Quantitative Detection of Micrometastases in Pelvic Lymph Nodes in Patients with Clinically Localized Prostate Cancer by Real-time Reverse Transcriptase-PCR

Hideaki Miyake,[1] Isao Hara,[1] Toshifumi Kurahashi,[1] Taka-aki Inoue,[2] Hiroshi Eto,[2] and Masato Fujisawa[1]

Abstract

Purpose: Routine pathologic examination can miss micrometastatic tumor foci in the lymph nodes of patients with prostate cancer, resulting in confusion during tumor staging and clinical decision-making. The objective of this study was to clarify the significance of micrometastases in pelvic lymph nodes in patients who underwent radical prostatectomy for prostate cancer.

Experimental Design: The expression of prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA) in 2,215 lymph nodes isolated from 120 patients with clinically localized prostate cancer was assessed by a fully quantitative real-time reverse transcriptase-PCR. We regarded specimens in which either PSA or PSMA mRNAs were positive as proof of the "presence of micrometastasis." Immunohistochemical staining of lymph node specimens with an antibody against PSA was also done.

Results: Pathologic examinations detected tumor cells in 29 lymph nodes from 11 patients, and real-time reverse transcriptase-PCR further identified micrometastasis in 143 lymph nodes from 32 patients with no pathologic evidence of lymph node involvement. The presence of micrometastatic cancer cells was confirmed by immunohistochemical staining in 61 lymph nodes from 17 patients with pathologically negative lymph nodes. The presence of micrometastases was significantly associated with other conventional prognostic variables, including serum PSA value, pathologic stage, Gleason score, and tumor volume. Biochemical recurrence was detected in 32 patients, 17 of whom were negative for lymph node metastasis by pathologic examination (including 4 patients with pathologically organ-confined disease), but were diagnosed as having micrometastasis. Biochemical recurrence–free survival rate in patients without micrometastasis was significantly higher than in those with micrometastasis irrespective of the presence of pathologically positive nodes. Furthermore, only the presence of micrometastasis was independently associated with biochemical recurrence regardless of other factors examined.

Conclusions: These findings suggest that ~30% of clinically localized prostate cancers shed cancer cells to the pelvic lymph nodes, and that biochemical recurrence after radical prostatectomy could be explained, at least in part, by micrometastases in pelvic lymph nodes.

Pelvic lymph node metastasis has been considered the most important predictive factor of disease recurrence in patients with clinically localized prostate cancer who have undergone radical prostatectomy. Patients with organ-confined prostate cancer have a good prognosis and a low risk of disease recurrence following radical prostatectomy, whereas biochemical recurrence, characterized by an increasing serum prostate-specific antigen (PSA) value, occurs in ~10% of patients in this category (1, 2). Because routine microscopic examination of lymphadenectomy specimens can miss small cancer foci, this finding might partially account for the presence of histologically undetectable micrometastases in the pelvic lymph nodes. In fact, various investigators have shown that higher sensitivity for detecting micrometastatic cancer cells in surgically removed pelvic lymph nodes at radical prostatectomy can be achieved by several molecular and histologic techniques targeting prostate-specific gene expression, including reverse transcriptase-PCR (RT-PCR) and immunohistochemical staining (3–6). To date, however, none of these methods have been introduced into clinical practice due to various limitations, such as a high false-positive rate and complicated procedures. Collectively, these findings suggest that an improved approach for detecting micrometastatic prostate cancer cells in the lymph nodes needs to be identified.

Recently, a real-time detection and quantitative PCR-based assay was developed (7). The advantage of this assay is the

Authors' Affiliations: [1]Department of Urology, Kobe University School of Medicine, Kobe, Japan and [2]Department of Urology, Hyogo Medical Center for Adults, Akashi, Japan

Received 12/13/05; revised 5/3/06; accepted 5/15/06.

