Table 4. Differentially Expressed Genes in Prevaccination Peripheral Blood Mononuclear Cells

Gene Symbol	Gene Name	Fold-Change[a]	P[b]	Expression[c]	Before and After[d]
PRKAR1A	Protein kinase, cAMP-dependent, regulatory, type I, alpha	−0.82	.049		
LRRN3	Leucine-rich repeat neuronal 3	−0.61	.008		
PCDH17	Protocadherin 17	−0.60	.002		
TTN	Titin	−0.60	.008		
LAIR2	Leukocyte-associated immunoglobulin-like receptor 2	0.60	.032		
RNASE3	Ribonuclease, RNase A family, 3	0.63	.020	G	#
CEACAM6	Carcinoembryonic antigen-related cell adhesion molecule 6	0.65	.010	G	#
AZU1	Azurocidin 1	0.66	.006	G	#
HIST1H4C	Histone cluster 1, H4c	0.71	.025		
PGLYRP1	Peptidoglycan recognition protein 1	0.72	.007	G	#
CEACAM8	Carcinoembryonic antigen-related cell adhesion molecule 8	0.78	.015	G	#
LCN2	Lipocalin 2	1.00	.005	G	#
MPO	Myeloperoxidase	1.04	.001	G	#
CAMP	Cathelicidin antimicrobial peptide	1.09	.007	G	#
DEFA1[e]	Defensin, alpha 1	1.17	.031	G	#
DEFA1[e]	Defensin, alpha 1	1.20	.018	G	#
DEFA1[e]	Defensin, alpha 1	1.26	.018	G	#
DEFA3	Defensin, alpha 3, neutrophil-specific	1.27	.017	G	#
DEFA1[e]	Defensin, alpha 1	1.27	.020	G	#
DEFA1[e]	Defensin, alpha 1	1.30	.015	G	#
CTSG	Cathepsin G	1.32	.003	G	#
DEFA4	Defensin, alpha 4, corticostatin	1.33	.002	G	#
ELA2	Elastase 2, neutrophil	1.36	.002	G	#

[a] Log_2 (short/long).
[b] Limma *P* value.
[c] Preferential expression in granulocyte (G).
[d] Commonly identified in both prevaccination and postvaccination peripheral blood mononuclear cells (#).
[e] Identified by multiple different probes on the gene chip.

Changes in the Gene Expression Profiles in PBMCs After Personalized Peptide Vaccination

To investigate how personalized peptide vaccination affected the gene expression profiles in PBMCs, we further compared them between before and after personalized peptide vaccination in the long-term (n = 16) and short-term survivors (n = 14). The changes were assessed by fold-change ranking (log_2 fold-change <-1.0 or >1.0) together with *P* values ($P < .01$). In the long-term survivors, only 1 gene, titin (*TTN*), was down-regulated (log_2 fold-change $= -1.04$, $P < .001$) after personalized peptide vaccination, whereas no genes were up-regulated. In contrast, as shown in Table 5, 41 genes (47 probes) were up-regulated after personalized peptide vaccination, whereas no genes were down-regulated in the short-term survivors. Notably, many of the 41 up-regulated genes in the short-term survivors were also identified as being differentially expressed in pre- and/or postvaccination PBMCs.

Selection of a Gene Classifier for Predicting Patient Prognosis After Personalized Peptide Vaccination

One of the most important applications of microarray-based gene expression data is the ability to predict clinical endpoints after treatments.[18-20] Thus, we examined whether the gene expression profile obtained by DNA microarray analysis of prevaccination PBMCs would be useful for predicting patient prognosis after personalized peptide vaccination. When a stepwise discriminant analysis method was used to choose a gene set from the 23 probes differentially expressed in the prevaccination PBMCs, a combination of 4 genes, *LRRN3, PCDH17, HIST1H4C*, and *PGLYRP1*, gave the best prediction of short-term survivors, with a sensitivity, specificity,

Table 5. Upregulated Genes After Vaccination in Peripheral Blood Mononuclear Cells From the Short-Term Survivors

Gene Symbol	Gene Name	Fold-Change[a]	*P*[b]	Expression[c]	Before and After[d]
RNASE2	Ribonuclease, RNase A family, 2	1.02	<.001		
SLC4A1	Solute carrier family 4, anion exchanger, member 1	1.06	.008	E	
HEMGN	Hemogen (HEMGN), transcript variant 2	1.08	.001	E	
CEACAM1	Carcinoembryonic antigen-related cell adhesion molecule 1	1.09	<.001	G	After
S100P	S100 calcium-binding protein P	1.09	.001		
ALS2	Amyotrophic lateral sclerosis 2	1.09	.001		
ARG1	Arginase, liver	1.10	<.001	G	After
SLPI	Secretory leukocyte peptidase inhibitor	1.12	<.001	G	After
OLR1	Oxidized low-density lipoprotein (lectin-like) receptor 1	1.14	<.001		After
RETN	Resistin	1.15	.005		
HBQ1	Hemoglobin, theta 1	1.16	.007	E	After
ALAS2[e]	Delta-aminolevulinate, synthase 2	1.19	.004	E	After
MMP9	Matrix metallopeptidase 9	1.22	<.001	G	After
RNASE3	Ribonuclease, RNase A family, 3	1.24	<.001	G	Before, after
HMGXB4	HMG box domain containing 4	1.24	.003		After
SELENBP1	Selenium-binding protein 1	1.24	.003		After
GYPE	Glycophorin E	1.36	.001	E	After
BPI	Bactericidal/permeability-increasing protein	1.36	<.001	G	After
TCN1	Transcobalamin I	1.38	<.001	G	
ORM1	Orosomucoid 1	1.38	<.001		
CEACAM6	Carcinoembryonic antigen-related cell adhesion molecule 6	1.40	<.001	G	Before, after
SNCA[e]	Synuclein, alpha	1.40	.001		After
MPO	Myeloperoxidase	1.44	.002	G	Before, after
SNCA[e]	Synuclein, alpha	1.44	<.001		After
HP	Haptoglobin	1.46	<.001	E	After
CD24	CD24 molecule	1.48	<.001	G	After
IFIT1L	Interferon-induced protein with tetratricopeptide repeats 1-like	1.55	.003		After
EPB42	Erythrocyte membrane protein band 4.2	1.56	.002	E	After
CTSG	Cathepsin G	1.56	.004	G	Before, after
ELA2	Elastase 2, neutrophil	1.74	.002	G	Before, after
PGLYRP1	Peptidoglycan recognition protein 1	1.77	<.001	G	Before, after
DEFA1[e]	Defensin, alpha 1	1.79	<.001	G	Before, after
CEACAM8	Carcinoembryonic antigen-related cell adhesion molecule 8	1.80	<.001	G	Before, after
HBM	Hemoglobin, mu	1.86	.005	E	After
DEFA4	Defensin, alpha 4, corticostatin	1.91	<.001	G	Before, after
ALAS2[e]	Delta-aminolevulinate, synthase 2	1.94	.005	E	After
CAMP	Cathelicidin antimicrobial peptide	2.03	<.001	G	Before, after
LCN2	Lipocalin 2	2.04	<.001	G	Before, after
OLFM4	Olfactomedin 4	2.05	<.001		After
DEFA3	Defensin, alpha 3, neutrophil-specific	2.12	<.001	G	Before, after
DEFA1[e]	Defensin, alpha 1	2.12	<.001	G	Before, after
DEFA1[e]	Defensin, alpha 1	2.16	<.001	G	Before, after
DEFA1[e]	Defensin, alpha 1	2.25	<.001	G	Before, after
ERAF	Erythroid associated factor	2.29	.002	E	After
CA1	Carbonic anhydrase I	2.45	<.001	G	After
HBD	Hemoglobin, delta	2.48	.001	E	After
DEFA1[e]	Defensin, alpha 1	2.73	<.001	G	Before, after

[a] $\log_2$ (postvaccination/prevaccination).
[b] Limma *P* value.
[c] Preferential expression in granulocytes (G) and erythroid cells (E).
[d] Identified as differentially expressed genes in prevaccination and/or postvaccination peripheral blood mononuclear cells.
[e] Identified by multiple different probes on the gene chip.

positive predictive value, negative predictive value, and accuracy of 85%, 75%, 77%, 83%, and 80%, respectively (Table 6). Importantly, when this 4-gene classifier was used in 13 new independent cancer patients as a validation test, prognosis was correctly predicted in 12 of the 13 patients with a sensitivity, specificity, positive predictive

Table 6. Selection of a Gene Classifier for Predicting Short-Term Survival

Training/Test	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Accuracy (%)
Training, n = 40	17/20 (85)	15/20 (75)	17/22 (77)	15/18 (83)	32/40 (80)
Test, n = 13	7/7 (100)	5/6 (83)	7/8 (88)	5/5 (100)	12/13 (92)

value, negative predictive value, and accuracy of 100%, 83%, 88%, 100%, and 92%, respectively, for the prediction of short-term survival (Table 6).

