Discrepancy Between Local and Central Pathological Review of Radical Prostatectomy Specimens

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Abbreviations and Acronyms

CR = central review
CRPC = Clinicopathological Research Group for Localized Prostate Cancer
ECE = extracapsular extension
GS = Gleason score
ISUP = International Society of Urological Pathology
LNI = lymph node involvement
LR = local review
PSA = prostate specific antigen
PSM = positive surgical margin
RP = radical prostatectomy
SVI = seminal vesicle invasion

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Purpose: Pathological assessment of radical prostatectomy specimens has not been uniform among pathologists. We investigated interobserver variability of radical prostatectomy specimen reviews between local and central pathologists.
Materials and Methods: We collated data from 50 institutions on 2,015 patients with cT1c-3 prostate cancer who underwent radical prostatectomy between 1997 and 2005. All radical prostatectomy specimens were retrospectively reevaluated by a central uropathologist. Gleason score, extracapsular extension, seminal vesicle invasion, lymph node involvement, positive surgical margin, year of diagnosis and pathology volume were recorded.
Results: The exact concordance rate of Gleason score between local and central review was 54.8%, and under grading and over grading rates at local review were 25.9% and 19.2%, respectively. Spearman's rank correlation coefficient was 0.61 for local and central radical prostatectomy Gleason score. The exact concordance rate of Gleason score 8–10 at local review was significantly lower than that of Gleason score 5–6, 3 + 4 and 4 + 3 at local review ($p = 0.011$, <0.001 and 0.006). Exact concordance rates between local and central review for extracapsular extension, seminal vesicle invasion, lymph node involvement and positive surgical margin were 82.5%, 97.6%, 99.6% and 87.5%, respectively. High volume institutions and recently diagnosed cohorts showed significantly higher exact concordance rates between local and central review for radical prostatectomy Gleason score and other pathological features (all $p < 0.001$).
Conclusions: High volume institutions and recent series show higher concordance between local and central review of radical prostatectomy pathology. However, concordance for high grade Gleason score, extracapsular extension and surgical margin status remains poor. Radical prostatectomy specimens should be reevaluated in a multi-institutional study for more accurate pathological data.

Key Words: pathology, prostatic neoplasms, prostatectomy

PATHOLOGICAL features of radical prostatectomy specimens such as Gleason score, extracapsular extension, seminal vesicle invasion, lymph node involvement and positive surgical margin are crucial observations for physicians to assess the prognosis of each patient. Various nomograms predicting PSA relapse after RP have been constructed based on these pathological features combined with preoperative PSA.[1–3] Therefore, ideally these features should be diagnosed uniformly among pathologists. However, there is concern about

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interobserver variability for pathological features of RP specimens, which would affect prognostic accuracy.

Interobserver variability for biopsy GS among pathologists is well documented.[4–9] Biopsy GS assigned by pathologists at an academic center has been reported as better correlated with RP GS than that by pathologists at community centers.[4,9] However, to our knowledge interobserver variability for RP GS has not been investigated in a large contemporary RP series.

There are only a few studies of interobserver variability for other pathological features of RP specimens.[10–12] It was reported that the exact concordance between local and central review of RP specimens for ECE, SVI and PSM was 57.5%, 94.0% and 69.4%, respectively, in patients with pT3/PSM.[11] On the other hand, expert uropathologists indicated good concordance when evaluating ECE (91.2%, $\kappa = 0.63$) and PSM (90.4%, $\kappa = 0.74$).[12]

We investigated the interobserver variability between local and central pathologists for RP pathological features in a large RP series of 2,015 patients. Central review for GS was based on the 2005 ISUP consensus. In addition, we analyzed the impact of the date of diagnosis and pathology volume on interobserver variability.

MATERIALS AND METHODS

Patient Population

The CRPC disease registry collates data on clinically localized prostate cancer accrued from 108 academic and community practices throughout Japan. Between 1997 and 2005 patients with clinically localized (cT1c–3) prostate cancer who underwent RP were enrolled in the CRPC registry after obtaining institutional review board approval from each center.

Of these CRPC patients pathological slides of biopsy and prostatectomy specimens were available from 50 institutions in 2,015 patients with no preoperative therapy. In all patients preoperative diagnosis was made by systemic biopsy (6 or more cores). Preoperative serum PSA was known for all patients. Clinical stage was determined by digital rectal examination and was assigned according to the 2002 American Joint Committee on Cancer staging system.

Pathological Assessment

Prostatectomy specimens from the patients were processed by a whole mount technique after formalin fixation at each institution.[13] All pathological slides of biopsy specimens were reviewed by a uropathologist (TS). All pathological slides of RP specimens were reviewed by 1 uropathologist (KK) who has reviewed more than 5,000 RP cases. GS was assigned according to the 2005 ISUP consensus, and categorized into 5 groups of 2–4, 5–6, 3 + 4, 4 + 3 and 8–10.[14] Global GS that considered the entire tumor within the prostate as 1 lesion was recorded for RP specimens since the GS of each tumor was not available in the original reports for most patients. The exact concordance rate for categorized Gleason score between original (local) and central review was investigated. Tertiary Gleason pattern in RP specimens was not reflected as primary or secondary pattern on the final RP GS.

The presence of ECE, SVI, LNI and PSM was recorded for all RP specimens. ECE level was further categorized as focal ECE and established ECE.[15] ECE was assigned as positive when tumor cells existed beyond the confines of the prostate.[16] Direct contact between tumor cells and adipose tissue was not needed to assign ECE. The presence of tumor cells at the inked margin of resection was considered a PSM. For specimens that had not been inked before formalin fixation the presence of tumor cells at the noninked margin of resection was considered a PSM. SVI was assigned as positive when tumor cells had invaded into the muscular coat of the extraprostatic seminal vesicle. The positive to negative rate for ECE, SVI, LNI and PSM was defined as No. centrally negative cases in locally positive cases/No. locally positive cases. The negative to positive rate was defined conversely.

Data from original pathological reports for RP GS, ECE, SVI, LNI and PSM were available in 1,774, 1,630, 1,639, 1,914 and 1,579 patients, respectively. All data for ECE, SVI, LNI and PSM were available in 1,526 patients. For influence of date of diagnosis we compared patients diagnosed by local pathologists in 1997 to 2003 with those diagnosed in 2004 to 2005. For pathology volume we defined high volume institutions as those contributing 100 or more patients to the CRPC registry and low volume institutions as those contributing less than 100 patients.