Grant support: Grant-in-Aid from the Ministry of Education, Science, and Culture of Japan.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Hideaki Miyake, Department of Urology, Kobe University School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Phone: 81-78382-6155; Fax: 81-78382-6169; E-mail: hideakimiyake@hotmail.com.

doi:10.1158/1078-0432.CCR-05-2706

specific detection of rare events; that is, sensitivity has been shown to allow for the detection of 10 to 100 pg of RNA from the target gene. Furthermore, it is highly reproducible and quantitative, significantly eliminating the risks of contamination encountered with other types of PCR-based assays, and requires no post-PCR product manipulation. Accordingly, the method has been widely used for accurately detecting occult micrometastatic tumor burden in resected lymph node specimens (8–12). For example, Van Trappen et al. used real-time RT-PCR targeting the cytokeratin 19 gene, and reported that ~50% of early stage cervical cancers shed tumor cells to the pelvic nodes, and the amount of cytokeratin 19 expression was related to the clinicopathologic features (8). To our knowledge, however, there has not been any study analyzing lymph node specimens obtained from patients with prostate cancer using real-time RT-PCR assay in order to clarify the significance of micrometastases in biochemical recurrence after successful radical surgery.

Expression of the PSA and prostate-specific membrane antigen (PSMA) genes is exclusively restricted to prostate epithelial cells (4), and this high specificity made it possible to identify metastatic prostate cancer cells among non–prostate cells. Moreover, these two genes are expressed heterogenously in prostate epithelial cells (4); thus, simultaneous targeting of these two specific antigens might promote the detection of metastatic prostate cancer cells with a wide phenotypic spectrum. Considering these findings, we did a fully quantitative real-time RT-PCR assay targeted against PSA and PSMA gene expression in 2,215 fresh pelvic lymph nodes obtained from 120 patients with clinically localized prostate cancer, then analyzed the clinical significance of occult micrometastasis of prostate cancer cells to pelvic lymph nodes.

Patients and Methods

Surgical specimens. This study was approved by the research ethics committee of our institution, and informed consent was obtained from all patients at the time of enrollment. Lymph node specimens were obtained from 120 patients with clinically localized prostate cancer who underwent radical retropubic prostatectomy and pelvic lymphadenectomy without neoadjuvant therapies between October 2001 and July 2004. Pelvic lymphadenectomy was done, targeting the obturator fossa and external iliac region by removing all fatty, connective, and lymphatic tissue. Lymph node samples were also available from seven female patients with invasive bladder cancer who underwent radical cystectomy. Each lymph node was bisected. One half was snap-frozen immediately and stored at −80°C until assessed, and the remainder was fixed in formalin, embedded in paraffin, and stained with H&E for histopathologic examination. In this series, all pathologic examinations were done by a single pathologist according to the Unio Internationale Contra Cancrum (tumor-node-metastasis) tumor stage classification (13). Biochemical recurrence was defined as a serum PSA level of ≥0.2 ng/mL; none of the patients received any additional therapies until their serum PSA levels reached ≥0.4 ng/mL.

Real-time RT-PCR assay. Total RNA was extracted from lymph node specimens using the acid guanidinium isothiocyanate, phenol chloroform method, and 1 μg of each total RNA was reverse-transcribed using an Oligo dT and Superscript preamplification system (Life Technologies, Rockville, MD). To analyze the expression levels of PSA, PSMA, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs, real-time quantitative PCR was done using Sequence Detector (ABI PRISM 7700; PE Applied Biosystems, Foster City, CA). The sequences of primers and probes for these genes were determined by Primer Express software (PE Applied Biosystems). Selected sequences of forward (F) and reverse (R) primers, and probes are as follows: PSA F, 5′ CGTGGATTGGTGCTGCAC 3′; PSA R, 5′-TGGGAATGCTTCTCGCACTC-3′; PSA probe, 5′-CCTGTCTCGGATTGTGGGAGGCTG-3′; PSMA F, 5′-TTCTGTTAAAAGCAGTGCTTCCAT-3′; PSMA R, 5′-CGTATTTCGAGGGAGAGAATAGCTA-3′; PSMA probe, 5′-CACGGCCTCTCTCACGGATTATAAGAACACA-3′; GAPDH F, 5′-GAAGGTGAAGGTCGGAGTC-3′; GAPDH R, 5′-GAAGATGGTGATGGGATTTC-3′; GAPDH probe, 5′-CAAGCTTCCCGTTCTCAGCC-3′. The probes used in this study consisted of an oligodeoxynucleotide with a 5′ FAM (6-carboxy-fluorescein) reporter dye and 3′ TAMRA (6-carboxy-tetramethylrhodamine) quencher dye. Each complementary DNA was analyzed by quantitative PCR in a 50 μL volume using Master Mix (PE Applied Biosystems). The thermal cycling conditions were composed of 50 cycles of amplification consisting of 15 s at 95°C and 1 min at 60°C.