Increase in the Prevaccination Plasma IL-6 Levels in the Patients With Poor Prognosis

Expression of cytokines, chemokines, and growth factors, which may result from proinflammatory and/or anti-inflammatory tumor microenvironments, gives a broad picture of the immunological status of cancer patients.[32-35] We therefore examined the levels of these soluble factors using a bead-based multiplex assay with prevaccination plasma samples from the long-term and short-term survivors. As shown in Figure 3, the plasma levels of proinflammatory cytokine IL-6 were significantly higher in the short-term survivors than in the long-term survivors ($P = .009$). However, the plasma levels of other cytokines, chemokines, or growth factors, including IL-1Rα, IL-1β, IL-2, IL-2R, IL-4, IL-5, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-α, IFN-γ, TNF-α, G-CSF, GM-CSF, IP-10, RANTES, Eotaxin, MIP-1α, MIP-1β, MCP-1, MIG, VEGF, EGF, HGF, and basic FGF, were not significantly different between the 2 groups (data not shown).

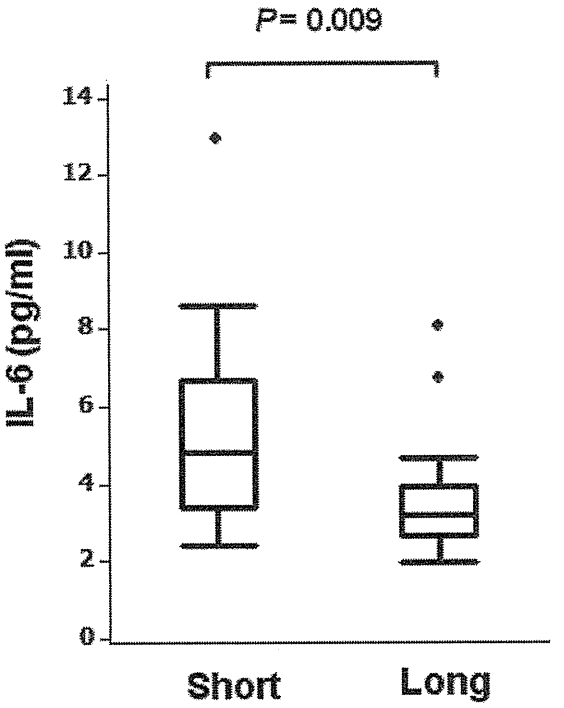

Figure 3. Increase in plasma interleukin (IL)-6 levels in the short-term survivors is shown. The levels of IL-6 assessed by bead-based multiplex assay in prevaccination plasma were compared between the short-term (n = 18) and long-term (n = 18) survivors. Box plots show median and interquartile range (IQR). The whiskers (vertical bars) are the lowest value within 1.5 × IQR of the lower quartile and the highest value within 1.5 × IQR of the upper quartile. Data not included between the whiskers were plotted as outliers with dots. Two-sided *P* value was calculated with Mann-Whitney test.

DISCUSSION

The identification of biomarkers to predict clinical responses to treatment is a challenging but important issue for the development of individualized therapies.[5-8] Although recent advances in high-throughput microarray technology have allowed gene expression profiling for subclassifications of patients in a variety of fields, including organ transplantation and autoimmune diseases,[18-20] little information is available regarding gene expression profiles in peripheral blood of patients treated with immunotherapies. In the current study, to identify promising biomarkers that are predictive of patient prognosis after personalized peptide vaccination, we examined gene expression profiles in PBMCs from 40 advanced castration-resistant prostate cancer patients who showed good or poor prognosis after personalized peptide vaccination. Our DNA microarray analysis in PBMCs identified distinctive genes that were differentially expressed between the long-term and short-term survivors. Interestingly, a statistical prediction model provided a 4-gene classifier that was able to predict patient prognosis with an accuracy of 92% in a validation test, suggesting that the identification of suitable patients for cancer vaccines may be possible with the profiling of a modest number of genes in peripheral blood samples. Because there were no significant differences in the other clinical and pathological

features of the patients enrolled in the current study, except for the number of vaccinations and overall survival, our findings seem to be quite informative for the further development of cancer vaccines.

In the current study, 4 genes, *LRRN3, PCDH17, HIST1H4C*, and *PGLYRP1*, were selected as the best combination for prediction of patient prognosis. *LRRN3* gene encodes a highly conserved transmembrane protein with multiple leucine-rich repeats, which is abundantly expressed in the developing and adult central nervous system. Polymorphisms in this gene were reported to be associated with autism spectrum disorder susceptibility.[36] *PCDH17* is 1 of the cadherin superfamily genes and is expressed predominantly in the nervous system. This molecule was reported to be a tumor suppressor gene candidate in squamous cell carcinomas.[37] *HIST1H4C* gene encodes a member of the histone H4 family, which forms the nucleosome structure of the chromosomal fiber, and may play a central role in transcription regulation, DNA repair and replication, and chromosomal stability.[38] *PGLYRP1* gene encodes a pattern recognition receptor related to innate immunity against bacteria, which is expressed primarily in the granules of granulocytes.[39] Although this information is available from the literature, little is known about the roles of these molecules in immune responses to cancer vaccines. Further studies remain to be done to elucidate them.

One of the most striking features of the differentially expressed genes is that many of the up-regulated genes in both prevaccination and postvaccination PBMCs from the short-term survivors were associated with gene signatures of granulocytes. This may possibly be reflected by the different frequencies of granulocytes in the PBMC fraction purified from peripheral whole blood on density gradient centrifugation using Ficoll-Paque. In healthy donors, normal granulocytes are usually separated from the PBMC fraction on Ficoll-Paque density gradient. However, patients with various types of cancers have been reported to show increased numbers of activated granulocytes in their peripheral blood, which are purified in the PBMC fraction.[40-42] Recently, these abnormal granulocytes have been defined as granulocytic myeloid-derived suppressor cells, which express higher levels of inhibitory molecules, such as ARG1 and inducible nitric oxide synthase,[41,42] and impair the immunological functions of T cells and other immune cells.[43-45] In addition, several studies have recently shown the critical roles for neutrophils, a main subset of granulocytes, in tumorigenesis.[46] Neutrophils have a significant impact on the tumor microenvironment by producing cytokines, chemokines, and other products, such as reactive oxygen species and proteinases, which regulate inflammatory cell activation/recruitment, tumor cell proliferation, angiogenesis, and metastasis.[47-49] For example, recent clinical studies have revealed that the presence of neutrophils in tumors was significantly associated with poor outcomes.[50,51] Unfortunately, because of the limited availability of blood samples, we have not fully characterized the granulocytes that were purified in the PBMC fraction, but it is highly possible that abnormal granulocytes in peripheral blood inhibit beneficial immune responses and lead to poor prognosis after peptide vaccines. The current study might provide a novel treatment approach capable of enhancing the clinical efficacy of cancer vaccines. Recently, chemotherapeutic drugs, such as gemcitabine and 5-fluorouracil, have been shown to selectively eliminate myeloid-derived suppressor cells in mice.[52,53] In addition, targeting of VEGF-mediated signaling using a tyrosine kinase inhibitor, sunitinib, has been reported to block expansion of $CD15^{+}CD14^{-}$ granulocytic myeloid-derived suppressor cells in patients with renal cell cancers.[54] It would thus be possible that accompanying treatments with such chemotherapeutic or molecularly targeted drugs before providing cancer vaccines suppress the gene signatures related to poor prognosis and improve patient outcomes after personalized peptide vaccination.