Statistical Analysis

Spearman's rank correlation coefficient (r) on the relationship of RP GS was generated. Simple kappa statistics were used for concordance between local and central review in ECE, SVI, PNI and PSM. The chi-square test was used for comparison of the exact concordance rate between local and central review for each pathological feature. All p values are 2-sided and $p < 0.05$ considered significant.

RESULTS

Preoperative Characteristics

Median patient age was 66 years (range 42 to 84) and median PSA was 8.5 ng/ml (range 0.5 to 85.9). A total of 1,327 patients (65.9%) had cT1c disease. For biopsy specimens the distribution of central biopsy GS 2–4, 5–6, 3 + 4, 4 + 3 and 8–10 was 0.1% (2), 33.6% (677), 27.4% (552), 19.0% (382) and 20.0% (402), respectively (table 1).

Concordance for RP GS

Table 2 shows concordance for RP GS between local and central review. Spearman's rank correlation coefficient was 0.61 for local and central RP GS. Overall exact concordance between central and local review was 54.8%, and the under grading and over grading rate in local review was 25.9% and 19.2%, respectively. When GS 3 + 4 and 4 + 3 were combined the exact concordance rate was 66.0%. All 67 cases with local review GS 2–4 were upgraded to GS

Table 1. *Preoperative clinicopathological characteristics*

No. tumor stage (%):	
T1c	1,327 (65.9)
T2a	363 (18.0)
T2b	163 (8.1)
T2c	132 (6.6)
T3	30 (1.5)
No. ng/ml PSA distribution (%):	
4.0 or Less	152 (7.5)
4.1–10.0	1,135 (56.3)
10.1–20.0	543 (26.9)
20.1 or Greater	185 (9.2)

5–6 or more by central review and 36 (53.7%) were upgraded to GS greater than 7. At local review the exact concordance rate of GS 8–10 was significantly lower than that of GS 5–6, 3 + 4 and 4 + 3 ($p = 0.011$, <0.001 and 0.006, respectively). The distribution of GS 2–4, 5–6, 3 + 4, 4 + 3 and 8–10 changed from 3.8%, 32.0%, 33.0%, 15.7% and 15.6% on local review to 0.0%, 26.0%, 40.8%, 23.3% and 9.9%, respectively, on central review.

Concordance for ECE, SVI, LNI and PSM

Concordance for ECE, SVI, LNI and PSM is shown in table 3. Positive rate for each pathological feature was similar between local and central review. Exact concordance rates (κ) between local and central review for ECE, SVI, LNI and PSM were 82.5% (0.59), 97.6% (0.82), 99.6% (0.93) and 87.5% (0.73), respectively. Exact concordance for patients with no ECE, focal ECE and established ECE by central review was 85.8% (946 of 1,102), 57.9% (121 of 209) and 85.0% (271 of 319), respectively.

Of 528 patients with positive ECE on local review 157 had negative ECE on central review (positive to negative rate 29.7%), whereas 129 of 1,102 patients with negative ECE on local review had positive ECE on central review (negative to positive rate 11.7%). For SVI, LNI and PSM the positive to negative rate was 21.5%, 3.9% and 15.6%, and the negative to positive rate was 0.9%, 0.3% and 10.9%, respectively. Of 1,526 patients the complete concordance rate for ECE, SVI, LNI and PSM was 73.5% (1,121 of 1,526).

Pathology Volume

For RP GS we identified 1,063 patients from 10 high volume institutions and 711 from 37 low volume institutions. As shown in table 4 high volume institutions had significantly higher exact concordance between local and central review for RP GS than low volume institutions (60.9% vs 45.9%, $p < 0.001$). Of 1,526 patients with all data for ECE, SVI, LNI and PSM available 962 were from 10 high volume institutions and 564 were from 34 low volume institutions. High volume institutions also had significantly higher exact concordance rates for all of these features than low volume institutions (77.1% vs 67.2%, $p < 0.001$).

Date of Diagnosis

Overall patients originally diagnosed in 2004 to 2005 had a significantly higher exact concordance rate between local and central review than those diagnosed in 1997 to 2003 for RP GS (63.1% vs 48.2%, $p < 0.001$) and all other pathological features (78.6% vs 68.1%, $p < 0.001$, table 4). This improvement in pathological concordance in more recently diagnosed patients was observed at high and low volume institutions.

DISCUSSION

Pathological features on RP specimens are important for physicians to predict the prognosis of each patient.[1,2] Adjuvant radiotherapy or hormonal therapy might be selected for patients with adverse pathological features on RP specimens such as ECE, SVI, PSM and LNI, although RP may offer long-term survival even to such patients.[17–20] However, in reality pathological assessment is not performed uniformly among pathologists and interobserver variability does exist.

For biopsy specimens of prostate cancer interobserver variability for biopsy GS has been abundantly investigated.[4–9] Recent educational efforts by the pathology community might have improved biopsy GS concordance between community hospitals and academic centers.[4] The ISUP consensus also improved GS correlation between biopsy and RP spec-

Table 2. *Concordance for radical prostatectomy Gleason score between local and central review*

	No. CR RP GS*						
RP GS (No. LR)	5–6	3 + 4	4 + 3	8–10	% Exact Concordance	% Under Grading by LR vs CR	% Over Grading by LR vs CR
2–4 (67)	31	25	8	3	0	100	0.0
5–6 (567)	318	189	48	12	56.1	43.9	0.0
3+4 (585)	99	363	112	11	62.1	21.0	16.9
4+3 (279)	10	85	163	21	58.4	7.5	34.1
8–10 (276)	3	62	82	129	46.7	0.0	53.3
Overall (1,774)	461	724	413	176	54.8	25.9	19.2

* No cases of GS 2–4 on central review.