Real-time quantitation was done based on TaqMan assay according to the manufacturer's instruction as described previously (14, 15). After the generation of a real-time amplification plot based on normalized fluorescence signals, the threshold cycle (Ct), which is the fractional cycle number at which the amount of amplified target reached a fixed threshold, was determined. The Ct was then used for kinetic analysis and was proportional to the initial number of target copies in the sample. The starting quantity of a sample was calculated after comparison to the Cts of a serial dilution of a positive control, human prostate cancer LNCaP cells (American Type Culture Collection, Rockville, MD). All serial dilutions were carried out in duplicate, and the reactions to generate standard curves were repeated twice (each time in triplicate). All clinical specimens were also analyzed in triplicate and the mean values were used for quantification. The coefficient of variation for triplicate reactions was <10%, and the coefficient of variation between assays was also <10%. In this series, except for samples in which PSA and/or PSMA were not amplified, the ranges of Ct values for PSA and PSMA were as follows: PSA, 17.2 to 43.4; PSMA, 13.3 to 41.1.

Both the precise amount and quality of total RNA added to each reaction mix are extremely difficult to assess; therefore, transcripts of the GAPDH gene were quantified as an internal reference according to a quantitative PCR assay. The quantification value of PSA or PSMA mRNA was described as each value relative to GAPDH mRNA. To exclude false positives, we used the mean relative mRNA value plus 2 SDs of PSA or PSMA mRNA expression in 148 lymph nodes from female patients with bladder cancer as the cutoff value for PSA or PSMA, respectively, and values above the cutoff value for PSA or PSMA mRNA were defined as PSA- or PSMA mRNA–positive, respectively. In this study, we regarded specimens in which PSA and/or PSMA mRNA were positive as proof of the "presence of micrometastasis."

Immunohistochemical staining. In cases diagnosed as having micrometastases according to real-time RT-PCR, despite the lack of positive findings on routine pathologic examinations, sections adjunct to the site of the original sections for H&E staining were cut from the original formalin-fixed, paraffin-embedded blocks, and examined to determine whether occult foci of prostate cancer cells were present by immunohistochemical staining with a monoclonal antibody against PSA (Dako, Carpinteria, CA) using standard immunohistochemical techniques as reported previously (16). After staining, the sections were counterstained with hematoxylin. All slides were reviewed by the same pathologist without knowledge of any clinicopathologic data, as described above.

Statistical analysis. Differences between the two groups were compared using the χ^2 test, unpaired t test, or Mann-Whitney U test. The biochemical recurrence–free survival rates were calculated by the Kaplan-Meier method, and the difference was determined by log rank test. Forward stepwise logistic regression analysis was used to determine the association between several variables and biochemical recurrence. The significance of several factors in the time to biochemical recurrence was assessed by the Cox proportional hazards regression model. All statistical calculations were done using StatView 5.0 software

Table 1. Outcomes of histologic examination and real-time RT-PCR assay

	Group A	Group B	Group C
No. of patients	11	32	77
No. of dissected lymph nodes	201	619	1,395
Histologic examination			
No. of positive patients	11	0	0
No. of positive lymph nodes	29	0	0
Real-time RT-PCR assay for PSA			
No. of positive patients	11	23	0
No. of positive lymph nodes	51	84	0
Real-time RT-PCR assay for PSMA			
No. of positive patients	11	29	0
No. of positive lymph nodes	71	112	0
Micrometastasis			
No. of positive patients	11	32	0
No. of positive lymph nodes	82	143	0

NOTE: Group A, patients with histologically confirmed lymph node metastases; group B, patients with micrometastases despite the lack of histologic evidence indicating nodal involvement; and group C, patients without any findings of lymph node metastases.

(Abacus Concepts, Inc., Berkeley, CA), and $P < 0.05$ was considered significant.