In addition to the granulocyte-related genes, other interesting genes were also differentially expressed between the long-term and short-term survivors. For example, leukocyte-associated immunoglobulin-like receptor 2 (*LAIR2*), a member of the immunoglobulin superfamily, was down-regulated in the prevaccination PBMCs of short-term survivors. Although not well studied, this molecule has been suggested to function as a proinflammatory mediator by suppressing the homologous immune inhibitor, leukocyte-associated immunoglobulin-like receptor 1 (*LAIR-1*), which is present on several types of mononuclear leukocytes.[55] In addition, another noticeable finding is that several erythroid-specific genes, such as hemoglobin families (*HBQ1, HBM, HBD), ALAS2, GYPE, EPB42, HP*, and *ERAF*, were up-regulated in the postvaccination PBMCs of short-term survivors. The precise roles of these differentially expressed genes in immune responses to cancer vaccines need to be determined.

Interestingly, when the gene expression profiles in PBMCs were compared between before and after personalized peptide vaccination, many of the differentially

expressed genes in prevaccination and/or postvaccination PBMCs, including granulocyte-related and erythroid-related genes, were up-regulated after personalized peptide vaccination in the short-term survivors, but not in the long-term survivors. This finding may be explained by the possibility that induction of granulocyte and erythroid gene signatures may be prevented by personalized peptide vaccination in the long-term survivors.

It should also be noted that the levels of the proinflammatory cytokine IL-6 in prevaccination plasma were significantly elevated in the short-term survivors. IL-6 is a multifunctional cytokine that regulates various aspects of immune responses, acute phase reactions, and hematopoiesis. In particular, IL-6 has been reported to be deeply involved in inflammation associated with cancer development and progression.[34] There have been many studies describing the correlation between IL-6 levels and prognosis in various types of cancers, including prostate cancer.[56-59] Interestingly, IL-6 has been also shown to rapidly generate myeloid-derived suppressor cells from precursors that are present in murine and human bone marrow or PBMCs, in the presence of other cytokines such as GM-CSF,[60,61] although in the current study, the expression levels of plasma IL-6 were not well correlated with expressions of granulocyte-related genes in the microarray analysis (data not shown). Although the role of IL-6 in the immune responses to cancer vaccines still remains to be clarified, it is possible that the blockage of IL-6 signaling would be beneficial for enhancing the therapeutic efficacy of cancer vaccines.

To the best of our knowledge, this is the first study to characterize gene expression profiles in peripheral blood and thereby identify biomarkers for predicting clinical outcomes after peptide vaccines. Our findings suggest that the widely available gene expression profiling in peripheral blood may permit future development of molecular-based personalized immunotherapies through discrimination between patients with good and poor prognoses. Although our experimental approaches were not novel, the ability to predict patient prognosis on the basis of relatively simple assays with easily available peripheral blood samples would be of importance. It may be possible that the current study would provide important information for defining eligibility and/or exclusion criteria for personalized peptide vaccination in castration-resistant prostate cancer patients. Nevertheless, because this is a retrospective study with a limited number of patients, all of whom received personalized peptide vaccination, clinical utility of the identified gene signatures and gene classifier needs to be confirmed in future larger-scale, prospective trials conducted in defined patient populations receiving or not receiving personalized peptide vaccination. In addition, the gene expression profiles identified in the current study remain to be verified by using other, independent methods for mRNA and/or protein quantification.

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Gleason Score Correlation Between Biopsy and Prostatectomy Specimens and Prediction of High-grade Gleason Patterns: Significance of Central Pathologic Review

Kentaro Kuroiwa, Taizo Shiraishi, and Seiji Naito, for the Clinicopathological Research Group for Localized Prostate Cancer Investigators

OBJECTIVES To investigate the significance of dedicated central pathologic review for Gleason score (GS) correlation between the biopsy and radical prostatectomy (RP) specimens and the prediction of high-grade Gleason patterns. A discrepancy in the GS between the biopsy and RP specimens has been reported.

METHODS The Clinicopathological Research Group for Localized Prostate Cancer disease registry collated the data from 1629 patients who had undergone RP from 1997 to 2005. All biopsy and RP specimens were retrospectively re-evaluated by 2 central uropathologists according to the International Society of Urological Pathology consensus. The GS correlation between the biopsy and RP specimens and the presence of high-grade Gleason patterns (4 or 5) were recorded. The GS was categorized into 5 groups (2-4, 5-6, 3 + 4, 4 + 3, and 8-10).

RESULTS Central review significantly increased the exact concordance rate and decreased the undergrading and overgrading rates between the biopsy and RP specimens compared with local review ($P < .05$ for all). In each GS or prostate-specific antigen group, the central review biopsy GS had a significantly greater exact concordance rate with the RP specimen GS compared with the local review biopsy GS ($P < .05$ for all). Regarding high-grade Gleason patterns in the RP specimens, central review showed significantly greater sensitivity, positive predictive value, and negative predictive value than local review ($P < .05$ for all).

CONCLUSIONS We have demonstrated that central review using the International Society of Urological Pathology consensus improves the GS correlation and better predicts high-grade Gleason patterns compared with local review. We recommend central pathologic review by dedicated uropathologists for multi-institutional studies using data from prostate biopsy and RP specimens. UROLOGY 77: 407–411, 2011. © 2011 Elsevier Inc.

The Gleason grading system, proposed by Gleason[1] and represented as the Gleason score (GS) for each case, is the most widely used histologic grading system for prostate cancer. The GS in both biopsy and radical prostatectomy (RP) specimens is a powerful prognostic factor.[2,3] Accurate GS correlation between the biopsy and RP specimens is mandatory for preoperative estimation of the disease and for the planning treatment of each patient. However, the biopsy GS has been reported to have been undergraded in 18%-60% and overgraded in 6%-25% of specimens compared with the RP specimen GS.[4-11] Investigator error is one important factor for the discrepancy; thus, pathologic assessment by dedicated uropathologists might improve the GS correlation between the biopsy and RP specimens. Modern GS assessment according to the 2005 International Society of Urological Pathology (ISUP) consensus, reflecting contemporary changes regarding prostate cancer and the Gleason grading system, has shown better GS correlation than the previous assessment.[12] Pathologic assessment by dedicated uropathologists in a single academic institution has also shown better GS correlation than outside assessment.[6,11] However, the usefulness of pathologic assessment by dedicated uropathologists using the ISUP consensus for a large RP series from multiple institutions has not yet been studied.

Although high-grade Gleason patterns (4 or 5) in RP specimens, either a primary/secondary pattern or a ter-

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From the Department of Urology, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan; and Department of Pathologic Oncology, Mie University Graduate School of Medicine, Tsu, Japan

Reprint requests: Kentaro Kuroiwa, M.D., Ph.D., Department of Urology, Kyushu University Graduate School of Medical Sciences, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582 Japan. E-mail: humeiten@hotmail.com

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tiary pattern, have been reported to be related to a poor outcome, it remains unclear how effectively the biopsy GS determined by pathologic assessment by dedicated uropathologists will predict for high-grade Gleason patterns in the RP specimens.[13-15]

In the present, large-scale, multicenter study, we used the pathologic assessment by dedicated uropathologists according to the ISUP consensus for the biopsy and RP specimens from a large RP series with high-grade biopsy GSs using data from the Clinicopathological Research for Localized Prostate Cancer (CRPC) disease registry. The CRPC collates data from patients with clinically localized prostate cancer accrued from 108 academic and community practices throughout Japan. From 1997 to 2005, approximately 5000 patients with clinically localized prostate cancer who had undergone RP were consecutively enrolled into the CRPC registry after obtaining institutional review board approval from each institution.