Table 3. *Concordance between local and central review*

LR	CR Pos	CR Neg	% Exact Concordance (κ value)	% Pos LR at RP	% Pos CR at RP	p Value	% Pos to Neg	% Neg to Pos
ECE:			82.5 (0.59)	32.4	30.7	0.291	29.7	11.7
Pos	371	157						
Neg	129	973						
SVI:			97.6 (0.82)	7.4	6.6	0.373	21.5	0.9
Pos	95	26						
Neg	13	1,505						
LNI:			99.6 (0.93)	2.7	2.8	0.767	3.9	0.3
Pos	49	2						
Neg	5	1,858						
PSM:			87.5 (0.73)	34.6	36.4	0.298	15.6	10.9
Pos	461	85						
Neg	113	920						

imens.[21] On the other hand, there are only a few reports regarding interobserver variability for pathological features on RP specimens.[10–12]

Significant discordance between general pathologists and uropathologists for RP GS has been reported in limited patients.[10] However, 22% of RP specimens were not processed by whole mount technique and detailed information for pathological assessment, such as categorization of GS and concordance in each GS, was not mentioned in that study. In our study using whole mount step sections and based on central review according to the ISUP consensus, the exact concordance rate between local and central review was 54.8%, and under grading or over grading in local pathology was observed in 25.9% and 19.2%, respectively. By central review the number of patients with RP GS 2–4, 5–6 and 8–10 decreased, and the number of those with RP GS 3 + 4 and 4 + 3 increased. Local pathologists in our study assigned RP GS 2–4 in 67 (3.8%) patients, whereas none did in central review. For biopsy specimens ISUP recommends that GS 2–4 should rarely, if ever, be diagnosed.[14] Because GS 2–4 was rarely assigned in contemporary RP series from academic centers, we would emphasize that GS 2–4 should seldom be assigned even in RP specimens.[4,21]

Table 4. *Impact of pathology volume and date of diagnosis on pathological concordance*

	No. RP GS Concordance (%)		No. ECE, SVI, LNI + PSM Concordance (%)
Pathology vol:			
Low	326/711	(45.9)	379/564 (67.2)
High	647/1,063	(60.9)	742/962 (77.1)
p Value	<0.001		<0.001
Date of diagnosis:			
1997–2003	473/981	(48.2)	511/750 (68.1)
2004–2005	500/793	(63.1)	610/776 (78.6)
p Value	<0.001		<0.001
Low/1997–2003	195/480	(40.6)	209/303 (62.8)
Low/2004–2005	131/231	(56.7)	170/231 (73.6)
p Value	<0.001		0.007
High/1997–2003	278/501	(55.5)	302/417 (72.4)
High/2004–2005	369/562	(65.7)	440/545 (80.7)
p Value	0.001		0.002

We also found poorer concordance between local and central review for high grade GS (8–10) compared with other GS (5–6, 3 + 4, 4 + 3). Of cases assigned as GS 8–10 by local review 53.3% were downgraded to 5–6, 3 + 4 or 4 + 3 by central review. This poor concordance in high grade GS may contribute to lower concordance between local and central review in our study compared with the previous study on biopsy GS that included only 32 high grade GS cases at local review (66.1% vs 76.5%).[4]

Unlike biopsy specimens with limited sample size, the discrepancy of GS in RP specimens among pathologists may reflect differences of interpretation itself for each Gleason pattern. We believe that this discrepancy can be effectively improved by educational effort as has been observed with biopsy specimens.[4] Indeed recently diagnosed RP specimens in our study showed higher GS concordance than those diagnosed earlier regardless of pathology volume. This improvement may be due to educational efforts by the pathology community as well as personal efforts of each pathologist even before the ISUP consensus. The ISUP consensus may result in further improvement for RP GS concordance between local and central review.

van der Kwast et al studied 552 RP specimens from multiple institutions, and observed poor concordance between local and central review for ECE (57.5%) and PSM (69.4%), and good concordance for SVI (94.0%).[11] Conversely good concordance was observed among 12 expert uropathologists for ECE (91.2%, $\kappa = 0.63$) and PSM (90.4%, $\kappa = 0.74$) in a small selected RP series.[12] We found excellent exact concordance for SVI (97.6%, $\kappa = 0.82$) and LNI (99.6%, $\kappa = 0.93$). The exact concordance rate in our study between local and central review for ECE (82.5%, $\kappa = 0.59$) and PSM (87.5%, $\kappa = 0.73$) was

better than that in a previous study comparing local and central pathologists (κ = 0.33 and 0.45).[11]

Although we should not simply compare κ values between studies because of the difference of data set, there may be some reasons for this difference. Since our study included more recently diagnosed patients, the effect of date of diagnosis as shown in our study may contribute to this higher concordance. Another contributing factor might be the fact that our patients underwent RP for cT1c–3 disease rather than more advanced disease (pT3 or PSM). The high κ values for ECE and PSM in our study as in a study with expert uropathologists (κ = 0.60 and 0.74) do not necessary mean that concordance in local and central pathologists is equivalent to that among expert uropathologists, because the study with expert uropathologists seems to include more difficult cases than our study.[12]

We found low concordance in patients with focal ECE at central review compared with those with established ECE (57.9% vs 85.0%). Since the prostate lacks a true histological capsule, and the boundaries between prostate and surrounding tissue are sometimes poorly defined especially with apical and anterior lesions, interobserver variability for ECE exists even among expert uropathologists.[12,22] ECE criteria for apical or anterior site remain to be established. Most cases in which definite judgment of ECE is difficult to make may have focal ECE.

We assigned PSM only when tumor cells touched the margin of resection. Most patients who were negative for PSM at local review and positive at central review were considered close to the margin by the central pathologist.

We also investigated the positive to negative and negative to positive rates for ECE, SVI, LNI and PSM. Although there were no differences regarding overall positive rates between local and central pathologists for each pathological feature, we found high positive to negative and negative to positive rates except for LNI. For SVI the positive to negative rate was 21.5% despite excellent overall concordance (97.6%).

It was previously reported that concordance between local and central review for ECE, SVI and PSM was essentially the same regardless of pathology volume.[11] We found that for GS and other pathological features local review at high volume institutions had higher concordance with central review than at low volume institutions. In high volume institutions there may be more communication between pathologists and urologists regarding pathological specimens, and pathologists may pay more attention to assessment for RP specimens. As in RP GS more recently diagnosed RP specimens in our study showed higher concordance for pathological features other than GS than those diagnosed at an earlier date in low and high volume institutions.

CONCLUSIONS

Although concordance between local and central pathologists was excellent for SVI and LNI, that for high grade GS, ECE and PSM was less satisfactory. This discrepancy may affect the outcomes of each pathological feature. High volume institutions showed higher concordance than low volume institutions. Although the concordance has recently improved, more educational and/or personal effort is warranted. For more precise pathological assessment we recommend central review for a study with RP specimens from multiple institutions.