Results

The expression of GAPDH mRNA in all lymph node specimens was confirmed. In 148 lymph nodes from seven female patients with bladder cancer, the mean values of relative PSA and PSMA mRNAs expression plus 2 SDs were 2.8 and 4.9, respectively, and these values were used as cutoff points for the positive expression of PSA and PSMA mRNA in lymph nodes from patients with prostate cancer in the subsequent study. Real-time RT-PCR assays in 2,215 pelvic lymph nodes from patients with clinically localized prostate cancer detected various amounts of relative expression levels of PSA and PSMA mRNAs (PSA: mean, 2.5; median, 0.6; range, 0-193; PSMA: mean, 4.4; median, 1.2; range, 0-792).

Twenty-nine of the 201 lymph nodes from 11 patients with prostate cancer showed histopathologic evidence of metastatic involvement, and real-time RT-PCR confirmed the expression of PSA and PSMA mRNAs in 29 and 28 nodes, respectively. In these 11 patients, positive PSA and/or PSMA mRNA expression was detected in an additional 53 histologically uninvolved lymph nodes; thus, a total of 82 lymph nodes were diagnosed as having occult micrometastases using real-time RT-PCR assay. Of the 2,014 nodes from the remaining 109 patients without histologic evidence of pelvic lymph node metastases, positive PSA and PSMA mRNA expression were detected in 84 nodes from 23 patients and 112 nodes from 29 patients, respectively. Among these, 53 nodes from 20 patients were judged positive for both PSA and PSMA mRNAs expression; therefore, a total of 32 patients were regarded as having micrometastases to pelvic lymph nodes. The relative expression levels of PSA and PSMA mRNAs in 225 nodes considered positive for micrometastases were as follows: (PSA) mean, 17.2; median, 15.1; range 1.9 to 193; (PSMA) mean, 32.4; median, 24.4; range, 3.1 to 792. These outcomes are summarized in Table 1 by dividing 120 patients into the following three groups: 11 with histologically detected lymph node metastases (group A), 32 with micrometastases despite the lack of histologic evidence indicating nodal involvement (group B), and the remaining 77 without any findings of lymph node metastases on histologic and real-time RT-PCR analyses (group C).

The incidence of micrometastases according to anatomic location was analyzed. Similar metastatic patterns of prostate cancer cells to the external iliac region and obturator fossa were observed between groups A and B, irrespective of the presence of histologically confirmed nodal involvement. We further compared clinicopathologic features among these three groups. As shown in Table 2, despite the absence of significant differences between groups A and B in several of the factors examined, preoperative serum PSA, pathologic stage, Gleason score, and tumor volume in groups A and B were significantly greater than those in group C.

The median follow-up period of the 120 patients included in this study was 38 months (range, 15-48 months). In this series, biochemical recurrence occurred in 8, 17, and 7 patients in groups A, B, and C, respectively (Table 3). The median intervals between radical prostatectomy and biochemical recurrence in groups A, B, and C were 6, 11, and 17 months, respectively. As

Table 2. Comparison of conventional prognostic indicators according to lymph node metastases detected by histologic examination and real-time RT-PCR assay

	Groups			*P*		
	A	B	C	A vs. B	B vs. C	C vs. A
No. of patients	11	32	77			
Preoperative serum PSA (ng/mL)*	25.3 ± 24.7	20.2 ± 18.7	9.8 ± 6.9	0.48	<0.0001	<0.0001
Pathologic stage (no. of patients)				0.23	0.0008	<0.0001
pT_2	1	11	55			
pT_3	9	20	22			
pT_4	1	1	0			
Gleason score*	8.1 ± 4.1	7.5 ± 3.9	6.1 ± 2.9	0.67	0.041	0.046
Tumor volume (cm^3)*	2.5 ± 1.7	2.0 ± 1.4	0.92 ± 0.65	0.34	<0.0001	<0.0001

NOTE: Group A, patients with histologically confirmed lymph node metastases; group B, patients with micrometastases despite the lack of histologic evidence indicating nodal involvement; group C, patients without any findings of lymph node metastases.
*Data are presented as mean ± SD.