MATERIAL AND METHODS

Patient Population

According to the CRPC data, the pathologic slides of the biopsy and RP specimens were available for 1650 patients with Stage cT1c-T3 disease and no preoperative therapy at 48 institutions that agreed to send the pathologic slides for central review. After excluding 21 patients (1.3%) without cancer cells in the biopsy specimens by central review, 1629 patients constituted the final cohort for the present study. In all patients, the diagnosis was made by systemic biopsy (≥6 cores). A total of 365 patients (22.4%) had only 6 cores taken at biopsy; 760 patients (46.7%) had ≥10 cores on taken at biopsy. The median number of biopsy cores taken was 8 (range 6-33). All RP specimens were processed using the whole mount technique at each institution. Preoperative information, including the serum prostate-specific antigen levels, and the original pathologic reports were available for all patients. The clinical stage was determined from the digital rectal examination findings and assigned according to the 2002 American Joint Committee on Cancer staging system.

Pathologic Analysis

The biopsy GS of each patient's original pathologic report was recorded as the local review biopsy GS. All pathologic slides and the biopsy and RP specimens were sent to, and reviewed by, 2 dedicated uropathologists (K.K. and T.S.) who were unaware of the original pathologic reports of each patient. In addition, the 2 uropathologists were unaware of the results from the biopsy specimens of each patient when reviewing the matching RP specimens, because the review of the RP specimens was separated from the review of the biopsy specimens. The Gleason pattern was assigned as the central review biopsy and RP GS according to the modified Gleason grading system using the ISUP consensus.[16] The GS was categorized into 5 groups (2-4, 5-6, 3 + 4, 4 + 3, and 8-10). For the biopsy specimens with multiple positive cores, a global GS was recorded, because the GS of each core was not available in most (>95%) of the original pathologic reports. For central review, the reporting rules for a secondary pattern occupying <5% and a tertiary pattern conformed to the ISUP consensus.[16] For the RP specimens, the global GS considering the entire tumor within the prostate as 1 lesion was recorded. A tertiary Gleason pattern in the RP specimens was not reflected as a primary or secondary pattern on the final RP GS. The presence of high-grade Gleason patterns (4 or 5), including tertiary patterns, in the RP specimens was recorded.

Statistical Analysis

Spearman's rank correlation coefficients for the GS in the biopsy and RP specimens were generated. The chi-square test was used for the comparison of the exact GS concordance rate between the local and central pathologic review and for the sensitivity, specificity, positive predictive value, and negative predictive value for the depiction of high-grade Gleason patterns. Two-sided P values were calculated; the significance level was set at 5%. All statistical analyses were performed using the Statistical Package for Social Sciences, version 17.0 (SPSS, Chicago, IL).

RESULTS

Clinical Characteristics

For the 1629 patients whose CRPC data were analyzed, the median age was 65 years (range 44-84), and the median prostate-specific antigen level 8.0 ng/mL (range 0.5-85.9). Of the 1629, patients, 1058 (64.9%) had Stage cT1c disease.

GS in Biopsy and RP Specimens

By central review, no patient (0%) had GS 2-4 disease in the biopsy specimens compared with 107 patients (6.6%) who had GS 2-4 by local review. Of the 107 patients with local review biopsy GS of 2-4, central review found a biopsy GS of 5-6, 3 + 4, 4 + 3, and 8-10 in 66 (61.7%), 35 (32.7%), 4 (3.7%), and 2 (1.9%), respectively. In the other GS groups, the distribution of the central biopsy GS was 5-6 in 545 (33.5%), 3 + 4 in 602 (37.0%), 4 + 3 in 257 (15.8%), and 8-10 in 225 (13.8%). The corresponding distribution by local review for the biopsy GS was 687 (42.2%), 379 (23.3%), 192 (11.8%), and 264 (16.2%; Table 1). Of the patients with a biopsy GS of 5-6, 3 (0.6%) of 545 by central review and 138 (20.1%) of 602 by local review had GS 5. Exact concordance between the local and central biopsy GS was observed for 841 patients (51.6%). The undergrading and overgrading rate for local review was 32.6% and 15.8%, respectively. Spearman's rank correlation coefficient for local biopsy GS and central biopsy GS was 0.607. The central review RP GS distribution for GS 5-6, 3 + 4, 4 + 3, and 8-10 was 423 (26.0%), 675 (41.4%), 363 (22.3%), and 168 (10.3%), respectively.

GS Correlation Between Biopsy and RP Specimens

Table 2 lists the correlation between the local review biopsy GS and central review RP GS. The exact concordance rate and the concordance rate within ±1 GS group was 41.3% (672 of 1629) and 81.7% (1331 of 1629), respectively. The undergrading and overgrading rate for

Table 1. Biopsy Gleason score correlation between local review and central review

Local Review Biopsy GS	Central Review Biopsy GS (n)					Exact Concordance Rate (%)	Local Review	
	2-4	5-6	3 + 4	4 + 3	8-10		Undergrading Rate (%)	Overgrading Rate (%)
2-4 (n = 107)	0	66	35	4	2	0.0	100.0	0.0
5-6 (n = 687)	0	388	233	50	16	56.5	43.5	0.0
3 + 4 (n = 379)	0	64	225	62	28	59.4	23.7	16.9
4 + 3 (n = 192)	0	13	60	84	35	43.8	18.2	38.0
8-10 (n = 264)	0	14	49	57	144	54.5	0	45.5
Total (n = 1629)	0	545	602	257	225	51.6	32.6	15.8

GS, Gleason score.

Table 2. Gleason score correlation between local review biopsy and central review prostatectomy specimens

Local Review Biopsy GS	Central Review RP GS (n)					Exact Concordance Rate (%)	Undergrading Rate in Biopsy (%)	Overgrading Rate in Biopsy (%)
	2-4	5-6	3 + 4	4 + 3	8-10			
2-4 (n = 107)	0	42	48	14	3	0.0	100.0	0.0
5-6 (n = 687)	0	282	286	97	22	41.0	59.0	0.0
3 + 4 (n = 379)	0	73	204	86	16	53.8	26.9	19.3
4 + 3 (n = 192)	0	16	65	85	26	44.3	13.5	42.2
8-10 (n = 264)	0	10	72	81	101	38.3	0.0	61.7
Total (n = 1629)	0	423	675	363	168	41.3	39.3	19.5

RP, radical prostatectomy; GS, Gleason score.

Table 3. Gleason score correlation between central review biopsy and prostatectomy specimens

Central Review Biopsy GS	Central Review RP GS (n)					Exact Concordance Rate (%)	Undergrading Rate in Biopsy (%)	Overgrading Rate in Biopsy (%)
	2-4	5-6	3 + 4	4 + 3	8-10			
2-4 (n = 107)	0	0	0	0	0	—	—	—
5-6 (n = 687)	0	335	173	27	10	61.5	38.5	0.0
3 + 4 (n = 379)	0	83	391	113	15	65.0	21.3	13.8
4 + 3 (n = 192)	0	2	76	160	19	62.3	7.4	30.4
8-10 (n = 264)	0	3	35	63	124	55.1	0.0	44.9
Total (n = 1629)	0	423	675	363	168	62.0	21.9	16.1

Abbreviations as in Table 2.

the biopsy specimens was 39.3% and 19.5%, respectively. Of the 107 patients with a biopsy GS of 2-4, all had an RP GS of ≥5-6, including 65 patients (60.1%) with a RP GS of ≥7. Spearman's rank correlation coefficient for the local biopsy GS and central RP GS was 0.459.