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

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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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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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Statins Reduce the Androgen Sensitivity and Cell Proliferation by Decreasing the Androgen Receptor Protein in Prostate Cancer Cells

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BACKGROUND. Statins (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors) are cholesterol-lowering drugs that are widely used to prevent and treat atherosclerotic cardiovascular disease. Recent epidemiological studies suggest that statins reduce serum prostate-specific antigen (PSA) levels and decrease the risk of prostate cancer. In the present study, we determined the molecular mechanisms related to the regulation of PSA, androgen receptor (AR) and cell proliferation in prostate cancer cell lines by statins.
METHODS. Western blotting, quantitative real-time polymerase chain reaction, cytotoxicity analysis and a cell proliferation assay were used to resolve the regulatory role of statins (mevastatin and simvastatin) in three prostate cancer cell lines, RWPE-1, 22Rv1, and LNCaP.
RESULTS. Western blotting revealed that both mevastatin and simvastatin downregulated AR and PSA protein. However, these statins did not downregulate AR mRNA expression, while they decreased PSA mRNA. The protease inhibitor MG132 inhibited the downregulation of AR protein which suggested that statins decreased AR protein levels by increasing AR proteolysis. Furthermore, statins reduced cell proliferation in AR positive cells but not in AR negative cells, suggesting that statins regulate cell proliferation via AR expression. In addition, cell proliferation assay at various concentrations of dihydrotestosterone (DHT) showed that statins decreased androgen sensitivity in LNCaP cells.
CONCLUSIONS. Statins decreased AR protein by proteolysis but not mRNA transcription. The drop in AR levels resulted in a reduction in androgen sensitivity and a decrease in cell proliferation in AR positive prostate cancer cells. *Prostate* © 2010 Wiley-Liss, Inc.

KEY WORDS: **statin; prostate cancer; androgen receptor; prostate specific antigen**

INTRODUCTION

Statins, also known as the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors, are drugs used for cholesterol reduction and are prescribed to treat hypercholesterolemia in 13 million patients in the United States [1]. Several studies in men with prostate cancer, who had a history of statin exposure, have shown risk reduction or improvement in progression free survival. Among patients in the Cancer Prevention Study II Nutrition cohort, the California Men's Health Study, the Veteran Affairs Medical Center Study in Oregon and the US Male Health Professional cohort, there were statistically significant

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reductions in the risk of developing advance prostate cancer among patients using cholesterol-lowering medications for 5 or more years [2–4]. Moreover, other reports have suggested that statins may decrease the levels of serum PSA, a prostate cancer biomarker, and thereby potentially impact on the risk of prostate cancer detection [5,6].

In the present study, we determined the molecular mechanisms related to the regulation of PSA, androgen receptor (AR) and cell proliferation in prostate cancer cell lines associated with the use of statins.

MATERIALS AND METHODS

Cell Culture

Human prostate cancer RWPE-1 (keratinocyte serum free medium), 22Rv1 (RPMI1640 medium), and LNCaP cells (RPMI1640 medium) were cultured in media which were purchased from Invitrogen (San Diego, CA), and contained 10% fetal bovine serum. LNCaP cells that had been propagated between 10 and 30 times were used. Castration-resistant derivatives of LNCaP cells, namely LNCaP-CxR cells (referred to as CxR cells) and hydrogen peroxide-resistant LNCaP cells, namely LNCaP-HPR50 cells (referred to as HPR50 cells) were established and maintained as described previously [7]. 22Rv1-AR-GFP cells, which were derived from 22Rv1 cells and stably expressed AR-Green Fluorescent Protein (GFP) protein, were established as described previously [8].Briefly, 22Rv1 cells were transfected with AR-GFP expressing plasmid that were kindly provided by Dr. Toshihiko Yanase (Fukuoka University, Fukuoka, Japan) [9]. They were cultured for 2 weeks in selection medium containing 500 μg/ml of geneticin (Nacalai Tesque, Kyoto, Japan). Protein expression in the clones that were obtained was verified using Western blotting and a fluorescence microscope (BIOZERO, Keyence, Tokyo, Japan). Isolated clones were maintained in the presence of 500 μg/ml of geneticin. The cell lines were maintained in a 5% CO_2 atmosphere at 37°C.

Antibodies and Reagents

Antibodies against AR (sc-815), PSA (sc-7316), and GFP (sc-8334) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-β-actin antibody, mevastatin, and simvastatin were purchased from Sigma (St. Louis, MO). MG132 and cycloheximide were obtained from Calbiochem (Gibbstown, NJ) and Nacalai Tesque, respectively.

Western Blotting

Whole-cell extracts were prepared as previously described [7,10,11]. The protein concentration was determined using a protein assay kit (Bio-Rad, Hercules, CA), based on Bradford's method. Whole-cell extracts (30 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to polyvinylidene difluoride microporous membranes (GE Healthcare Bio-Science, Piscataway, NJ) using a semi-dry blotter. The blotted membranes were incubated for 1 hr at room temperature with a primary antibody. Membranes were then incubated for 40 min at room temperature with a peroxidase-conjugated secondary antibody. The bound antibody was visualized using an ECL kit (GE Healthcare Bio-Science) and membranes were exposed

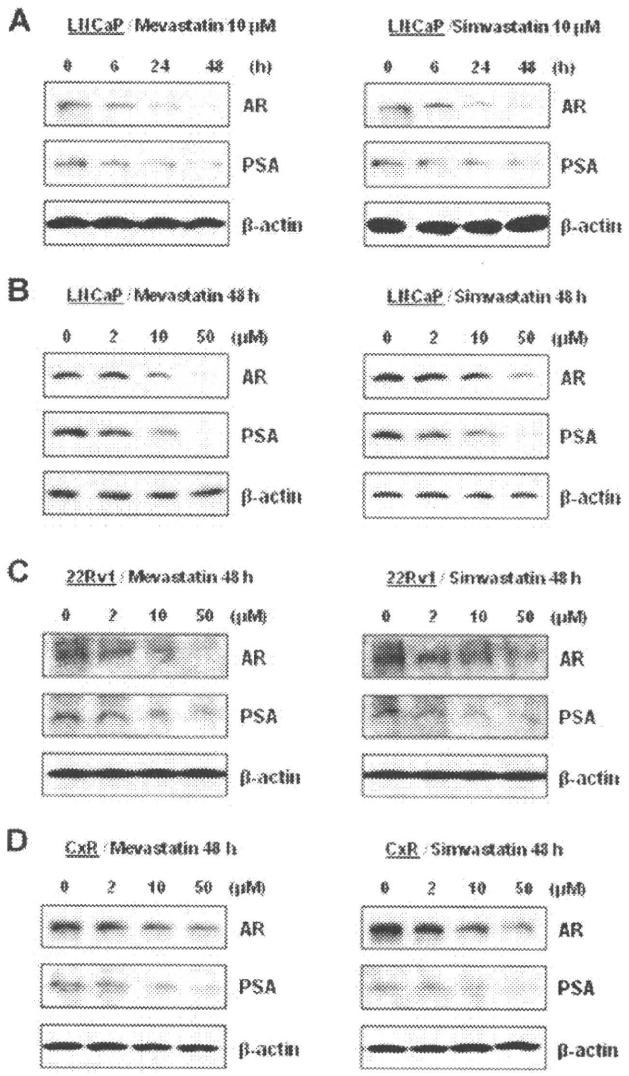

Fig. 1. Downregulation of AR and PSA protein by mevastatin and simvastatin. Mevastatin and simvastatin were added to media containing (**A–D**), LNCaP (A,B), 22Rv1 (C), and CxR (D) cells at the concentrations and for the durations indicated. The whole-cell extracts were subjected to SDS–PAGE. Western blotting was performed using the antibodies indicated.

to high performance chemiluminescence film (GE Healthcare Bio-Science).