Table 3. Incidence of biochemical recurrence according to lymph node metastases detected by histologic examination and real-time RT-PCR assay

	Groups			*P*		
	A	B	C	A vs. B	B vs. C	C vs. A
No. of patients	11	32	77			
No. of patients with biochemical recurrence (%)	8 (72.7)	17 (53.1)	7 (9.1)	0.26	<0.0001	<0.0001
Mean time to biochemical recurrence after radical prostatectomy (mo)*	9.5 ± 9.9	14.9 ± 7.6	21.0 ± 9.9	0.86	0.032	0.0004
Pathologically organ-confined disease						
No. of patients with biochemical recurrence/total no. of patients	0/0	4/11	2/55	—	0.0006	—
Pathologically extraprostatic disease						
No. of patients with biochemical recurrence/total no. of patients	8/11	13/21	5/22	0.54	0.092	0.0056

NOTE: Group A, patients with histologically confirmed lymph node metastases; group B, patients with micrometastases despite the lack of histologic evidence indicating nodal involvement; group C, patients without any findings of lymph node metastases.
*Data are presented as mean ± SD.

shown in Fig. 1, biochemical recurrence–free survival rates in groups A and B were significantly lower than that in group C. However, there was no significant association between the number of positive nodes for micrometastases as well as quantitative values of PSA and PSMA expression with biochemical recurrence (data not shown). In addition, of the 66 patients with pathologically organ-confined disease, only 6 developed biochemical recurrence, among whom 4 were diagnosed as having micrometastases in the pelvic lymph nodes (Table 3).

To evaluate the association between several clinicopathologic factors with biochemical recurrence, multivariate analysis using a stepwise logistic regression model was done. As shown in Table 4, only the presence of micrometastasis was independently related to whether or not biochemical recurrence occurred. Furthermore, multivariate analysis using the Cox regression hazard model showed that only the presence of micrometastasis was independently associated with biochemical recurrence–free survival, irrespective of other factors examined in this study (Table 4).

To further confirm the presence of micrometastatic diseases in pelvic lymph nodes, immunohistochemical stainings were done with a monoclonal antibody against PSA in 143 lymph nodes from 32 patients diagnosed as having micrometastases using real-time RT-PCR assays (despite the lack of pathologic evidence of nodal involvement). Sixty-one of the 143 lymph nodes (from 17 patients) were evidently stained with PSA antibody. Representative results are shown in Fig. 2.

Discussion

Lymph node metastasis is the most useful factor predicting poor prognosis in patients undergoing radical prostatectomy for clinically localized prostate cancer. However, ~30% of such patients without evidence of pathologic nodal involvement will develop biochemical disease recurrence (1, 2). Although the etiology of biochemical disease recurrence following radical prostatectomy is likely multifactorial, a significant proportion of these recurrences might be due to occult metastases to pelvic lymph nodes undetected by routine pathologic examinations. Several investigators have assessed whether microscopic foci of prostate cancer cells are present in histologically uninvolved pelvic nodes using molecular and histochemical approaches (3–6), but the clinical significance of micrometastases in pelvic nodes remains controversial. Because accurate staging of prostate cancer facilitates the prediction of therapeutic outcomes and appropriate tailoring of adjuvant therapies to the individual patient, we investigated PSA and PSMA mRNA expression in 2,215 pelvic lymph nodes dissected at radical prostatectomy from 120 patients with clinically localized prostate cancer using quantitative real-time RT-PCR assay, evaluated the sensitivity of this assay for detecting occult lymph

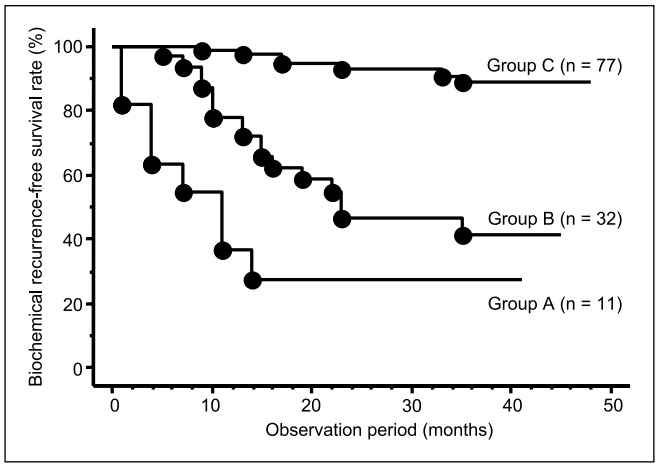

Fig. 1. Comparison of biochemical recurrence – free survival rates in groups A, B, and C using the Kaplan-Meier method. The biochemical recurrence – free survival rates in groups A and B were significantly lower than that in group C ($P = 0.059$, group A versus group B; $P < 0.0001$, group B versus group C; $P < 0.0001$, group C versus group A using the log-rank test).