Table 3 lists the correlation between the central biopsy GS and the central RP GS. The exact concordance rate and the concordance rate within ±1 GS group was 62.0% (1010 of 1629) and 94.4% (1537 of 1629), respectively. The undergrading and overgrading rate for the biopsy specimens was 21.9% and 16.1%, respectively. Central review had a significantly greater exact concordance and lower undergrading and overgrading rates than did the local review ($P < .05$ for all). Spearman's rank correlation coefficient for central biopsy GS and central RP GS was 0.687. In each GS group, the central review biopsy GS (GS 5-6, 61.5%; 3 + 4, 65.0%; 4 + 3, 62.3%; and 8-10, 65.1%) had a significantly greater exact concordance rate than did the local review biopsy GS (GS 5-6, 41.0%; 3 + 4, 53.8%; 4 + 3, 44.3%; and 8-10, 38.3%; $P < .05$ for all). In each prostate-specific antigen group, the central review biopsy GS (<4.0 ng/mL, 56.6%; 4.1-10 ng/mL, 64.1%; 10.1-20 ng/mL, 60.7%; and >20 ng/mL, 56.4%) had a significantly greater exact concordance rate than the local review biopsy GS (<4.0 ng/mL, 56.6%; 4.1-10 ng/mL, 64.1%; 10.1-20 ng/mL, 60.7%; and >20 ng/mL, 56.4%; $P < .05$ for all).

High-Grade Gleason Patterns (4 or 5)

The number of patients with Gleason pattern 4 or 5 in the biopsy GS as a primary or secondary pattern was 846 (51.9%) in the local review and 1084 (66.6%) in the central review.

Overall, 1371 patients (84.2%) had Gleason pattern 4 or 5 on RP specimens on the central pathology review of the RP specimens. Of these, 1206 (88.0%) had Gleason pattern 4 or 5 as the primary or secondary pattern. The remaining 165 (12.0%) with RP GS 3 + 3 had a high-grade Gleason pattern of <5% on the RP specimens.

Table 4 lists the correlation of high-grade Gleason patterns between the biopsy GS and RP specimens. The central review GS had significantly greater sensitivity and a significantly greater positive and negative predictive values ($P < .05$ for all).

Table 4. High-grade Gleason patterns (4 or 5) in biopsy Gleason score and prostatectomy specimens

Review	High-Grade GP in Biopsy GS	High-Grade GP in RP Specimens (n)		Sensitivity	Specificity	PPV	NPV
		Positive	Negative				
Local	Positive	797	49	0.581	0.810	0.942	0.140
	Negative	574	206				
Central	Positive	1052	32	0.767	0.876	0.970	0.415
	Negative	319	226				
P value				<.001	.053	.003	<.001

GP, Gleason pattern; NPV, negative predictive value; PPV, positive predictive value; other abbreviations as in Table 2.

COMMENT

In the pretreatment setting for prostate cancer in which clinicians can only use the biopsy information for histologic grade, a more accurate GS correlation between the biopsy and RP specimens must result in more precise evaluation of the disease, regardless of the treatment type planned. However, studies investigating the GS correlation between the biopsy and RP specimens have shown considerable discrepancy—especially of undergrading in biopsy specimens.[4-11] Although the number of patients involved in these studies has varied from 28 to 1455, very few men had high-grade biopsy GSs.[8-10] The present study included the largest number of patients with high-grade biopsy GS (local review 264, central review 168) for investigating the correlation of the GS between the biopsy and RP specimens. Pathology error and sampling error are thought to be the main reasons for the discrepancy.

Steinberg et al[11] previously reported that pathologists at an academic center had a better GS correlation than those at community sites. According to their recent study of 1455 patients, Fine and Epstein[6] reported that the exact GS concordance rate was improved in both community sites (from 34% to 70%) and an academic center (multiple pathologists; from 58% to 76%) compared with the rate in their older study. The effects of education and pathologists' efforts in the United States might have contributed to this improvement.

The present study had some differences from that conducted by Fine and Epstein.[6] First, each Gleason pattern was assigned according to the ISUP consensus, which was published in 2005 after their study period (2002-2003). Second, we used the global GS, considering the entire tumor within the prostate as 1 lesion for both the biopsy and the RP specimens because the GS of each core was not available in most (>95%) of the original pathologic reports. The use of the global GS should be considered a weakness of the present study. In the study by Fine and Epstein,[6] the RP GS was recorded from the dominant tumor or highest grade tumor. However, it was not clearly reported whether the global or highest core GS had been used for the biopsy specimens. Although almost all preoperative nomograms have used the highest core grade of the given case when multiple cores with different GSs are present, and urologists have tended to use the greatest GS to determine their treatment plan, some clinicians might use the global GS. ISUP did not actually specify that the highest core GS should be used for the biopsy GS in each case.[2,16,17] Third, the present study included significantly more patients with greater biopsy and RP specimens than the previous study. In the present study, 67% of the biopsy and 74% of the RP specimens had a GS of ≥7 compared with the previous 26% and 23%, respectively.[6,11] This might have resulted from patient selection bias and ethnic differences in the patients with prostate cancer, because the present cohort of patients underwent RP at academic or community institutions in Japan.[18] In addition to the differences in the distribution of GS, the division of GS 7 into 3 + 4 and 4 + 3 might explain the relatively low exact concordance rate in our study. When GS 3 + 4 and 4 + 3 were combined as 1 entity, the exact concordance rate was high (73.6%) in the present study. However, a GS of 3 + 4 and that of 4 + 3 have different biologic behavior and should not be combined into 1 category.[19]

Reflecting contemporary changes regarding prostate cancer and the Gleason grading system, the ISUP proposed a modified Gleason grading system in 2005.[16] The ISUP consensus has been reported to minimize biopsy undergrading and improve the GS correlation compared with the previous system.[12] In the present study, including patients who underwent RP from 1997 to 2005, biopsy GS 2-4 was originally diagnosed at each institution in 14.6% of all patients compared with 1.6% in another study.[6] ISUP recommended that a GS 2-4 should rarely, if ever, be considered, because of the poor correlation with the RP GS. Most expert uropathologists would not have assigned a GS of 2-4 even before the ISUP consensus.[20] In our study, all locally reviewed biopsy GS 2-4 specimens were upgraded by the central review and 61% actually had a RP GS of ≥7, including 3 patients with a RP specimen GS of 8-10. In addition, no RP specimens in the present study was graded with a GS of 2-4. For the GS categories other than 2-4, we also showed that central review using the ISUP consensus gave a more accurate GS correlation than local review, including biopsy GS 8-10. However, the exact concordance rate was far from perfect (100%) and was less satisfactory even when a central review using the ISUP consensus was done. The actual GS of each patient can be apparent only after RP has been performed. We believe this is an advantage for RP compared with other

treatment modalities that offer patient surveillance and adjuvant treatment according to the biopsy GS only.

High-grade Gleason patterns, either a primary/secondary pattern or a tertiary pattern, in RP specimens have been related to a poor outcome.[13-15] We have demonstrated that the central review biopsy GS using the ISUP consensus is superior to the local review biopsy GS in terms of predicting high-grade Gleason patterns in the RP specimens. It has been reported that the highest core GS has the largest effect on a significant upward shift of the biopsy GS among the reporting rules of the ISUP consensus.[21] Because we used a global biopsy GS for the central review, the difference in the interpretation of each Gleason pattern between the local review and central review might explain our results for high-grade Gleason patterns.

CONCLUSIONS

This is the first study to investigate the significance of dedicated pathologic reassessment using the ISUP consensus for biopsy and RP specimens from academic and community practices. Central pathologic review resulted in a more accurate GS correlation and prediction of high-grade Gleason patterns. We believe that more educational effort is needed for both pathology and urology communities to disseminate the ISUP consensus. We recommend central pathology review by dedicated uropathologists for a study of prostate biopsy and RP specimens from patients at multiple institutions, although the central review will cost more and is time-consuming. We should carefully interpret multicenter study data that have not included a central review. In addition, the exact concordance rate was far from perfect (100%) and was not satisfactory even when a central review using the ISUP consensus was done. Also, the actual GS of each patient can be apparent only when RP has been performed.