RNA Isolation and RT-PCR

RNA isolation and RT-PCR was performed as described previously [7,10,11]. Briefly, total RNA was prepared from culture cells using RNeasy mini kits (Qiagen, Hilden, Germany). First-strand cDNA was synthesized from 1.0 μg of total RNA using a Transcriptor First-Strand cDNA Synthesis Kit (Roche Applied Science, Indianapolis) according to the manufacturer's protocol.

Quantitative Real-Time PCR

Quantitative real-time PCR was performed as described previously [7,10,11]. Briefly, the synthesized cDNA was diluted 1:2 and 2.0 μl of the diluted mixture was used. Quantitative real-time PCR with a TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA) was performed using ABI 7900HT. The expression level of each target gene was corrected for the corresponding GAPDH expression level. The results are representative of at least three independent experiments.

Cytotoxicity Analysis

Cytotoxicity analysis was performed as previously described [7,8]. Briefly, RWPE-1, 22Rv1, LNCaP, CxR, and HPR50 cells (2.5×10^3) were seeded into 96-well plates. The following day the indicated concentrations of mevastatin, simvastatin, and dihydrotestosterone (DHT) were added. After 72 hr, the surviving cells were stained using the Alamar Blue assay (TREK Diagnostic Systems, Cleveland, OH) for 180 min at 37°C. Absorbance in each well was measured using a plate reader (ARVO^{TM} MX; Perkin Elmer, Inc., Waltham, MA).

Statistical Analysis

The Mann–Whitney's *U*-test was used for statistical analysis, and significance was set at the 5% level.

RESULTS

First, using Western blotting we examined whether or not mevastatin and simvastatin downregulated AR and PSA protein expression. As shown in Figure 1, both AR and PSA expression were downregulated in a time and dose dependent manner in LNCaP (A and B), 22Rv1 (C) and CxR (D) cells after the addition of mevastatin and simvastatin. However, quantitative

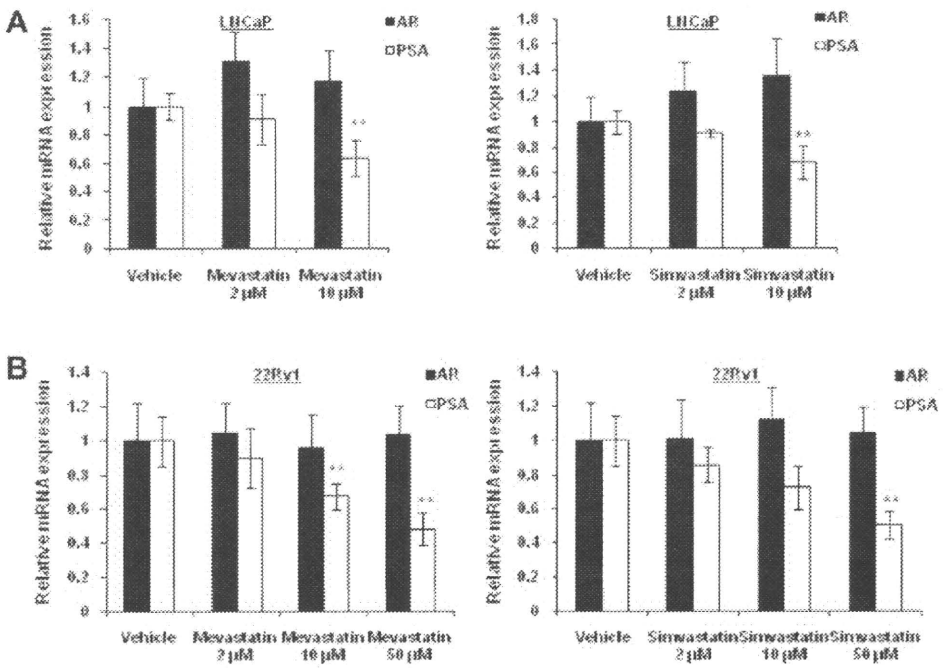

Fig. 2. Downregulation of PSA mRNA expression but not AR mRNA expression by mevastatin and simvastatin. LNCaP (**A**) and 22Rv1 (**B**) cells were exposed to the concentration of mevastatin and simvastatin indicated for a period of 48 hr. Then, the extraction of total RNA and the synthesis of cDNA were performed. Quantitative real-time PCR was performed using the primers and probes for AR, PSA, and GAPDH. The transcript level of the target transcript was corrected with the corresponding GAPDH transcript level. All values represent at least three independent experiments. The AR and PSA transcript levels of cells applied to the vehicle were defined as 1. Boxes: mean values; Bars ± SD, $^{**}P < 0.05$ (compared with that of vehicle).

real-time PCR of AR, PSA, and GAPDH indicated that while mRNA expression of PSA was downregulated by statins, AR was not downregulated. This finding indicated that AR expression was not transcriptionally regulated by mevastatin and simvastatin (Fig. 2). The downregulation of PSA can be explained by the fact that PSA was transcriptionally regulated by AR, as has been previously reported [10]. To confirm that statins regulate AR expression by regulating the protein level rather than the transcriptional level, the proteasome inhibitor MG132 was applied in our experiments. As shown in Figure 3, mevastatin and simvastatin promote AR protein degradation in LNCaP (A) and CxR (B) cells, but this was prevented by MG132. The protein level of PSA was also similarly regulated by the AR protein levels (Fig. 3A,B). Furthermore, the application of cycloheximide, a translational inhibitor, did not affect the down regulation of AR in the presence or absence of dihydrotestosterone (DHT), indicating that statins did not regulate AR expression at the translational level (Fig. 3C). Finally, exogenously introduced expression vector AR-GFP [9] was also downregulated by mevastatin, which again supports the data that statins downregulated AR by proteolysis (Fig. 3D).