Table 4. Multivariate analyses of various factors in relation to biochemical recurrence

Variables	*P* value	
	Stepwise logistic regression model	Cox proportional hazards regression model
Serum PSA, ng/mL (<10 vs. ≥10)	0.55	0.61
Pathologic stage (pT_2 vs. pT_3 or pT_4)	0.23	0.51
Gleason score (6 or 7 vs. 8, 9, or 10)	0.14	0.10
Tumor volume, cm^3 (<1.0 vs. ≥1.0)	0.44	0.33
Micrometastasis (negative vs. positive)	0.040	0.032

node metastases, and analyzed various clinicopathologic factors according to the assay findings.

In this series, standard pelvic lymphadenectomy targeting the external iliac region and obturator fossa for all 120 patients was done, and the mean number of lymph nodes removed at radical prostatectomy in these patients was 18.5. Based on an autopsy study, approximately 20 lymph nodes have been shown to serve as a guideline for optimal and representative pelvic lymph node dissection (17), suggesting that the procedure for pelvic lymphadenectomy done in this study, which met this requirement, would be suitable. We also examined 148 pelvic lymph nodes obtained from seven female patients undergoing radical cystectomy for invasive bladder cancer to determine the appropriate cutoff points for the positive expression of PSA and PSMA mRNAs on real-time RT-PCR. Although it is a potentially crucial point to reduce the false positivity of real-time RT-PCR, it is usually difficult to establish cutoff points on this assay for diseases lacking specific markers. However, PSA and PSMA gene expressions are highly restricted to prostate epithelial cells (4); that is, although it is inevitable to detect extremely low levels of PSA and PSMA expressions considering the principle of this assay, lymph nodes from females theoretically do not express these genes, indicating that the cutoff points used in this study were properly determined. Furthermore, in order to avoid underestimating the significance of micrometastases of prostate cancer cells, the expression levels of both PSA and PSMA mRNAs in each node, which were shown to be heterogenously expressed in prostate cancers (4), were measured, and nodes diagnosed as positively expressing PSA and/or PSMA mRNA were judged to be the presence of micrometastatic cancer foci. Collectively, these findings suggest that the present study was carried out under ideal conditions, which contributes to the reliability of the current outcomes.

We diagnosed the presence of occult micrometastasis in 225 lymph nodes from 43 patients using real-time RT-PCR assay, including 29 histologically involved nodes from 11 patients. This proportion of micrometastases to pelvic lymph nodes was significantly high compared with that reported in previous studies evaluated by RT-PCR (3, 4), suggesting that the real-time RT-PCR assay used in this study was more sensitive than conventional RT-PCR. In addition, the differences in the procedures between real-time RT-PCR and conventional RT-PCR contribute to the enhanced specificity; that is, it does not require post-PCR manipulation, and quantitation and calculation are all automated. In fact, immunohistochemical staining with PSA antibody detected micrometastatic cancer foci in approximately half of the pelvic nodes diagnosed as positive for micrometastasis despite the lack of histologic findings.

Characterization of clinicopathologic features according to nodal status showed that there were no significant differences in several conventional prognostic factors between patients with histologically detected nodal involvement and those with nodes positive for micrometastases despite the lack of histologically positive findings. Anatomic locations of micrometastatic nodes were also similar between these two patient groups. In addition, the proportion of patients positive for micrometastases was closely related to several poor prognostic indicators (data not shown). These findings strongly suggest that even with the lack of histologic confirmation, some of the