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ORIGINAL ARTICLE

Biochemical outcome of small-volume or insignificant prostate cancer treated with radical prostatectomy in Japanese population

Yasuhiro Hashimoto · Akiko Okamoto · Atsushi Imai · Tohru Yoneyama · Shingo Hatakeyama · Takahiro Yoneyama · Takuya Koie · Noritaka Kaminura · Chikara Ohyama

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Abstract
Background We investigated the biochemical outcome of small-volume prostate cancers [tumor volume (TV) < 0.5 mL, SVCa] and insignificant prostate cancers (TV <0.5 mL without any Gleason pattern 4/5 elements, InsigCa) treated with radical prostatectomy.
Methods Between April 2000 and May 2010, 609 patients with prostate cancer underwent radical prostatectomy at Hirosaki University Graduate School of Medicine. Of these, 237 were excluded from the study because of preoperative adjuvant therapy. The remaining 372 patients underwent routine histopathological and TV evaluations. Biochemical recurrence (BCR) was defined as the presence of prostate-specific antigen (PSA) levels greater than 0.2 ng/mL after prostatectomy.
Results The median patient age was 68 years (range 48–78 years) and the median preoperative PSA level was 7.50 ng/mL. The mean follow-up period was 45.9 months and the mean TV was 2.16 mL. Sixty patients (16.3%) had SVCa and 14 (3.7%) had InsigCa. The 5-year BCR-free survival rate for patients with SVCa was 67.3% and that for patients with a TV of 0.5 or greater was 87.1%. A significant difference was seen between the groups using the log-rank test ($P = 0.008$). We could not identify any BCR in patients with InsigCa.
Conclusion Despite the limited number of cases, patients with InsigCa did not develop BCR whereas 12.9% of those with SVCa developed BCR after radical prostatectomy within 5 years. Accurate prediction of the biochemical outcome of SVCa remains difficult and further studies are needed.

Keywords Prostate cancer · Small-volume cancer · Insignificant cancer · Tumor volume

Y. Hashimoto · A. Okamoto · A. Imai · T. Yoneyama · S. Hatakeyama · T. Yoneyama · T. Koie · N. Kaminura · C. Ohyama
Department of Urology, Hirosaki University Graduate School of Medicine, 5 Zaifu Cho, Hirosaki 036-8562, Japan

Y. Hashimoto (✉) · A. Okamoto · A. Imai
Kidney Research Institute Hirosaki Hospital, 90 Yamazaki Kozawa, Hirosaki 036-8243, Japan
e-mail: bikkuri@opal.plala.or.jp

Introduction

Screening of prostate-specific antigen (PSA) levels and extensive biopsy procedures have clearly shown stage migration of prostate cancer and a significant number of small-volume, low-risk prostate cancers have been identified [1–3]. However, such screening strategies have led to much earlier identification of localized prostate cancer and thus also over-detection and over-treatment [4].

The European Randomized Study of Screening for Prostate Cancer (ERSPC) and the Göteborg randomised population-based prostate-cancer screening trial were representative randomized prostate cancer screening trials [5, 6]. These studies concluded that prostate cancer mortality was reduced by PSA screening, but the risk of over-diagnosis was substantial. It is therefore doubtful whether all prostate cancer patients should undergo radical treatment.

Recently, active surveillance protocols have been proposed for patients with low-grade, low-stage prostate cancer, and minimally invasive treatment is emerging as a feasible approach for small volume, low-grade prostate cancer [7, 8]. Advances in prostate mapping and imaging (such as 3T-MRI, dynamic contrast-enhanced MRI, MR spectroscopy) and focal therapies such as high-intensity

Table 1 Clinicopathological characteristics of prostate cancer patients

Variable	
No. of patients	372
Age (years)	67.1 ± 5.2 (median 68)
Range (years)	48–78
PSA (ng/ml)	9.59 ± 6.65 (median 7.16)
Range (ng/ml)	0.5–60.12
Clinical stage (%)	
T1c	238 (63.9%)
T2a	93 (25.0%)
T2b	26 (6.9%)
T2c	15 (4.0%)
Biopsy Gleason sum (%)	
6 or less	54 (14.5%)
7	267 (71.7%)
8	24 (6.4%)
9	27 (7.2%)
Postoperative follow-up period (months)	45.9 ± 21.7
Range (months)	4.2–127.8
Pathological stage (%)	
T2a/b	51 (13.7%)
T2c	167 (44.8%)
T3a	135 (36.2%)
T3b	16 (4.3%)
T4	3 (0.8%)
Gleason score (%)	
6	16 (4.3%)
7	243 (65.3%)
8	38 (10.2%)
9	75 (20.1%)
Tumor volume (mL)	2.29 ± 2.63
Range (mL)	0.02–25.71
Surgical margin	
(−)	253 (68.0%)
(+)	119 (31.9%)
BCR (%)	92 (24.7%)

focused ultrasound (HIFU), cryotherapy, and photodynamic therapy have all led to the increasing availability of various therapeutic options for small-volume, low-grade prostate cancer [9–13].

Small-volume prostate cancer is often defined as a tumor volume (TV) of 0.5 mL or less in the entire prostate and patients with small-volume cancer are considered to have a good prognosis [14, 15]. However, in a recent study, Oort et al. [16] analyzed detailed histopathological features of small-volume prostate cancer (TV <0.5 mL, SVCa) and insignificant prostate cancer (TV <0.5 mL without any Gleason pattern 4/5 elements, InsigCa) and found that 1 in 10 patients with SVCa developed biochemical recurrence (BCR) after radical prostatectomy (RP). Furthermore, to the best of our knowledge, not much data is available on the clinical features of InsigCa and SVCa in the Japanese population.

To evaluate the oncological outcomes of SVCa and InsigCa in the Japanese patient population, we retrospectively examined the relationship between biochemical status and clinicopathological features among 609 patients with prostate cancer treated with RP at our clinic.

Materials and methods

Study population

Between April 2000 and May 2010, 609 patients with prostate cancer were treated with RP and dissection of the obturator lymph node at the Hirosaki University Graduate School of Medicine. For low-intermediate risk patients assessed according to D'Amico's criteria, RP was performed without preoperative adjuvant therapy but in high risk patients RP was performed with preoperative adjuvant therapy [17].

Of the 609 patients, 237 were excluded from the study because of preoperative adjuvant treatment. The remaining 372 eligible patients underwent routine histopathological and TV evaluations.

Evaluation of tumor characteristics

All RP specimens were fixed with 10% formalin, stained, and cut serially into 5-mm thick transverse slices. All slices were macroscopically photographed and subdivided into halves or quadrants to fit routine cassettes for further processing. The apex and base were sectioned sagittally for evaluation of the surgical margin. After histological staining, all specimens were evaluated by an experienced pathologist, and the macroscopic slide photographs were evaluated to allow reconstruction of the tumor extent.

Total cancer volume was determined by computer-assisted image analysis (NIH Image, developed and maintained by the National Institutes of Health, Bethesda, MD, USA) and was defined as the sum of the volumes of individual cancer foci [18]. Tumors were initially staged according to the TNM classification, which is generally used for tumor classification at the time of surgery, but retrospectively all pT2 tumors were restaged according to the 2002 TNM staging criteria [19].

SVCa were defined as cancers with a TV less than 0.5 mL, whereas InsigCa were defined as cancers with a TV <0.5 mL without any Gleason pattern 4/5 elements. BCR

Table 2 Pathological characteristics of patients with a TV >0.5 mL and those with SVCa

Tumor characteristics	SVCa	TV >0.5 mL	*P* value
Number	60 (16.4%)	312 (83.5%)	
Tumor volume (mL)	0.24 ± 0.14	2.69 ± 2.70	
Range (mL)	0.02–0.49	0.51–25.71	
TNM 2002			
T2a/b	28 (46.6%)	23 (7.3%)	(pT2 vs. pT3 or pT4)
T2c	23 (38.3%)	144 (46.1%)	
T3a	9 (15.0%)	126 (40.3%)	$P < 0.001$*
T3b		16 (5.1%)	
T4		3 (0.9%)	
Gleason score (%)			
6 or less	14 (16.3%)	2 (0.6%)	(GS ≤7 vs. GS ≥8)
7	41 (77.5%)	202 (64.7%)	
8	4 (4.0%)	34 (10.8%)	$P < 0.001$*
9	1 (2.0%)	74 (23.7%)	
Positive surgical margin (%)	7 (11.6%)	112 (35.8%)	$P = 0.002$*
PSA failure (%)	7 (11.6%)	85 (27.2%)	$P = 0.008$#

* Chi-squared test

\# Log-rank test

Table 3 Pathological characteristics of patients with InsigCa

Tumor characteristics	InsigCa
Number	14 (3.7%)
Tumor volume (mL)	0.16 ± 0.14
Range (mL)	0.02–0.42
TNM 2002	
T2a/b	14 (100%)
T2c	
T3a	
T3b	
T4	
Gleason score (%)	
6 or less	14 (100%)
7	
8	
9	
Positive surgical margin (%)	0 (0%)
PSA failure (%)	0 (0%)

was defined as the presence of PSA levels greater than 0.2 ng/mL after RP.