Next, the inhibitory effect of statins on cell growth was examined as shown in Figure 4. Mevastatin and simvastatin suppressed cell growth in AR positive cells (LNCaP and 22Rv1) in a dose dependent manner, but this inhibitory effect was limited in AR negative RWPE-1 cells (Fig. 4A,B). Additionally, statins inhibited cell growth, not only in LNCaP cells but also in castration-resistant LNCaP derivative CxR cells [7] and hydrogen peroxide-resistant LNCaP derivative HPR50 [7]. These data possibly suggest that the inhibitory effect of statins is harbored in castration resistant prostate cancer cells.

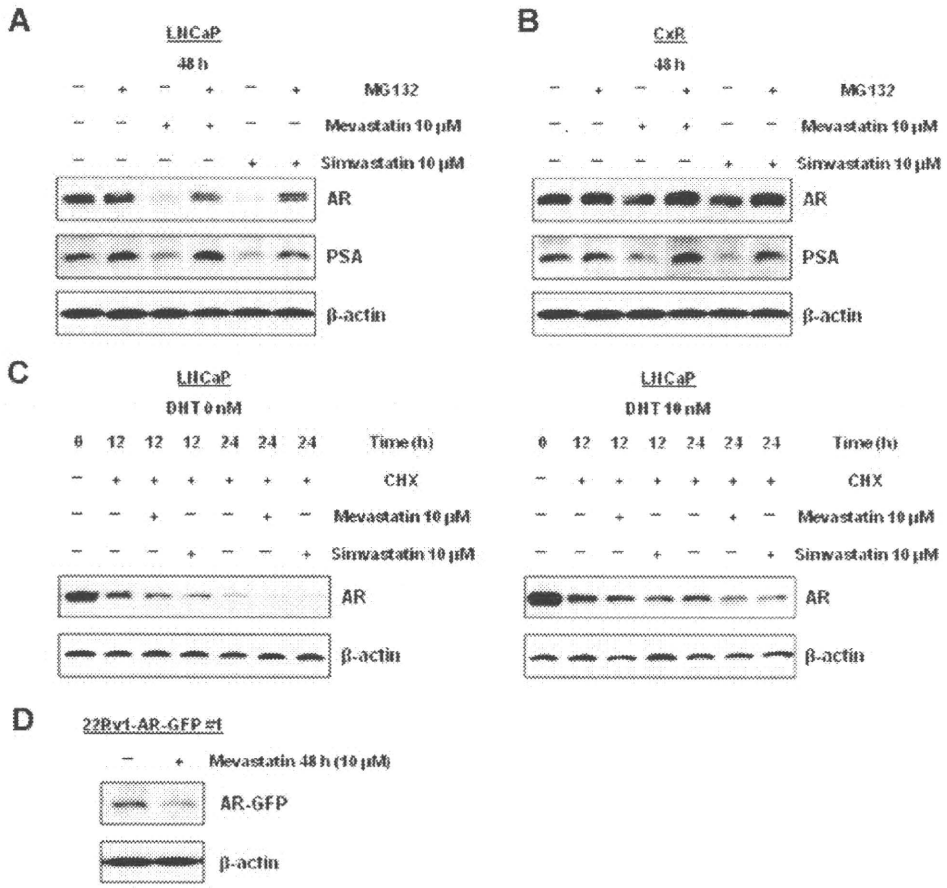

Fig. 3. Promotion of AR degradation by mevastatin and simvastatin. LNCaP (**A**) and CxR (**B**) cells were exposed to the concentrations of mevastatin and simvastatin indicated for a period of 48 hr, with or without 5 μM of MG132. The whole-cell extracts were subjected to SDS–PAGE. Western blotting was performed using the antibodies indicated. LNCaP cells (**C**) were exposed to 10 μM of mevastatin and simvastatin, with or without 1 μg/ml of cycloheximide for the durations indicated in the absence (**left panel**) or presence (**right panel**) of 10 nM of DHT. The whole-cell extracts were subjected to SDS–PAGE. Western blotting was performed using the antibodies indicated. 22Rv1-AR-GFP cells (**D**) were exposed to 10 μM of mevastatin for 48 hr. The whole-cell extracts were subjected to SDS–PAGE. Western blotting was performed using antibodies against GFP and β-actin.