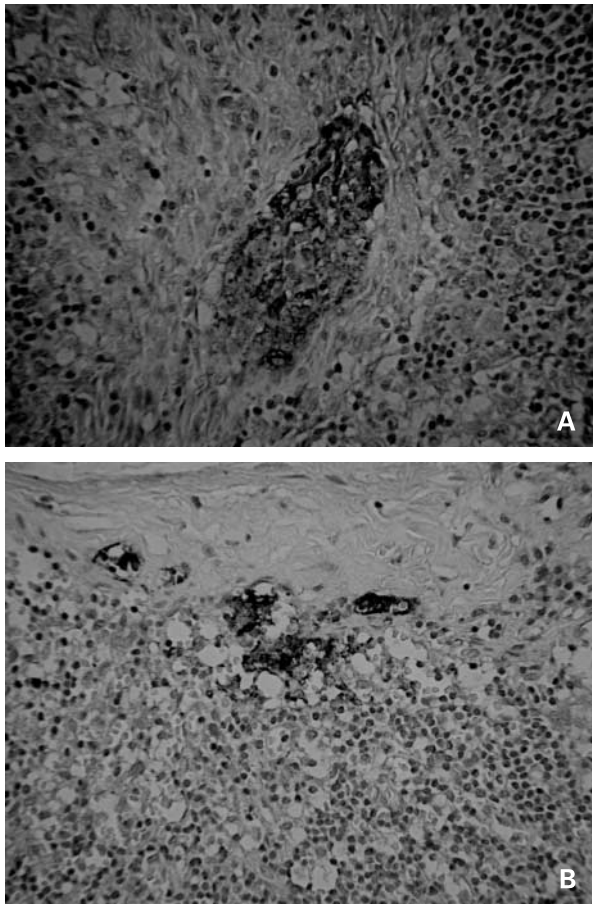

Fig. 2. Representative results of immunohistochemically detected micrometastatic cancer foci using a monoclonal antibody against PSA in histologically uninvolved lymph node specimens (*A* and *B*).

micrometastatic diseases diagnosed by the current real-time RT-PCR assay have biological characteristics similar to those of histologically positive nodal diseases. This hypothesis was supported by the incidence of biochemical recurrence following radical prostatectomy. Although the follow-up period of this study was too short to draw conclusions concerning the prognosis, there were no significant differences in the incidence of biochemical recurrence between these two groups. In addition, biochemical recurrence in four patients with pathologically organ-confined disease (diagnosed as positive for micrometastases) also supports this hypothesis. Furthermore, the presence of micrometastasis was independently associated with whether or not biochemical recurrence occurred—as well as the time to biochemical recurrence. Although longer follow-up periods are absolutely necessary to draw a definitive conclusion, the present findings suggest that some micrometastases in pelvic lymph nodes may, at least in part, contribute to the development of biochemical recurrence following radical prostatectomy.

To further address the significance of micrometastases in prostate cancer, several problems should be elucidated. For example, it would be of interest to investigate whether histologically undetectable or dormant micrometastatic disease in the lymphatic system will always progress to clinically significant recurrence after variable disease-free recurrence. If not, it will be necessary to develop a diagnostic system differentiating significant micrometastatic diseases from insignificant disease. Recent studies have reported the possible effect of lymphadenectomy on the survival of patients with pathologically confirmed positive nodes who underwent radical prostatectomy (18, 19). If there is a survival benefit in pelvic lymph node dissection for such patients, it would be interesting to evaluate whether removing micrometastatic nodes affects the prognosis. Recently, several investigators showed the usefulness of novel approaches for detecting occult prostate cancer metastases in lymph nodes (20, 21). For example, Shariat et al. reported that a splice variant–specific RT-PCR targeting the human glandular kallikrein gene can detect biologically and clinically significant micrometastases of prostate cancer in histopathologically normal lymph nodes (21). The assessment of these issues may facilitate the determination of a more appropriate procedure for lymphadenectomy considering the findings on molecular staging.

In conclusion, the results of this study showed the usefulness of quantitative real-time RT-PCR targeting the expression of PSA and PSMA genes for identifying micrometastatic tumor foci in pelvic lymph nodes from clinically localized prostate cancer at radical prostatectomy. Although longer follow-up periods are absolutely necessary to draw a definitive conclusion, the present findings suggest that some micrometastases in pelvic lymph nodes may, at least in part, contribute to the development of biochemical recurrence after radical prostatectomy.

