCR and HYAL-1 staining in transrectal ultrasound (TRUS) biopsy specimens. In addition, a software program was used for HYAL-1 staining and evaluation of specimens was performed by 3 pathologists. The BCR cut-off level was 0.4 ng/dL and the median follow-up period was 103 months (28). Furthermore, HYAL-1 staining was statistically significant in predicting recurrence in univariate and multivariate analysis of RP specimens. Posey et al. and Ekici et al. reported the prognostic potential of histological markers for predicting BCR in prostate cancer patients, such as HA, HYAL-1, CD44v6, and microvessel density (29,30). The prognostic value of each following item was less than that of the Gleason score: microvessel density, N-acetyltransferase 2 gene polymorphism, Ki-67, p53, p27, P21, bcl-2, PTEN mutation, Pim-1 kinase, EZH2, Bax, DNA ploidy, methylation of the GSTP1 gene, HER-2/neu, E-cadherin, CD44, retinoblastoma proteins, apoptotic index, androgen receptor status, acid phosphates, and IGF-1 expression (31-33). Although HYAL-1 is an important prognostic factor for colon, breast, endometrial, and bladder cancer, there are not enough data about prostate cancer. While HYAL-1 expression was a significant prognostic factor for recurrence in prostate cancer patients in the abovementioned study, our results did not confirm this. HYAL-1 expression was not significant in predicting BCR. Percentage of tumor on biopsy cores, positive surgical margin, clinical stage, final specimen Gleason sum of ≥7, seminal vesicle invasion, capsular penetration, and extraprostatic expansion were the factors that were related to BCR in multivariate analysis in the present study. We think that the minor differences of staining and grading methods, difference of secondary antibodies, and number of pathologists do not explain this discrepancy. However, selection of a PSA cut-off value of 0.2 ng/dL as BCR instead of 0.4 ng/dL, a relatively shorter follow-up period, and a smaller number of patients with Gleason scores of ≥8 in addition to the heterogenic nature of prostate cancer may have had an impact on our results.

HYAL-1 expression is not predictive of BCR in patients with prostate cancer and did not contribute to the use of contemporary clinical and pathologic parameters (preoperative PSA, TRUS biopsy Gleason sum and primary Gleason pattern, percentage of tumor core in biopsy, Gleason sum for RP, and primary and secondary Gleason grade) in predicting BCR. Apparently, additional molecular markers are required to improve our understanding of the prognosis and nature of prostate cancer.

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