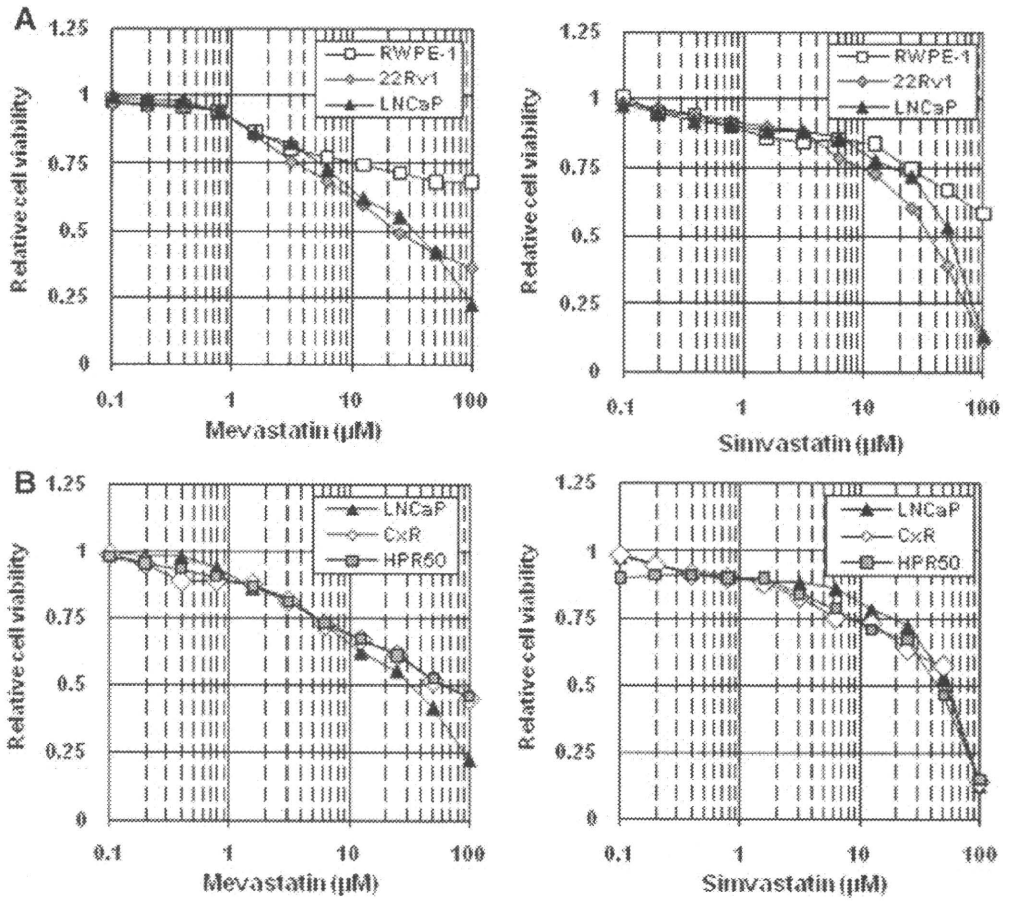

Fig. 4. The growth inhibitory effect of statins on AR negative (RWPE-1) and positive (LNCaP and 22Rv1) cell lines. RWPE-1, 22Rv1, and LNCaP cells (**A**) were seeded into 96-well plates. On the following day, various concentrations of mevastatin or simvastatin were added. After 72 hr the cell survival rates were evaluated using cytotoxicity analyses. Cell survival in the absence of mevastatin or simvastatin was set as 1. All values are representative of at least three independent experiments. Data represent mean value of LNCaP, CxR, and HPR50 cells (**B**) were subjected to cytotoxicity analyses as described in (A).

Finally, the influence of statins on androgen sensitivity was investigated as shown in Figure 5. LNCaP cell proliferation was increased in a dose dependent manner up to 10 nM of DHT, but cell growth of LNCap became saturated at concentrations of DHT exceeding 10 nM (Fig. 5). However, this growth saturation disappeared when 10 μM of mevastatin and simvastatin was added, suggesting that statins decreased androgen sensitivity in LNCaP cells (Fig. 5).

DISCUSSION

Our in vitro experiments clearly showed that statins decreased the level of AR protein by proteolysis, which resulted in a reduction in androgen sensitivity and cell proliferation in AR positive prostate cancer cells. These observations could support epidemiological evidence that indicated that PSA levels declined significantly after the initiation of statin treatment in a cohort of 1214 men in the Durham Veterans Affairs Medical Center 5 study and a cohort of 962 men in the University of Rochester Medical Center study [12].

Several molecular mechanisms had been investigated in relation to the correlation between statin exposure and prostate cancer cell proliferation. Sekine et al. [13] reported that simvastatin suppressed proliferation and induced apoptosis in PC-3 cells, and that the expression of insulin-like growth factor 1 receptor (IGF-1R) was suppressed by simvastatin. This IGF-1R pathway might be important in the inhibitory effect of simvastatin on prostate cancer cell proliferation, especially AR negative prostate cancer cells such as PC-3 cells [13]. Unfortunately, Sekine et al. failed to examine the correlation between statins and AR. As has previously been reported, PC-3 cells are AR-negative cells [14]. In addition, Hong et al. [15] reported that lovastatin inhibited AR-positive LNCaP cell proliferation. Their study focused on Chinese red yeast rice that contains a mixture of eight different monacolins, and monacolin K was identical to lovastatin. They found

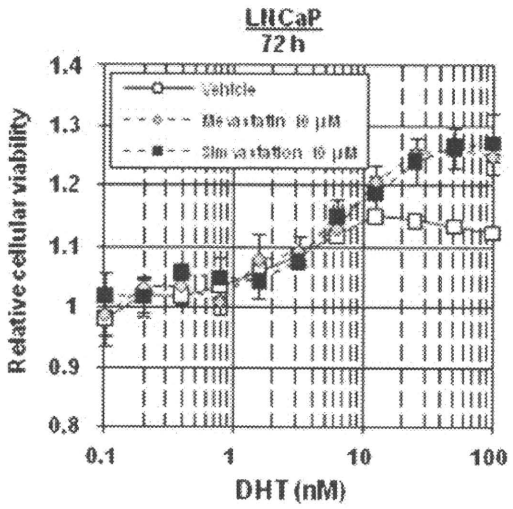

Fig. 5. Decreased androgen sensitivity by mevastatin and simvastatin. LNCaP cells were seeded into 96-well plates with vehicle, 10 μM of mevastatin or simvastatin. On the following day various concentrations of DHT were added. After 72 hr the cell survival rates were analyzed by cytotoxicity analyses. Cell survival in the absence of DHT was set as 1. All values are representative of at least three independent experiments. Data represent means ± SD.

that Chinese red yeast rice inhibited cholesterogenesis and had an inhibitory effect on LNCaP cells. They observed a similar statin induced growth inhibitory effect on LNCaP cells to that observed in the present study, but they failed to examine the precise molecular mechanism involved.

On the other hand, statins may influence prostate cancer cell growth by changing steroid sex hormone biosynthesis. Statins could alter the balance of steroid hormones by two methods. They could reduce the levels of cholesterol, a required intermediate in steroid synthesis, by affecting cytochrome P450 which is an enzyme complex involved in steroid-hormone metabolism [16]. Circulating androgen levels have been reported to be unchanged in statin users [17,18]. However, it has been suggested by Carruba that intraprostatic hormone levels are more important than circulating levels of hormone in prostate carcinogenesis [19]. As of yet, no published research has addressed the effects of statins on either intraprostatic sex-steroid levels or on circulating estrogen levels in men [20].

AR proteins undergo systematic protein degradation via the ubiquitin-proteasome pathway (UPP) [21]. Degradation via the UPP involves two discrete and successive steps. The first is a covalent attachment of multiple ubiquitin molecules to the AR protein to form a polyubiquitin chain and the second is degradation of the tagged protein by the 26S proteasome or, in certain cases, by lysosomes/vacuoles [21]. The precise molecular mechanism related to how statins affect the UPP are still unknown and further investigations are needed to elucidate the correlations between the UPP and downregulation of AR by statins.

CONCLUSIONS

This is the first report that has demonstrated that statins can downregulate AR protein in AR positive prostate cancer cells by proteolysis, resulting in a reduction in androgen sensitivity and cell proliferation.

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BRACHYTHERAPY

Differences between intraoperative ultrasound-based dosimetry and postoperative computed tomography-based dosimetry for permanent interstitial prostate brachytherapy

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ABSTRACT

PURPOSE: To compare the results of intraoperative ultrasound (US)-based dosimetry with those of postimplant computed tomography (CT)-based dosimetry after ^{125}I prostate brachytherapy.