References

1. Walsh PC, Partin AW, Epstein JI. Cancer control and quality of life following anatomical radical retropubic prostatectomy: results at 10 years. J Urol 1994;152:1831–6.
2. Catalona WJ, Smith DS. Cancer recurrence and survival rates after anatomic radical retropubic prostatectomy for prostate cancer: intermediate-term results. J Urol 1998;160:2428–34.
3. Okegawa T, Nutahara K, Higashihara E. Detection of micrometastatic prostate cancer cells in the lymph nodes by reverse transcriptase polymerase chain reaction is predictive of biochemical recurrence in pathological stage T2 prostate cancer. J Urol 2000;163:1183–8.
4. Potter SR, Mangold LA, Shue MJ, et al. Molecular and immunohistochemical staging of men with seminal vesicle invasion and negative pelvic lymph nodes at radical prostatectomy. Cancer 2000;89:2577–86.
5. Ferrari AC, Stone NN, Eyler JN, et al. Prospective analysis of prostate-specific markers in pelvic lymph nodes of patients with high-risk prostate cancer. J Natl Cancer Inst 1997;89:1498–504.
6. Wawroschek F, Wagner T, Hamm M, et al. The influence of serial sections, immunohistochemistry, and extension of pelvic lymph node dissection on the lymph node status in clinically localized prostate cancer. Eur Urol 2003;43:132–7.
7. Gibson UE, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. Genome Res 1996;6:995–1001.
8. Van Trappen PO, Gyselman VG, Lowe DG, et al. Molecular quantification and mapping of lymph-node micrometastases in cervical cancer. Lancet 2001;357:15–20.
9. Mrras M, Mikhitarian K, Walters C, et al. Quantitative real-time RT-PCR detection of breast cancer micrometastasis using a multigene marker panel. Int J Cancer 2001;93:162–71.
10. Yoshioka S, Fujiwara Y, Sugita Y, et al. Real-time reverse transcriptase-polymerase chain reaction for intraoperative diagnosis of lymph node micrometastasis: clinical application for cervical lymph node dissection in esophageal cancers. Surgery 2002;132:34–40.
11. Kubota K, Nakanishi H, Hiki N, et al. Quantitative detection of micrometastases in the lymph nodes of gastric cancer patients with real-time RT-PCR: a comparative study with immunohistochemistry. Int J Cancer 2003;105:136–43.
12. Inokuchi M, Ninomiya I, Tsugawa K, Terada I, Miwa K. Quantitative evaluation of metastases in axillary lymph nodes of breast cancer. Br J Cancer 2003;89:1750–6.
13. Sobin LH, Wittekind CH, editors. TNM classification of malignant tumors. 5th ed. New York: Wiley-Liss; 1997.
14. Das H, Koizumi T, Sugimoto T, et al. Quantitation of Fas and Fas ligand gene expression in human ovarian, cervical and endometrial carcinomas using real-time quantitative RT-PCR. Br J Cancer 2000;82:1682–8.
15. Kurahashi T, Hara I, Oka N, Kamidono S, Eto H, Miyake H. Detection of micrometastases in pelvic lymph nodes in patients undergoing radical cystectomy for locally invasive bladder cancer by real-time reverse transcriptase-PCR for cytokeratin 19 and uroplakin II. Clin Cancer Res 2005;11:3773–7.
16. Kurahashi T, Muramaki M, Yamanaka K, Hara I, Miyake H. Expression of the secreted form of clusterin protein in renal cell carcinoma as a predictor of disease extension. BJU Int 2005;96:895–9.
17. Weingartner K, Ramaswamy A, Bittinger A, et al. Anatomical basis for pelvic lymphadenectomy in prostate cancer: results of an autopsy study and implications for the clinic. J Urol 1996;156:1969–73.
18. Bader P, Burkhard FC, Markwalder R, et al. Disease progression and survival of patients with positive lymph nodes after radical prostatectomy. Is there a chance of cure? J Urol 2003;169:849–54.
19. Cheng L, Zincke H, Blute ML,et al. Risk of prostate carcinoma death in patients with lymph node metastasis. Cancer 2001;91:66–73.
20. Haas CJ, Wagner T, Wawroschek F, Arnholdt H. Combined application of RT-PCR and immunohistochemistry on paraffin embedded sentinel lymph nodes of prostate cancer patients. Pathol Res Pract 2005;200:763–70.
21. Shariat SF, Kattan MW, Erdamar S, et al. Detection of clinically significant, occult prostate cancer metastases in lymph nodes using a splice variant-specific rt-PCR assay for human glandular kallikrein. J Clin Oncol 2003;21:1223–31.