Statistical analysis

Kaplan–Meier curves were used to evaluate the risk of BCR. The log-rank test and chi-squared test were used for comparisons between groups. The significance level for all analyses was set at $P < 0.05$. SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Table 1 summarizes the clinicopathological characteristics of patients with prostate cancer included in this study. The median patient age at the time of surgery was 68 years (range 48–78 years), and the median preoperative PSA level was 7.50 ng/mL (range 0.50–60.12 ng/mL). The mean follow-up period was 45.9 months, and the mean TV was 2.16 mL.

A total of 60 patients (16.4%) were identified as having SVCa and 14 (3.7%) as having InsigCa. Tables 2 and 3 summarize the histopathological characteristics of the patients with SVCa and InsigCa. The 5-year BCR-free survival rate for patients with SVCa was 67.3% and that for patients with a TV of 0.5 mL or greater was 87.1%; a log-rank test showed that the difference between the two groups was significant ($P = 0.008$; Fig. 1). We could not identify a BCR in patients with InsigCa. Using multivariate Cox proportional hazards model analysis, significant differences were observed for PSA, Gleason score, and surgical margin status, but not for TV (Table 4).

Discussion

The characteristics most commonly used to predict progression of prostate cancer after prostatectomy are Gleason score, histopathological stage, positive surgical margins, and TV [20, 21]. The TV in prostate cancer is clearly associated with prognosis but whether it is an independent prognostic factor or not remains controversial.

Stamey et al. examined the medical records of 379 patients who underwent RP between 1983 and 1992 and found that 14% of patients with a TV of 0.5–2.0 mL, 39% of those with a TV of 2.0–6.0 mL, 61% of those with a TV of 6.0–12.0 mL, and 97% of those with a TV of 12 mL or more were regarded as having lapsed into BCR. They also reported a strong correlation between tumor size and BCR [22]. Furthermore, recent studies have shown a strong correlation between TV and BCR [23, 24]. Kikuchi et al. examined 1302 cases of prostate cancer surgery and found that, while the mean TV before 1995 had been 2.16 mL, since 1995 it was significantly smaller at 1.25 mL.

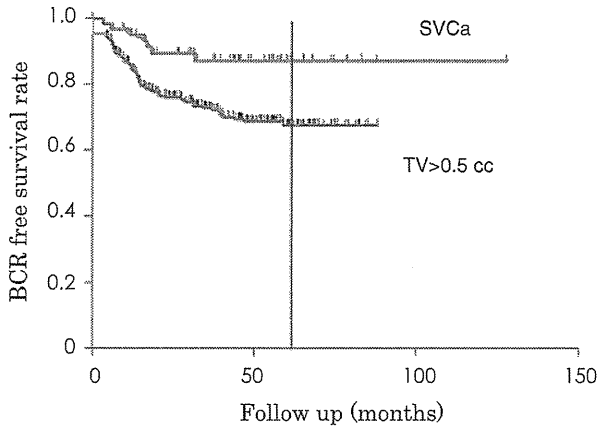

Fig. 1 Kaplan–Meier curves for BCR-free survival. The 5-year BCR-free survival rate for patients with SVCa was 67.3% and that for patients with a TV of 0.5 mL or more was 87.1%. There was a significant difference between the two groups when they were compared with a log-rank test ($P = 0.008$)

Furthermore, after conducting a multivariate analysis, they did not consider TV to be an independent prognostic factor [25].

In the present study, the mean TV was 2.16 mL, and a significant relationship between TV and BCR was observed on univariate analysis. The 5-year BCR-free survival rate for patients with SVCa was 67.3% and that for patients with a TV of 0.5 mL or greater was 87.1%. A significant difference was observed between the two groups by the log-rank test. However, the results of multivariate analysis suggest that TV is inferior to the incidence of BCR, Gleason score, and surgical margin status as BCR prognostic factors. The number of patients with InsigCa was low, and no patients with InsigCa developed BCR after prostatectomy.

Oort et al. [16] analyzed detailed histopathological features of SVCa and InsigCa and found that 1 in 10 patients with SVCa developed BCR after prostatectomy. These findings support our results.

In the present study, only 14 of 372 (3.7%) patients had InsigCa and, of these, none developed BCR. The incidence of InsigCa in our study was significantly less than that in Epstein et al.'s study [26]. Furthermore, the mean TV was 2.16 mL, which was higher than that in Kikuchi et al.'s study (1.3 mL) [25]. These results suggest that the TV in our series of patients was quite large.

As a consequence of stage migration, it is expected that in the future the number of patients with SVCa will increase [27–29]. SVCa is presently considered to be a favorable risk factor; however, about 12% of patients in the present study with SVCa developed BCR.

On multivariate analysis, TV was inferior to the initial PSA level, Gleason score, and surgical margin status as a prognostic factor for PSA failure. Because of stage migration, measurement of TV may become less important.

Table 4 Multivariate analysis of several parameters for BCR

	Hazard ratio	95% confidence interval	*P* value
Tumor volume (mL) (>0.5 vs. ≤0.5)	1.399	0.632–3.094	0.408
PSA (ng/mL) (>7.5 vs. ≤7.5)	1.654	1.041–2.626	0.033
Pathological stage (pT2 vs. pT3 or pT4)	1.58	0.856–2.914	0.143
Gleason score (≤7 vs. 8 or 9)	1.869	1.198–2.917	0.006
Surgical margin [(−) vs. (+)]	3.222	1.791–5.796	<0.001

In conclusion, the TV in prostate cancer remains quite large in Japan, larger than in the USA. The biochemical outcome is not identical in SVCa and InsigCa. Furthermore, accurate prediction of the biochemical outcome in SVCa remains difficult. Our study involved a limited number of cases and a relatively short follow-up period and so we intend to increase the number of cases and follow up the patients for a longer period in a future study.

Conflict of interest The authors have no conflicts of interest to declare.

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特別講演

がんペプチドワクチン：第1世代の実用化と第2世代への展開

久留米大学免疫・免疫治療学講座
伊東恭悟
久留米大学医学部泌尿器科学講座
守屋普久子・末金茂高・松岡啓
久留米大学先端癌治療研究センター臨床研究部門
野口正典

PEPTIDE-BASED CANCER VACCINE: FROM THE FIRST TO SECOND GENERATION

KYOGO ITOH
Department of Immunology and Immunotherapy,
Kurume University School of Medicine, Kurume, Japan
FUKUKO MORIYA, SHIGETAKA SUEKANE AND KEI MATSUOKA
Department of Urology, Kurume University School of Medicine, Kurume, Japan
MASANORI NOGUCHI
Clinical Research Division, Research Center for Innovative Cancer Center,
Kurume University School of Medicine, Kurume, Japan

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特　別　講　演

がんペプチドワクチン：第1世代の実用化と第2世代への展開

久留米大学免疫・免疫治療学講座
伊　東　恭　悟*
久留米大学医学部泌尿器科学講座
守屋普久子・末金茂高・松岡　啓
久留米大学先端癌治療研究センター臨床研究部門
野　口　正　典