METHODS AND MATERIALS: Subjects comprised 160 patients who underwent prostate brachytherapy using ^{125}I seed implants. Prescribed dose was set as 145 Gy to the periphery of the prostate. Implantation was performed using an intraoperative interactive technique. Postimplant dosimetry was performed on Days 1 and 30 after implantation using CT. Dosimetric results for the prostate, urethra, and rectum were compared among intraoperative US and CT on Day 1 (CT_1) and Day 30 (CT_{30}).

RESULTS: Mean minimal dose received by 90% of prostate volume was 133.7%, 115.6%, and 125.8% of the prescribed dose on US, CT_1, and CT_{30}, respectively: This value temporarily decreased on Day 1 and increased on Day 30. Other parameters for the prostate and urethra showed similar trends. Conversely, mean rectal volume receiving 100% of the prescribed dose was 0.69, 0.46, and 1.02 mL on US, CT_1, and CT_{30}, respectively. Rectal parameters tended to be underestimated on US relative to CT_{30}-based dosimetry. A positive linear relationship was identified between US and CT observations for every prostate parameter and the dose covering 30% of the urethra.

CONCLUSIONS: Our results demonstrate significant differences between dosimetric parameters obtained by US, CT_1, and CT_{30}. However, significant correlations also exist between US and CT, at least in prostate and urethral parameters. Clarification of the degrees of difference might make US planning more feasible.

Keywords: Prostate cancer; Brachytherapy; Dosimetry; ^{125}I; Ultrasound; Computed tomography

Introduction

Ultrasonography (US)-guided transperineal interstitial permanent prostate brachytherapy for prostate cancer is quickly growing in popularity as a therapeutic option for patients with early stage, localized prostate cancer (1–3). With prostate brachytherapy, treatment planning is performed using US, whereas postimplant analysis is performed using computed tomography (CT). Inherent dosimetric differences thus exist between the US plan and postimplant CT analyses because of the different modalities, timings, and body positions used.

Although one of the purposes of postimplant dosimetric analysis is to provide feedback to the clinician for improving implantation technique, few data have been reported regarding differences between these two modalities, making such feedback difficult to interpret. We believe that the lack of information regarding differences between

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Conflicts of interest: Any actual or potential conflicts of interest do not exist in this report.

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preplan and postimplant analysis represents a crucial issue. The present study investigated differences in dosimetry between intraoperative US and postimplant CT analysis on Day 1 (CT_1) and Day 30 (CT_{30}).

Methods and materials

Patients

Subjects comprised 160 patients treated using intraoperative planning technique, with 43 patients treated at Iwate Medical University Hospital and the remaining 117 patients treated at Kitasato University Hospital. According to our treatment protocol criteria, patients with clinical stage T1c or T2a, prostate-specific antigen level ≤10 ng/mL, and Gleason score ≤7 are basically treated with permanent prostate brachytherapy as monotherapy. Patient characteristics are summarized in Table 1.

Intraoperative planning and implantation

Total activity and number of ^{125}I seeds for implantation were determined from preoperative prostate US using a nomogram (4). Intraoperative, real-time, interactive treatment planning was performed. Two radiotherapy planning systems (Interplant version 3.2; CMS, Tokyo, Japan and Variseed version 7.2; Varian Medical Systems, Palo Alto, CA) were used for planning and postimplant analysis, and all doses were defined using TG43 criteria (5). Both of these systems include a built-in optical encoder in the probe-stepping mechanism that permits real-time images from US to be spatially registered against the positions of the probe and template, allowing instant operator feedback on probe position within the prostate.

Transrectal US imaging was performed in the operating room and images were imported into the planning systems. The prostate, urethra, and anterior part of the rectum were contoured at 5-mm intervals. A Foley catheter or bubbled jelly was used to identify the urethra. Regarding rectal contouring, the anterior one-third of the wall was contoured because the US field is restricted to this area. Treatment planning was then performed and a dose–volume histogram (DVH) generated. Needles were inserted according to the treatment plan. After needle insertion, transrectal US imaging was performed once again and refinement of contour and treatment plan was done. Then, seeds were implanted to peripheral portion of the prostate using a Mick applicator (Mick Radio-Nuclear Instruments, Mount Vernon, NY). Dosimetry was updated according to the estimated position of deposited seeds along the needle track. If necessary, second refinement of treatment plan was performed before implantation to the central zone of prostate. The final US-based dosimetry and DVH were obtained in the operating room at the end of the procedure.

Table 1
Patient characteristics

Age (y)	68 (51–81)
Initial PSA (ng/mL)	6.0 (2.7–29.1)
Gleason score	
≤6	86
7	72
8	2
T stage	
T1c	126
T2a	23
T2b	10
T2c	1
Neoadjuvant hormone	
Yes	46
No	114

Values represent median (range) or number.
PSA = prostate-specific antigen.

Postoperative dosimetry

CT with 3-mm slice thickness was performed for postoperative evaluation at 1 day and 30 days after implantation. The prostate, urethra, and rectum were contoured on each of the CT slices by the same physician in each hospital. A Foley catheter was used to identify the urethra on CT_1. However, we virtually contoured the urethra without catheter on CT_{30} by referring to US or CT_1 images. Thus, urethral doses on CT_{30} might not be reliable measurements. The entire rectum, including sphincter muscle and filling was outlined on the same slice to prostate. All seeds were identified and accounted for in CT-based dosimetry analysis using Interplant or Variseed. Dosimetric parameters, including the dose covering 90% of prostate volume (pD_{90}), prostate volume covered by 100% of the prescription dose (pV_{100}), prostate volume covered by 150% of the prescription dose (pV_{150}), dose covering 90% of the urethra (uD_{90}), dose covering 30% of the urethra (uD_{30}), rectal volume covered by 100% of the prescription dose (rV_{100}), and rectal volume covered by 150% of the prescription dose (rV_{150}) were calculated. DVH parameters were compared among intraoperative US, CT_1, and CT_{30}.

Because US, CT_1, and CT_{30} were performed in different timing, this comparison was inevitably influenced by volumetric change of prostate caused by edema. In addition, probe insertion could change the shape of prostate, urethra, and rectal wall in US-based dosimetry. Thus, our study was inevitably influenced by the effect of edema and probe insertion. However, with regard to feedback from CT-based analysis to US-based planning, we believe that simple comparison, including these influences in the same way as clinical practice is most useful.

Statistics

SPSS version 11.01.j (SPSS Japan, Tokyo, Japan) statistical software was used for data analysis. Dependent *t* tests