抄録：
がんワクチン療法は1991年にがん関連抗原同定法が報告されてから20年間を経て，欧米において医薬品承認される時代を迎え，我が国においても今後3～5年間には承認される可能性が高い。久留米大学で開発中の再燃前立腺がんに対するテーラーメイドペプチドワクチンも高度医療に承認され，また承認申請のための後期臨床試験（企業治験）が間もなく開始される。このように2000年初めより開発されてきたいわゆる第1世代のがんワクチンは実用化時代に入りつつあるといえる。今後，さらに第2世代ともいえる新しいタイプのがんペプチドワクチンの基礎及び臨床研究も進展している。本稿では，がんペプチドワクチンについてその概要を記載したのち，第1世代のがんペプチドワクチン実用化の現状や課題と第2世代への展開について記述する。
（西日泌尿．74：107-117，2012）

キーワード：がんワクチン，ペプチドワクチン，前立腺がん，テーラーメイドワクチン，実用化

1．免疫学の歴史からみたペプチドワクチン療法

ワクチンの歴史は1796年エドワード・ジェナーの種痘に始まる。その後，コレラワクチンなど感染症予防ワクチンが次々と実用化されていった。また免疫学も1884年マクロファージの発見に始まり，中和抗体，血液型，補体が発見され，1940年代からは細胞性免疫にかかわる多くの発見がなされ，1984年の利根川博士のT細胞抗原受容体の同定へと続いた。一方，1954年長野博士らによるインターフェロンの発見は，1980年代のがん治療薬としてのサイトカイン実用化につながり，1975年のケラー・ミリシュタイン博士による単クローン抗体作成技術の発見は，抗体によるがん治療の時代を生み出した。すなわち，がん治療分野では1997年に白血病に対する抗CD20単クローン抗体（リツキサン）が初めて実用化され，その後は毎年のように新規の抗体が実用化されている。このように免疫学の歴史からみた場合，大きな基礎研究成果から20～25年を経て，医薬品として実用化されてきたことが分かる。

さてがんワクチンであるが，免疫学の急速な進歩があったために1980年代には，がん免疫を担当する細胞としては，マクロファージ，ナチュラルキラー（NK）細胞，キラーT細胞及びヘルパーT細胞であることが解明されていた。液性因子としては各種サイトカインや抗体であること，及び，その中で中心的な役割を果たすのはT細胞であることも1980年代には明らかになっていた（**図1左**）。そして，1988年頃になり「がん細胞上の白血球抗原上に結合する9～10個のがん抗原由来のアミノ酸（ペプチド）が患者の免疫系（T細胞）により，がん細胞として認識される」らしいことが予想されるにいたった（**図1右上**）。しかし，T細胞はどのようにがん細胞のどこを認識するのか？その抗原は何か？などについては，まったくの謎であったが，1991年になりBoon博士らが，ヒトのがん関連抗原の同定法を報告し，がん

* 〒830-0011　久留米市旭町67
TEL　0942-31-7551，FAX　0942-31-7699
E-mail：kyogo@med.kurume-u.ac.jp

ワクチンの世界に大きな進展をもたらした[1]。すぐに，その技術（cDNA expression cloning technique）を用いて次々とヒトがん関連抗原が同定された[2)~5)]。そして現在ではがん関連抗原として1000種類以上が同定され，がん細胞上の白血球抗原上に結合する9～10個のがん関連抗原由来のアミノ酸（ペプチド）としては数千にも及ぶことが明らかになっている。それらを大別すると，がん細胞に特異的（遺伝子変異などにより発現）に，もしくは，正常細胞に比べて過剰発現している抗原が大多数を占める。また，胎児性抗原や精巣のみ発現されている抗原，ウイルス由来抗原なども挙げられる（**図1右下**）。

同定された各種がん抗原やペプチドを用いてのがんワクチン臨床試験は主としてメラノーマを対象として1992年頃より開始されたが，それらの臨床試験では失望をもたらす結果になったことが報告された[6)]。しかし，我々はテーラーメイド型にして従来では認められなかった優れた臨床効果を報告した[7)]。そして，つい最近，我々は，無作為比較試験にて標準治療に比べて，ペプチドワクチンが，優れた臨床効果をもたらすことを報告した[8)]。このようにペプチドワクチンの実用化研究は着実に進展している。その成果の一部は国内発の研究である。抗体技術の画期的発見から23年後に最初の抗体医薬が実用化されたことから，1991年から24年後である2015年頃には，最初のペプチドワクチンが実用化されると，国内外から期待されている。

2．ペプチドワクチン療法の原理

がん細胞の目印となる，がん関連抗原は，がん細胞の中で産生と分解を繰り返している。そのがん関連抗原は分解されると短い蛋白質断片（ペプチド）となるが，その一部は主要組織適合抗原（ヒトではヒト白血球抗原，human leukocyte antigen, HLA）分子に結合して，HLA・ペプチド複合体を形成し，がん細胞の細胞膜表面に提示される（**図1右**）。そして複合体が，100個以上の数としてまとまって提示された場合にのみ，宿主のT細胞にとって，がん細胞であることの目印になる。正常細胞では，そのようなHLA・がん関連抗原ペプチド複合体の数は100個以下であるために，T細胞抗原受容体を介してT細胞により認識されることはない。がん細胞ではその数が100個以上あるために，がん細胞として認識される。がん細胞上のHLA-クラスⅠ分子は10000個以上あるため，少なくとも100個の異なるがん抗原ペプチドを提示できることになる。哺乳類では，細胞内での非自己としての危険シグナルをこのような仕組みで，宿主免疫系（T細胞）に伝えることができるために，免疫系による非自己細胞の排除が成立している。すなわち，宿主（T細胞）は，がん細胞と，そうでない正常細胞を，T細胞抗原受容体を介して識別している。T細胞が非自己として認識した場合には速やかに増殖・分化（活性化，賦活化）する。活性化T細胞は，がん細胞を殺傷し（主にキラーT細胞），また抗体を産生させるシグナルをB細胞に指令する（主にヘルパーT細胞）と共に，各種サイ

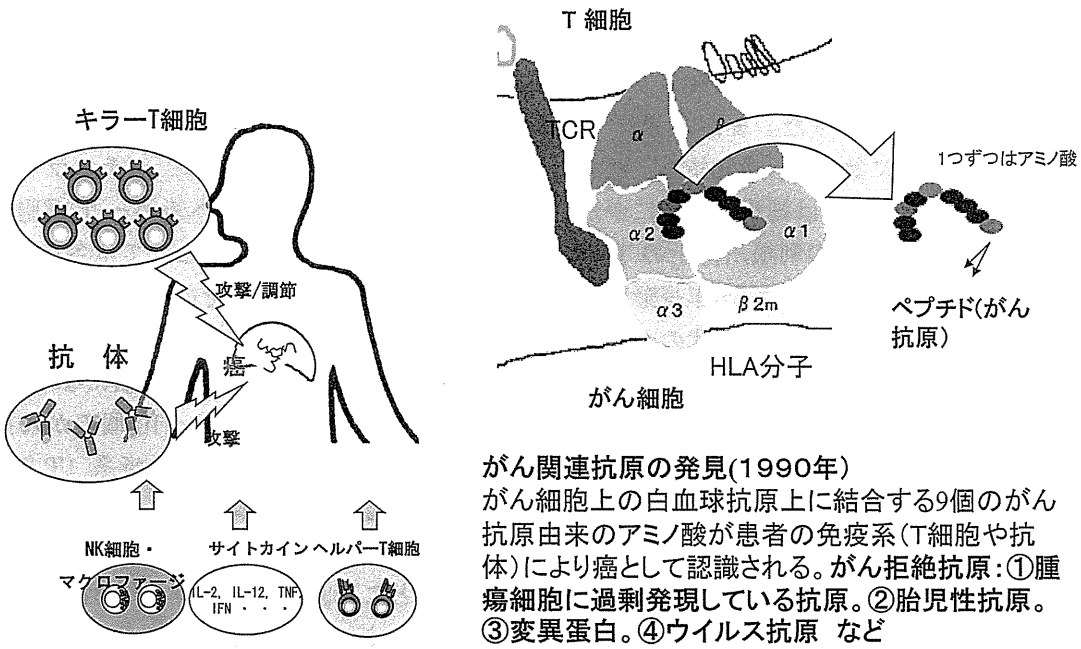

図1　がん免疫系・がん関連抗原の発見